Antioxidant enzymes and oxidative stress adaptation to exercise training: Comparison of endurance, resistance, and concurrent training in untrained males

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Abstract

The aim of this study was to compare the effect of endurance training (ET), resistance training (RT), and concurrent training (CT) on circulating antioxidant capacity and oxidative stress. For this purpose, 30 men aged 21.7 ± 2.4 years were assigned to the following three training groups: ET, which included continuous running with incremental intensity that was increased up to 80% of maximal heart rate (n = 10); RT, which included a beginning load of 50% of one repetition maximum (1RM) that was increased up to 80% of 1RM (n = 10); and CT, which included ET and RT programs every other day during the week (n = 10). Activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in erythrocytes and total antioxidant capacity (TAC) and malondialdehyde (MDA) level in plasma were measured. The results showed that SOD significantly increased by 21.85% (p = 0.020), 9.54% (p = 0.032), and 14.55% (p = 0.038) in the ET, RT, and CT groups, respectively. Furthermore, the activity of erythrocyte GPx significantly increased in the ET (p = 0.018) and CT (p = 0.042) groups. The TAC increased significantly in the ET (p = 0.040) and CT (p = 0.049) groups compared with the pretest values. The MDA level significantly decreased in the ET group by 32.7% (p = 0.028), by 32% in the RT group (p = 0.025), and by 29.1% (p = 0.047) in the CT group. However, there was no significant difference in the interaction of time and group between variables of SOD and GPx enzymes and TAC of plasma and MDA in the ET, RT, and CT groups (p < 0.05). It can be concluded that all three training types induced the same changes in redox state (increased SOD activity and reduction in MDA levels), but at different rates.

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Keywords: Antioxidant defense; Combined exercise training; Erythrocyte; Malondialdehyde

Introduction

For every 25 molecules reduced by normal mitochondrial respiration in aerobic organisms, incomplete reduction occurs in one molecule.1 This generates a reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, or hydroxyl radical, which are highly reactive molecules that can attack cellular components and damage cellular structure.1 While performing a strenuous exercise, the metabolic rate in the skeletal muscle increases up to 100 times above the resting levels, with marked increases in oxygen consumption and elevated superoxide anion production in the mitochondria.2,3 It has been reported that if either aerobic or anaerobic exercise is of sufficient intensity and duration, the result is increased oxidative modification of proteins, nucleic acids, and lipids.4,5 During aerobic metabolism, skeletal muscles produce significant amounts of superoxide anion due to electron leakage from the electron transfer chain, which further leads to the production of ROS such as hydroxyl radical, hydrogen peroxide, and hydroperoxides.6-8 These ROS are highly reactive and can attack cellular components, leading to cellular damage.7,8 Therefore, it is essential to evaluate the antioxidant capacity in response to exercise training.
transport chain. In anaerobic exercise, however, other pathways of reactive oxygen and nitrogen species (RONS) generation exist, including ischemia reperfusion, xanthine oxidase production, prostanoid metabolism, phagocytic respiratory burst activity, disruption of iron-containing proteins, and altered calcium. It has been postulated that because physical exercise causes an augmented generation of ROS in skeletal muscle, exercise training might upregulate enzyme and nonenzymatic antioxidant defense systems. Radovanovic et al. reported that CT increased oxidant enzyme and nonenzymatic antioxidant defense systems. Radaš et al. suggested that endurance training (ET) exerts a beneficial (protective) effect on oxidative damage independent of age.

By contrast, recent evidence indicates that chronic resistance training (RT) may provide a protective effect similar to aerobic exercise. Although there are some conflicting reports about the effect of RT and ET on oxidative stress and antioxidant defense mechanisms, few studies compared the effect of associated ET, RT, and concurrent training (CT) on oxidative stress with these two types of exercise performed in isolation. Radovanovic et al. reported that CT increased erythrocyte malondialdehyde (MDA) and plasma catalase activities, and thus this type of training was considered undesirable with regard to oxidative damage. However, whether this negative effect was due to CT or simply due to the increase in exercise load is not clear because there was no ET-only control, and the training load was not matched. To this point, no studies have been conducted that are appropriately designed to compare the effect of CT on systemic oxidation. Therefore, this study was carried out to measure the respective effects of 8 weeks of RT, ET, and CT on the activity of antioxidant enzymes in erythrocytes, and MDA level and total antioxidant concentration in plasma in sedentary young men.

