Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 38–47
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Modulation of insulin resistance in ovariectomized rats by endurance exercise training and estrogen replacement

Vitoon Saengsirisuwan*, Somrudee Pongseeda, Mujalin Prasannarong, Kanokwan Vichaiwong, Chaivat Toskulkao

Exercise Physiology Laboratory, Department of Physiology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand

Received 10 January 2008; accepted 6 August 2008

Abstract

Estrogen is known to play a role in fat metabolism, but its role in carbohydrate metabolism remains controversial. We investigated alterations in carbohydrate and fat metabolism after prolonged estrogen deprivation by determining body weight, food intake, visceral fat content, serum lipids, glucose tolerance, and insulin action on glucose transport activity in isolated soleus and extensor digitorum longus muscles. In addition, effects of endurance exercise training with or without estrogen replacement on metabolic alterations occurring under estrogen deficiency were examined. Female Sprague-Dawley rats were ovariectomized (OVX) or sham-operated (SHAM). The OVX rats remained sedentary, received 5 μg of 17β-estradiol (E_2), performed exercise training (ET), or underwent both estrogen treatment and exercise training (E_2 + ET) for 12 weeks. Compared with SHAM, OVX animals had greater final body weights, visceral fat content, and serum levels of total and low-density lipoprotein cholesterol ($P < .05$). Exercise training and E_2 significantly reduced body weights (6% and 25%), visceral fat (37% and 51%), and low-density lipoprotein cholesterol level (19% and 26%). Exercise training alone improved whole-body glucose tolerance (29%), which was enhanced to the greatest extent (51%) in the ET rats that also received E_2. Insulin-stimulated glucose transport activity in OVX group was lower than that in SHAM by 29% to 44% ($P < .05$). Exercise training and E_2 corrected the diminished insulin action on skeletal muscle glucose transport in OVX animals, which was partly due to elevated glucose transporter–4 protein expression. These findings indicate that 12 weeks of ovariectomy caused metabolic alterations mimicking features of the insulin resistance syndrome. Furthermore, these metabolic disturbances were attenuated by ET or E_2, whereas the beneficial interactive effects of ET and E_2 on these defects were not apparent.

1. Introduction

Estrogen insufficiency in postmenopausal women is a status of permanent cessation of menstruation resulting from loss of ovarian follicular activity. Postmenopausal women are at risk for increased incidence of metabolic and cardiovascular abnormalities including obesity, type 2 diabetes mellitus, and cardiovascular disease [1]. Alterations in lipid metabolism and body fat distribution with estrogen deficiency are thought to be substantial causal factors [2], which are believed to contribute to an increased prevalence of insulin resistance syndrome in postmenopausal women compared with premenopausal population [3]; and this postmenopausal worsening of metabolic profile may in turn contribute to higher risk of cardiovascular disease.

Ovariectomized (OVX) animals have been used as models for studying the effects of ovarian hormone deficiency. It is well established that an increased daily energy consumption [4,5], body mass [4-6], and fat mass [7] occur as a result of ovariectomy. However, there are conflicting data on whether ovariectomy also results in impaired energy and substrate utilization. Ovariectomy in rats leads to decreased [8,9] or unchanged tolerance to glucose challenge [4,10]. Decreased insulin-stimulated glucose transport in skeletal muscle of OVX rodents has been reported [8,11]. On the other hand, Hansen et al [12] found that moderate duration of ovariectomy does not alter insulin action on muscle glucose transport but instead leads to a decline in contraction-stimulated glucose transport activity.

* Corresponding author. Tel.: +66 2 201 5504; fax: +66 2 354 7154.
E-mail address: scvss@mahidol.ac.th (V. Saengsirisuwan).

0026-0495/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.08.004

Hormone replacement therapy is one of the most effective therapies used to reduce unfavorable postmenopausal complications such as osteoporosis [13] despite its adverse effects, such as an increased incidence of breast cancer and a transient increase in venous thromboembolism [14]. In addition, hormone replacement therapy has been demonstrated to attenuate hypercholesterolemia [15], coronary heart disease [16], and risk of developing type 2 diabetes mellitus [17]. In OVX rodents, estrogen replacement decreases fat accumulation, improves serum lipid profiles [18,19], and restores insulin action on muscle glucose transport [8,11].

It is well established that endurance exercise training enhances whole-body insulin sensitivity and insulin action on muscle glucose disposal in healthy individuals [20] and in normal rodent models [21,22]. Moreover, previous studies clearly demonstrated that endurance exercise training is an effective intervention that leads to enhancements of insulin-mediated glucose metabolism in animal models of obesity-associated insulin resistance [23,24] as well as in humans with glucose intolerance [25,26]. For example, improved insulin action on the glucose transport process after exercise training in the obese Zucker rat, an animal model of severe skeletal muscle insulin resistance, occurs primarily through an up-regulation of glucose transporter (GLUT)–4 protein expression [27,28] and enhanced GLUT-4 translocation to the sarcolemma [29]. Whereas increasing evidence demonstrated the benefits of exercise training on insulin action in the obese Zucker rat, the effects of exercise training on insulin action in conditions with metabolic disturbances caused by prolonged estrogen deprivation have not been investigated. Furthermore, although it is clear that both endurance exercise training and hormone replacement individually improve insulin action in insulin-resistant skeletal muscle, at present, there is no information regarding how these 2 interventions might interact to modulate insulin action in prolonged estrogen-deficient animals.

In this context, the objectives of this study were to assess whether the estrogen-deprived condition leads to phenotypic characteristics of the insulin resistance syndrome by comparing central body fatness, whole-body glucose tolerance, insulin action on muscle glucose transport, and serum lipid profile in female rats after a 12-week period of ovariectomy with those of sham-operated animals. This investigation also examined the hypothesis that endurance exercise training and hormone replacement in combination could be beneficial to insulin action in OVX animals to a greater extent than either intervention used individually.

2. Material and methods

2.1. Animals and interventions

Eight-week–old female Sprague-Dawley rats, weighing between 180 and 200 g, supplied by the National Laboratory Animal Center, Thailand, were individually housed in 8 × 10–in hygienic hanging cages at the Center of Animal Facilities, Faculty of Science, Mahidol University. They were given regular rat chow (Perfect Companion, Samutprakarn, Thailand) and water ad libitum. The housing unit was maintained at 22°C with a reversed 12/12-hour light/dark cycle (light on from 6:00 PM to 6:00 AM) so that exercise training occurs during the dark cycle when the animals are most active. Animal procedures were approved by the Institution of Animal Care and Use Committee, Faculty of Science, Mahidol University, in accordance with those of the National Laboratory Animal Center, Thailand. Rats were randomly assigned to either sham operation (SHAM, n = 10) or bilateral ovariectomy (n = 40). Surgical procedure was performed under anesthesia through bilateral skin incision at the lower back as previously described [30]. Animals were allowed to recover for 7 days after surgery. Ovariectomized animals were further divided into 4 groups: OVX control (OVX, n = 10), OVX given 17β-estradiol (OVX + E_2, n = 10), OVX undergoing endurance exercise training (OVX + ET, n = 10), or OVX undergoing endurance exercise training and given 17β-estradiol (OVX + ET + E_2, n = 10).

