Effect of high intensity interval training under hypoxic conditions in a normobaric environment on moderately trained university students’ antioxidant status

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Abstract

Purpose: The effects of high intensity interval exercises on antioxidant defence system are not clear. Since there is an evident lack of studies focused on oxidative stress in moderately trained males following high intensity interval training, we investigated oxidative stress markers (malondialdehyde [MDA], catalase [CAT], glutathione peroxidase [GPX], superoxide dismutase [SOD]) by completing a high intensity interval training program (HIITP) under hypoxic and normoxic conditions in a normobaric environment.

Material: The study was carried out on moderately trained university students who had regular exercising habits. The participants completed 8-week wingate based high intensity interval training under normoxic and hypoxic conditions (2500 m.) in the normobaric environment. They were instructed to maintain their normal dietary practices during the study not to take any antioxidant containing vitamin tablets.

Results: The interaction effect (time×group) for SOD (p=0.230), CAT (p=0.736), GPX (p=0.517), and MDA (p=0.596), revealed no significant change in repeated response.

Conclusions: Although 8 weeks of high-intensity interval training significantly affected only SOD and GPX (p<0.05), the normoxic and hypoxic conditions did not present any significant change between treatments.

Keywords: interval training, superoxide dismutase, catalase, glutathione peroxidase, malondialdehyde.

Introduction

In recent decades, intensive research in the field of oxidative damage indicates that exercise exacerbates the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), some of which are free radicals [1, 2]. A free-radical is any specie capable of existence with one or more unpaired electron [3]. ROS/RNS refer to oxygen or nitrogen containing free-radicals and their non-free-radical derivatives [4]. ROS are generated by regular metabolic process in vivo and can initiate a cascade of free-radical formation and damage to macromolecules [5]. Oxidative stress is an inevitable consequence of aerobic life, and there is growing evidence that the endogenous generation of ROS plays a major role in aging and many pathological conditions [6]. In resting state the body is equipped with both non-enzymatic and enzymatic antioxidant defence system to scavenge the potentially harmful effects of ROS [7, 8]. This system includes antioxidant enzymes such as glutathione peroxidase (GPX), catalase (CAT), and superoxide dismutase (SOD), and non-enzymatic molecules including vitamin E, vitamin C, vitamin A precursor, thiol-containing compounds e.g. glutathione (GSH). These antioxidant defence systems preserve homeostasis for normal cell functions at rest and under normal physiological conditions. However, during strenuous exercise, pathogenic processes and aging, ROS production may overwhelm antioxidant defence capacity causing cell and tissue damage [9, 10].

Chronic aerobic exercise has emerged as a promising means of reducing oxidative stress. Mechanisms responsible for beneficial effects of chronic aerobic exercise are training induced regulation of SOD [11] and CAT [4] and reduced mitochondrial reactive oxygen species [12]. It has been proposed that chronic high intensity interval training (HIIT) may elicit greater health benefits than traditional chronic aerobic exercise, so HIIT has gained importance, since it is more effective on developing aerobic capacity. While it provides fast and effective adaptation, it also shortens exercise time. Nowadays, HIIT and its other forms are most effective training methods used to improve aerobic and anaerobic capacity, cardiovascular system and metabolic functions [13, 14]. HIIT provides new and favorable contributions in respect to health and performance and positive adaptation for both sedentaries and athletes. When it is compared with traditional aerobic exercise, it has been drawing interest, since it uses time more economically and effectively, improves aerobic and anaerobic systems besides the metabolic functions and physical performance [15, 16]. High intensity training can produce oxidative stress and antioxidant elements of organisms are affected with this challenge [17]. Few studies have investigated oxidative stress in response to both aerobic and anaerobic exercise bouts [18, 19], especially oxidative stress, which is experienced following sporting competitions [20].

Apart from abovementioned effects, intermittent hypoxia (IH) occurs in many pathophysiological conditions. The molecular mechanisms associated
with IH, however, have received little attention [21]. The consequences of oxidative stress under hypoxic conditions, when physical effort is limited by the availability of oxygen to the working muscles, are of great interest to sport science [22-24]. Indeed, physical training under hypoxic conditions is frequently used to improve physical performance [25, 26] and training in hypoxic conditions has become an important element of preparing elite athletes [27, 28]. The training is thought to be most effective when it is performed at an altitude of 2, 000 to 2, 500 m [29, 30].

There have been studies concerning effects of HIIT and IH applications on antioxidant markers [12, 31]. These studies investigated acute effects and were applied to elite athletes and thus there is a lack of studies investigating effects of this training method on antioxidant markers of active individuals for a longer time. Herein, we evaluated the antioxidant status after 8 week wingate style HIIT protocols in untrained healthy men. Plasma samples were collected for the measurement of CAT, SOD, GPX and MDA activities and total antioxidant status as a general marker of antioxidant defences.

