

Effects of Gender, Endurance Training and Acute Exhaustive Exercise on Oxidative Stress in the Heart and Skeletal Muscle of the Rat

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Abstract

Effects of gender differences and endurance training on exhaustive exercise induced-oxidative stress have been a question that has not been clarified in the literature. The aim of this study was to determine the effects of sex, acute exhaustive exercise and chronic aerobic exercise training on oxidative stress in the heart and the skeletal muscle. The study was carried out with 12 week-old male (n = 24) and female (n = 24) young adult Wistar rats. They were randomly divided into four groups: untrained, trained, untrained exhausted and trained exhausted. The rats in the trained group swam for 60 min/day, five days per week for eight weeks. Thereafter, one-half of the trained and one-half of the untrained rats were randomly selected into the trained and untrained exhaustive exercise groups, respectively. They were killed immediately after one last exhaustive swimming exercise. In the heart, endurance training decreased malondialdehyde (MDA) levels in the female rats at rest, but did not change in the male rats in the heart; MDA levels were also increased in female rats at rest in the gastrocnemius tissues. In the trained female rats, exhaustive exercise decreased MDA levels in the heart and gastrocnemius tissues. The nitric oxide (NO) levels in the heart in the untrained female rats were higher than in the male rats after exhaustive exercise. Training decreased the NO levels in both sexes in the gastrocnemius tissue at rest. In the heart, the untrained female rats had higher total glutathione (GSH) levels than in the male rats at rest. Also, exhaustive exercise decreased the GSH levels in the trained female rats. In the gastrocnemius, untrained female rats showed higher GSH levels than in the male after exhaustive exercise. The superoxide dismutase activities in the gastrocnemius were similar between the female and male rats. The results suggested that gender was a major determinant of changes in MDA, NO and GSH levels in the heart and gastrocnemius tissues after the exhaustive exercise or endurance training. Also, the responses to oxidative stress induced by acute exercise or training in the heart and gastrocnemius muscle tissues are different.

Key Words: gender differences, endurance training, exhaustive exercise, oxidative stress, heart, skeletal muscle

Introduction

Oxidative stress is an imbalance between reactive oxygen or nitrogen species and is the antioxidant

defense capacity of the cell. A large number of studies have reported that acute physical activity, especially under circumstances such as unaccustomed intensity and/or duration (7, 43) increases the produc-

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Received: March 16, 2011; Revised: July 12, 2011; Accepted: August 8, 2011.

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tion of reactive oxygen/nitrogen species and leads to oxidative stress. Also, exercise-induced oxidative stress depends on the exercise intensity. High-intensity exercises cause more lipid peroxidation compared to moderate- or low- intensity exercises (30, 41). However, regular physical training can reduce acute exercise-induced oxidative stress and muscle damage while increasing antioxidant capacity (33, 37).

The gender is a profound determinant of aging and lifespan, but little is known about sex differences in free radical homeostasis. Differences in reactive oxygen species (ROS) homeostasis contribute to sex divergence in survival (1). Sex hormones have been suggested to play a role in oxidative stress, because estradiol inhibits the formation of lipid peroxidase in some tissues (18). However, another study indicated that the effect of exercise on oxidative stress was independent of change in estrogen metabolism (40). Several studies have reported that the adaptation to changes in antioxidant capacity is affected by gender difference. It was also reported that males and females had different reactions to exercise-induced free radical production (13, 14, 18, 19, 31, 49). On the contrary, several studies have reported no differences in exercise-induced oxidative stress between male and female (5, 21, 36). However, the effects of gender difference, training and exhaustive exercise on oxidative stress in different tissues are still unclear.

Furthermore, exercise-induced oxidative stress may elicit different responses depending on tissue type and antioxidant capacity of the tissue. The antioxidant capacity is usually high in liver and kidney but low in lung and heart (10). Muscle and heart appear to respond to oxidative stress quite differently than other organs possibly due to the difference in mitochondrial biogenesis and the occurrence of oxidant-induced degeneration (29). Recently, although there have been many studies on the oxidative stress induced by exhaustive exercise, there have been no studies on effects of training and gender on acute exhaustive exercise-induced oxidative stress in the heart and skeletal muscle tissues. The purpose of the present study was to examine the effects of training and gender differences on exhaustive exercise-induced oxidative stress.

