

TATJANA TRIVIĆ<sup>1</sup>, PATRIK DRID<sup>1</sup>, SLAVKO OBADOV<sup>1</sup>, SERGEJ OSTOJIC<sup>2</sup>

<sup>1</sup> Faculty of Sport and Physical Education, University of Novi Sad (Serbia)

<sup>2</sup> Faculty of Sport and Tourism, Novi Sad Metropolitan University (Serbia)

Corresponding author: Tatjana Trivic. Faculty of Sport and Physical Education, Lovcenska 16, 21000 Novi Sad  
phone: +381 21 450 188, mail: ttrivic@yahoo.com

## Effect of endurance training on biomarkers of oxidative stress in male wrestlers

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**Key words:** athletes, aerobic activity, antioxidant enzyme activity

**Abstract:** In this research we evaluate the influence of continuous endurance training program on oxidative stress in male athletes. Participants in this study were wrestlers (age  $21.9 \pm 3.5$ ,  $11.7 \pm 4.0$  sport experience  $11.7 \pm 4.0$ , height  $176.7 \pm 6.6$  cm, body mass  $79.6 \pm 13.5$  kg) from Serbian national team. All of them ( $n=14$ ) have had same training program in period of four weeks, which included training regime on 75-85%  $HR_{max}$  continuous aerobic activity, in duration of 60 min, 3 days per week. Blood samples were collected in the morning before and day after the training program. After the training program we examined activity of the enzymes: superoxide dismutase (SOD), glutathione reductase (GSH-R), glutathione peroxidase (GSH-Px), catalase (CAT) and total antioxidant activity (TAC). After applied the endurance training program TAC decreased significantly ( $p<0.05$ ), but at a referent level which could not have a negative effect on health or performance of athletes.

### Introduction

A well structured training regime is the best way to achieve good results. The result of training in both aerobic [Chavi *et al.* 2007] and anaerobic activities [Bailey *et al.* 2007] induce elevated oxidative processes. Endurance athletes consume large amounts of oxygen which increases the production of reactive oxygen species (ROS) and leads to oxidative stress [Wiliams *et al.* 2006].

Endurance training is a generic term for any repeated physical activity which is improving the ability of an individual to sustain exercise performance for prolonged periods of time. Endurance training type is traditionally important in sports such as athletics and cycling as they predominantly rely on the aerobic energy system for the regeneration of ATP. Wrestlers must produce powerful movements and repeat them several times in a short period with or without rest. In order to maintain the same amount of power with each effort, a certain level of endurance is required.

Endurance training for example will provoke increase of oxidative enzymes and number of mitochondria in the muscles [Saltin, Astrand 1967; Kayar *et al.* 1986].

It is necessary to say that alongside with changes inside the muscles, there are adaptation reactions of surrounding tissues as well. If the increase of free radicals is greater than the ability to neutralize them, the radicals will attack cellular components.

Antioxidants are molecules that can neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition. They can operate in three different ways: reduce energy of free radicals, prevent their occurrence or to stop a chain reaction of oxidant. Our body produces several enzymes, including superoxide dismutase (SOD), glutathione reductase (GSH-R), glutathione peroxidase (GSH-Px), and catalase (CAT) which neutralize harmful free radicals effects.

In a training process it is necessary to develop sport specific abilities, which are going to contribute the sport result. Successful performance in wrestling fights demands high technical and tactical ability, power, strength and endurance of athletes. Wrestling symbolizes characteristic metabolic stress of organism [Kraemer *et al.* 2004; Nemet *et al.* 2004; Utter *et al.* 1998]. Research has shown [Kraemer 2002; Utter *et al.* 2002] that wrestling is one of the more demanding sports as far as metabolic and absolute muscle force and strength are concerned.

The purpose of the present investigation was to determine the effect of endurance training on biomarkers of oxidative stress and change in total antioxidant activity in wrestlers without supplementation within a period of four weeks. We hypothesized that oxidative stress would be elevated following the endurance training considering that wrestlers did not intake supplements during training treatment within a period of 4 weeks.