**Material and Methods**

**Subjects**

In this study, 16 recreationally active university students, aged 20-29 (23.50±2.52) were involved in this study. Only males were included to avoid any distortion in the hormonal response to physical exercise caused by sex differences. Anthropometric characteristics of the subjects are summarized in Table 1. The exclusion criteria for study were drugs and medicines intake as well as suffering from some illness and smoking habit. None of the subjects participated regularly in sport competitions and they did not engage in any form of vigorous exercise for 24 hours before the study was performed. They were instructed to maintain their normal dietary practices during the study and not to take any antioxidant containing vitamin tablets.

**Table 1. Anthropometric characteristics of the subjects**

<table>
<thead>
<tr>
<th>Variables (n=16)</th>
<th>Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23.50±2.52</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.00±6.19</td>
</tr>
<tr>
<td>Body Weight (kg)</td>
<td>70.60±9.03</td>
</tr>
</tbody>
</table>

**Physiological measurements**

The body mass was measured using calibrated digital scales and height was measured using stadiometer. The age of athletes were accurately recorded as years. Blood samples were taken from the participants three times; the first one prior the training, the second one in 4th week and the third one after the training.

**Exercise Protocol**

16 participants were randomly assigned to normoxic or hypoxic groups and then they were completed 8-week high intensity interval training on normoxic and hypoxic conditions (2500 m) in the normobaric environment. The hypoxic conditions were provided with Hypoxica Submit II exercise package (Made in USA). All participants completed 8 weeks of wingate style cycling training, 3 days/week, consisting of incremental repeats 4 to 7 every two weeks × 30s all-out effort with 4 min rests.

**Measurement of oxidative status**

Erythrocyte MDA level were measured as previously described by Dahle et al. [32], SOD level was measured as previously described by Durak et al. [33], GPX level were measured as previously described by Paglia and Valantina [34], CAT level was measured as previously described by Aebi [35].

**Statistical analysis**

Results of all variables are expressed as mean and standard deviation. The Shapiro-Wilk Test of normality was used to determine if the data normally distributed. Baseline differences of antioxidant variables were calculated with Independent Sample T-Test between groups. Then the two way repeated measures ANOVA was used to compare differences in three measurement results using time and conditions factor (interaction effect: time×group). The post-hoc analysis was performed to specify pairwise differences. All analyses were set at p=0.05 significance level.

**Results**

The baseline measurements of SOD, CAT, GPX, and MDA presented no significant differences in normoxic and hypoxic groups (p>0.05). The interaction effect (time×group) for SOD (p=0.230), CAT (p=0.736), GPX (p=0.517), and MDA (p=0.596), revealed no significant change in repeated response (baseline, after 4 weeks and 8 weeks) (Table 2). Although 8 weeks of high-intensity interval training effected significantly only SOD and GPX (p<0.05), the normoxic and hypoxic conditions did not present any change between treatments. The post-hoc analysis showed that the high-intensity interval training effect differed 4 weeks and 8 weeks for SOD (p=0.037), baseline and 8 weeks for GPX (p=0.014) responses. The rate of nonsignificant increase on SOD was 13.5% in the hypoxic group, 8.65% in the normoxic group after 8 weeks (Figure 1). This trend was not same for GPX response in hypoxic condition. The GPX decreased by 2.33%. However, GPX was increased by 7.33% in the normoxic group (Figure 2). When we looked our study, we monitored that MDA levels increased both 4th and 8th week. (p<0.05)

**Discussions**

To our knowledge, this is the first investigation to compare the differential effects of HIIT in hypoxia vs HIIT in normoxia on antioxidant status in a normobaric environment. There are studies stating that both methods changed oxidative stress markers [31, 36-38].

There are numerous reports that provide reasonable
support to the notion that exercise increases the production of ROS [39]. Little is known, regarding the extend of oxidative stress when comparing aerobic and anaerobic exercise modes [40]. On the occurrence of exercise, stress is not clear. However, the principal factor responsible for oxidative damage during exercise is the increase in oxygen consumption [41]. It appears that anaerobic types of exercise, which involves less oxygen circulation throughout the body than aerobic exercise is associated with an increased ROS generation level through other pathways [42, 43] suggesting that oxygen consumption per se is not the major cause of exercise induced oxidative damage [41].

Different types of exercises may have different effects on oxidative stress [34]. Which is defined as a situation in which an increased level of ROS generation overwhelms

### Table 2. The baseline measurements.

<table>
<thead>
<tr>
<th>Indicators</th>
<th>HIIT (Hypoxia)</th>
<th>HIIT (Normoxia)</th>
<th>Timex Treatment</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4 Weeks</td>
<td>8 Weeks</td>
<td>Baseline</td>
<td>4 Weeks</td>
</tr>
<tr>
<td>SOD (U/ml)</td>
<td>1930.16 ± 1183.74</td>
<td>2046.95 ± 745.15</td>
<td>2231.46 ± 714.93</td>
<td>2449.82 ± 799.13</td>
</tr>
<tr>
<td>CAT (KU/ml)</td>
<td>29691.75 ± 5232.78</td>
<td>27724.50 ± 6578.55</td>
<td>28685.25 ± 8418.99</td>
<td>32848.50 ± 8687.06</td>
</tr>
<tr>
<td>GPX (U/ml)</td>
<td>6.47±1.67 ± 21.41</td>
<td>8.13±1.64 ± 33.56</td>
<td>6.27±2.14 ± 24.18</td>
<td>5.73±1.31 ± 60.22</td>
</tr>
<tr>
<td>MDA (nmol/ml)</td>
<td>263.12 ± 21.41</td>
<td>274.71 ± 33.56</td>
<td>275.72 ± 24.18</td>
<td>263.12 ± 60.22</td>
</tr>
</tbody>
</table>

Notes. SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase; MDA: malondialdehyde. n=16

**Figure 1.** The oxidative stress status of CAT and GPX on moderately trained university students. Values are expressed as mean ± S.D. CAT: catalese; GPX: glutathione peroxidase; n=16.

