RESEARCH ARTICLE

ARAŞTIRMA

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The effect of regularly performed moderate-intensity exercise program on thiol/disulfide homeostasis, and ischemia-modified albumin

Düzenli olarak uygulanan orta şiddetteki egzersiz programının tiyol/disülfid homeostazı ve iskemi modifiye albümin üzerine etkisi

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ABSTRACT

Aim: Thiol/disulfide homeostasis is an indicator of oxidative stress and antioxidant capacity. Ischemia-modified albumin (IMA) is an important marker for both oxidative stress and ischemia. We aimed to evaluate the possible effects of regularly performed moderate-intensity exercise on thiol/disulfide homeostasis, and IMA levels.
Methods: Sprague Dawley rats were used. The study was composed of an Exercise group (EG, n=9) and Control group (CG, n=6). A 10-weeks swimming exercise was performed. Thiol/disulfide homeostasis measurement method was used in this study. IMA levels were measured by a cobalt-albumin binding method.
Results: In the EG, total thiol levels were significantly higher compared to the CG ($p<0.01$). The disulfide/total thiol ratio was lower in the EG compared to the CG ($p<0.01$). We observed that there was a slight increase in IMA levels in EG ($p=0.18$). This increase was not statistically significant.
Conclusion: Regularly performed moderate-intensity exercise has increased native and total thiol levels. Increase of thiol levels can prevent oxidative stress. Regularly performed moderate-intensity exercise programs appear to provide favourable effects on oxidative stress.

Key words: Swimming, oxidative stress, thiol/disulfide homeostasis, ischemia-modified albumin

ÖZ

Amaç: Tiyol/disülfid homeostazı, oksidatif stresin ve antioksidan kapasitenin bir göstergesidir. İskemi-modifiye albümin (İMA), hem oksidatif stres hem de iskemi için önemli bir belirteçtir. Düzenli olarak uygulanan orta şiddetteki egzersizin tiyol/disülfid homeostazı ve IMA seviyeleri üzerine olası etkilerini değerlendirmeyi amaçladık.
Yöntemler: Sprague-Dawley sıçanlar kullanıldı. Çalışma, Egzersiz grubu (EG, n=9) ve Kontrol grubundan (KG, n=6) oluşturuldu. 10 haftalık bir yüzme egzersizi yaptırıldı. Bu çalışmada tiyol/disülfid homeostazı ölçüm yöntemi kullanıldı. İMA seviyeleri, bir kobalt-albümin bağlama yöntemiyle ölçüldü.
Bulgular: EG'da, total tiyol seviyeleri KG ile karşılaştırıldığında anlamlı derecede daha yüksekti ($p<0.01$). Disülfid/total tiyol oranı EG'da KG ile karşılaştırıldığında daha düşüktü ($p<0.01$). EG'da IMA seviyelerinde hafif bir artış olduğunu gözlemledik ($p=0,18$). Bu artış istatistiksel olarak anlamlı değildi.
Sonuç: Düzenli olarak uygulanan orta şiddetteki egzersiz, nativ ve total tiyol seviyelerini artırdı. Tiyol seviyelerinin artması oksidatif stresi önleyebilir. Düzenli olarak uygulanan orta şiddetteki egzersiz programlarının oksidatif stres üzerinde olumlu etkiler sağladığı görülmektedir.

Anahtar Kelimeler: Yüzme, oksidatif stres, tiyol-disülfit homeostazı, iskemi-modifiye albumin

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Introduction

Exercise plays an important role in maintaining physical fitness and health [1]. Swimming exercises are generally of an aerobic nature and involve large muscle groups of the body. In physical exercises, as the energy demand of the body increases, oxygen consumption levels increase as well, compared to resting consumption [2]. Regularly performed swimming exercise is considered to be a good model of endurance exercise training [3]. Skeletal muscle metabolism changes in adaptation to endurance exercise [4]. Specifically, endurance exercises increase the oxidative capacity of skeletal muscles and this increase in metabolic rate can lead to a 100-fold increase in oxygen consumption in these muscles [5].

