PRE-ADMINISTRATION OF VITAMIN C REDUCES EXERCISE-INDUCED OXIDATIVE STRESS IN UNTRAINED SUBJECTS

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Abstract – We investigated the effects of a 40 min bout of bicycle exercise, and the effects of vitamin C administration 12 h before exercise, on the serum markers of oxidative stress in young untrained subjects. Increased levels of malondialdehyde, the marker of the lipid peroxidation, and a decrease in specific activity of the antioxidant enzyme glutathione peroxidase that were observed as a result of exercise, pointed to the presence of exercise-induced oxidative stress. These markers were reduced by pre-administration of vitamin C. The results suggest that physically active subjects could increase their daily dietary vitamin C intake in order to reinforce their antioxidant defenses prior to exercise training.

Key words: bicycle exercise; oxidative stress; vitamin C.

INTRODUCTION

It is now generally accepted that there is an important correlation between various physical exercises and oxidative stress (Reid et al., 2001). However, when it comes to the exact mechanism governing the interaction between contracting skeletal muscles and the oxidative metabolism, there are many controversies. Some authors have reported that intense and prolonged exercise can result in free radical generation and oxidative damage (Powers and Jackson, 2008, Reid et al., 1992), while there are reports stating that exercise decreases oxidative stress and inflammation (Asghar et al., 2007), and in vivo experiments demonstrating that regular exercise is a very effective way to reduce oxidative stress, especially in old rats (Goto et al., 2004). In addition, there are many contradictions in this area of research regarding the levels of some oxidative stress markers, as they have been shown to either increase (Ohno et al., 1994, Evelo et al., 1992), decrease (Tiidus et al., 1996, Ji et al., 1993) or remain unchanged after exercise (Powers and Jackson, 2008). Despite some recent opinions stating that exercise-induced oxidative stress might be prevented by some antioxidant interventions (Sun et al., 2010, Araújo et al., 2011), there is still insufficient knowledge about the possible protective effects of antioxidants against exercise-induced oxidative damage.

In the present work, we studied the effects of a 40 min bout of bicycle exercise, and the effects of vitamin C administration 12 h before the exercise, on several serum markers of oxidative stress, superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and malondialdehyde (MDA).
MATERIALS AND METHODS

The study included 28 untrained volunteers aged between 19 and 30 (average age 22.64±1.66), all of them males, from the Alexandru Ioan Cuza University of Iaşi. Their demographical features are presented in Table 1.

The subjects were physically evaluated in order to determine the characteristics of the study groups, such as maximal oxygen uptake (VO\textsubscript{2max}) and maximal aerobic power (MAP). Before physical assessments, the subjects warmed up taking part in joint gymnastics. During the tests, subjects were provided with water for hydration.

*The maximal oxygen uptake (VO\textsubscript{2max})*

This is considered the maximum rate at which oxygen can be taken in by the body through transport from the environment to the active muscularity. The assessment of VO\textsubscript{2max} is generally done by standardized effort tests (Grant et al., 1995). The subject pedaled on a Vision Fitness E3200 bicycle at a rate of 80 rotations per minute (RPM) with a load of 143 W (corresponding to the fourth level of resistance for the bike). Blood pressure was measured at the beginning and end of the test. This is a progressive effort test on minute levels, the power increasing on each level by 25 W. The subject must carry out the maximum number of levels possible. A pedaling frequency of 80 RPM and a steady breathing rate throughout the whole test must be kept. The effort ceases when the subject can no longer keep up the pace of the previous level he reached. Leaning on the bicycle horns is allowed according to subject’s preference, except on forearms. Contact of the foot with the pedal is done on the sole.

*Maximal aerobic power (MAP)*

This parameter indicates the maximum physical workout scheme of the muscles involved in the effort, being reached simultaneously with VO\textsubscript{2max}. During a progressive effort test, VO\textsubscript{2max} and MAP increase gradually, but there are cases of individuals with similar VO\textsubscript{2max} and different MAP (Arts et al., 1993). The average values of these two parameters are presented in Table 2. To safely conduct the evaluations, professional medical staff and an intervention team from the Romanian Red Cross (Iaşi division) were present in the sports hall. The sports hall in which the effort tests were held has an area of 68.5 m\textsuperscript{2}, a volume of 230 m\textsuperscript{3} with ventilation through a 1 m\textsuperscript{2} window. During the research, some atmospheric parameters (temperature, humidity and atmospheric pressure) were continuously monitored (Table 3). After the physical assessment of the subjects, one week later, the experiment was performed using the Vision Fitness E3200 bicycle between 9 a.m. and 4 p.m. Serum was collected before and immediately after the exercise. Parameters such as the heart rate, blood pressure and the oxygen saturation were monitored before and after the exercise. The exercise required 3 min of cycling at 30% of MAP, followed by 37 min at 50% of MAP. A week later, twelve hours before the experimental effort, the subjects received one 1000 mg tablet of vitamin C. The administered tablets contain traces of hydroxypropyl methylcellulose, microcrystalline cellulose, stearic acid, silicon dioxide and magnesium stearate. After the slow assimilation interval (12 h later), the subjects performed the same experimental effort described above for 40 min. Serum was again collected immediately after the effort in 9 ml vacutainers, allowed to clot and centrifuged immediately, aliquoted into Eppendorf tubes and stored at -40°C until measurement. After collecting biological samples, the subjects remained in the hall to be monitored for at least 15 min. The study was conducted according to provisions of the Helsinki Declaration and all subjects signed an informed consent for participation in this study.