The SHAM rats were injected subcutaneously with 0.1 mL of corn oil, whereas the OVX rats were randomly divided into 2 groups and subcutaneously injected with 0.1 mL of corn oil with or without 5 μg 17β-estradiol (Sigma Chemical, St Louis, MO) 3 times per week for 12 weeks as previously described [30]. Exercise-trained rats ran in the morning on a motorized treadmill 5 days per week throughout the 12-week experimental period. During the first 2 weeks, animals were accustomed to exercise by running at low exercise intensity (15 m/min at 0% grade for 5-15 min/d). The duration of training, speed, and slope of the running track were gradually increased to 60 min/d by week 6, continuously rotating through the following 15-minute cycles: 18 m/min for 3 minutes, 22 m/min for 5 minutes, 25 m/min for 5 minutes, and 27 m/min for 2 minutes at 4% grade. A steam of air blown on the backs of the animals was used to encourage animals to run. Animals that received combined treatment underwent exercise training while also receiving estrogen replacement as described above.

Body weight was monitored every other day. The amount of food intake of each animal was measured over a 24 hour period using a metabolic cage, and the measurements were conducted at least 3 times per week. Each animal was initially provided with 30 g of pellet rat chow. At the same time on the following day, the amount of food intake of each animal was calculated from the weight of food remaining in feeding sieve and discarded chow.

2.2. Oral glucose tolerance tests

After a 12-week period of treatments, oral glucose tolerance test (OGTT) was performed on each animal as follows. In the evening (6:00 PM) of the day before the test, rats were food-restricted to 4 g of chow. On the day of the test (9:00 AM), approximately 24 hours after the last E_2 treatment

and/or the final exercise bout, tail blood was collected before glucose feeding (1 g/kg body weight) by gavage and 15, 30, 60, and 90 minutes after glucose challenge. Blood sample was mixed with anticoagulant (18 mmol/L final concentration of EDTA) and centrifuged at 13 000*g* at 4°C for 1 minute. Plasma was kept at −80°C and used for determination of glucose (Gesellschaft fur Biochemica und Diagnostica, Wiesbaden, Germany) and insulin (Linco Research, St Charles, MO). Immediately after OGTT, each animal was given 2.5 mL of sterile 0.9% saline subcutaneously to replace fluid loss.

2.3. Assessment of muscle glucose transport activity

Five days after OGTT, animals were food-restricted as described above. At 8:00 AM, approximately 24 hours after the last E_2 treatment and/or the last training session, animals were weighed and anesthetized with an intraperitoneal administration of a mixture of ketamine (50 mg/kg body weight; Calypsol Inj, Gedeon Richter, Hungary) and xylazine (10 mg/kg body weight; Xylaz, Farvet Laboratories, Bladel, the Netherlands). Soleus (SOL) and extensor digitorum longus (EDL) muscles were isolated. Whole muscle was divided into 2 portions of approximately 20 to 25 mg and incubated for 60 minutes at 37°C in 3 mL of oxygenated Krebs-Henseleit buffer (KHB) supplemented with 8 mmol/L D-glucose, 32 mmol/L D-mannitol, and 0.1% radioimmunoassay-grade bovine serum albumin (BSA) (Sigma Chemical). One strip of EDL and 1 strip of SOL were incubated in the absence of insulin, and the other strips were incubated in the presence of a maximally effective concentration of insulin (2 mU/mL; Human R, Eli Lilly, Indianapolis, IN). The flasks were continuously gassed with a mixture of 95% O_2 and 5% CO_2 throughout the incubation and transport study procedure. After the first incubation period, muscle strip was rinsed for 10 minutes at 37°C in 3 mL of oxygenated KHB containing 40 mmol/L D-mannitol, 0.1% BSA, and insulin, if previously present. Muscle strip was then incubated for 20 minutes at 37°C in 2 mL of KHB containing 1 mmol/L 2-[1,2-^{3}H]deoxyglucose (2-DG, 300 μCi/mmol; PerkinElmer Life Sciences, Boston, MA), 39 mmol/L [U-^{14}C]mannitol (0.8 μCi/mmol, PerkinElmer Life Sciences), 0.1% BSA, and insulin, if previously present. At the end of the incubation period, muscle was removed and trimmed of excess fat and connective tissue, immediately frozen with liquid nitrogen, and weighed. The frozen muscles were solubilized in 0.5 mL of 0.5 N NaOH, and then 10 mL of scintillation cocktail (Ultima Gold, PerkinElmer Life Sciences) was added. The specific intracellular accumulation of 2-DG was determined as described previously [31] using mannitol to correct for extracellular accumulation of 2-DG. Glucose transport activity was measured as the intracellular accumulation of 2-DG (in picomoles per milligram muscle wet weight per 20 minutes). The contralateral SOL and EDL muscles were also removed, trimmed of fat and connective tissue, quickly frozen in liquid nitrogen, and used for determination of GLUT-4 protein level.

2.4. Measurements of serum lipids

After muscle tissues were removed, blood was collected from abdominal vein into 5 mL syringe. Whole blood was allowed to clot and then centrifuged at 3000*g* for 20 minutes to obtain serum, which was kept at −80 °C until determination of serum lipids. Serum levels of total cholesterol (TC), high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL) were determined using Dimension RxL Max (DADE Behring, Marburg, Germany).

2.5. Determination of heart, uterine, and visceral fat weights

Immediately after completion of blood collection, heart was detached; and blood remaining in the heart chambers was drained. Heart was frozen in liquid nitrogen and weighed. Visceral fat was collected from the superficial area covering the alimentary tract, and the uterine was removed and weighed.

2.6. Muscle GLUT-4 protein content

Portions of muscles were homogenized as previously described [32]. These homogenates were used for determination of total protein (bicinchoninic acid method, Sigma Chemical) and then were solubilized in Laemmli buffer [33]. Samples were separated in 12% precast polyacrylamide gel (Pierce, Perbio Science, Northumberland, England) and transferred electrophoretically onto nitrocellulose paper. Protein blots were incubated with polyclonal antibody against GLUT-4 (Santa Cruz Biotechnology, Santa Cruz, CA) and subsequently were incubated with horseradish peroxidase–conjugated (immunoglobulin G–horseradish peroxidase) secondary antibody (Cell Signaling Technology, Beverly, MA). Protein bands were visualized by enhanced chemiluminescence (Cell Signaling Technology) on hyperfilm (Amersham Biosciences, Buckinghamshire, England). Images were digitized and band intensities were quantified using an Image Master TotalLab Software, version 3.0 (Amersham Pharmacia Biotech, Uppsala, Sweden).

