Golestan University of Medical Sciences

Jorjani Biomedicine Journal

Online ISSN: 2645-3509

Interactive effect of aerobic training and estrogen consumption on serum levels of catalase and glutathione peroxidase enzymes in ovariectomized rats

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Article Type:
Original article

Article History:

Received: 17 Jun 2020
Revised: 5 Mar 2020
Accepted: 1 May 2020

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DOI: 10.29252/jorjanibiomedj.8.2.38

Abstract

Background and objective: Aging and menopause are associated with decreased antioxidant function, however, the role of exercise and estrogen consumption in the health of these people has been shown. The aim of the present study was to investigate the interactive effect of aerobic training (AT) and estrogen (Es) on serum levels of catalase (Cat) and glutathione peroxidase (Gpx) enzymes in ovariectomized rats.

Methods: In this experimental study, 45 ovariectomized rats were randomly divided into 5 groups of 9 rats, including (1) control (C), (2) estrogen solvent (sesame oil) (Sh), (3) AT, (4), Es,and (5) AT+Es. Rats in groups 3 and 5 were trained for eight weeks and three sessions, and groups 4 and 5 randomly received 30 micrograms of estradiol valerate daily for eight weeks. Data analysis was analyzed using one-way analysis of variance and Tukey's post hoc test at the significance level of 0.05.

Results: Cat levels in the AT (P= 0.006), Es (P= 0.005) and AT + Es (P = 0.001) groups were significantly higher than the control group. Cat levels in the AT + Es group were also significantly higher than in the Es group (P = 0.01). Gpx levels in the AT (P = 0.001), S (P = 0.001) and AT + Es (p = 0.001) groups were significantly higher than the control group, while in the S (P = 0.001) and AT + Es (P = 0.001) groups, they were significantly higher than the AT group.

Conclusion: It seems that aerobic training combined with estrogen consumption synergistically improves the function of the antioxidant system in ovariectomized rats. However, it seems that the signaling pathway of interventions requires further study.

Keywords: Training; Estrogen; Catalase; Glutathione peroxidase; Ovariectomy

Introduction

Menopause is a natural period that affects almost a third of a woman's life. During this period of a woman's life, hormonal changes stop menstruation forever)1). Ovariectomy is a standardized experimental model of menopause in rodents to examine menopause in women. Ovariectomized animals are a model for studying the effects of ovarian hormone deficiency. Ovariectomized rats are a desirable model with clinical features of estrogen (Es) deficiency or menopause that are used to achieve research goals. Also, Es plasma levels have significantly reduced after 6 weeks for ovariectomized rats (2). Recent studies have confirmed a relation between oxidative stress and a decrease in ovarian hormones (3). Antioxidants are the most important way to fight free radicals and regenerate damaged cells. The most important antioxidant enzymes made in the body are catalase (Cat) and glutathione peroxidase (Gpx).Glutathione enzyme uses glutathione to neutralize hydrogen peroxide, which is a toxic substance, and converts it to water(4). Research has shown that Ovariectomized rats are at risk for osteoporosis and hypertrophy of the heart, cardiovascular disorders, uterine atrophy, and an imbalance between free radicals and antioxidant defenses in various tissues of the body (5). Es has antioxidant properties. This property belongs to the hydroxyl group of its phenolic ring, which acts as an electron donor, destroying free radicals and neutralizing lipid oxidation.Es also leads to the over-regulation of the gene for antioxidant enzymes such as superoxide dismutase and Gpx(6)Es deficiency after menopause causes oxidative stress, which in turn causes a variety of injuries, such as high blood pressure, hot flashes, and the risk of osteoporosis(7). In order to eliminate Es deficiency, special measures have been considered, the most effective of which is hormone therapy (6). Menopausal women use Es to protect against the effects of this hormone deficiency (8,9).

Studies have been performed on the effect of Es or estradiol in ovariectomized rats, for example, eight weeks of estradiol use reduced liver enzymes in ovariectomized rats (10); however, estradiol injection had no significant effect on the weight and visceral fat weight of ovariectomized rats (11).On the other hand, in addition to estrogen during menopause, researchers believe that exercise as a non-invasive intervention has beneficial effects on the overall health of the body during menopause and old age (12);There have been many studies on the effect of exercise on the body's antioxidant system, with researchers believing that a session of exercise is associated with increased fat metabolism and energy substrates with increased oxidative stress. Nonetheless, long-term, low-to-moderate intensity exercise through adapting at the molecular cellular level induces increased antioxidant mRNA and antioxidant capacity (13).