Methods

Participants

Thirty untrained males with no experience of formal physical activity volunteered to participate in this study and were assigned to one of three homogeneous groups: resistance (RT; n = 10), endurance (ET; n = 10), and concurrent (CT; n = 10). Table 1 presents the physical characteristics of the study participants.

![Table 1: Physical characteristics of participants at the start of the study.]

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Body mass (kg)</th>
<th>Height (cm)</th>
<th>Body fat (%)</th>
<th>BMI (kg/m²)</th>
<th>1RM (kg) chest press</th>
<th>VO2max (mL/kg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.2 ± 2.1</td>
<td>72.6 ± 3.8</td>
<td>173.4 ± 3.6</td>
<td>19.3 ± 6.1</td>
<td>24.1 ± 2.1</td>
<td>33.7 ± 3.7</td>
<td>41.3 ± 3.5</td>
</tr>
<tr>
<td>21.1 ± 2.1</td>
<td>71.4 ± 3.8</td>
<td>175.4 ± 3.6</td>
<td>19.5 ± 2.3</td>
<td>23.1 ± 2.1</td>
<td>30.8 ± 6.2</td>
<td>41.3 ± 2.9</td>
</tr>
<tr>
<td>22.8 ± 2.9</td>
<td>73.5 ± 5.8</td>
<td>174 ± 5.5</td>
<td>20.3 ± 2.8</td>
<td>24.2 ± 2.6</td>
<td>32.5 ± 4.1</td>
<td>40.7 ± 4</td>
</tr>
<tr>
<td>0.984 ± 0.411</td>
<td>1.65 ± 0.210</td>
<td>0.593 ± 0.560</td>
<td>0.615 ± 0.610</td>
<td>0.739 ± 0.487</td>
<td>0.165 ± 0.848</td>
<td>0.616 ± 0.609</td>
</tr>
</tbody>
</table>

1RM = one repetition maximum; BMI = body mass index; VO2max = maximum oxygen consumption.

The participants attended an information and familiarization session in which the details of all training procedures were explained. The criteria of exclusion were cardiovascular and pulmonary disease, smoking, obesity, and hormonal abnormalities. None of the participants reported taking exogenous anabolic-androgenic steroids, drugs, medication, or dietary supplements that affected their redox potential or physical performance. The experimental procedures were approved by the Institutional Review Committee of Islamic Azad University (Sanandaj Branch) and were in accordance with the Declaration of Helsinki.

Functional and physiological measurements

Participants attended the laboratory on three occasions at baseline. During their first visit, 10 mL of peripheral blood were obtained from the antecubital region of the left arm with the participants in a seated position after 20 minutes of rest. In the second visit, participants’ height and weight (Seca, Model 220, Germany) were measured, and their body fat percentages were estimated by measuring skin-fold thickness (Lafayette, Model 01127, USA). The participants were then segregated into three groups according to body fatness, because it has been reported that there is a correlation between the exercise-induced change in oxidative stress and body fat. Then, one repetition maximum (1RM) on a bench press was measured. Participants were required to perform 10 repetitions at 50% of 1RM, estimated according to the capacity of each participant. After 3 minutes, subsequent trials were performed for 1RM with progressively heavier weights until the 1RM was determined within three attempts, with 3–5 minutes of rest between trials. On the third visit, maximal oxygen consumption (VO2max) was determined by indirect, open-circuit calorimetry (MedGraphics, CPX/D) according to Bruce protocol while the participants exercised to volitional fatigue on a motorized treadmill (TechnoGym, Model Run Race, Italy).

