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# Exercise-Induced Changes in Redox Status of Elite Karate Athletes

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## Abstract

**Regular training has been claimed to increase the activity of antioxidant enzymes and, consequently, augments the resistance to oxidative stress; however, large volumes of training performed by elite sportsmen could lead to a chronic oxidative stress state. The aim of our study was to assess the oxidative status of elite athletes at both the beginning of the preparatory and the competition training phases, so that the influence of the three months of programmed physical activity on redox status could be determined. The chronic effects of exercise on the redox state of the athletes were compared to the effects of a single bout of karate training. Thirty elite karate athletes, 16-30 years old, were subjected to maximal graded exercise test to estimate their aerobic capacity; blood sampling was also performed to measure levels of superoxide anion radical ($O_2^-$), hydrogen peroxide ($H_2O_2$), superoxide dismutase activity and catalase (CAT) activity. The only significant change after the three-month training process was found in the significantly decreased CAT activity (X ± SE: 7.95 ± 0.13 U/g Hb × $10^3$ in the preparatory period, 6.65 ± 0.28 U/g Hb × $10^3$ in the competition stage; $P < 0.01$). After a single karate training session, there was a statistically significant decrease of $O_2^-$ (X ± SE: 32.7 ± 4.9 nmol/ml in the preparatory period, 24.5 ± 2.5 nmol/ml in the competition stage; $P < 0.05$) and increase of $H_2O_2$ (X ± SE: 11.8 ± 1.0 nmol/ml in the preparatory period, 14.2 ± 0.9 nmol/ml in the competition stage; $P < 0.01$), as well as significant CAT increase (X ± SE: 6.6 ± 0.6 U/g Hb × $10^3$ in the preparatory period, 8.5 ± 0.5 U/g Hb × $10^3$ in the competition stage; $P < 0.05$). Although the three-month training process induced, at the first sight, negative changes in the redox state, expressed through the decrease in CAT activity, adequate response of the antioxidant system of our athletes to acute exercise was preserved.**

**Key Words: oxidative stress, elite athletes, karate, phases of training**

## Introduction

The topic of exercise-induced oxidative stress has been receiving considerable scientific attention for more than 30 years, and hundreds of original investigations have been published. Although there is some inconsistency present in the literature, it is clear that both acute aerobic and anaerobic exercises have the potential to result in an increased free radical production, which may or may not result in an acute

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oxidative stress (20). The extent of redox homeostasis disturbance induced by acute bout of exercise depends on many factors, *inter alia*, exercise mode, intensity and duration, and participant's state of training, gender, age and nutrition habits (20).

Although exercise-induced reactive oxygen species (ROS) production represents a potential detriment to physiological functions, there is also an alternative role for ROS production in regards to favorable exercise-induced adaptations (20). According to the principle of hormesis, and the basic principle of exercise-stress-adaptation, the rise in ROS production does not have to be considered detrimental, since it represents stimuli for an upregulation in endogenous antioxidant defenses (20, 34). This provides adaptive protection from ROS during subsequent training sessions as well as during non-exercise-related conditions (16).

Karate is a polystructural acyclic sport that consists of many repetitions of short sequences (bursting techniques and hopping movements) interrupted by recovery periods (37). Although karate is characterized by high-intensity intermittent activities, aerobic metabolism also plays an important role in the physiological profile of an elite karate athlete (5, 14, 38). Owing to the needs of aerobic and anaerobic demands during karate training, elite-class karate athletes are usually getting mixed training combining both demands (37).

Compared to the number of investigations regarding the effects of predominantly aerobic or anaerobic training on oxidative stress, little research has been carried out on exercise-induced changes of redox status in interval-trained athletes. Also, there is only a small number of investigations on exercise-induced oxidative stress in the population of martial art athletes, especially in karate athletes which may be because karate is a relatively young sport. Karate is recognized by International Olympic Committee but it is not included in the Olympic program.

The primary objective of our study was to establish the redox status of elite karate athletes in different periods of training process, *i.e.* in the beginning of the preparatory period and in the beginning of the competition stage, so that the influence of three months of programmed physical activity on redox status could be assessed. Furthermore, we compared the effects of chronic exercise with the effects of a single karate training session.