In this way, the results indicate a decrease in the production of free radicals by increasing the activity of antioxidant substances such as Gpx and superoxide dismutase following endurance training (14).Goto and Radak (2013) have reported that strenuous exercise leads to increased oxidative stress and injury, and in contrast, moderate-intensity exercise promotes optimal adaptation and protective effect against oxidative stress (15).Also, 12 weeks of aerobic training with an intensity of 65 to 75% of heart reserved rate decreased malondialdehyde (MDA) and increased total antioxidant capacity (TAC) in middle-aged women (12).However, six weeks of exercise had no significant effect on changes in hepatic superoxide dismutase (SOD) changes in

doxorubicin-poisoned elderly rats (16).Human studies during menopause are associated with concerns, however, measuring serum oxidative stress-antioxidant function is important in assessing general body condition; in addition, contradictory results have been reported regarding the effect of exercise on oxidative stress and antioxidant systems.

Regarding the contradictory results associated with the effect of exercise training on the activity of antioxidant enzymes and, on the other hand, reduction of these enzymes during menopause and insufficient research on the combined effect of AT with Es consumption during menopause, the aim of the present study was to investigate the effect of 8 weeks of aerobic training (AT)and Es consumption on serum levels of Catand Gpxenzymes in ovariectomized rats.

Materials and Methods

Subjects

In this experimental study, with post-test design and control group, 45 Sprague Dawley female rats with an age range of 8 to 10 weeks and an average weight of 220.24±20.16 were obtained from the Center for Breeding and Proliferation of Laboratory Animals of Islamic Azad University, Marvdasht Branch. All rats were kept for a week in the university's Animal Sports Physiology Laboratory to adapt to the environment. It should be noted that during the study period rats were kept in standard conditions in cages made of transparent polycarbonate with autoclave capability, and the use of sterile wood grater to absorb urine and feces of rats, temperature 22-24˚ C and relative humidity 55- 60% and the light-dark cycle was maintained every 12 hours. Subsequently, during the adjustment period on the eighth day, the rats' ovaries were removed (Ovarian removal operation). To remove the ovaries through the abdomen, first, rats were anesthetized with 50 mg / ml of ketamine solution and 20 mg / ml of xylazine. Then the site of the operation was disinfected with betadine scrub and then a 3 cm incision was made in the abdomen on the white line in the middle of the abdomen from the kidney downwards. After incisions were made in the muscular layers and peritoneal membranes, the ovaries and uterus were observed and separated with surgical scissors. Then the relevant suture was sutured with a single simple suture pattern with a 3-zero Vikril thread and an animal skin with a Nilen-2 surgical suture. OTC solution is used at the surgical site to prevent infection. After ovariectomy, the animals were kept under controlled conditions for 4 weeks with the aim of creating osteoporosis (17). After ovariectomy, the animals were kept under controlled conditions for 4 weeks with the aim of creating osteoporosis (18). Subsequently, rats were randomly divided into the following groups(nine animals per group) including :(1) control (C), (2) Es solvent (sesame / almond oil) (Sh), (3) Aerobic training (AT), (4), Es, (5), AT + Es. Group 3 and 5 were trained for eight weeks and three sessions, and groups 4 and 5 received 30 micrograms / kg of estradiol valerate dissolved in 0.2 ml of sesame oil per day for eight weeks (18(.

Aerobic training protocol

Aerobic training was performed in 5 sessions per week with increasing running on the rodent treadmill for eight weeks. In this way, the running activity started at 15 meters per minute for 15 minutes on a zero-degree treadmill in the first session and continued in such a way that in the last 4 weeks, the activity speed reached 26 meters per minute,

the duration of each training session was increased to 60 minutes, and the inclination of the treadmill was increased to 10 degrees. In addition, in each training session, about 5 minutes for warming up and 5 minutes for cooling downwere considered (19). The warming and cooling program was implemented at a speed of 8 meters per minute and a slope of zero degrees.

Measure the antioxidant enzymes

Biochemical evaluation: About 48 hours after the last session of AT and Es consumption, rats were anesthetized using ketamine and xylazine and the blood samples were taken directly from the heart. The activity of the Cat enzyme was evaluated based on changes in the concentration of hydrogen peroxide (10 ml) at a wavelength of 240 nm and according to the Aebi method (20).In this method, the Cat enzyme in the serum sample decomposed hydrogen peroxide, leading to reduced absorption of this substance at a wavelength of 240 nm over a period of 2 minutes. And from the difference of absorption240 AΔ per unit time, enzyme activity was calculated using standard curve. The amount of enzyme that breaks down the amount of one micromole of hydrogen peroxide at pH of 7 and the temperature of 25˚C was considered to be one unit of enzyme activity. On the other hand, the activity of Gpx was calculated using the following formula and Beer-Lambert's law (21).