Oxidative stress (OS) causes the production of reactive oxygen species (ROS) and ROS induces the oxidation of disulfide groups to sulfhydryl containing amino acids [6]. In case of tissue damage, thiol groups can take part in the defence mechanism by reacting with free radicals. Thiol groups may be involved in the antioxidant defence system of the body by reacting with free radicals, having the potential to prevent tissue damage [7]. Under oxidative stress conditions, thiol groups are reversibly oxidized and converted into disulfide bonds and these can turn into thiol groups. Disruption of this homeostasis may lead to different diseases such as diabetes mellitus, cancer and cardiovascular diseases [7]. The intensity and duration of the exercise affects redox balance; regularly performed moderate-intensity exercise programs appear to provide favourable effects on oxidative stress [8].

Ischemia modified albumin (IMA) results from the damage caused by ROS at the N-terminus of albumin, and this conversion in albumin leads to a decrease in its metal binding capacity. IMA has been evaluated in various states of ischemia, such as exercise-induced myocardial ischemia, acute coronary syndromes, after a percutaneous coronary intervention [9,10]. Previous studies have shown that IMA is an ideal biomarker for ischemia and increased OS. Our study aimed to examine the effects of regularly performed moderate-intensity exercise on thiol/disulfide homeostasis and IMA levels.

Material and method

Experimental design

Male Sprague Dawley rats (300 to 350g, n=15) were housed 3 to 5 individuals per cage under controlled conditions (12-12 h light-dark cycle, room temperature 20±22°C, humidity 55 to 60%) with chow and tap water available ad libitum. All experimental procedures were approved by the Bezmialem Foundation University Ethics Committee (2021/127). Rats were randomly divided into two groups: Exercise Group (EG, n=9) and Control group (CG, n=6). The control group subjects remained in their cages throughout the study.

Exercise training program

The pre-training period in the swimming exercise group lasted for seven days. The first day lasted only 20 minutes, in the following days, duration of the exercise was increased 10 min per day until the duration of 60 minutes a day was achieved. Swimming sessions were applied as 60 min/day, 5 days/week, for 8 weeks. On the 9th week, rats swam twice a day, and on the 10th-week rats swam three times a day with sessions of 60 min duration [11]. A cylindrical beaker filled with water (35 cm deep and 50 cm wide) was used for swimming exercises. The temperature of the water was kept between 30°C and 32°C. The body weights were measured before and after exercise program.

Anaesthesia of the rats was ensured by intraperitoneally administered ketamine (45 mg/kg) and xylazine (5 mg/kg) and rats were sacrificed at the end of the tenth week of the swimming exercise protocol. Blood samples were collected by the intracardiac puncture and centrifuged at 3.000 rpm for 10 minutes at room temperature, just after the collection. After centrifugation, serum samples were separated to be frozen and stored at -80°C.

Biochemical analysis

Until now, thiol and disulfide groups were measured in one direction. Erel and Neselioglu have developed a new method that measures the levels of both variables, either separately and together. In our study, disulfide concentrations were calculated using half the difference between total thiol and natural thiol levels. The percentage of disulfide/total thiol, the percentage of disulfide/native thiol and the percentage of native thiol/total thiol, were then calculated [12]. Albumin levels were measured using the bromocresol green method. Cobalt-albumin binding assay, which is a colorimetric method, was used to measure IMA levels [13].

Statistical analysis

Statistical analyses were performed using the SPSS 13.0 (SPSS Inc. Chicago, IL, USA). Quantitative data was given as mean±SD or medians (interquartile ranges, IQR). Normal distribution and differences between variances were determined using Kolmogorov-Smirnov and Levene tests, respectively. Student's t-test and Mann Whitney U tests were used to comparing the groups. A p-value of <0.05 was considered statistically significant.

Results

Effect of exercise on body weight

After the exercise program, bodyweight of the EG decreased significantly compared to CG (352.38±20.74g vs 411.67±30.02g, $p<0.05$).