## Materials and Methods

*Subjects*

The research was carried out in a group of 30 male elite karate athletes, 16 to 30 years old. All of them had been professional athletes for more than 5 years and were members of the national team for at least 2 years. All participants were healthy (there were no chronic or acute diseases, and no active sports injuries), had no special nutritional habits, did not use medications or supplements, and were non-smokers. All participants, and their parents if they were under 18, gave a written informed consent. The study was approved by the Ethical Committee of the School of Medicine, University of Belgrade.

*Protocol*

*Chronic Effects of Exercise on Redox Status of Athletes*

Examination was conducted in the first week of the preparatory period that lasted three months (the first examination) and in the first week of the competition stage (the second examination). Both examinations were performed using the same protocol. The examinations started at 8 AM in the morning and consisted of blood sampling and maximal exercise test. Three days before the first examination, subjects were asked to keep a diary of daily food intake, and to avoid heavy physical activities for 24 h and consumption of alcohol and caffeine for 48 h before the test; they also should not have breakfast before the examination. Before the second examination, subjects were asked to try to repeat the three-day menu that had been noted in their diary of daily food intake. The average dietary intake was calculated using the food composition database of the Italian National Institute of Nutrition.

After the athletes had filled in the standard sports medicine questionnaire and passed a standard sports medicine examination, a blood sample was taken from the subjects' antecubital vein to determine the levels of pro/antioxidants before breakfast. Blood was analyzed immediately after sampling all the athletes.

After breakfast, athletes were subjected to a maximal progressive exercise test on a bicycle ergometer *Kettler AX1* to measure their relative aerobic capacity (maximal oxygen consumption - $VO_{2max}$ (ml/kg/min)) and to estimate their anaerobic threshold (AT). Load was set to 2 W/kg, and increased every 3 min for 50 W; subjects were instructed to ride at 60 rpm. Aerobic capacity was estimated using *Cosmed Fitmate Pro*, an apparatus for direct oxygen consumption measurement. We hypothesized that the $VO_{2max}$ was reached when the oxygen consumption was at its plateau (the time when increasing of workload cannot affect an increase in oxygen consumption) (23). Anaerobic threshold was determined automatically by the software of the *Cosmed Fitmate Pro* apparatus. Immediately after finishing the maximal exercise

test, another blood sample was taken to determine the levels of lactic acid.

Preparatory period was a specially-designed training program composed of physical conditioning and perfecting of specific karate techniques. It consisted of 3 mesocycles, which differed in training volume, training intensity and training goals. Intensity was gradually increased from 60% to 100% of $Vo_{2max}$, training sessions lasted from 60 to 120 min. Athletes had trainings 5 times a week, except in the third preparatory mesocycle when the number of trainings doubled to twice a day. By the end of the precompetition mesocycle, a few preparatory matches were organized.

*Acute Effects of Exercise on Redox Status of Athletes*

The chronic effects of exercise were compared to the effects of a single bout of exercise, which was the third part of the examination on the same group of karate athletes (30). The third examination took place 36 h after the second examination during which athletes had no physical activity and followed their previously mentioned dietary diary. The training was implemented in regular term at 8 PM and lasted 90 min. Warm-up lasted 15 min, the main part of the training 60 min and cool down 15 min. The main part of the training consisted of kata Kitei and kumite sparring, *i.e.* elements of Fuku-go karate discipline. Kata Kitei includes techniques of defense and attack taken from three main karate styles: Shoto-kan, Goju-ryu and Shito-ryu. Kata Kitei, the average duration of which was 90 sec, was performed using the interval method which was defined by 3 units of work and one unit of rest. After the kata was performed 3 times, kumite sparring took place. Sparring lasted 3 min. Four series of this complex routine (3 × kata and 1 × kumite) were performed. Heart rate (HR) was monitored throughout the training session. The average HR after three performed katas was 168.5 c/min, while after kumite it was 165.9 c/min. After 1 min of rest, the average HR was 128.9 c/min.