Materials and Methods

Animal Care

Experiments were carried out with male ($n = 24$) and female ($n = 24$) young adult Wistar rats (12 weeks of age). The rats were housed in individual cages in a temperature-controlled room ($23 \pm 1^\circ\text{C}$, with $50 \pm 5\%$ relative humidity) maintained on a cycle of 12 h light, 12 h dark. They were fed rat chow and water *ad libitum* throughout the study period. The

rats were weighed and the body mass gain throughout this study was determined. Animal experiments were performed in accordance with the guidelines issued by Ethical Committee of Gazi University Faculty of Medicine. All experimental procedures were carried out at Experimental Research Centre, Faculty of Medicine, Gazi University.

Experimental Design

After a week of acclimation, both male (M) and female (F) rats were randomly assigned to one of the following four subgroups: untrained, trained, untrained exhausted and trained exhausted. Swimming was selected as an endurance exercise model, because it is physically less traumatic than treadmill-based exercise models for the rats. Besides, slight electrical shock is used as a stimulator to make the rats run in treadmill-based exercise models, and this electrical stimulation itself might cause an oxidative stress (38).

For familiarization to the swimming exercise, the rats in the trained and exhausted groups were accustomed to swimming with repeated short-term swimming sessions for a week before the experiment. After the eight-week exercise training session, all animals were killed by decapitation. In order to avoid the effect of the last exercise session and to be able to measure the adaptive or maladaptive changes, the rats were killed two days after the last training session.

Exercise Training of Rats

The rats in the exercise-training groups swam for 60 min/day, five days/week for eight weeks. The exercise was performed in a cylindrical plastic container that was 150 cm in diameter and 90 cm in height, filled to a depth of 70 cm with water, and maintained at a temperature between 30°C and 33°C . All exercises were performed at the same time of day for each training group and were continuously supervised.

Exhaustive Exercise

The rats in the exhaustive exercise groups were killed immediately after the exhaustive swimming exercise. Exhaustion was defined as that point at which the animal could not remain at the water surface. Swimming time varied between 120 and 270 min.

Tissue Preparation and Biochemical Analysis

At the end of the experiment, the rats were anesthetized intraperitoneally by ketamine–HCl and xylazine cocktail (100 mg/kg and 5 mg/kg, respectively). Heart, soleus and gastrocnemius muscles were care-

fully isolated from all rats. Isolated muscle samples were quickly frozen in liquid nitrogen, and stored at -80°C until analyses of oxidant/antioxidant system markers.

Measurement of Malondialdehyde (MDA)

Tissue samples were obtained after measuring lesion areas and frozen immediately in liquid nitrogen then kept in -70°C deep-freeze until the assay. Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS). Samples were homogenized in ice-cold trichloroacetic acid (1 g tissue in 10 ml 10% trichloroacetic acid) in a tissue homogenizer (Heidolph Diax 900, Germany). Following centrifugation of the homogenate at 3,000 rpm for 10 min (Hermle Z 323 K, Germany), 750 μl of supernatant was added to an equal volume of 0.67% (m/v) thiobarbituric acid and heated at 100°C for 15 min. The absorbances of the samples were measured at 535 nm. Lipid peroxide levels are expressed in terms of MDA equivalents using an extinction coefficient of 1.56×10^5 mol/cm (11).

Measurement of Nitric Oxide (NO)

NO levels were measured by Griess assay (32, 44). Prior to NO determination, the tissues were homogenized in five volumes of phosphate buffer saline (pH 7.4) and centrifuged at 2,000 g for 5 min. NaOH 0.25 ml, 0.3 M was added to 0.5 ml of the supernatant. After incubation for 5 min at room temperature, 0.25 ml of 5% (w/v) ZnSO_4 was added for deproteinization. This mixture was then centrifuged at 14,000 rpm for 5 min and supernatants were used for the assays (32). A nitrate standard solution was serially diluted. After loading the plate with samples (100 μl), addition of vanadium III chloride (VCl_3) (100 μl) to each well was rapidly followed by addition of Griess reagents, sulphanilamide (SULF) (50 μl) and N-(1-naphtyl) ethylenediamide dihydrochloride (NEDD) (50 μl). After the incubation at 37°C (usually 30-45 min), samples were measured spectrophotometrically at 540 nm.