2.7. Determination of protein carbonyl content

The level of protein carbonyl formation is an indicator of tissue oxidative damage [34]. Protein carbonyl contents were assessed by using a spectrophotometric assay as described previously [35]. In brief, pieces of frozen tissues (30-50 mg) were homogenized in 0.75 mL of 50 mmol/L phosphate buffer (pH 7.4) containing 1 mmol/L EDTA, 0.1% digitonin, and protease inhibitors (40 μg/mL phenylmethylsulfonyl fluoride, 5 μg/mL leupeptin, 7 μg/mL pepstatin, 5 μg/mL aprotinin). Nucleic acids were removed with 1% streptomycin sulfate. The extracted soluble protein was then reacted with 10 mmol/L 2,4-dinitrophenylhydrazine in 2.5 mol/L HCl for 1 hour in the dark at room temperature. Proteins were precipitated with

Table 1
Initial and final body weight; total energy intake; and uterine, heart, and fat weight in SHAM and OVX rats that received E_2 and ET

	SHAM	OVX	OVX + E_2	OVX + ET	OVX + E_2 + ET
BW (g)					
Initial weight	239.64 ± 2.73	236.29 ± 3.30	241.38 ± 3.96	235.95 ± 3.31	235.85 ± 3.52
Final weight (BW)	288.91 ± 2.97	322.54 ± 5.35*	254.65 ± 5.34*,†	306.68 ± 7.29*,†	247.89 ± 3.78*,†
BW gain/d (g)	0.96 ± 0.05	1.43 ± 0.07*	0.50 ± 0.04*,†	1.12 ± 0.06*,†	0.49 ± 0.05*,†
Total energy intake (kcal × 10^3)	3.99 ± 0.16	4.53 ± 0.15*	3.91 ± 0.12†	4.25 ± 0.10	3.83 ± 0.14†
UW (mg)	455.76 ± 41.72	146.93 ± 18.16*	810.58 ± 44.61*,†	173.31 ± 26.07*	695.48 ± 34.36*,†
UW/kg BW	1.58 ± 0.14	0.46 ± 0.06*	3.18 ± 0.18*,†	0.57 ± 0.09*	2.81 ± 0.14*,†
HW (mg)	1005.36 ± 18.13	933.89 ± 46.45	891.52 ± 17.92*	978.14 ± 24.80	889.57 ± 44.72*
HW/kg BW	3.48 ± 0.06	2.90 ± 0.14*	3.50 ± 0.07†	3.19 ± 0.08†	3.59 ± 0.18†
FW (g)	9.88 ± 0.74	12.62 ± 0.84*	6.08 ± 0.63*,†	7.89 ± 0.89†	5.91 ± 0.44*,†,§
FW/kg BW	34.19 ± 2.16	39.12 ± 2.15	23.86 ± 2.64*,†	25.71 ± 3.46*,†	23.83 ± 1.85*,†

Values are means ± SE for 10 animals per group. BW indicates body weight; UW, uterine weight; HW, heart weight; FW, fat weight.
* $P < .05$ vs SHAM group.
† $P < .05$ vs OVX group.
§ $P < .05$ vs OVX + ET.

10% trichloroacetic acid, and the pellets were washed with 1:1 absolute ethanol/ethyl acetate mixture to remove free 2,4-dinitrophenylhydrazine and lipid contaminants. Final precipitates were dissolved in 6 mol/L guanidine HCl and incubated at 37°C for 10 minutes. The carbonyl contents of these samples were assessed by a spectrophotometric assay at 370 nm with an absorption coefficient of 22 000/(mol/L)/cm, whereas protein contents were quantified by reading the absorbance at 280 nm using a BSA standard curve.

2.8. Statistical analysis

All values are expressed as means ± SE. Differences among groups were determined by 1-way analysis of variance with a post hoc Fisher protected least significant difference test. The effects of endurance exercise training, estrogen replacement, and interaction of both interventions in the 4 OVX groups were analyzed by 2-way analysis of variance. Statistical analyses were performed using SPSS 16.0 (SPSS, Chicago, IL). A value of P less than .05 is considered to be statistically significant.

3. Results

3.1. Body and tissue weights

Estrogen deprivation by ovariectomy led to significant weight gain ($P < .001$) compared with SHAM animals (Table 1). Body weight gain observed in OVX rats was significantly reversed by estrogen replacement (25%) or by endurance exercise (6%). However, combined intervention of estrogen and endurance exercise training did not lead to a further reduction in body weight. Effectiveness of ovariectomy was reflected by uterine weights (absolute and relative) of OVX animals being 3- to 4-fold lower than those of SHAM rats (Table 1). Estrogen replacement resulted in 1.5- to 1.8-fold increase in uterine weights of OVX animals when compared with those of SHAM rats. Exercise training resulted in heart weight to body weight ratio of OVX animals being significantly enhanced by 13% (Table 1). As central obesity is one of the major phenotypes of metabolic syndrome, visceral fat weights of the animals were determined. Compared with SHAM rats, a 29% increase ($P < .05$) in visceral fat content was observed in OVX animals (Table 1), which was suppressed ($P < .001$) by intervention of estrogen replacement or exercise training. In addition, combined estrogen replacement and exercise training brought about the greatest reduction in visceral fat weights, with a significant interactive effect when compared with the individual intervention (Table 1).

3.2. Food intake

Total energy intake was calculated by multiplying energy in each gram of rat chow by average daily food intake calculated from at least 3 collections in 1 week during the 12-

Table 2
Serum levels of TC, HDL, and LDL; HDL/TC; and LDL/TC in SHAM and OVX rats that received E_2 and ET

	SHAM	OVX	OVX + E_2	OVX + ET	OVX + E_2 + ET
TC (mmol/L)	2.25 ± 0.09	3.10 ± 0.09*	3.20 ± 0.13*	2.61 ± 0.08*,†	3.16 ± 0.16*
HDL (mmol/L)	0.53 ± 0.03	0.62 ± 0.02	0.98 ± 0.05*,†	0.55 ± 0.02	0.87 ± 0.04*,†
LDL (mmol/L)	1.57 ± 0.07	2.34 ± 0.07*	1.85 ± 0.10*,†	1.97 ± 0.06*,†	1.97 ± 0.10*,†
HDL/TC	0.24 ± 0.01	0.20 ± 0.00*	0.31 ± 0.01*,†	0.21 ± 0.01*	0.28 ± 0.01*,†
LDL/TC	0.70 ± 0.01	0.76 ± 0.01*	0.58 ± 0.03*,†	0.76 ± 0.01*	0.62 ± 0.01*,†

Values are means ± SE for 10 animals per group.
* $P < .05$ vs SHAM group.
† $P < .05$ vs OVX group.