Biochemical analysis

Disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol and IMA/albumin ratios of CG and EG, are presented in Table 1. Native thiol, total thiol, native thiol/total thiol ratio and albumin levels, were significantly higher in the EG compared to the CG (p values <0.01, <0.01, <0.01, and p value=0.04, respectively). Total and native thiol levels in the groups are shown as whiskers graphs in Figure 1. In comparison to the CG, the EG had significantly higher native thiol levels (243.4±28.69 vs 179.6±20 µmol/L, $p<0.01$) and higher total thiol levels (298.6± 30.50 vs 240.2±24.53 µmol/L, $p<0.01$). Albumin levels were significantly higher in the EG (2.95±0.56 vs 2.37±0.52 g/dL, $p<0.04$). IMA/albumin ratio decreased in the EG compared to the CG, although this decrease was not statistically significant ($p=0.17$). Although not statistically significant, there was a slight increase in IMA levels in the EG ($p=0.18$). Disulfide/total thiol and disulfide/native thiol ratios were significantly lower in the EG compared to the CG (p values <0.01, both). Native thiol/total thiol ratio increased significantly in the EG compared to the CG ($p<0.01$). The disulfide level was lower in the EG compared to the CG, however this decrease was not statistically significant ($p=0.19$). No other significant difference in the measured parameters was found between the groups.

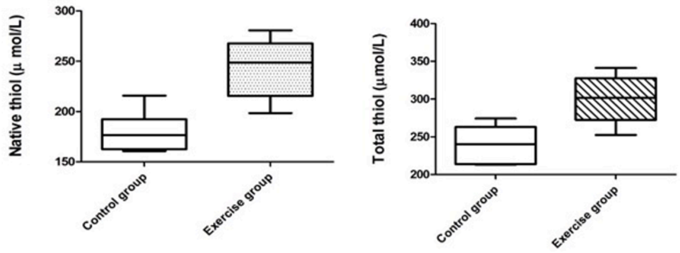

Figure 1. Box-and-whisker plot of native thiol and total thiol levels in the exercise and control groups (Whiskers: 10 and 90 percentile). The middle lines, upper and lower margin of boxes represent medians.

Discussion

The relationship between exercise and oxidative stress is complex. The occurrence of different responses to exercise varies depending on the duration and intensity of the exercise. One study examined the effect of a high-intensity exercise program on oxidative stress in post pubertal boys and girls. High-intensity exercise has been shown to cause oxidative stress and increase skeletal muscle damage in these cases [14].

Physical exercises cause an increase in metabolic demands of the body and to meet this demand, the body increases its oxygen consumption. As a result of this process, exercise causes an increase in the formation of free radicals, the amount of which depends on the duration and intensity of the exercise. As the intensity of the exercise increases, oxidant levels in the body increase in line with the intensity [15]. Munoz et al. performed cycling exercises with two different intensities in male cyclers, showing that short-term cycling exercises cause an increase in oxidative stress parameters [16]. In another study, Goto et al. applied training with different intensities (25%, 50%, 75%VO2Max) [17]. They showed that a 75% intensity exercise significantly increased oxidative stress parameters. In addition, they stated that mild intensity exercise caused a decrease in oxidative stress parameters.

Regularly performed moderate-intensity exercise programs are beneficial in reducing oxidative stress and protecting health. Acute and exhausting exercise programs can cause excessive ROS production, an increase in oxidative stress and an upregulation of antioxidant defence systems. Therefore, regular moderate exercise programs cause a decrease in oxidative stress, provides protection against diseases and improve quality of life [8].

In addition, regular exercise programs cause a decrease in body weight [18]. Oscai et al. showed that rats subjected to a regular endurance exercise program had lower body weight than controls [19]. In our study, we showed that regularly performed moderate-intensity swimming exercises caused a significant decrease in body weight ($p < 0.05$). The other response was observed in the antioxidant defence system: regular exercise provides more resistance to oxidative stress damage. Generally, antioxidant capacity increases in athletes and rats who exercise regularly [18].

Different results were obtained in studies evaluating the relationship between exercise and antioxidant parameters. This difference appears to occur depending on the model, duration, intensity and type of exercise and the methods used to measure antioxidant parameters. Therefore, it is crucial to use simple, dependable and sensitive methods [20]. Inayama et al. have shown that after a marathon race, plasma protein thiol concentrations were decreased and they suggested protein sulfhydryls were oxidized during the competition [21]. Oxidative stress causes thiol groups are reversibly oxidized and converted to disulfide bonds, which can convert to thiol groups [7]. Thiol groups can react with free radicals to create an antioxidant defence system. This antioxidant defence mechanism is maintained by thiol/disulfide homeostasis and disruption of this homeostasis can lead to various disorders such as diabetes, cancer and cardiovascular diseases [20].