Measurement of Total Glutathione (GSH)

The total GSH levels were determined by the Ellman method with some modifications (3). Briefly, after centrifugation of the homogenates at 3,000 rpm for 10 min, 0.5 ml of supernatant was added to 2 ml of 0.3 M $\text{Na}_2\text{HPO}_4 \cdot 2 \text{H}_2\text{O}$ solution. A 0.2 ml solution of dithiobisnitrobenzoate (0.4 mg/ml 1% sodium citrate) was added and after mixing, the absorbance at 412 nm was immediately measured using a spectrophotometer (UV 1208, Shimadzu, Japan) at room temperature.

The GSH levels were calculated using an extinction coefficient of 13600 mol/cm.

Measurement of Superoxide Dismutase (SOD)

SOD assay kit was obtained from Cayman Chemical (catalog no. 706002, Cayman Chemical Co., Ann Arbor, MI, USA) and the assays were conducted according to manufacturer's instructions. The assay uses a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The SOD assay measures all three types of SOD (Cu/Zn-, Mn- and Fe-SOD).

Statistical Analysis

Statistical analysis was performed using SPSS for Windows (version 15.0, SPSS Inc. Chicago, IL, USA). One-way ANOVA with *post-hoc* Bonferroni test was used to compare group means. A three-way ANOVA was performed to examine the main effects of gender, exercise training, acute exhaustive exercise ($2 \times 2 \times 2$) and interaction on the measured variables. Body-weight data were analyzed by repeated-measures two-factor analysis of variance. Repeated-measures ANOVA followed by Bonferroni *post-hoc* analysis was used to examine differences between the groups. Statistical significance was set at a $P < 0.05$ level and data are expressed as means \pm SEM.

Results

Body weight of the rats at 12th week was not different among the groups. Changes in body weight of the rats during the experimental period were significantly different between female and male rats (time effect: $F = 280.9$, $P = 0.00$; time-group interaction effect: $F = 4.63$, $P = 0.00$; group effect: $F = 13.9$, $P = 0.00$). Male rats weighed more than female rats in all groups (Table 1).

The effects of gender, endurance training and exhaustive exercise on MDA and NO levels in the heart and gastrocnemius muscle tissues are shown in Fig. 1. The MDA level in the heart was significantly affected by gender ($F = 6.47$, $P = 0.01$) and training ($F = 14.91$, $P = 0.00$), but no significant effect of exhaustion was found ($F = 1.52$, $P = 0.22$). The MDA levels in the heart and gastrocnemius tissues were significantly different among the groups ($P < 0.05$) (Fig. 1). There was a significant interaction effect between gender and endurance training ($F = 5.84$, $P = 0.02$), gender and exhaustive exercise ($F = 8.43$, $P = 0.01$), also endurance training and exhaustive exercise ($F = 5.72$, $P = 0.02$) for the MDA level in the heart. The female

Table 1. Changes in body weight of rats during the experimental period

			n	Beginning of Experiments	End of Experiments
Female	Untrained	Rest	5	199.8 ± 6.7	244.6 ± 6.9
		Exhausted	5	204.6 ± 11.6	249.0 ± 11.3
	Trained	Rest	7	206.7 ± 7.1	252.4 ± 8.3
		Exhausted	7	207.3 ± 8.0	252.1 ± 5.7
Male	Untrained	Rest	5	232.0 ± 6.9	325.2 ± 6.1
		Exhausted	6	226.7 ± 10.7	320.3 ± 8.9
	Trained	Rest	7	240.6 ± 11.4	327.9 ± 9.7
		Exhausted	6	246.2 ± 17.9	334.7 ± 9.9

Values are means ± SEM. Changes in body weight of the male rats were significantly higher than in the female group. A significant time effect ($F = 280.9$, $P = 0.00$), time-group interaction effect ($F = 4.63$, $P = 0.00$) and group effect ($F = 13.9$, $P = 0.00$) were observed for body weight.