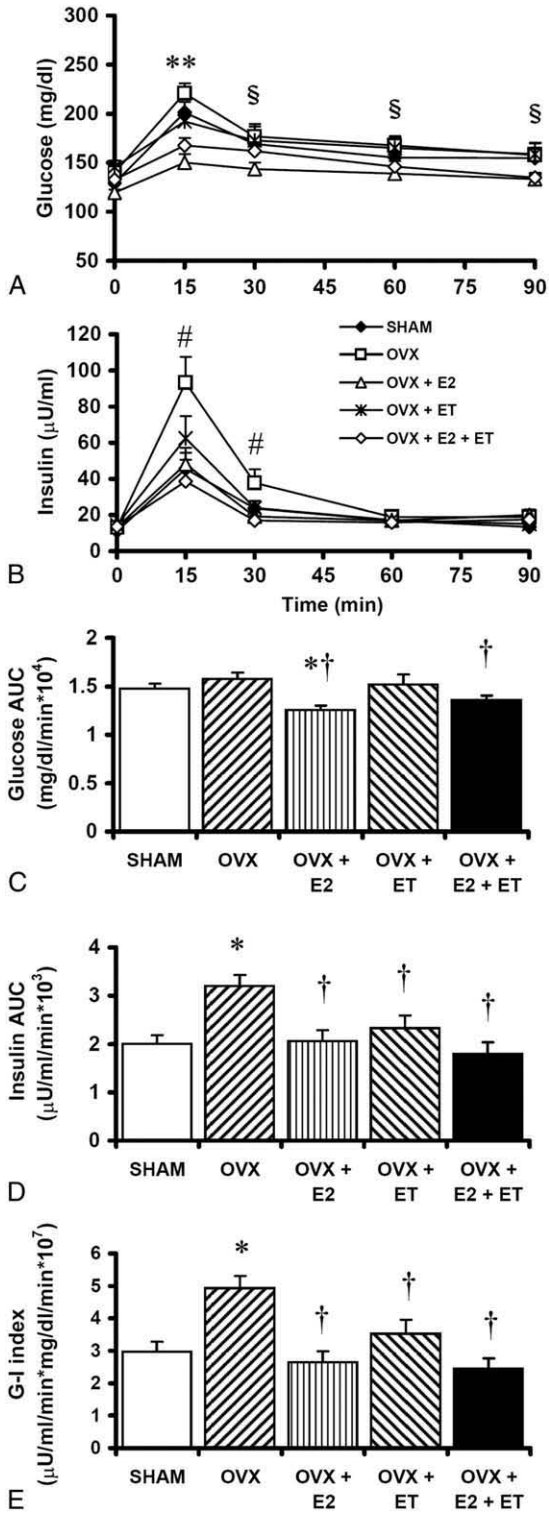

Fig. 1. Glucose (A) and insulin (B) responses during an OGTT, AUC for glucose (C) and insulin (D), and the G-I index (E) in SHAM and OVX rats that received E_2, ET, and E_2 + ET. Data for AUC were calculated from glucose (A) and insulin (B) responses. The G-I index was the product of glucose AUC and insulin AUC for each individual animal. Values are means ± SE for 10 animals per group. $^{*}P < .05$ vs SHAM group; $^{\dagger}P < .05$ vs OVX group; $^{\#}P < .05$ OVX vs all other experimental groups; $^{**}P < .05$ OVX vs OVX + E_2, OVX + ET, OVX + E_2 + ET; $^{\S}P < .05$ OVX vs OVX + E_2, OVX + E_2 + ET.

week experimental period. Total energy intake was significantly higher in OVX rats compared with SHAM and OVX animals that received estrogen replacement (Table 1). Ovariectomized rats that underwent exercise training consumed a comparable total energy intake compared with sedentary OVX rats.

3.3. Serum lipid profile

Serum levels of TC, HDL, and LDL, and the HDL and LDL to TC ratio are demonstrated in Table 2. Compared with SHAM rats, ovariectomy led to a significant increase in TC (38%) and LDL (50%), with an insignificant increase in HDL. Estrogen treatment produced an increase in HDL (63%) and a lowering in LDL (26%) levels. Exercise training alone significantly reduced TC (18%) and LDL (19%) levels without any significant change in HDL. Combined intervention brought about the same lipid profile as estrogen replacement alone.

3.4. OGTT responses

Plasma levels of glucose and insulin in response to OGTT are shown in Fig. 1. The basal levels of insulin and glucose at zero time point were not significantly different among the groups. After glucose challenge, plasma insulin levels in OVX rats were significantly higher than those in the other experimental groups at the 15-minute (34%-59%) and 30-minute time points (37%-55%) (Fig. 1B). These differences were not observed after 30 minutes. At the 15-minute time point, plasma glucose levels in OVX rats were significantly higher than those in the OVX groups that underwent ET and/or received E_2 (14%-33%) and were approximately 10% higher than those in the SHAM group ($P > .05$). Significant differences in plasma glucose levels between the OVX group and the OVX groups that received E_2 were still detected at the 30-minute time point (19%) and lasted throughout the test (Fig. 1A). The total areas under the curves (AUCs) for glucose and insulin were calculated. The product of glucose AUCs and insulin AUCs is defined as the *glucose-insulin index* (G-I index) [24]; and this index was used to reflect whole-body insulin sensitivity, with a low G-I index indicating a high whole-body insulin sensitivity. Whereas glucose AUCs are not significantly different among all experimental groups, ovariectomy resulted in a significant rise in insulin AUCs compared with SHAM group (Fig. 1D). As a result, the G-I index in OVX group was highest among the 5 experimental groups. The G-I index was significantly reduced as a result of estrogen replacement (44%) or exercise training (29%), with the greatest reduction (51%) found in the group that received combined intervention (Fig. 1E). Nevertheless, the interaction between estrogen replacement and exercise training was not statistically significant.

3.5. Muscle glucose transport

Basal and insulin-stimulated glucose transport activities in isolated SOL and EDL muscles are shown in Fig. 2. Basal