*Determination of SOD*

Superoxide dismutase (SOD) activity was measured by the percentage reaction inhibition rate of enzyme with WST-1 (2-(4-Iodophenyl)- 3-(4-nitrophenyl)-5- (2,4-disulfophenyl)- 2H-tetrazolium, monosodium salt) substrate (a water-soluble dye) and xanthine oxidase using a SOD Assay Kit (FLUKA, 19160) according to the manufacturer’s instructions. Each end-
point assay was monitored by absorbance at 450 nm (the absorbance wavelength for the colored product of WST-1 reaction with superoxide anions) after 20 min of reaction time at 37°C. The percent inhibition was normalized by mg protein and presented as SOD activity units.

**Determination of GPX and CAT**

The glutathione peroxidase (GPX) activity was measured using the GPX cellular activity assay kit CGP-1 (SIGMA). This kit uses an indirect method based on the oxidation of glutathione (GSH) to oxidized glutathione (GSSG) catalyzed by GPX, which is then coupled with recycling GSSG back to GSH utilizing glutathione reductase (GR) and NADPH. The decrease in NADPH at 340 nm during oxidation of NADPH to NADP is indicative of GPX activity. Catalase (CAT) activity was determined according to the method of Sinha et al. (1972).

**Determination of MDA**

Malondialdehyde (MDA) levels were determined by thiobarbituric acid reactive substance (TBAR) assay. The signal was read against an MDA standard curve, and the results were expressed as nmol/ml (Ciobica et al., 2011).

**Data analysis**

The levels of oxidative stress markers (SOD, GPX, CAT and MDA) were statistically analyzed using one-way analysis of variance (ANOVA). All results are expressed as mean ± the standard error of the mean (SEM). Post hoc analysis was performed using Tukey’s honestly significant difference test in order to compare the groups. F values for which p<0.05 were regarded as statistically significant.

**RESULTS**

We observed a significant group difference in terms of specific activity of SOD, the first line of defense against reactive oxygen species (ROS) (F(2,79)=3, p = 0.038) (Fig. 1). Post hoc comparisons showed a significant difference between the baseline group and the after-exercise (effort) group (p=0.013), as well as between the baseline and vitamin C+effort group (p=0.034). However, no significant differences were observed between the effort group and vitamin C+effort group (p=0.82) (Fig. 1). A significant group difference (F(2,79)=12, p < 0.0001) (Fig. 2) GPX activity was observed. Post hoc analysis showed significant differences between the baseline group and after-effort group (p=0.032), between the baseline and the vitamin C+effort group (p=0.01), as well as between the effort group and vitamin C+effort group (p <0.0001) (Fig. 2). A significant group difference in CAT activity was observed (F(2,79)=5, p = 0.007) (Figure 3). Post hoc comparisons showed a significant difference between the baseline group and after-effort group (p=0.01), as well as between the effort group and vitamin C+effort group (p=0.015). However, no significant differences were observed between the baseline and the vitamin C+effort group (p=0.8) (Fig. 3).

MDA, the main marker for lipid peroxidation processes, exhibited a significant group difference (F(2,79)=32, p < 0.0001) (Fig. 4). Post-hoc analysis revealed significant differences between the baseline and after-effort group (p<0.0001), between the baseline and the vitamin C+effort group (p=0.048), as well as between the effort group and vitamin C+effort group (p < 0.0001) (Fig. 4).

![Graph](image-url)  
**Fig. 1.** The specific activity of SOD in baseline, effort and effort after pre-treatment with vitamin C groups. The values are mean ± SEM (n=28). *p < 0.034 vs. baseline.
Discussion

In the present study, we observed an increase in oxidative stress as a result of 40 min bicycle exercising in untrained subjects, as shown by the increased levels of MDA and by the decrease in specific activity of the antioxidant enzyme GPX, when compared to baseline. We also showed that the oxidative stress levels were reduced by the pre-administration of vitamin C, as demonstrated by the decreased levels of MDA and the increase in GPX activity of GPX.

It is generally believed that the barrier between the benefits of exercising in good health and chronic exercise, which could represent a form of oxidative stress through the alteration of the balance between antioxidants and ROS, is extremely fragile (Ji et al., 1999). Moreover, it is believed that low levels of ROS are required for normal and physiological force production in skeletal muscle, while high levels of ROS would result in contractile dysfunction and consequent muscle weakness and fatigue (Powers and Jackson, 2008). The ROS resulting from exercise can be mainly produced from the mitochondrial electron transport chain, xanthine oxidase and various inflammatory processes (Stefanescu et al., 2012).