Blood collection and erythrocyte isolation

After a 10-hour fasting between 8 and 10 AM and before the determination of functional and physiological measurements, a 10-mL blood sample was obtained from the antecubital vein. The procedure was repeated 72 hours after the completion of the training program (at the end of the 8th session). Immediately, 150 µL of ethylenediaminetetraacetic acid (Merck, Germany) was added to the blood samples and the mixture was centrifuged at 2500–2700 rpm for 7 minutes. The plasma and the surface layer were then separated from erythrocytes. Plasma samples were used for measurement of total antioxidant capacity (TAC) and MDA. In addition, activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured in erythrocytes. Erythrocytes were washed three times with 0.9% NaCl solution (normal saline) at 4°C. Distilled water was added to the erythrocytes to obtain a solution of approximately 50% dilution. To achieve complete lysis of erythrocytes, samples were stored at room temperature for 5–10 minutes. The mixture was then centrifuged for 10
minutes at 4°C. The clear supernatant solution was then divided into equal volumes of 0.5 mL and stored in Eppendorf tubes at −70°C for SOD and GPx analysis.

**Biochemical analyses**

Erythrocyte SOD activity was measured using a commercially available kit (RANSOD, catalog number SD 125, Randox UK).\(^\text{16}\) The activity of erythrocyte GPx was measured using a commercially available kit (RANSEL, catalog number D 505, Randox UK), which uses the method suggested by Paglia and Valentine.\(^\text{17}\) Activities of SOD and GPx were expressed relative to the hemoglobin concentration. Similarly, a commercially available kit (catalog number NX 2332, Randox UK) was used to measure TAC in the plasma.\(^\text{8}\) In addition, MDA was measured based on the method suggested by Buege and Aust.\(^\text{19}\)

**RT protocol**

The RT protocol was performed with progressive loading three times/week on nonconsecutive days for 8 weeks, and included circuit training. The movements included in this training were chest press, lateral pull down, leg extension and flexion, biceps and triceps curl, squat, and sit-ups (with 90- and 180-second intervals between sets and cycles, respectively). The training started with 50% of 1RM at Week 1 with 10 repetitions (3 sets in each exercise). The load for each exercise was increased by approximately 5% each week such that the intensity of training reached 80−85% of 1RM by the end of the 8th week. Participants’ 1RM was measured again at the end of the 4th week and the prescribed loads were adjusted accordingly.

**ET protocol**

The ET protocol was performed in the form of continuous running with progressive intensity three times/week on nonconsecutive days for 8 weeks. The training started with 50% of maximal heart rate (HR\(_{\text{max}}\)), which was increased by 5% each week in such a way that the intensity of the training reached 80−85% of HR\(_{\text{max}}\) by the end of the 8th week. Participants at the start of the study and analysis of dietary data were done with one-way analysis of variance (ANOVA) (Table 1). Dependent variables were compared using two-way, repeated measures (pretraining and post-training) ANOVA to investigate the influence of time and the training intervention. A significant interaction between group and time indicates a significant effect of training on the dependent variable of interest. The dependent t test was used for comparing pretest and post-test in groups if time and training were significant. A p value <0.05 was taken to be significant. All statistical analyses were carried out using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

**Results**

**Nutrition**

All participants completed a detailed daily food diary in which they recorded all food and drinks consumed during the 3 consecutive days before beginning the training protocol. At the end of the training period, participants again recorded their food intake for 3 consecutive days. Diet was analyzed using Food Processor software (ESHA Research, Salem, MA, USA) with regard to the antioxidant and macronutrient content.

**Statistical analyses**

All data are presented as mean ± standard deviation. First, the Shapiro−Wilk test was used to determine the normality of data distribution. Homogeny of physical characteristics of participants at the start of the study and analysis of dietary data were done with one-way analysis of variance (ANOVA) (Table 1). Dependent variables were compared using two-way, repeated measures (pretraining and post-training) ANOVA to investigate the influence of time and the training intervention. A significant interaction between group and time indicates a significant effect of training on the dependent variable of interest. The dependent t test was used for comparing pretest and post-test in groups if time and training were significant. A p value <0.05 was taken to be significant. All statistical analyses were carried out using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA).