## Material and methods

### Study subjects

Fourteen healthy wrestlers (age  $21.9 \pm 3.5$  years, sport experience  $11.7 \pm 4.0$ , height  $176.7 \pm 6.6$ cm, body mass  $79.6 \pm 13.5$ kg) participated in this study. All participants were members of Serbian national wrestling team in a consistent training (10 hours per week) for the past 4 years. All subjects were informed about the requirements of the study and gave their consent when accessing the survey. Before taking part in the research a detailed medical examination was conducted during which it was established that all subjects are no smokers, have excellent health, without cardiovascular, respiratory and endocrine system disease.

### Procedure

Blood samples were taken from male wrestlers ( $n=14$ ) in the morning at 7.00 am one day before the program in "Eurolab" laboratory. The second blood samples were taken one day after the training program which included a period of four weeks. The participants did not take food twelve hours before both blood testings.

Athletes had no special nutrition program or supplements during a four-week training period.

### Blood collection and biochemical analysis

Blood samples were taken from the antecubital vein inside of the elbow into plain vacutainer tubes. The site is cleaned with germ-killing medicine (antiseptic). The health care provider wraps an elastic band around the upper arm to apply pressure to the area and make the vein swell with blood. Furthermore, serum samples were used to determine the activity of the enzymes: superoxide dismutase (SOD), glutathione reductase (GSH-R), glutathione peroxidase (GSH-Px), catalase (CAT) and total antioxidant activity (TAC). The primary antioxidant status was characterized SOD, CAT, GSH-R, GSH-Px and TAC which was measured

by a spectrophotometry method.

### Training protocol

All subjects trained three times per week for 4 weeks. The endurance training in wrestlers consisted of 60 min of aerobic training. The intensity varied from 75–85% of  $HR_{max}$ . All endurance training was accompanied with SUNTO TEAM POD system which enables wireless communications with athletes during the training and held athletes in the desired area of the work load.

All training sessions were supervised by a certified strength and condition coach.

TRAINING SCHEDULE		
	8:30-9:30	19:30-21:00
<b>Monday</b>	Training D	Training A
<b>Tuesday</b>	Training D	Training D
<b>Wednesday</b>	Training D	Training A
<b>Thursday</b>	Training D	Training C
<b>Friday</b>	Training D	Training A
<b>Saturday</b>	Training D	Rest
<b>Sunday</b>	Rest	Rest

*Training A, development of endurance*

*Training D, wrestling training*

*Training C, football*

### Statistical analysis

All values were presented as the mean (SD). Differences in the pre-values and post-values for each biochemical parameter were examined by t-test for independent samples. The differences were considered statistically significant at  $p < 0.05$ .

### Results

The analysis of oxidative stress parameters and components of antioxidant protection in relation to applied training treatment in the studied athletes showed that statistically significant differences were not obtained in the activities of antioxidant enzymes (SOD, CAT, GSH-R, GSH-Px).

The endurance training did not result in statistically significant changes in antioxidant enzyme activity, although there were some alterations. Endurance training induced a statistically significant decrease in TAC ( $p < 0.05$ ) in wrestlers after the final measurement. Data of this research (Table 2.) suggest that athletes under endurance training program exhibit low TAC values, but at the reference level, which do not cause negative effect on their sport performance.

**Table 2. Antioxidant enzyme activity and total antioxidant status after applying endurance training in a period of 4 weeks.**

Parameter	Group	Initial measurement	Final measurement
SOD U/gHb	Wrestling	1240±56	1272±70
CAT kU/gHb	Wrestling	319±60	332± 47
GSH-R U/gHb	Wrestling	11.5±0.9	11.5±0.8
GSH-Px U/gHb	Wrestling	70.8±1.3	70.9±1.4
TAC mmol/L	Wrestling	1.34± 0.05	1.32±0.04 <sup>a</sup>

Values are presented as Mean ± SD

Significance of means differences between initial and final measurement within groups:  $p < 0.05^a$

## Discussion

Number of conditions may lead to an imbalance of oxidants production and antioxidant defense, and these do create condition of oxidant stress [Bloomer *et al.* 2005]. The level of oxidative damage may be related to the intensity and duration of physical exercise [Degoutte *et al.* 2006].