One unit of activity of Gpx is defined as the amount of enzyme that oxidizes one micromole of nicotinamide to adenine diphonochloid phosphate to nicotinamide adenine diphonochloid phosphate once positively (its oxidized form) per unit time (minute) at 25˚C.n the present study, the activity of Gpx in serum samples was expressed as micromole nicotine, adenine dinucleotide oxidized phosphate per minute per milliliter of serum.

Statistical analysis

The Shapiro-Wilk test was used to investigate the normal distribution of the data. To compare the differences between the study groups, one-way analysis of variance test and to determine the place of the differences between the groups, Tukey's post hoc test were used at the significance level of (P<0.05(in SPSS software.

$$\text{GPx activity} = \frac{A_{340}/\text{min.}}{0.00377\ \mu M^{-1}} \times \frac{\text{V total (mL)}}{\text{V sample (mL)}} \times \text{Sample dilution} = \mu\text{mol/min/mL (IU/mL)}$$

Results

Levels of Cat and Gpx in rats in the research groups are shown in Figures 1 and 2. The results of one-way analysis of variance showed a significant difference in Cat (P= 0.001) and Gpx levels (P = 0.001) in ovariectomized rats.

The results of Tukey's post hoc test showed that the Cat levels in the C and Sh groups did not differ significantly (P = 0.99). The results of the showed that there is no significant difference between the Cat levels in the C and Shgroups (P = 0.99). However, Cat levels in the AT (P = 0.006), Es (P = 0.005) and AT + Es (P = 0.001) groups were significantly higher than the control group. There was no significant difference between the Es (P = 0.93) and AT + Es groups (P = 0.109) compared to AT. However, Cat levels in the AT + Es group were significantly higher than the Es group (P = 0.01) (Figure. 1).

There was no significant difference in the Gpx levels in C and Sh groups (P = 0.96). However, Gpx levels in the AT (P = 0.001), Es (P = 0.001) and AT + Es (P = 0.001) groups were significantly higher than the control group. Gpx levels in the Es (P = 0.001) and AT + Es (P = 0.001) groupswere significantly higher than the AT group. However, there was no significant difference in the Gpx levels of the Es group compared to the AT + Es group (P = 0.28) (Figure. 2).

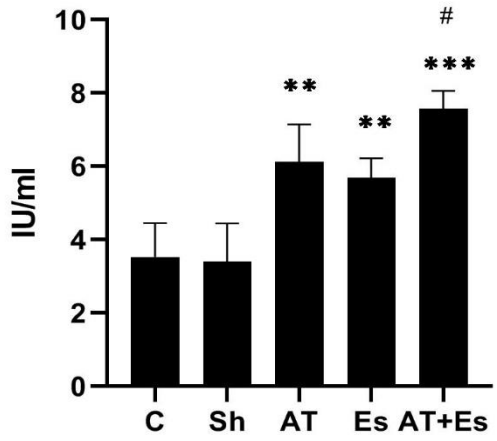

Figure 1. Catalase levels in the research groups
C: Control; Sh: Estrogen solvent (sesame/almond oil);
AT: Aerobic training; Es: Estrogen; AT + Es; Aerobic training + Estrogen
*** (p = 0.001) and ** (p <0.01) significant increase compared to the C and SH groups
# (P = 0.01) Significant increase compared to the Es group.

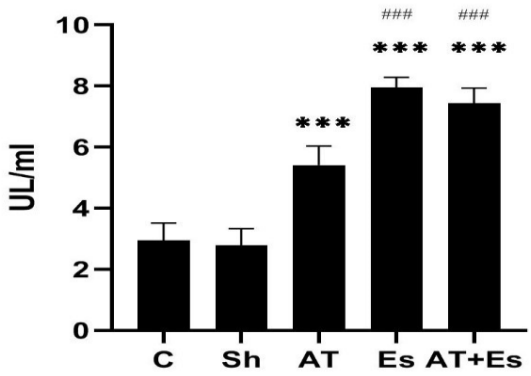

Figure 2. Gpx levels in the research groups
C: Control; Sh: Estrogen solvent (sesame/almond oil); AT: Aerobic training;
Es: Estrogen; AT + Es; Aerobic training + Estrogen
*** (p=0.001) significant increase compared to the C and SHgroups
(p = 0.001) Significant increase compared to the AT group

