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The effects of gender and exercise on malondialdehyde, nitric oxide and total glutathione levels in rat liver

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This study examined the effects of gender, acute exhaustive exercise and endurance training on the levels of malondialdehyde (MDA), nitric oxide (NOx) and total glutathione (GSH) in rat liver. The study was carried out with 12-week-old male and female Wistar rats. Rats were randomly divided into four subgroups: untrained-control, trained-control, untrained-exhausted, and trained-exhausted. Endurance training consisted of swimming 1 h each day, 5 days a week, for 8 weeks. Rats in exhausted exercise group were forced to swim until exhaustion. Levels of MDA and GSH in the liver were affected by both gender and acute exhaustive exercise. There were no significant interactions between gender, acute exhaustive exercise and endurance training on MDA, NOx or GSH levels in rat liver. In contrast to MDA and GSH levels, the level of NOx was not affected by acute exhaustive exercise or gender. Acute exhaustive exercise increased lipid peroxidation in the liver, especially in females. As a result, we conclude that gender, exhaustive exercise and endurance training do not have an effect on liver MDA, NOx and GSH levels.

Key words: Oxidative stress, acute exercise, exercise-training, gender, liver.

INTRODUCTION

Despite the many known health benefits of exercise, there is strong evidence suggesting that exhaustive and/or strenuous exercise can cause oxidative stress in both animals and humans (Ji, 1995; Viña et al., 2000). Exercise-induced free radical production is lower during low-intensity exercise than during high-intensity exercise. The free radical production that occurs during low-intensity exercise does not exceed the body’s antioxidant capacity; thus, no free radical damage is observed. However, free radical production and oxidative stress are both greatly increased during high-intensity exercise (Aguiló et al., 2003; Finaud et al., 2006; Goto et al., 2003). The liver is a complex organ that plays a key role in the detoxification and excretion of wastes and toxins. The liver also serves essential functions in the synthesis of blood proteins and coagulation proteins and in the storage of glycogen, iron and vitamins. Thus, a healthy liver is required to maintain normal physiological functioning and homeostasis (Beyer et al., 2010). The liver is the main organ responsible for maintaining the proper levels of blood nutrients that are required by peripheral tissues, including muscle. The liver is uniquely situated to perform this task because all of the nutrients that are absorbed by the intestines, except fatty acids, are released into the portal vein, which drains directly into the liver.

During intensive exercise, this task becomes more complex because a tremendous increase in energy production is required in order to maintain homeostasis (Lavoie, 2005). Lipid peroxidation terminates upon the conversion of lipid hydroperoxides into aldehydes and other carbonyl compounds. The amount of malondialdehyde (MDA), an aldehyde, can be measured using the thiobarbituric acid reactive substances (TBARS) test, a commonly used method for determining lipid peroxide levels (Halliwell et al., 1992). NOx is a versatile, inorganic free radical produced in biological systems (Valance and Collier, 1994). Depending on its micro-environment, NOx can react with a number of different oxygen metabolites and can convert these metabolites into various reactive nitrogen products, including peroxyxinitrite, which plays a role in oxidative damage (Stamler et al., 1992). Aerobic organisms are protected from oxygen toxicity by antioxidants. GSH is
one of the major antioxidants that limit the damage caused by oxidative stress, and it plays a crucial role in the defense of the body’s immune system. GSH is found in every part of the body, but it is most highly expressed in the liver (Itoh et al., 1998; Ji, 2000; Yamamoto et al., 2002). The liver is a large organ with a high metabolic rate; consequently, it exhibits the highest level of resting oxygen consumption of all organs in the body.

Recent studies have shown that reactive oxygen species (ROS), which are produced by the liver and neutrophils, may be responsible for hepatocyte damage (Giusti et al., 1995). Prominent liver responses to exercise include the activation of glycogenolysis, the conversion of glucogenic amino acids into pyruvate or citric acid cycle intermediates to supply glucose through gluconeogenesis, and fatty acid degradation to acetyl-CoA and then to ketone bodies for export to peripheral tissues via the bloodstream. All of these functions require specific cellular adaptations of the liver, an understanding of which has recently begun to emerge (Lavoie, 2005). The effects of acute exhaustive exercise and regular endurance training on the liver and other tissues have been examined by many previous studies. However, whether endurance training or gender alters the oxidative damage caused by acute exhaustive exercise in the liver tissue has not been determined. This study examined the effects of acute exhaustive exercise and endurance training on liver MDA, NOx and total GSH levels in both males and females.