However, as mentioned before, there are many contradictory results regarding the connections that might exist between exercising and oxidative stress. A possible explanation for these different results could be differences that might appear between trained and untrained subjects. Senturk et al. (2001) demonstrated that exercise-induced oxidative stress affects erythrocytes in sedentary rats, but not in exercise-trained rats.

We also obtained increased levels of SOD in our subjects. According to some authors, this might be a compensatory mechanism for coping with the en-
hanced production of ROS during endurance exercise (as shown in our results by the increased levels of MDA), since it has been demonstrated that acute exercise results in a large increase in enzyme activities and this activation can be viewed as a defensive mechanism of the cell in the face of an increased oxidative challenge (Ji et al., 1995). There are reports describing increased levels of SOD in similar situations, which found that endurance exercise will result in an increase in mitochondrial superoxide dismutase and catalase activities (Somani et al., 1995). In our study, the levels of CAT were also increased in exercise group compared to baseline.

It seems that in order to protect the tissues against potential ROS damage, antioxidant enzymes adaptively respond during exercise training by increasing their activities in the tissues and organs (Oztasan et al., 2004). Regarding the relevance of vitamin administration in this area of research, we decided to administrate vitamin C, which is a water-soluble antioxidant that could scavenge superoxide, hydroxyl or lipid hydroperoxide radicals and thus could prevent some oxidative damages (Fang et al., 2002).

Previous results regarding the relevance of vitamin C in this area of research gave contradictory results, since it was reported to reduce the running time to exhaustion (Packer et al., 2006) by modulating some effects at the mitochondrial level, while other authors found that vitamin C dietary supplementation could not prevent training-induced oxidative stress (Gohil et al., 1996). Although some studies demonstrated that vitamin C administration could in fact decrease muscle fatigue (Ji et al., 1999), other authors insisted that overdosing with this specific vitamin could affect the heart, especially in the case of acute prolonged exercise, mainly through a pro-oxidant reaction with transitional metal ions (Halliwell and Gutteridge, 2007).

We showed that the oxidative stress levels are reduced by the pre-administration of vitamin C, as demonstrated by the decreased levels of MDA and the increase in the activity of GPX when compared to both baseline and effort without antioxidant administration groups. In addition, it was previously shown that vitamin C could play an important role in the recycling of vitamin E, a process that will result in the formation of a vitamin C (semi-ascorbyl) radical (Powers et al., 2008).

Regarding the mechanisms of vitamin supplementation in exercise-induced oxidative stress, it is

<table>
<thead>
<tr>
<th>Feature</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>22.64 ± 1.66</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.29 ± 3.69</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.16 ± 8.82</td>
</tr>
</tbody>
</table>

**Table 1.** The demographical features of the experimental group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VO2max (mL/kg/min)</td>
<td>45.31 ± 5.29</td>
</tr>
<tr>
<td>Maximal aerobic power (W)</td>
<td>287.93 ± 41.02</td>
</tr>
</tbody>
</table>

**Table 2.** The physiological parameters of the experimental group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
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<tbody>
<tr>
<td>Temperature (ºC)</td>
<td>20.85 ± 0.11</td>
</tr>
<tr>
<td>Atmospheric pressure (mmHg)</td>
<td>743.37 ± 6.79</td>
</tr>
<tr>
<td>Humidity (%)</td>
<td>40.42 ± 0.69</td>
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**Table 3.** Atmospheric parameters for conducting the research.
generally accepted that they could reduce the production of ROS, inhibiting lipid peroxidation and enhance antioxidant defenses (i.e. the production of antioxidant enzymes and endogenous antioxidants). The results we present here show a significant increase in the specific activity of SOD and GPX as a result of vitamin C pre-administration.

There are recent studies that demonstrated the relevance in this area of research of a combination of mitochondrial targeting nutrients in the rat, considering that besides increasing the levels of oxidative stress, long-term exercise could also result in mitochondrial dysfunctions. Sun et al. (2010) demonstrated that these compounds could represent an effective strategy to prevent fatigue or enhance recovery, despite some dual effects in their usage, which are mainly dependent on the so-called adaptation process. The process of adaptation mainly refers to the fact that long term, the cells may induce antioxidant enzyme production in order to cope with the exercise-induced oxidative stress. These aspects are of course limited by the antioxidant capacity of the individual, as well as the specific tissue involved.

Additionally, when it comes to this specific process of adaptation, one of the advanced features proposed for its mechanism was reported by Senturk et al. (2001), which described it as an adaptive mechanism aimed to ensure the prevalence of a younger erythrocyte population by leading to increased erythrocyte destruction.

Due to the limitations of our study, one of the most important aspects to mention the fact that we collected the blood immediately after the exercise finished, while the true levels of ROS during the exercise are much more harder to collect, since they decrease rapidly in the first 1 or 2 min after the end of the exercise bout (Ji et al., 1999). However, in the present experimental protocol we used the same patients in all of our experimental situations, while also determining the balance between ROS levels, represented by MDA, as a parameter of the lipid peroxidation processes vs. the activity of the main antioxidant enzymes.

REFERENCES