rats had higher MDA levels than the male rats at rest in the untrained groups in the heart. Also, exhaustive exercise increased MDA levels in male rats, but decreased in female rats. Endurance training decreased MDA levels in the female at rest, but did not change in the male rats in the heart and also increased in female rats at rest in the gastrocnemius muscle. In trained female rats, exhaustive exercise decreased MDA levels in the heart and gastrocnemius muscle tissues ($P < 0.05$). The NO level was significantly affected by exhaustive exercise in the heart ($F = 5.57$, $P = 0.02$) and in the gastrocnemius muscle ($F = 5.67$, $P = 0.02$), and by training in the gastrocnemius muscle ($F = 39.14$, $P = 0.00$). The NO levels in untrained female rats were higher than in the male rats after exhaustive exercise in the heart. Endurance training decreased NO levels in both sexes at rest and in female after exhaustive exercise in the gastrocnemius muscle tissue. There was, however, no significant interaction effect among sexes, endurance training and acute exhaustive exercise for MDA and NO in the heart and gastrocnemius muscle tissues (Fig. 1).

The effects of gender, endurance training and exhaustive exercise on GSH and SOD in the rat heart and gastrocnemius muscle tissues are shown in Fig. 2. The GSH level in the heart tissue was significantly affected by gender ($F = 7.30$, $P = 0.01$), but no significant effect by training ($F = 0.75$, $P = 0.39$). There was a significant interaction effect between gender and exhaustion ($F = 3.40$, $P = 0.07$), and endurance training ($F = 5.41$, $P = 0.03$), also endurance training and exhaustive exercise ($F = 4.73$, $P = 0.04$) for the GSH levels in the heart tissue. The GSH levels in the heart tissue were significantly different among the groups ($P < 0.05$). In the heart tissue, the GSH levels in untrained female rats were higher than those of the untrained male rats in the rest groups ($P < 0.05$). Also, exhaustive exercise decreased GSH levels in

trained female rats ($P < 0.05$). There was a significant effect of gender on the GSH in the gastrocnemius muscle tissues ($F = 9.17$, $P = 0.00$). In the gastrocnemius muscle, GSH levels were not significantly different between the female and male rats at rest, but female rats had higher GSH levels than the male after exhaustive exercise in untrained groups ($P < 0.05$). Exhaustive exercise increased GSH levels in the trained male rats ($P < 0.05$), but it did not alter in the female rats (Fig. 2). The SOD activity in the heart was significantly affected by the training program ($F = 7.97$, $P = 0.01$). Endurance training decreased SOD activities in male rats at rest in the heart ($P < 0.05$), but SOD activities did not change in the female rats. However, in gastrocnemius muscle tissues, the SOD activities were not significantly different among the groups ($P < 0.05$). There was also no significant interaction effect among sexes, endurance training and acute exhaustive exercise for GSH and SOD in the heart and gastrocnemius muscle tissues (Fig. 2).

Discussion

One of the major findings of the present study is that the gender was a significant determinant of the effects of acute exhaustive exercise and endurance training on the MDA, NO and GSH levels in the heart and gastrocnemius muscle tissues. Another important finding is that the responses of the heart muscle to exercise-induced oxidative stress are different from the gastrocnemius muscle. Muscle contraction results in the generation of reactive oxygen and nitrogen species (RONS) depend on the intensity, frequency and duration of the exercise. Strenuous exercise causes oxidation of protein, lipid and DNA, release of cytosolic enzymes and other signs of cell damage; however, only exhaustive exercise is detrimental (15). In the present study, MDA and NO levels in the gas-