METHODS

Animal care

The experiments were carried out with 12-week-old male (n=24) and female (n=24) Wistar rats. The rats were obtained from the Gazi University Experimental Research Centre (GÜDAM). Rats were housed in pathogen-free conditions at 23±1°C and were kept on a 12-h reversed light-dark cycle. They were provided standard rat chow and water ad libitum throughout the entire study period. All protocols for the current study were approved by Gazi University's Local Ethical Committee for Laboratory Animals.

Experimental design

Both male and female rats were randomly assigned to one of the following four subgroups: untrained-control, trained-control, untrained-exhausted, and trained-exhausted. The rats in the untrained-exhausted and all trained groups were accustomed to swimming with repeated 20 min swimming sessions for 1 week prior to the experiment. The rats in the two trained groups swam for 60 min/day, 5 days/week for 8 weeks. Swimming was performed in a plastic container and continuously supervised. The water temperature was set to 32°C. All exercises were performed at the same time of day for each training group. At the end of the exercise-training period, the trained and untrained rats were evenly divided between the control and the exhausted groups. Rats in the two exhausted groups were sacrificed immediately after acute exhaustive swimming, which was defined as the point at which the animal could not remain at the water surface. Rats in the control groups were sacrificed at rest.

Biochemical analysis

At the end of the experiment, rats were anesthetized with ketamine-HCl and xylazine (100 and 5 mg·kg^-1, respectively, intraperitoneally). Animals were subsequently decapitated, and their livers were quickly removed, washed and stored at -80°C until analysis.

Measurement of malondialdehyde

Lipid peroxidation was quantified by measuring the formation of TBARS (Casini et al., 1986). Lipid peroxide levels are expressed in terms of MDA equivalents using an extinction coefficient of 1.56 x 105 mol^-1 cm^-1.

Measurement of nitric oxide

The total nitric oxide level in liver was measured using a vanadium III chloride (VCl3)/Griess assay. Prior to NOx determination, liver tissue was homogenized in five volumes of phosphate-buffered saline (pH=7) and centrifuged at 2,000 g for 5 min. The supernatant was collected, and 0.25 ml of 0.3 M NaOH was added to 0.5 ml of the supernatant.

After incubating the reaction for 5 min at room temperature, 0.25 ml of 5% (w/v) ZnSO4 was added for deproteinization. This mixture was then centrifuged at 14,000 rpm for 5 min, and the supernatants were collected and used for the subsequent assays (Miranda et al., 2001). A standard nitrate solution was made via serial dilution. After loading the plate with 100 µl of each sample, VCl3 (100 µl) was added to each well, and the Griess reagents sulfanilamide (SULF) (50 µl) and N-(1-naphthy) ethylendiamine dihydrochloride (NEDD) (50 µl) were added immediately thereafter. Samples were incubated (usually 30 to 45 min), and the absorbance was measured at 540 nm using an ELISA reader.

Measurement of total glutathione

Total levels of GSH were determined using a modified Ellman method (Aykaç et al., 1985). Samples of liver tissue were homogenized in nine volumes of 10% trichloroacetic acid (TCA) and centrifuged at 3,000 g for 10 min. Two milliliters of 0.3 M Na2HPO4 and 0.25 ml solution of dithiobisni-trobenzoate (0.4 mg/ml 1% sodium citrate) were added to each well, and the Griess reagents sulfanilamide (SULF) (50 µl) and N-(1-naphthy) ethylendiamine dihydrochloride (NEDD) (50 µl) was then centrifuged at room temperature for 10 min. GSH levels were determined by measuring the absorption at 412 nm using a spectrophotometer (UV 1208, Shimadzu, Japan).

Statistical analysis

Statistical analyses were performed using SPSS version 15.0. Statistical significance was set at a level of P<0.05, and data were expressed as the mean ± SEM. One-way ANOVAs with Bonferroni’s post-hoc tests were used to compare group means. A three-way ANOVA was performed to examine the main effects of gender, training, and acute exercise (2x2x2) as well as any possible interactions between these variables.

RESULTS

The levels of MDA rat liver were significantly affected by gender (F=17.17, P=0.00) and acute exercise (F=35.49, P=0.00), but endurance training did not have a significant effect (F=0.02, P=0.88). MDA levels were significantly different among the groups (F=7.55, P=0.00). The MDA
levels observed in untrained male rats were higher than in untrained female rats in the exhausted group. There were also significant differences in MDA levels between the control and exhausted groups of both untrained and trained females.