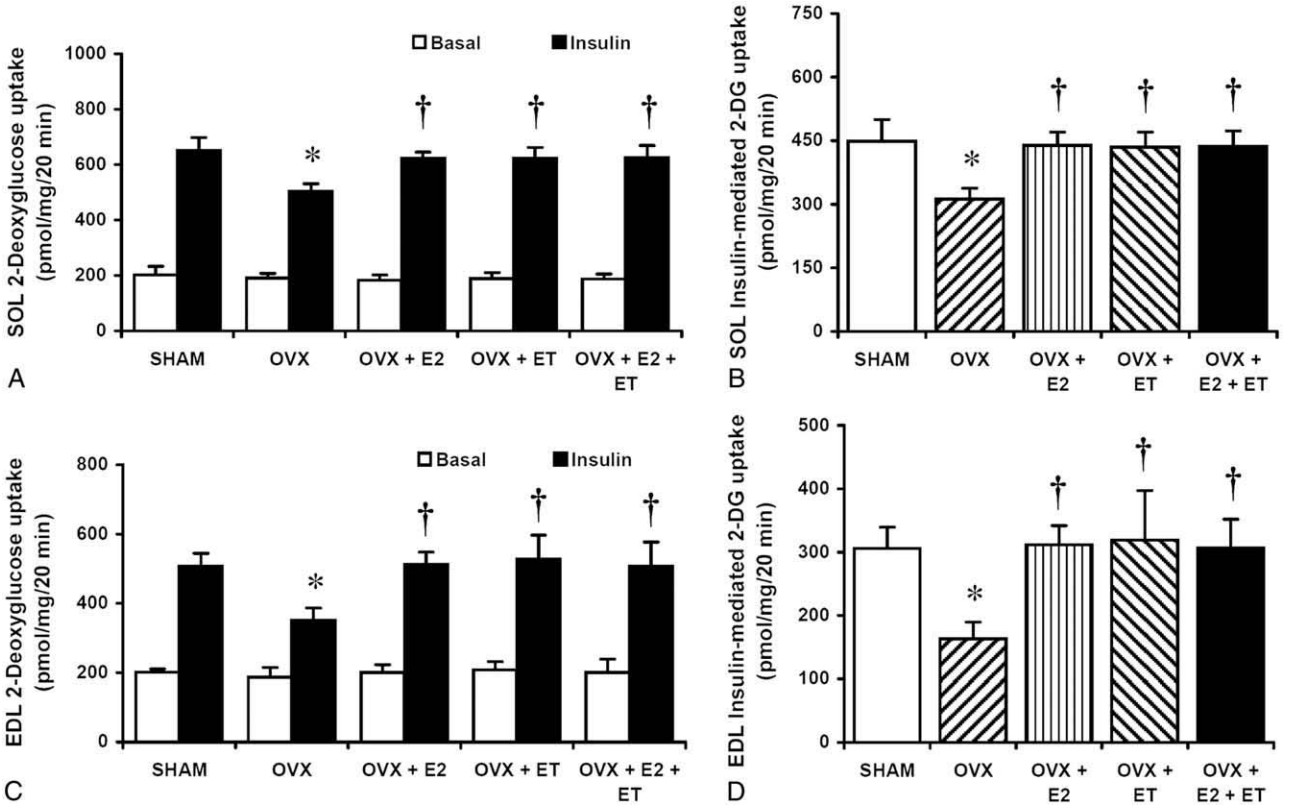

Fig. 2. In vitro rates of 2-DG uptake in SOL (A) and EDL (C) muscles in the absence (blank bars) and presence (filled bars) of insulin (2 mU/mL) and net increase above basal level for 2-DG uptake due to insulin in SOL (B) and EDL (D) muscles of SHAM and OVX rats after receiving E_2, ET, and E_2 + ET. Values are means ± SE for 10 animals per group. $^{*}P < .05$ vs SHAM group; $^{\dagger}P < .05$ vs OVX group.

2-DG uptake was not different among experimental groups in both types of muscle. Compared with SHAM group, insulin-stimulated and insulin-mediated 2-DG transport rate in SOL muscle from OVX rats was reduced by 29% and 43%, respectively. Estrogen supplement enhanced insulin-stimulated (24%) and insulin-mediated (40%) 2-DG uptake. Exercise training improved both parameters to about the same levels as estrogen treatment. In EDL muscle, ovariectomy led to a similar response in insulin-stimulated glucose transport system as observed in SOL muscle. Ovariectomy lowered insulin-stimulated and insulin-mediated 2-DG uptake by 44% and 87%, respectively. Impaired insulin-stimulated and insulin-mediated 2-DG glucose transport activity in EDL muscle was corrected by estrogen replacement (46% and 90%, respectively) or exercise training (50% and 95%, respectively). However, combined estrogen supplementation and exercise training under estrogen-deprived condition did not produce a further improvement in insulin action on glucose transport in both types of skeletal muscle.

3.6. GLUT-4 protein level

A 12-week period of ovariectomy reduced total GLUT-4 protein level by 29% and 32% in SOL and EDL, respectively (Fig. 3). Intervention with estrogen or exercise training alone

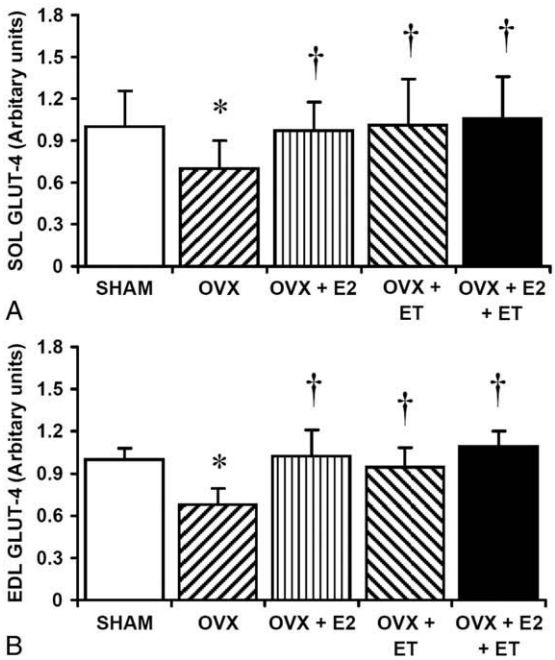

Fig. 3. Effects of E_2, ET, and E_2 + ET on whole muscle level of GLUT-4 protein in SOL (A) and EDL (B) muscles from OVX rats. Data of SHAM group were used as control. Values are means ± SE for 10 animals per group. $^{*}P < .05$ vs SHAM group; $^{\dagger}P < .05$ vs OVX group.

Table 3
Protein carbonyl content in SOL and EDL muscle in SHAM and OVX rats that received E_2 and ET

	SHAM	OVX	OVX + E_2	OVX + ET	OVX + E_2 + ET
SOL (nmol/mg protein)	3.32 ± 0.22	3.20 ± 0.10	3.22 ± 0.07	3.29 ± 0.10	3.16 ± 0.18
EDL (nmol/mg protein)	3.76 ± 0.21	3.85 ± 0.24	3.70 ± 0.22	3.63 ± 0.28	3.51 ± 0.20

Values are means ± SE for 10 animals per group.

significantly restored GLUT-4 content in SOL (27% and 31%, respectively) and EDL (34% and 26%, respectively), whereas the combined intervention did not promote in an additive manner GLUT-4 level in insulin-resistant skeletal muscle induced by estrogen-deprived condition.

3.7. Protein carbonyl level

There is no significant difference in protein carbonyl content in both types of skeletal muscle among the experimental groups (Table 3), suggesting that oxidative damage of proteins is not responsible for adaptive changes in insulin action on muscle glucose transport as the result of ovariectomy, estrogen replacement, or endurance exercise training.