Discussion

The results of the present study showed that AT had a significant effect on increasing the levels of Cat and Gpx in ovariectomized rats. The results of the present research are consistent with the results of research by Hsu et al. (2008), Khassaf et al. (2003), Venojärvi et al. (2013), (22-24). Afzalpour et al. reported that 10 weeks of AT with an intensity of 70 to 65% of maximum heart rate strengthens the antioxidant system and increases the levels of superoxide dismutase, Cat (25). The results of the present study are

not consistent with the results of Decastro et al. (2009), Daud et al. (2006) and Fisher-Wellman (2009) (26-28). However, in a studyobserved a decrease in the amount of antioxidant enzyme after the training period, which is not consistent with our recent findings (29). Incompatibility in results can be attributed to differences in the statistical population and type of exercise. Researchers in the pathogenesis of some sex hormone-related diseases have stated that oxidative stress as a factor in infertility can produce reactive species oxygen. If the body is unable to compensate the homeostasis system, it reduces the antioxidant capacity and enzymes Cat and Gpx (30). Bureau et al. (2002) stated that the total antioxidant capacity of postmenopausal women is significantly reduced (31). It seems thatthe beneficial effects of regular exercise on reducing and preventing oxidative stress-related diseases are due to the strengthening of the body's anti-oxidative system as a result of regular, prolonged, moderate-intensity exercise (24).Exercise can affect oxidative stress processes by several mechanisms, including oxygen depletion from the electron transport chain, prostanoid fuel, the activity of xanthine oxidases, macrophages, and increased catecholamine activity (32).However, adapting to exercise simultaneously increases antioxidant enzymes and increases the production of free radicals, which can counteract the adverse effects of oxidative stress caused by exercise and lead to greater utilization of Gpx in the oxidative defense system. Therefore, increasing the activity of oxidative enzymes is a well-known adaptation to endurance training (32).

The results of the present study showed that Es consumption had a significant effect on the increase in Cat and Gpx levels of ovariectomized rats. Studies show that with increasing aging and decreased levels of sex hormones, the antioxidant effects of these hormones also decrease, so in such people, prescribing Es and exogenous sex hormones both improves the functions associated with these hormones and has antioxidant effects (33). It seems exogenous estradiol in animal without ovaries increases Es receptor mRNA and improves gonadotropin axis function in the hypothalamus-pituitary. As a result, this improves cell metabolism in pathways similar to insulin-like growth receptor factor (IGF-1) and the growth hormone (GH), and causes the transcription of multiple genes, such as antioxidant enzymes (9-6, 33). In this regard, researchers have reported that Es consumption improves the metabolism of fats, glucose and atrogenic indices in ovariectomized rats (34). Studies also show that an increase in endogenous Es is associated with a decrease in body fat mass, which can lead to the availability of fatty acids, weight loss and decrease ROS levels (35).

The results of the present study showed that AT and Es consumption increased the levels of Cat and Gpx in ovariectomized rats. Also, the interaction of training and Es was more significant on the increase of Cat and Gpx. Studies show that exercise with a mechanism to reduce oxidative stress (24) improves the performance of the electron transport chain, the prostanoid fuel, the activity of xanthine oxidases, macrophages, increases the activity of catecholamines (32) and increases antioxidant mRNA and antioxidant capacity (13).Also, consumption of estrogen increases Es receptor mRNA, improves hypothalamus-pituitary axis function, cell metabolism, IGF-1 and GH, and causes the transcription of multiple genes, such as antioxidant enzymes (9-6, 33). This mechanism leads to increased levels of antioxidant enzymes. Therefore, it

seems that aerobic training and estrogen intake with a similar mechanism lead to increased antioxidant capacity in rats.

In this regard, researchers have shown that AT combined with the use of estradiol reduces the MDA in the uterine tissue of ovariectomizedrats (33).Researchers have also shown that exercise training and Es therapy interactively reduce the weight of fat and improve the metabolism of ovariectomized (36, 37).Therefore, it seems that AT and Es consumption increase the activity of antioxidants in similar ways to some extent, although different signaling pathways are observed in these two interventions according to previous studies. Therefore, it seems that the lack of review of this signaling pathway for more reliability is one of the limitations of the present study. Therefore, it is suggested that different pathways should be considered in future studies.

Due to the complexity of the mechanism of oxidative stress and antioxidants and their different functions, it seems that one of the limitations of the present study is the lack of measurement of oxidative stress markers and also the ratio of oxidative stress to antioxidants. Future studies are recommended in which oxidants stress and antioxidants should be examined simultaneously.

Conclusion

It seems that aerobic training combined with estrogen consumption will synergistically improve the function of the antioxidant system in ovariectomizedrats. However, it seems that the signaling pathway of interventions requires further study.

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How to cite:

Salehi O.R, Ghabezi S, Khajehlandi A, Mohammadi A. Interactive effect of aerobic training and estrogen consumption on serum levels of catalase and glutathione peroxidase enzymes in ovariectomized rats. Jorjani Biomedicine Journal. 2020; 8(2): 38-47.