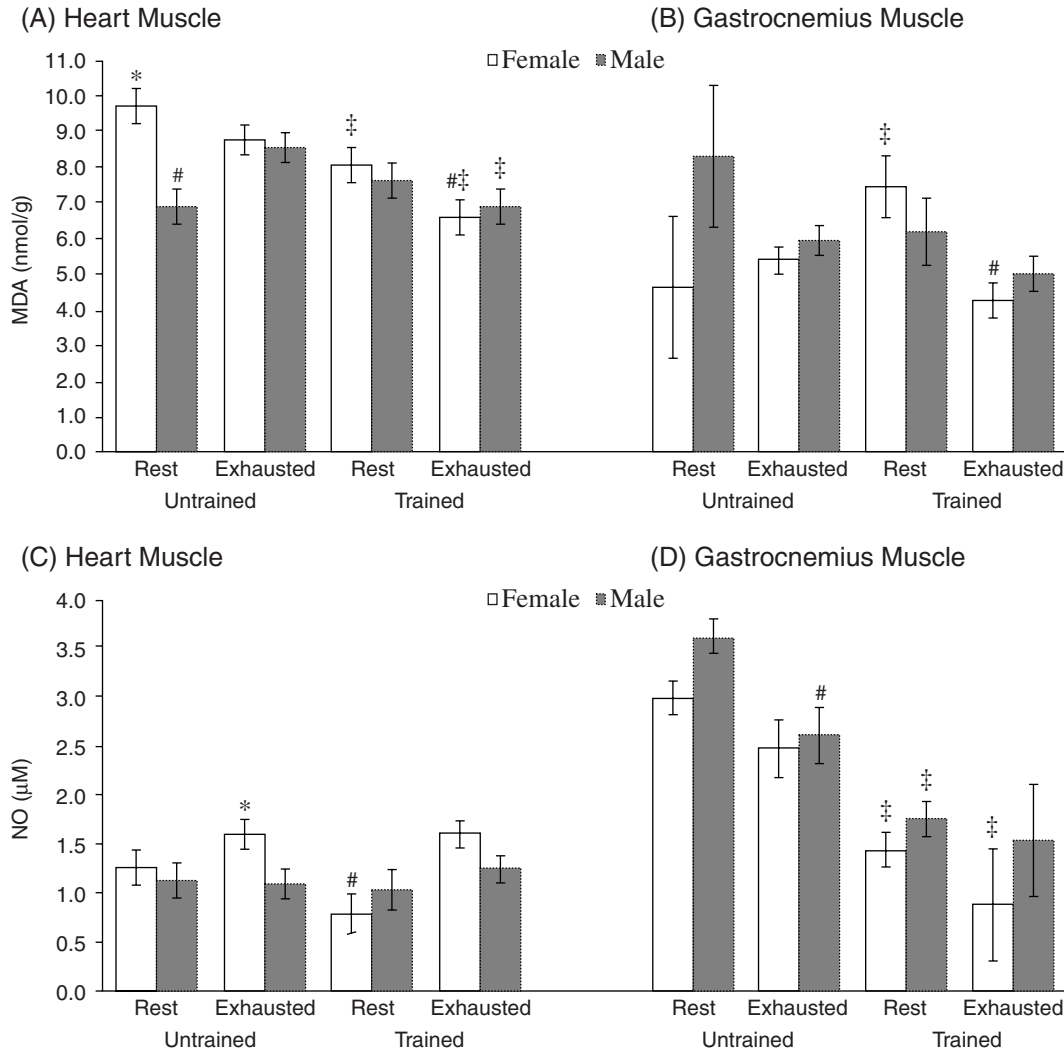


Fig. 1. The effects of sex, endurance training and exhaustive exercise on malondialdehyde (MDA) and nitric oxide (NO) in the heart and gastrocnemius muscle tissues. No significant interaction effect among the sex, endurance training and acute exhaustive exercise was observed on the MDA levels (A) in the heart ($F = 2.40$, $P = 0.13$) and (B) the gastrocnemius muscle ($F = 3.90$, $P = 0.06$), on the NO levels (C) in the heart ($F = 0.16$, $P = 0.69$), (D) the gastrocnemius muscle ($F = 0.70$, $P = 0.41$). The data were analyzed by a three way ANOVA for the effects of sex, endurance-training and acute exhaustive exercise. The data are presented as means \pm SEM. * $P < 0.05$ significantly different between male and female rats of rest or exhausted groups. # $P < 0.05$ significantly different between rest and exhausted rats of male or female groups. ‡ $P < 0.05$ significantly different between untrained and trained rats of male or female groups.