4. Discussion

The major findings of the present investigation emphasize the role of estrogen in the regulation of glucose homeostasis. We have shown that OVX rats exhibited 4 major characteristics of the insulin resistance syndrome, namely, increased visceral fat content, dyslipidemia, impaired glucose tolerance, and defect in insulin-mediated glucose uptake in skeletal muscle. Furthermore, we report a novel observation that endurance exercise training effectively attenuated muscle insulin resistance induced by estrogen-deprived condition. The beneficial effects of exercise training on glucose transport system were comparable to those by estrogen replacement, but the interactive effects by these 2 intervention strategies were not observed.

It is well recognized that ovariectomy results in a substantial decrease in circulating 17β-estradiol level and that OVX animals become hyperphagic and gain weight [4,36], reflecting a state in which positive energy balance occurs because of increased energy intake. As a state of positive energy balance was still observed in studies in which a limited amount of food is given to OVX animals [37], the accelerated rate for energy storage that occurs during an estrogen-deprived state is also due to decreased basal metabolic rate [37,38], decreased thermogenesis [38,39], and reduced voluntary activity [38]. Furthermore, increased energy flux is accompanied by concomitant adaptation of peripheral lipid metabolism that includes induction of pathways involved in fat accumulation, increased hepatic lipid production, and elevated levels of circulating lipoproteins [40]. In the present study, we observed elevated body weight gain, increased visceral fat accumulation, and elevation of circulating levels of TC and LDL. Moreover, our results, together with previous studies [4-6,18], demonstrated that increased weight gain, central adiposity, and dyslipidemia after ovariectomy were reversed by estrogen supplement, findings clearly indicating that estrogen plays an essential role in the control of energy balance and that obesity occurring under estrogen-deprived condition is the combined result of a decline in energy expenditure and an increase in energy intake.

Excessive caloric intake is an essential causal factor leading to insulin-resistant state and diabetes. This notion has been demonstrated in animal models with defect in leptin function [41-43]. As OVX animals are hyperphagic, whether insulin-resistant condition also develops in the OVX animals has been a matter of controversy. Elevated fasting insulin level, indicating whole-body insulin resistance, is observed 5 weeks after ovariectomy [44], whereas insulin sensitivity assessed by a glucose challenge after an 8-week period of ovariectomy is not affected [4]. Our study demonstrated that fasting insulin level in OVX rats was not significantly different from that in SHAM animals; but during a glucose challenge, plasma levels of glucose and insulin in the OVX rats were significantly higher than those in SHAM, which brought about a significant increase in G-I index, indicating a state of whole-body insulin resistance in OVX rats. Because whole-body glucose disposal after an oral or intravenous load is accomplished primarily by skeletal muscle, it was important to determine whether impaired glucose tolerance caused by withdrawal of ovarian hormones is associated with an alteration in insulin action at the tissue level. Glucose transport activities were examined under basal and insulin-stimulated condition in slow-twitch SOL muscle and fast-twitch EDL muscle. Consistent with previous studies [8,11], we demonstrated a substantial decrease in insulin-stimulated glucose transport rate in both types of skeletal muscle. Although these results are contrary to a report by Hansen et al [12], who observed no significant changes in insulin action after a 6-week period of ovarian hormone deficiency, 1 major difference between the 2 studies is the duration of sex hormone deficiency.

Furthermore, we have investigated possible mechanisms that potentially help explain impaired insulin-stimulated glucose transport activity in skeletal muscle. As estrogen is recognized to have antioxidant properties due to the presence of a phenolic group in the steroid structure, prolonged estrogen deprivation may enhance cellular oxidative damage. Previous studies [22,45] using insulin-resistant obese and insulin-sensitive lean Zucker rats indicated that protein oxidative damage in muscle tissue may contribute to insulin resistance. Thus, the level of protein carbonyls, a

tissue marker of a continuous state of oxidative stress, was measured in the present study. Nevertheless, our results indicate that estrogen-deficient condition does not produce protein oxidative damage in muscles from OVX rats. Therefore, these results suggest that skeletal muscle insulin resistance caused by estrogen deficiency was not associated with protein oxidative damage. On the other hand, ovariectomy brought about a significant reduction in GLUT-4 protein level in muscle tissue; and this defect could be reversed by estrogen supplementation, indicating that the dampened action of estrogen on GLUT-4 protein expression may account, at least in part, for muscle insulin resistance under estrogen-deprived state. Although our findings are contradictory with previous reports that showed GLUT-4 protein in OVX rats is unaffected [12,46], a recent study has demonstrated the role of estrogen in regulation of muscle GLUT-4 protein in estrogen receptor knockout mice, showing that reduction in GLUT-4 contributes to the insulin resistance observed in ER$\alpha^{-/-}$ mice [47]. Future research is required to elucidate whether other proteins such as those in the insulin signaling cascade will be affected by the compromised action of estrogen. In addition, because a significant body of literature implicates the causal role of lipid intermediates in impairments of insulin signaling (for a review, see Savage et al [48]), the possibility that elevated lipid intermediates in OVX rats contribute to ovariectomy-induced skeletal muscle insulin resistance by interrupting the insulin signaling pathway remains to be verified.

In addition to increased caloric intake resulting in a state of positive energy balance in OVX rats, it was reported that estrogen-deprived condition also leads to reduced voluntary activity [38]. We found that the animals that underwent endurance exercise training had a comparable level of food intake as that of sedentary OVX rats, but the final weight of the exercise-trained animals was significantly less than that of the sedentary OVX animals. These findings indicate that the exercise training program used in our study sufficiently enhanced energy expenditure. In addition, long-term exercise training substantially reduced visceral fat weight, suggesting that the increased energy expenditure by exercise training prevented fat accumulation and/or enhanced fat utilization in this animal model of insulin resistance.

Beneficial effects of exercise training on lipid profile are reflected in reduced serum levels of TC [20] and LDL [49] as well as an increase in HDL [50], although changes in these parameters are not always consistently observed [51,52]. In the present study, regular treadmill exercise running effectively lowered the levels of TC and LDL in OVX rats. It is of interest to note that the level of HDL did not differ between OVX animals undergoing exercise training and those remaining sedentary. High-density lipoprotein cholesterol is enhanced by regular exercise in studies using animals [53] and human subjects [54]. Nevertheless, there are reports showing that HDL was not improved after exercise training under estrogen-deprived condition, including those using OVX animals [18,19] and postmenopausal women [51,55].

Several lines of evidence support the notion that repeated bouts of physical activity enhance whole-body glucose utilization, improve insulin sensitivity in skeletal muscle [21], and prevent deterioration of glucose tolerance produced by high-energy diet in obese Zucker rats [22,45]. These latter findings showed an increased GLUT-4 protein content and expression of some of the insulin signaling molecules, such as insulin receptor substrate−1 [32]. Our current investigations raise a possibility that this may be also applicable to the insulin-resistant state induced by ovariectomy. In addition, we examined the potential interactive effects of endurance exercise training and estrogen replacement. Although we observed a significant interaction between exercise training and estrogen replacement on visceral fat content, these 2 interventions did not work in an additive fashion on glucose transport activity, suggesting that endurance exercise training and estrogen may share common mechanisms to correct defects in skeletal muscle insulin action caused by estrogen deficiency. This notion is supported by observations that transcripts encoding estrogen signaling in skeletal muscle [56,57], cardiac muscle, and liver [58] are enhanced by regular exercise. Nevertheless, as the uterine weights of OVX animals receiving estrogen replacement denoted that these animals could be hyperestrogenic, it is also plausible that the potential interactive effects are overruled by the hyperestrogenic condition.