When the condition is such that there is an increased production of free radicals with reduced ability of their removal and neutralization, one can talk about oxidative stress state, which can lead to patho-physiological changes. The production of free radicals and associated ROS increases markedly during sustained endurance exercise [Child *et al.* 1998]. However, in this research there was no evidence that endurance exercise enhanced the levels of any antioxidant enzyme activity.

Several studies have used total antioxidant capacity as a method to detect increases in oxidative stress after exercise [Ginsburg *et al.* 2001; Santos-Silva *et al.* 2001]. In this study we found that plasma total antioxidant activity decreased in response to applied endurance training treatment. Although the antioxidant capacity of the body is decreased with endurance training, it appears that even these decreases is in reference level after a 4-week training treatment and there is no need for ingesting supplements.

While some reports suggest a potential beneficial role of antioxidant supplementation in relation to a bout of resistance exercise [Goldfarb *et al.* 2005; Phillips *et al.* 2003], others indicate no benefit [Beaton *et al.* 2002; Viitala *et al.* 2004].

Whether these changes have negative effects in the organism in a prolonged period of this type of training needs further investigation. Future research must take into account multiple parameters and data analyses in order to establish the influence of the antioxidant scavenging and free radical producing system on different types of exercise.

## Conclusion

The findings of the present study partially refute our initial hypotheses. To conclude, our results suggest that endurance training in male wrestlers did not have an affect on antioxidant enzymes activity, but the endurance training program affected a decrease in TAC. Evidence from the present study suggests that the applied endurance training treatment does not require additional supplementation with antioxidants correlated with a controlled lifestyle and proper nutrition, but decrease in TAC indicate that athletes need to ingest a diet rich in antioxidants.

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## **Efekt treningu wytrzymałości przy biomarkerach stresu oksydacyjnego u zapaśników**

**Słowa kluczowe:** sportowcy, ćwiczenia aerobowe, enzym przeciwutleniający

### **Streszczenie**

Celem badania była ocena wpływu programu szkoleniowego dotyczącego ciągłej wytrzymałości na stres tlenowy wśród sportowców płci męskiej. Uczestnikami tego badania byli zapaśnicy z serbskiej drużyny narodowej (wiek  $21.9 \pm 3.5$ , doświadczenie sportowe  $11.7 \pm 4.0$ , wzrost  $176.7 \pm 6.6$ , masa ciała  $79.6 \pm 13.5$ ). Wszyscy z nich ( $n=14$ ) przeszli ten sam program szkoleniowy trwający 4 tygodnie, który obejmował system treningowy  $75-85\%_{HR_{max}}$  ciągłych ćwiczeń aerobowych trwających 60 minut, 3 dni w tygodniu. Próbkę krwi zostały pobrane rano dzień przed i po szkoleniu, po czym przebadano aktywność enzymów: dysmutacji podtlenkowej (SOD), reduktazy glutationowej (GSH-R), peroksydazy glutationowej (GSH-Px), katalazy (CAT) oraz całkowitej aktywności przeciwutleniającej (TAC).

Rezultatem treningu aerobowego i anaerobowego jest wzrost procesów oksydacyjnych. Duża ilość tlenu wchłaniana przez sportowców zwiększa produkcję reaktywnych form tlenu (ROS) i prowadzi do stresu tlenowego. Zapaśnicy muszą wykonywać wiele ruchów w krótkim czasie, często bez odpoczynku. Aby otrzymać tę samą siłę działania wymagany jest pewien poziom wytrzymałości.

Po zastosowaniu wytrzymałościowego programu treningowego całkowita aktywność przeciwutleniająca (TAC) znacznie się zmniejszyła ( $p<0.05$ ), ale na poziomie referencyjnym, który nie mógł mieć wpływu na zdrowie lub wydolność sportowców. Dowody zawarte w obecnym badaniu sugerują, iż zastosowane działanie w czasie treningu wytrzymałościowego nie wymaga dodatkowej suplementacji w postaci antyoksydantów skorelowanej z kontrolowanym stylem życia i odpowiednimi składnikami odżywczymi, aczkolwiek malejący poziom TAC wskazuje, że sportowcy potrzebują diety bogatej w przeciwutleniacze.