trocnemius muscle was affected by acute exhaustive exercise. However, contrary to expectations, the MDA and NO levels in the exhausted groups were lower than the rest group in trained rats. In contrast, the MDA level in the heart tissue was not affected by acute exhaustive exercise. In agreement with the present findings, Gul *et al.* (17) have reported that the MDA level in the heart tissue was not affected in rats that ran on the treadmill until exhaustion. Similarly, Liu *et al.* (29) have observed that level of MDA in the heart and skeletal muscle tissues are not different between the control and exhausted rats. On the contrary, after exhaustive swimming exercise,

increases in MDA levels in the heart tissue have been reported (2, 45). The results of the present study with regard to the levels of MDA and NO indicate that heart and gastrocnemius muscle tissues have a strong enzymatic and non-enzymatic antioxidant defense against exhaustive exercise-induced oxidative stress, especially in the trained rats.

MDA levels in the heart and NO levels in gastrocnemius muscle tissues were affected by training. In general, the endurance training reduced the levels of MDA and NO in both the rest and exhausted rats. In most of studies related to the effects of training on oxidative stress have reported that endurance training

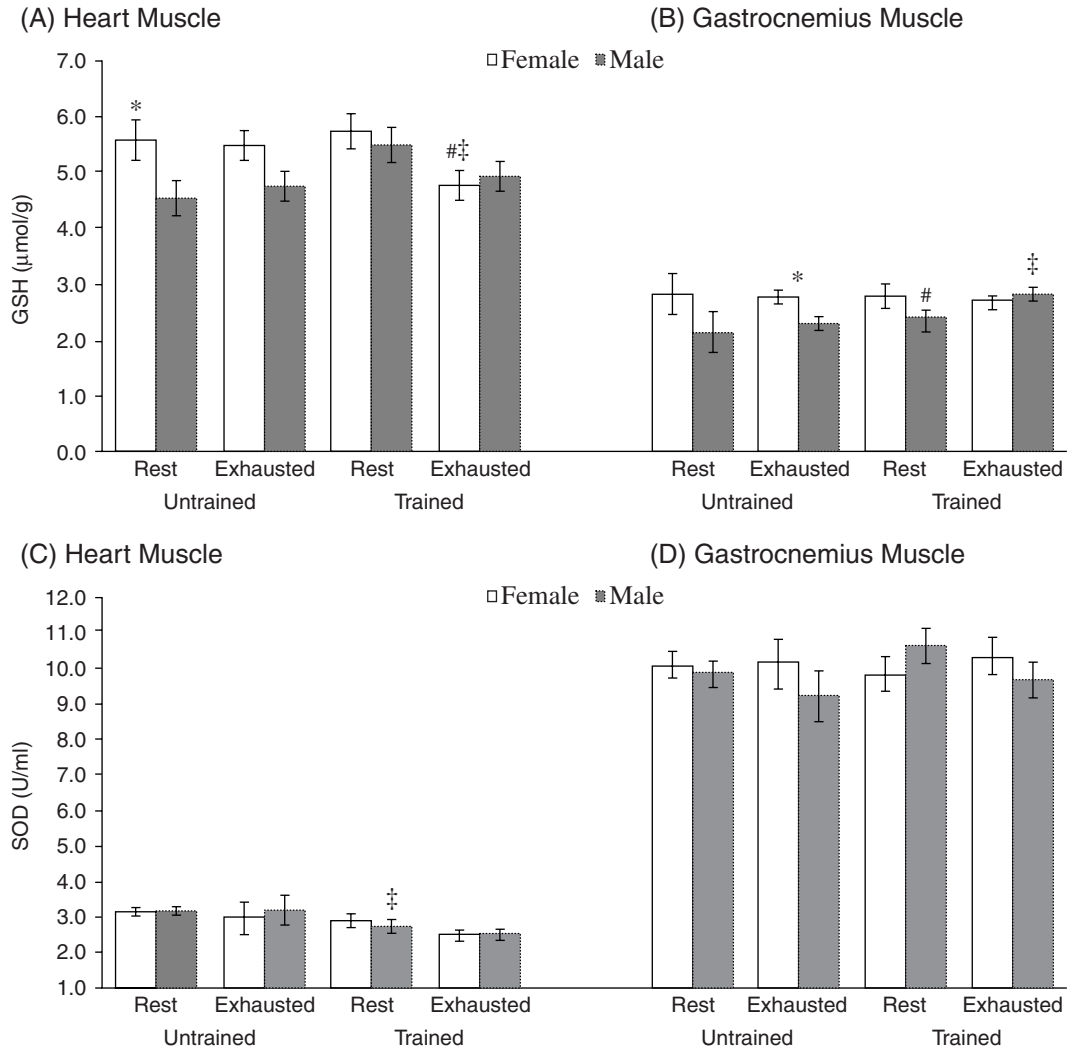


Fig. 2. The effects of sex, endurance training and exhaustive exercise on total glutathione (GSH) and superoxide dismutase (SOD) in the heart and gastrocnemius muscle tissues. No significant interaction effect among the sex, endurance training and acute exhaustive exercise was observed on the GSH levels (A) in the heart ($F = 0.01$, $P = 0.92$) and (B) the gastrocnemius muscle ($F = 0.52$, $P = 0.48$), on the SOD activity (C) in the heart ($F = 0.00$, $P = 1.00$), (D) the gastrocnemius muscle ($F = 0.03$, $P = 0.86$). The data were analyzed by a three way ANOVA for the effects of sex, endurance-training and acute exhaustive exercise. The data are presented as means \pm SEM. * $P < 0.05$ significantly different between male and female rats of rest or exhausted groups. # $P < 0.05$ significantly different between rest and exhausted rats of male or female groups. ‡ $P < 0.05$ significantly different between untrained and trained rats of male or female groups.