In conclusion, we have presented evidence that prolonged (12 weeks) ovariectomy leads to the development of a systemic metabolic condition representing phenotype features of the insulin resistance syndrome including increased visceral fat content, abnormal serum lipid profile, impaired glucose tolerance, and defective insulin-mediated skeletal muscle glucose transport. Moreover, we have provided new evidence that whole-body and skeletal muscle insulin resistance is effectively corrected by endurance exercise training alone and estrogen replacement alone. Despite this, we could find no evidence that exercise training could additively modulate insulin action in OVX animals that also received estrogen replacement.

Acknowledgment

This work was supported by grants from The Commission on Higher Education, Thai Ministry of Education, and The Thailand Research Fund (MRG5180322). We thank Dr Prapon Wilairat for constructive comments on the manuscript.

References

[1] Sternfeld B, Bhat AK, Wang H, Sharp T, Quesenberry Jr CP. Menopause, physical activity, and body composition/fat distribution in midlife women. Med Sci Sports Exerc 2005;37:1195-202.
[2] Kannel WB, Wilson PW. Risk factors that attenuate the female coronary disease advantage. Arch Intern Med 1995;155:57-61.
[3] Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome: prevalence and associated

risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. Arch Intern Med 2003;163:427-36.
[4] Latour MG, Shinoda M, Lavoie JM. Metabolic effects of physical training in ovariectomized and hyperestrogenic rats. J Appl Physiol 2001;90:235-41.
[5] Richard D, Rochon L, Deshaies Y. Effects of exercise training on energy balance of ovariectomized rats. Am J Physiol 1987;253: R740-5.
[6] Laudenslager ML, Wilkinson CW, Carlisle HJ, Hammel HT. Energy balance in ovariectomized rats with and without estrogen replacement. Am J Physiol 1980;238:R400-5.
[7] Rachoń D, Vortherms T, Seidlová-Wuttke D, Wuttke W. Effects of dietary equol on body weight gain, intra-abdominal fat accumulation, plasma lipids, and glucose tolerance in ovariectomized Sprague-Dawley rats. Menopause 2007;14:1-8.
[8] Kumagai SA, Holmang A, Bjorntorp P. The effects of oestrogen and progesterone on insulin sensitivity in female rats. Acta Physiol Scand 1993;149:91-7.
[9] Rincon J, Holmäng A, Wahlström EO, Lönnroth P, Björntorp P, Zierath JR, et al. Mechanisms behind insulin resistance in rat skeletal muscle after oophorectomy and additional testosterone treatment. Diabetes 1996;45:615-21.
[10] Holmäng A, Björntorp P. The effects of testosterone on insulin sensitivity in male rats. Acta Physiol Scand 1992;146:505-10.
[11] Puah JA, Bailey CJ. Effect of ovarian hormones on glucose metabolism in mouse soleus muscle. Endocrinology 1985;117: 1336-40.
[12] Hansen PA, McCarthy TJ, Pasia EN, Spina RJ, Gulve EA. Effects of ovariectomy and exercise training on muscle GLUT-4 content and glucose metabolism in rats. Am J Physiol 1996;80:1605-11.
[13] Stevenson JC. Justification for the use of HRT in the long-term prevention of osteoporosis. Maturitas 2005;51:113-26.
[14] Scarabin PY, Oger E, Plu-Bureau G. Differential association of oral and transdermal oestrogen-replacement therapy with venous thromboembolism risk. Lancet 2003;362:428-32.
[15] Zegura B, Guzic-Salobir B, Sebestjen M, Keber I. The effect of various menopausal hormone therapies on markers of inflammation, coagulation, fibrinolysis, lipids, and lipoproteins in healthy postmenopausal women. Menopause 2006;13:643-50.
[16] Collins P, Flather M, Lees B, Mister R, Proudler AJ, Stevenson JC, et al. Randomized trial of effects of continuous combined HRT on markers of lipids and coagulation in women with acute coronary syndromes: WHISP Pilot Study. Eur Heart J 2006;27:2046-53.
[17] Skouby SO, Endrikat J, Düsterberg B, Schmidt W, Gerlinger C, Wessel J, et al. A 1-year randomized study to evaluate the effects of a dose reduction in oral contraceptives on lipids and carbohydrate metabolism: 20 microg ethinyl estradiol combined with 100 microg levonorgestrel. Contraception 2005;71:111-7.
[18] Shinoda M, Latour MG, Lavoie JM. Effects of physical training on body composition and organ weights in ovariectomized and hyperestrogenic rats. Int J Obes 2002;26:335-43.
[19] Wu J, Wang X, Chiba H, Higuchi M, Nakatani T, Ezaki O, et al. Combined intervention of soy isoflavone and moderate exercise prevents body fat elevation and bone loss in ovariectomized mice. Metabolism 2004;53:942-8.
[20] Kiens B, Lithell H, Mikines KJ, Richter EA. Effects of insulin and exercise on muscle lipoprotein lipase activity in man and its relation to insulin action. J Clin Invest 1989;84:1124-9.
[21] James DE, Kraegen EW, Chisholm DJ. Effect of exercise training on whole-body insulin sensitivity and responsiveness. J Appl Physiol 1984;56:1217-22.
[22] Saengsirisuwan V, Perez FR, Kinnick TR, Henriksen EJ. Effects of exercise training and antioxidant R-ALA on glucose transport in insulin-sensitive rat skeletal muscle. J Appl Physiol 2002;92:50-8.
[23] Becker-Zimmermann K, Berger M, Berchtold P, Gries FA, Herberg L, Schwenen M. Treadmill training improves intravenous glucose tolerance and insulin sensitivity in fatty Zucker rats. Diabetologia 1982;22:468-74.
[24] Cortez MY, Torgan CE, Brozinick Jr JT, Ivy JL. Insulin resistance of obese Zucker rats exercise trained at two different intensities. Am J Physiol 1991;261:E613-9.
[25] Arciero PJ, Vukovich MD, Holloszy JO, Racette SB, Kohrt WM. Comparison of short-term diet and exercise on insulin action in individuals with abnormal glucose tolerance. J Appl Physiol 1999;86: 1930-5.
[26] Hughes VA, Fiatarone MA, Fielding RA, Kahn BB, Ferrara CM, Shepherd P, et al. Exercise increases muscle GLUT-4 levels and insulin action in subjects with impaired glucose tolerance. Am J Physiol 1993; 264:E855-62.
[27] Banks EA, Brozinick JT, Yaspelkis BB, Kang HY, Ivy JL. Muscle glucose transport, GLUT-4 content, and degree of exercise training in obese Zucker rats. Am J Physiol 1992;263:E1010-5.
[28] Brozinick Jr JT, Etgen Jr GJ, Yaspelkis 3rd BB, Kang HY, Ivy JL. Effects of exercise training on muscle GLUT-4 protein content and translocation in obese Zucker rats. Am J Physiol 1993;265: E419-27.
[29] Etgen GJ, Jensen J, Wilson CM, Hunt DG, Cushman SW. Exercise training reverses insulin resistance in muscle by enhanced recruitment of GLUT-4 to the cell surface. Am J Physiol 1997;272:E864-9.
[30] Wattanapermpool J. Increase in calcium responsiveness of cardiac myofilament activation in ovariectomized rats. Life Sci 1998;63: 955-64.
[31] Henriksen EJ, Halseth AE. Early alterations in soleus GLUT-4, glucose transport, and glycogen in voluntary running rats. J Appl Physiol 1994; 76:1862-7.
[32] Saengsirisuwan V, Perez FR, Sloniger JA, Maier T, Henriksen EJ. Interactions of exercise training and alpha-lipoic acid on insulin signaling in skeletal muscle of obese Zucker rats. Am J Physiol Endocrinol Metab 2004;287:E529-36.
[33] Laemmli UK. Cleavage of structural proteins during assembly of the head of bacteriophage T4. Nature London 1970;227:680-5.
[34] Davies KJ. Protein damage and degradation by oxygen radicals. I. General aspects. J Biol Chem 1987;262:9895-901.
[35] Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. Methods Enzymol 1994;233: 357-63.
[36] Picard F, Deshaies Y, Lalonde J, Samson P, Labrie C, Bélanger A, et al. Effects of the estrogen antagonist EM-652.HCl on energy balance and lipid metabolism in ovariectomized rats. Int J Obes Relat Metab Disord 2000;24:830-40.
[37] 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 U S A 2000;97:12729-34.
[38] Musatov S, Chen W, Pfaff DW, Mobbs CV, Yang XJ, Clegg DJ, et al. Silencing of estrogen receptor alpha in the ventromedial nucleus of hypothalamus leads to metabolic syndrome. Proc Natl Acad Sci U S A 2007;104:2501-6.
[39] Wade GN, Gray JM, Bartness TJ. Gonadal influences on adiposity. Int J Obes 1985;9:83-92.
[40] Deshaies Y, Dagnault A, Lalonde J, Richard D. Interaction of corticosterone and gonadal steroids on lipid deposition in the female rat. Am J Physiol 1997;273:E355-62.
[41] Wang J, Yuen VG, McNeill JH. Effect of vanadium on insulin sensitivity and appetite. Metabolism 2001;50:667-73.
[42] Liang Y, Chen X, Osborne M, DeCarlo SO, Jetton TL, Demarest K. Topiramate ameliorates hyperglycaemia and improves glucose-stimulated insulin release in ZDF rats and db/db mice. Diabetes Obes Metab 2005;7:360-9.
[43] Beck B. Neuropeptides and obesity. Nutrition 2000;16:916-23.
[44] Lui M, Xu X, Rang W, Li Y, Song H. Influence of ovariectomy and 17beta-estradiol treatment on insulin sensitivity, lipid metabolism and post-ischemic cardiac function. Int J Cardiol 2004;97: 485-93.