We observed no significant interaction between gender and acute exercise (F=1.06, P=0.31), between gender and endurance training (F=1.50, P=0.23), between acute exercise and endurance training (F=0.06, P=0.81), or between gender, acute exercise and endurance training (F=0.20, P=0.66) on MDA levels in rat liver (Figure 1).

We observed no significant main effects of gender, acute exercise or endurance training and no significant interactions between these variables (P>0.05) on liver NOx levels, except for an interaction between acute exercise and endurance training (F=7.24, P=0.01). However, NOx levels in the liver were not significantly different between the groups (F=2.04, P=0.07) (Figure 2). The levels of GSH in rat liver were significantly affected by gender (F=40.84, P=0.00), acute exercise (F=106.61, P=0.00), and endurance training (F=4.80, P=0.03).

We also observed significant differences in GSH levels.
Figure 3. The effects of gender, acute exercise and endurance training on total glutathione (GSH). Open columns and solid columns show female and male rats. *P<0.05 significantly different between control and exhausted groups of males or females. †P<0.05 significantly different between males and females of control or exhausted groups. ‡P<0.05 significantly different between untrained control and trained exhausted rats of males or females.

between groups (F= 23.95, P=0.00). There were significant differences in GSH levels between male and female rats in both the exhausted-untrained and trained-control groups (P<0.05). GSH levels were significantly lower in the acute exhaustive exercise groups compared to the control groups in both untrained and trained male and female rats (P<0.05). Our results reveal significant interactions between gender and acute exercise (F=6.90, P=0.01) and between gender and endurance training (F=12.40, P=0.00) on the GSH levels. However, we observed no significant interactions between acute exercise and endurance training (F=0.15, P=0.70) or between gender, acute exercise and endurance training (F=0.01, P=0.91) on the levels of GSH in rat liver (Figure 3).

**DISCUSSION**

The objective of this study was to determine the effects of gender, acute exhaustive exercise and endurance training on MDA, NOx and total GSH levels in the rat liver. The major findings of this study were that the levels of MDA and GSH in the liver were affected by both gender and acute exhaustive exercise. In addition, we demonstrated that these factors had opposing effects on the levels of MDA and GSH in the rat liver. However, we observed no significant interactions between acute exhaustive exercise, endurance training and gender on the levels of MDA, NOx and GSH in liver tissue. Whether oxidative damage occurs in response to acute exercise depends on the intensity of the exercise. High-intensity acute exercise causes more oxidative damage than low-intensity exercise (Lovlin et al., 1987), an effect that is especially true when an individual is not accustomed to either the intensity or the duration of the exercise (Viña et al., 2000). The amount of oxidative damage caused by exercise can differ between various tissues and organs (Liu et al., 2002). Exhaustive exercise, such as swimming, affects the level of MDA in the livers of both trained and untrained rats.

In this study, we found that liver MDA levels in exhausted female rats were significantly higher than the levels in control female rats, regardless of training. Similar results were obtained in male rats, but the differences in males did not reach statistical significance. Similar to our results, Liu et al. (2000) found that the levels of MDA in the livers of female rats that had been run on a treadmill until exhaustion were significantly higher than in control female rats. The levels of MDA in the livers of male rats that had been run until exhaustion are also significantly higher than the levels in control male rats (Huang et al., 2009; Kon et al., 2007). In addition, liver MDA levels increased significantly in rats that had performed 30 min of swimming exercise when compared to control rats (Turgut et al., 2003). Many studies examining a variety of different tissues have reported that regular aerobic exercise can reduce the oxidative damage caused by acute exhaustive exercise (Aksoy et al., 2006; Miyazaki et al., 2001; Oh-ishi et al., 1997; Oztasan et al., 2004).

However, the results from the current study did not find
a significant effect of endurance training on liver MDA levels in response to acute exhaustive exercise. Rather, the levels of MDA in the livers of both male and female rats from the trained and untrained groups were similar in the control and exhaustion groups. Similar results were obtained in a study performed by Liu et al. (2000) who reported similar MDA levels in the livers of female rats that had undergone 8 weeks of training (that is running) and control female rats. Choi and Cho (2007) reported that the levels of MDA in the livers of trained and untrained male rats were similar at rest, during acute exercise and after acute exercise.