**Nutrition**

Before training, the daily energy intake (proportions of carbohydrate, fat, and protein, respectively) of the ET (57% ± 5%, 28% ± 4%, 15% ± 3%), RT (51% ± 3%, 31% ± 2%, 18% ± 3%), and CT (54% ± 3%, 33% ± 7%, 13% ± 3%) groups were not significantly different to each other. Although individual and total macronutrient intake increased during training in all the groups, there was no significant difference between the groups. Nutrient analysis of the dietary records of the ET, RT, and CT groups before and after the training period using one-way ANOVA is presented in Table 2.

**Maximum strength**

Before starting the study, there was no significant difference between the ET, RT, and CT groups in maximum strength (1RM of bench press). Nevertheless, after 8 weeks of RT, strength in the RT group in bench press movement significantly increased from 33.3 to 44.2 kg by 32.7% (\(p < 0.001\)). In the CT group, 1RM of bench press significantly increased from 32.4 to 39.3 kg by 21.2% (\(p = 0.037\)). However, these results did not show any significant change in the ET group.
The results showed no significant difference between the ET, RT, and CT groups in VO2max before starting the training program. However, after the training course, VO2max significantly increased from 41.3 to 47.5 mL/kg/minute (15%, p = 0.003) in the ET group. In addition, in the CT group, the VO2max nonsignificantly increased by 5.8% from 40.7 to 43.1. However, no significant difference was found between pretest and post-test results in the RT group. There was also no significant difference between the ET, RT, and CT groups in body fat percentage before and after the 8-week training.

Moreover, results of one-way ANOVA test demonstrated no significant difference between SOD and GPx activities in erythrocytes and also between TAC and MDA plasma levels in the ET, RT, and CT groups before starting the training course.

Repeated measures ANOVA did not reveal a significant group–time interaction for erythrocyte SOD and GPx activities. Similarly, no significant group–time interaction was apparent for MDA and TAC levels in plasma. This issue showed that there was no significant difference between the effects of ET, RT, and CT on the aforementioned dependent variables.

By contrast, the results do indicate the main effects of training (time). Post hoc testing (dependent t test) showed that the activity of erythrocyte SOD was significant following training in the ET (p = 0.020), RT (p = 0.032), and CT (p = 0.038) groups (Fig. 1A). Furthermore, the activity of erythrocyte GPx significantly increased with ET (p = 0.018) and CT (p = 0.047) in MDA and nonsignificantly increased with RT (p = 0.049) compared with the pretest value (Fig. 1C). A significant decrease was found between pretest and post-test ET (p = 0.028), RT (p = 0.025), and CT (p = 0.047) in MDA (Fig. 1D).

**Discussion**

The aim of this study was to test the effect of CT on changes of antioxidant and oxidative stress indices. To this end, 30 untrained men received the aforementioned training programs for 8 weeks. The results showed that 8 weeks of ET,
RT, and CT in men decreased oxidative stress and increased enzymatic and nonenzymatic antioxidant capacity. However, as there was no training-group interaction, no significant difference was observed in the effectiveness of these trainings.

It has been reported that there is a correlation between the exercise-induced change in oxidative stress and body fat. Results showed that 8 weeks of exercise training was not able to produce a significant change in body composition and body fat. Therefore, a change in body composition was not behind the training-induced changes in oxidative stress and MDA.