reduces exercise-induced oxidative stress damage (33, 35, 37). On the other hand, Gul *et al.* (17) found that MDA levels in heart tissue were not affected by eight weeks of training in male rats. Also, in heart and muscle tissues, increase in MDA levels in trained female rats has been reported (29). The interaction effects between exhaustive exercise and training on MDA levels show that endurance training reduces lipid peroxidation caused by exhaustive exercise in the heart tissue. In the trained groups, exhausted exercise decreased MDA levels in female rats. However, from these results, it has not been determined whether the change of MDA and NO levels comes

from a decrease of free radical generation during exercise, or from changes in the activities of the antioxidant enzymes.

The effect of gender on oxidative stress at rest is quite questionable. Several human studies have reported data that suggest that the MDA levels in men are higher than in women at rest (6, 24). However, other studies have reported that MDA levels were not different between male and female (16, 34). In the present study, we found that MDA was affected by gender in the heart tissue. Also, interaction effect was determined between gender and exhaustive exercise, endurance training on MDA in heart tissues.

Interestingly, the untrained female rats had higher MDA levels in the heart than the male rats at rest. Also, exhausted exercise increased MDA levels in male rats, but decreased in the female. Endurance training decreased MDA levels in females at rest, but did not change in males. However, the effect of gender on MDA and NO levels in gastrocnemius muscle was not significant. Effect of exhaustive exercise, endurance training and gender on MDA levels in heart tissue was different from gastrocnemius muscle tissues. The mitochondria in the female exhibited higher antioxidant capacity and lower oxidative damage than in male rats at rest (9). Another experimental study in mouse suggested that the female has a greater survival advantage when challenged with oxidative stress-induced cell death in heart compared to males (48). Oxidative stress by acute exhaustive exercise or endurance training elicits different responses depending on the organ tissue type and its endogenous antioxidant levels (29). Also, the differences in endogenous antioxidant capacity at rest may also contribute to gender differences in oxidative stress response to acute and chronic exercise.