[45] Saengsirisuwan V, Kinnick TR, Schmit MB, Henriksen EJ. Interaction of exercise training and lipoic acid on skeletal muscle glucose transport in obese Zucker rats. J Appl Physiol 2001;91:145-53.
[46] Campbell SE, Febbraio MA. Effect of the ovarian hormones on GLUT4 expression and contraction-stimulated glucose uptake. Am J Physiol 2002;282:E1139-46.
[47] Barros RP, Machado UF, Warner M, Gustafsson JA. Muscle GLUT4 regulation by estrogen receptors ERbeta and ERalpha. Proc Natl Acad Sci U S A 2006;103:1605-8.
[48] Savage DB, Petersen KF, Shulman GI. Disordered lipid metabolism and the pathogenesis of insulin resistance. Physiol Rev 2007;87: 507-20.
[49] Green JS, Stanforth PR, Rankinen T, Leon AS, Rao Dc D, Skinner JS, et al. The effects of exercise training on abdominal visceral fat, body composition, and indicators of the metabolic syndrome in postmenopausal women with and without estrogen replacement therapy: the HERITAGE family study. Metabolism 2004;53:1192-6.
[50] Durstine JL, Thomson PD. Exercise in the treatment of lipid disorders. Cardiol Clin 2001;19:1-19.
[51] Mohanka M, Irwin M, Heckbert SR, Yasui Y, Sorensen B, Chubak J, et al. Serum lipoproteins in overweight/obese postmenopausal women: a one-year exercise trial. Med Sci Sports Exerc 2006;38:231-9.
[52] Stefanick ML, Mackey S, Sheehan M, Ellsworth N, Haskell WL, Wood PD. Effects of diet and exercise in men and postmenopausal women with low levels of HDL cholesterol and high levels of LDL cholesterol. N Engl J Med 1998;339:12-20.
[53] Burneiko RC, Diniz YS, Galhardi CM, Rodrigues HG, Ebaid GM, Faine LA, et al. Interaction of hypercaloric diet and physical exercise on lipid profile, oxidative stress and antioxidant defenses. Food Chem Toxicol 2006;44:1167-72.
[54] Haddock BL, Hopp HP, Mason JJ, Blix G, Blair SN. Cardiorespiratory fitness and cardiovascular disease risk factors in postmenopausal women. Med Sci Sports Exerc 1998;30:893-8.
[55] Klebanoff R, Miller VT, Fernhall B. Effects of exercise and estrogen therapy on lipid profiles of postmenopausal women. Med Sci Sports Exerc 1998;30:1028-34.
[56] Lemoine S, Granier P, Tiffoche C, Berthon PM, Rannou-Bekono F, Thieulant ML, et al. Effect of endurance training on oestrogen receptor alpha transcripts in rat skeletal muscle. Acta Physiol Scand 2002;174: 283-9.
[57] Wiik A, Gustaffson T, Esbjornsson M, Johansson O, Ekman M, Sundberg J, et al. Expression of oestrogen receptor α β is higher in skeletal muscle of highly endurance-trained than of moderately active men. Acta Physiol Scand 2005;184:105-12.
[58] Paquette A, Wang D, Gauthier MS, Prud'homme D, Jankowski M, Gutkowska J, et al. Specific adaptations of estrogen receptor α β transcripts in liver and heart after endurance training in rats. Mol Cell Biochem 2007;306:179-87.