The SOD activity in erythrocytes increased by 21.8% in the ET, 14.5% in the CT, and 9.5% in the RT group; however, there was no statistically significant difference between the three groups. Several studies on sedentary individuals and athletes have produced differentiating effects of training on the basal activity of erythrocytes antioxidant enzymes such as SOD. Factors such as the initial training status, the training protocols, and different isoenzymes of SOD have been pointed out as conditioning the basal erythrocyte antioxidant enzyme activities. For example, Higuchi et al. suggested that the MnSOD enzyme is the main factor for increasing the total SOD activity because the Cu,ZnSOD isoenzyme was not largely affected by training. These results contradict the results of our study because MnSOD isoenzyme is a mitochondrial enzyme, and therefore, an increase in the activity of SOD in the current study should be due to the increase in the activity of Cu,ZnSOD isoenzymes. The increase in activity of SOD suggests that training produced oxidative stress in the erythrocytes in all treatments. However, it should be considered that the oxidative stress created during resistance and endurance exercises could be through different mechanisms. Despite a lesser oxygen requirement during RT compared with aerobic activity, production of free radicals during resistance activity is significant and caused by xanthine oxidase pathway, respiratory burst of neutrophils, catecholamine autoxidation, local muscle ischemia, and conversion of the weak superoxide to the strong hydroxyl radical by lactate, which caused oxidative stress.

It is not clear whether the increase in enzymatic activity in the CT group was caused by adaptation effects in response to ET or RT and also it is not known which one has a greater effect.

These data demonstrate that GPx enzyme activity significantly increased by 36.9% in the ET, significantly by 33.3% in the CT, and nonsignificantly by 20.8% in the RT group. Although catalase and GPx perform similar actions by converting hydrogen peroxide to H2O2, GPx and catalase deal with high accumulation of H2O2 and low accumulation of H2O2, respectively. It seems that ET increased production of H2O2 enough to stimulate significant increases in GPx activity. By contrast, in this study, RT did not significantly increase GPx activity. In all likelihood, erythrocytes were less exposed to the production of H2O2 during RT.

Fig. 1. Pretraining and post-training values between the three groups over 8 weeks of training, with the values as mean and standard deviation: (A) superoxide dismutase, (B) glutathione peroxidase, (C) plasma total antioxidant capacity, and (D) malondialdehyde. * Significant difference from pretraining. CT = concurrent training; ET = endurance training; GPx = glutathione peroxidase; MDA = malondialdehyde; RT = resistance training; SOD = superoxide dismutase; TAC = total antioxidant capacity.
The effects of RT in SOD enzyme and decrease in the need for TAC for defense against oxidative stress.

Finally, the data show that all of ET, RT, and CT significantly decreased resting MDA levels (by 32.7%, 32%, and 28%, respectively), although no significant difference was found between these three groups. Decrease of MDA resting level was caused either by the increase in antioxidants activity or by the decrease in the production rate of free radicals and an associated decrease in oxidative stress. Because the SOD activity increased in all the three ET, RT, and CT groups, an increase in antioxidant activity is clearly one of the factors for the decrease of plasma MDA. However, it has been reported that the ability of neutrophils to produce superoxide anion and the decrease of antioxidant activity increased in all the three ET, RT, and CT groups, an associated decrease in oxidative stress. Because the SOD activity significantly decreased resting MDA levels (by 32.7%, 32%, and 28%, respectively), although no significant difference was found between these three groups. Decrease of MDA resting level was caused either by the increase in antioxidants activity or by the decrease in the production rate of free radicals and an associated decrease in oxidative stress. Because the SOD activity increased in all the three ET, RT, and CT groups, an increase in antioxidant activity is clearly one of the factors for the decrease of plasma MDA. However, it has been reported that the ability of neutrophils to produce superoxide anion decreases by performing ET. Moreover, an increase in the production of ROS during ET, RT, and CT might increase the resistance of cell membrane-rich unsaturated fatty acids to lipid peroxidation reactions. In this regard, Petibois and Déleris point out that ET can decrease the sensitivity of erythrocytes to oxidative stress.

Conclusion

This study shows that all three of ET, RT, and CT types could decrease oxidative stress. However, the potential mechanism mediating this phenomenon may vary such that some antioxidants significantly change with RT, and some more so with ET.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

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