Kayacan et al. applied treadmill exercise training to rats (5 min/week for 10 weeks) and measured thiol and disulfide levels. It was seen that the disulfide levels were significantly lower in the EG. This study demonstrated that moderate exercise reduced oxidative stress [20].

We investigated the effects of regularly performed moderate-intensity swimming exercise on thiol/disulfide homeostasis and to the best of our knowledge, this is the first study of its kind. We have shown that in the exercise group, thiol levels increased and disulfide levels decreased. Disulfide/thiol ratios were significantly lower in the EG compared to the CG (Table 1). These results show that a regularly performed moderate-intensity exercise program is effective in reducing oxidative stress, by reducing disulfide levels and increasing thiol levels.

Table 1. Thiol, disulfide levels, and IMA levels in the exercise and control groups.

	Control Group (n=6)	Exercise Group (n=9)	p value
Albumin (g/dl)	2.37± 0.52	2.95 ± 0.56	0.04
IMA (IU/mL)	0.51 ± 0.02	0.55 ± 0.05	0.18
IMA/Albumin Ratio	0.23 ± 0.06	0.19 ± 0.04	0.17
Native thiol (μmol/L)	179.6 ± 20	243.4 ± 28.69	< 0.01
Total thiol (μmol/L)	240.2 ± 24.53	298.6 ± 30.50	< 0.01
Disulfide (μmol/L)	30.33 ± 4.46	27.64 ± 2.96	0.19
Disulfide/Native thiol (%)	16.94 ± 2.34	11.47 ± 1.68	< 0.01
Disulfide/Total thiol (%)	12.62 ± 1.31	9.3 ± 1.11	< 0.01
Native Thiol/Total thiol (%)	74.77 ± 2.63	81.40 ± 2.23	< 0.01

IMA: Ischemia modified albumin, p<0.05 was considered significant for statistical analyses and only significant statistics were shown bold in the table.

The mechanisms that cause changes in IMA levels during exercise are not yet clear. IMA levels change depending on the type of exercise applied [21]. After a marathon run, Apple et al. showed that the level of IMA had not changed immediately [22]. Other researchers have reported a mild decrease in mean IMA concentration after a marathon run [23]. In a study on plasma, IMA levels of patients with coronary artery disease were measured after a treadmill exercise. Decreased IMA levels were observed, and levels returned to initial concentrations 60 min after the exercise [9]. IMA levels were measured in healthy individuals after the hand-grip test. IMA levels were found to decrease at 1, 3 and 5 min after the forearm ischemia, whereas afterwards, IMA levels returned to baseline. The same changes have been seen in the IMA/albumin ratio [24].

Bhagwan et al. showed that IMA levels increase in patients with coronary ischemia [25]. Bar-Or et al. showed that after ischemia, IMA levels returned to baseline level within 6 hours [13]. In the present study, as we investigated albumin and IMA levels, we observed that in the EG, albumin levels were significantly higher compared to the CG (Table 1) and that there was a slight increase in IMA levels in the EG. Additionally, we showed that IMA/albumin ratios in both groups were similar (Table 1). Finally, we found that the exercise program applied can cause a slight increase in the IMA plasma level.

This study showed that a swimming exercise program applied as 60 min/day for 10 weeks can prevent oxidative stress. In this context, we can say that the regularly performed moderate exercise will result in an increase in the antioxidant capacity thiol/disulfide ratio.

Limitations

Our study had some limitations. Studies that have evaluated exercise and antioxidant parameters together, showed that a range of variables affected the findings. These variables depend on variations in experimental design (exercise intensities, duration, type, etc.) and methods used to assess oxidative stress.

Conclusion

In our study, we demonstrated that regularly performed moderate-intensity exercise decreases oxidative stress and increases antioxidant capacity with increasing thiol groups, without creating an ischemic load. In addition, in this study, a new method of thiol/disulfide measurement was used to evaluate oxidative stress. This measurement method is proposed as a useful and practical method.

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