**Figure 2.** The oxidative stress of MDA and SOD on moderately trained university students. Values are expressed as mean ± S.D. MDA: melondialdehyde; SOD: superoxide dismutase; n=16.
the antioxidant defence capacity, resulting in oxidative damage to lipids, proteins and DNA [41, 43].

A review of literature on changes in oxidative stress markers and physical parameters following the long duration HIIT training in hypoxia indicates a lack of information in this subject area. For this reason, we investigated the oxidative status of moderately trained males during 8 week by completing HIIT program.

It was reported that the activities of antioxidant enzymes including SOD, CAT and GSH peroxidase (GPX) increased with an acute bout of exercise in skeletal muscle, heart and liver [38].

SOD, CAT, GPX and MDA activities in response to exercise are variable. When we looked at our study, we observed that interaction effect (time×group) for SOD, CAT, GPX and MDA revealed no significant in repeated response (p<0.05) (baseline, after 4 weeks and 8 weeks). SOD and GPX activities effected significantly by HIIT. But the normoxic and hypoxic conditions were not present between treatments. HIIT effect were differ 4 weeks and 8 weeks for SOD (p=0.037), baseline and 8 weeks for GPX (p=0.014) responses. This trend was not same for GPX response in hypoxic condition. However, GPX was increased by non significantly in the normoxic group. MDA levels increased both 4th and 8th week.

Currently, there is limited information on the effects of HIIT on the development of oxidative stress in humans. Previous studies were short-term and applied on rats and in normoxic conditions. Similarly, Wozniak et al. evaluated the influence of exercise in high-altitude conditions (about 2000 m a.s.l.) on SOD and CAT activities in 10 kayakers and 10 rowers. They found a significant increase of SOD and CAT activities in erythrocytes after the 4th, 10th and 18th day of training [44]. It is known that continuous and intermittent efforts under hypoxic conditions increase oxidative stress [23, 24, 45]. Bailey et al. demonstrated that 60 min of simulated training under hypoxic conditions significantly increased the levels of serum lipid peroxides, with a simultaneous reduction of antioxidant enzyme activities [46]. Gonzalez et al. [47] and Pialoux et al. [48] reported diminished MDA levels in the plasma of swimmers after an acute hypoxic swimming test (10 min at 4, 800 m) as well as in cyclists who spent 13 days at an altitude of 2, 500 to 3, 000 m, and trained at 1200 m above sea level.

SOD is one of the main antioxidant enzymes that degrade superoxide radicals [46]. Increase in SOD enzyme activity corresponds with enhanced resistance to oxidative stress. Groussard et al. found that SOD activity decreased after a single sprint anaerobic exercise [40]. Not all studies reported decrease in SOD in response to exercise. It has been reported that 8-week moderate intensity of aerobic training did not elevate SOD activity. Furthermore, it has been revealed a decrease in SOD levels an acute bout of exercise in skiers participating in a graded treadmill test to exhaustion and elevated erythrocyte SOD activity immediately post exercise when the sprinters performed a sprint exercise [49].

CAT activity in response to exercise is variable. Following about of submaximal exercise a decrease in erythrocyte CAT activity reported in trained cyclists. Furthermore, it has been reported that sprinters who performed a sprint type exercise did not have altered erythrocyte CAT activity [49].

GPX activity is a key component of the glutathione homeostasis and its response to exercise is variable [35]. Higher in oxygen consumption during exercise activates the enzyme GPX to remove hydrogen peroxide. In response to an acute bout of HIIT, elevated erythrocyte GPX activity has been found after a sprint exercise but no change when runners performed an endurance exercise [49].

**Conclusion.**

The present study is the first to report improvements in oxidative status after 4 and 8 week high intensity interval training. We speculate that changes in these parameters might represent an increase in ROS after high intensity interval training.

In comparison with other investigators, we believe the present study provided the first direct analysis of effect of high intensity interval training on moderately trained males’ antioxidant status hypoxic conditions in a normobaric environment after training. The results of this study also suggest that interaction effect (time×group) for SOD, CAT, GPX and MDA revealed no significant in repeated response. However, it was observed that 8 weeks of high-intensity interval training affected significantly only SOD and GPX. The normoxic and hypoxic conditions were not present between treatments. The limitation of our study was no standardization of dietary habits of the participants. It can be advised to monitor dietary habits of the participants and to apply the same applications to elite athletes for the future studies.

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**Conflict of interest**

There were no conflicts of interest.
References


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