It is well known that GSH is a major non-enzymatic endogenous antioxidant and has been reported to play an important role in protecting the skeletal and heart muscles from exercise-induced oxidative stress (20, 42). Prolonged physical exercise depletes GSH in the body; also exercise training maintains GSH levels or GSH redox status in the heart and skeletal muscle (25, 28). In the present study, total GSH levels in rat heart and gastrocnemius muscles were not affected by exhaustive exercise and endurance training. However, in the heart, the interaction was significant. Endurance training increased levels of GSH in response to exhaustive exercise in tissues. This situation can be explained in part by a higher gamma-glutamyl transpeptidase activity in this tissue, the GSH is actively used in heart and skeletal muscles during prolonged exercise and that GSH deficiency is tolerated by the heart and skeletal muscles, possibly compensated for by an increased GSH uptake from the plasma (26, 27). Previous studies have demonstrated inter-gender difference in antioxidant capacity for different organs, and this may partially account for longer lifespan of the female (9, 23). In the present study, the levels of total GSH in the heart and gastrocnemius muscles were affected by gender. In the heart, GSH levels were higher in untrained female rats compared to male rats at rest. GSH levels in the gastrocnemius were not different between the female and male rats at rest, but female rats showed higher GSH levels than the male after exhausted exercise in the untrained groups. Exhaustive exercise increased GSH levels in trained male rats, but it did not alter in female rats. Similarly, Goldfarb *et al.* (14) stated

that women have higher resting plasma antioxidant levels than men. However, oxidative stress markers increased similarly in both sexes in response to exercise of similar intensity and duration. Barp *et al.* (4) have suggested that myocardial antioxidant enzyme activities affected by sex hormones and estrogen may have an antioxidant role in heart muscle, while testosterone does not. Borrás *et al.* (8, 9) and Viña *et al.* (46) emphasized that mitochondria from the female produced less hydrogen peroxide than those from the male and have higher levels of mitochondrial reduced glutathione, SOD and glutathione peroxidase than the male. Also, oxidative damage is higher in the male than in the female. These differences between male and female, when present, has been attributed to estrogen action. The estrogens increase antioxidant levels leading to a lower efflux of ROS from the mitochondria. Also, estrogen does not act as antioxidants; rather, it acts by regulating the expression of antioxidant genes (46, 47). However, another study suggested that differences in oxidative stress could not be caused by differences in estrogen levels. Instead, other possibilities, such as differences in body size, could be responsible for such differences (39). The female has a stronger antioxidant defense system against oxidative damage than the male (23) and the adaptation to altered antioxidant capacity induced by exercise appears to be affected by gender differences (49).

On the other hand, total SOD activity was not affected by gender and exhaustive exercise in the tissues, but effects of endurance training on total SOD activities in the rat heart was found. SOD activities in the gastrocnemius were similar between female and male rats at rest after exhaustive exercise and training. The endurance training caused a decrease in SOD activities in the heart. Parallel to the present results, Gul *et al.* (17) and da Rocha *et al.* (12) found that SOD activities in the heart were not affected by endurance training in male rats. However, increased SOD activities in the heart have been reported in young and mid-aged female rats after 12-week endurance training program (22). The total SOD activities in the heart and gastrocnemius muscle showed a similar response to exhaustive exercise and training for both sex groups. Inconsistencies in findings of the studies may be partly explained by the different study designs, tissues and also from the different oxidative stress parameters and methodologies used for their determinations. A limitation of the study is that we examined only three markers of oxidative stress. However, it is possible that other markers of oxidative stress may provide additional information about the effects of sex differences and exercise training on exercise-induced oxidative stress in the heart and muscle tissues.

In conclusion, evidence from the current study suggests that gender was a major determinant of changes in MDA, NO and GSH levels after exhaustive exercise or endurance training. Also, the main and interactions effects of acute exhaustive exercise, endurance training and gender indicate that responses to oxidative stress in the heart and gastrocnemius muscle tissues are different. In general, most of the experimental studies related to exercise-induced oxidative stress and antioxidant changes are performed on either male or female, and results based on one sex are generalized to both. The present results suggest that gender differences should be considered when evaluating the effects of acute exercise and/or training on oxidative stress and antioxidant status.

Acknowledgments

This study was supported by the Selçuk University, Scientific Research Projects Coordinatorship (S.U.-BAP, Konya, Turkey) (Project no: 09401080).

Conflict of Interest

There is no conflict of interest in the present study.

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