In contrast, Taysi et al. (2008) reported that acute exhaustive exercise increased the levels of MDA in the livers of untrained rats but not trained rats. Kakarla et al. (2005) reported decreased levels of oxidative stress in the livers of untrained rats but not trained rats. Kakarla et al. (2005) reported decreased levels of oxidative stress in the livers of female rats that had been repeatedly forced to train (that is run) over the course of 12 weeks. In another study, the TBARS levels of male rats that had been subjected to repeated forced swimming exercise over a period of 9 weeks were significantly lower than the TBARS levels in the control group (Metin et al., 2010). Husain and Somani (1997) reported that the liver levels of MDA decreased as a result of the 6.5 weeks of training. The many differences reported by these various studies may result from the fact that different training programs and acute exercises were performed at different times. It is also possible that the differential methodologies performed in the analyses of these data are responsible for the observed conflicting results.

In this study, we demonstrate that levels of MDA in rat liver are affected by gender. The MDA levels observed in female rats that have been subjected to exhaustive exercise was higher than the levels observed in male rats under the same conditions. In contrast to these results, Huh et al. (1994) previously reported that the levels of liver MDA in male rats were significantly higher than in female rats. This report concluded that the observed gender difference in lipid peroxidation resulted from the suppression of free radical production in females by estradiol. Another study carried out in male rats reported that estradiol protects hepatocytes from oxidative damage due to its antioxidant effects (Liu et al., 2002). Joo et al. (2004) reported that the free radicals produced during exercise at times when estradiol levels are high in women can be removed more easily than free radicals produced when estradiol levels are low. In this study, we found that the levels of liver MDA in both trained and untrained female rats that have been forced to exercise exhaustively were higher than the MDA levels observed in control females. However, we did not observe significant differences in male rats, a result that is inconsistent with human studies reporting no significant effects of gender on the level of oxidative stress caused by exercise (Bloomer et al., 2007; Pepe et al., 2009).

NOx, which is primarily produced in the liver, maintains the hepatic microcirculation and the integrity of the endothelial layer. On the other hand, inducible NOx synthase can be either beneficial or harmful (Chen et al., 2003). The variations in tissue levels of NOx in response to acute exercise may differ from tissue to tissue (Miyauchi et al., 2003). In the current study, we did not observe significant main effects of gender, acute exhaustive exercise or endurance training on the levels of NOx in the liver, but we did observe a combined effect of exhaustive exercise and endurance training on NOx levels. We observed no gender differences in NOx levels under any conditions. The levels of NOx in the livers of untrained rats tended to decrease as a result of acute exhaustive exercise but tended to increase in trained rats. However, these effects were not statistically significant. In contrast to the results of the current study, Qian et al. (2001) and Xiao et al. (2003) reported that the levels of NOx in the livers of rats that had been repeatedly forced to perform swimming exercise for 3 months were significantly higher than in rats that had not exercised.

Glutathione is the primary antioxidant synthesized in the liver. From the liver, it is transported into other tissues where it plays a major role in detoxification and the minimization of oxidative damage (Hardy et al., 2002; Meister and Anderson, 1983). In this study, the total level of GSH in the liver was affected by acute exhaustive exercise. Exhaustive exercise decreased the levels of liver GSH in both untrained and trained rats of both genders. Leeuwenburgh and Ji (1996) found that the levels of GSH in the livers of male rats that were fed 28 and 48 h before exhaustive exercise were significantly lower than the levels observed in control rats. However, they reported no significant differences in GSH levels in rats that were not fed. Similarly, Villa et al. (1990) reported that acute exhaustive exercise significantly decreased the levels of GSH in the livers of male rats. Ohkuwa et al. (1997) found that the levels of GSH in the livers of physically active and sedentary young rats decreased after exercise. In contrast to these results, exhaustive exercise, such as forced running, significantly increased the total levels of GSH in the livers of both young and old male rats (Bejma and Li, 2000). The observed decrease in liver GSH levels after acute exhaustive exercise may occur as a result of the transfer of GSH, which is synthesized in the liver, into the plasma and other tissues during exercise (Lew et al., 1985; Pyke et al., 1986). Previous reports have suggested that the levels of MDA and GSH in the liver are inversely proportional (Ji, 2000), a claim that is supported by the results of our study.

We observed no significant differences in the levels of GSH in the livers of any of the groups. Consistent with these findings, Ogonovszky et al. (2005) reported that three different (moderate, intense, excessive) swimming training programs performed at different intensities for 8 weeks did not cause any significant changes in the levels of GSH in the rat liver. Furthermore, endurance training
REFERENCES