*Biochemical Assays*

Venous blood was drawn with a 5-ml syringe and mixed with 100 µl of 3 M perchloric acid, 400 µl of 20 mM EDTA in a 10 ml test tube. After incubation on ice for 20 min, it was centrifuged at 15,000 rpm for 5 min. ROS were determined in the plasma and antioxidant enzymes in red blood cells (RBCs). Biochemical parameters were measured spectrophotometrically.

*Superoxide Anion Radical Determination*

The level of superoxide anion radical ($O_2^-$) was measured using NBT (Nitro Blue Tetrazolium) reaction in Tris-buffer combined with the plasma samples and read at 530 nm (1).

*Hydrogen Peroxide ($H_2O_2$) Determination*

The protocol for measurement of $H_2O_2$ was based on the oxidation of phenol red in the presence of horseradish peroxidase (POD) (31). Two hundred µl samples with 800 µl PRS (phenol red solution) and 10 µl POD were combined (1:20). The level of $H_2O_2$ was measured at 610 nm.

*Determination of Activities of Antioxidant Enzymes*

Isolated RBCs were washed three times with 3 volumes of ice-cold 0.9 mM NaCl and hemolysates containing about 50 g Hb/l, prepared according to McCord and Fridovich (26), and were used for the determination of catalase (CAT) activity. CAT activity was determined according to Beutler (7). Lysates were diluted with distilled water (1:7 v/v) and treated with chloroform-ethanol (0.6:1 v/v) to remove haemoglobin (45), then 50 µl catalase buffer, 100 µl sample and 1 ml 10 mM $H_2O_2$ were added to the samples. Detection was performed at 360 nm. Distilled water was used as a blank probe. Superoxide dismutase (SOD) activity was determined by the epinephrine method of Misra and Fridovich (27). A hundred µl lysate and 1 ml carbonate buffer were mixed, and then 100 µl epinephrine was added. Detection was performed at 470 nm.

*Statistics*

All descriptive statistical parameters of the analyzed characteristics were used to calculate arithmetic mean (X) with dispersion measures (standard deviation, SD, and standard error, SE) and median. Depending on distribution checked by Shapiro-Wilk, Paired Samples *t*-test or Wilcoxon signed rank sum test was used to assess the differences between parameters gathered in two different time-points.

## Results

Subjects in our study were young athletes with 20.9 ± 4.1 years of age, with solid sport experience of 8.7 ± 3.6 years. Parameters of aerobic capacity and lactate levels before and after the three-month training process are presented in Table 1. The average daily dietary intake in the preparatory and competition training phases is shown in Table 2.

Regarding oxidative status changes from preparatory to competition stage, the only significant change was found in the activity of CAT, which significantly

**Table 1. Some physiological parameters obtained during exercise testing of athletes**

| | X ± SD (Med) | Test |
|---|---|---|
| $Vo_{2max}$ (ml/kg/min) | | |
| Preparatory period | 53.9 ± 6.6 (52.5) | $P > 0.05$ |
| Competition period | 54.6 ± 6.7 (54.3) | |
| AT (% of $Vo_{2max}$) | | |
| Preparatory period | 85.4 ± 5.4 (86.4) | $P > 0.05$ |
| Competition period | 84.9 ± 7.1 (84.9) | |
| HRmax (c/min) | | |
| Preparatory period | 199.4 ± 5.0 (200.0) | $P < 0.05$* |
| Competition period | 195.4 ± 7.4 (193.0) | |
| HR at AT (c/min) | | |
| Preparatory period | 183.4 ± 8.6 (186.0) | $P > 0.05$ |
| Competition period | 178.5 ± 9.5 (178.0) | |
| Lactates in rest (mM) | | |
| Preparatory period | 2.4 ± 0.8 (2.4) | $P < 0.01$** |
| Competition period | 1.9 ± 0.7 (1.6) | |
| Lactates after GXT (mM) | | |
| Preparatory period | 14.9 ± 3.9 (15.0) | $P > 0.05$ |
| Competition period | 12.4 ± 3.0 (12.5) | |

HR, heart rate; HRmax, maximal heart rate achieved during GXT.

**Table 2. Macronutrient intake and supply with nutritional antioxidants in the preparatory and competition training phases**

| | X ± SD (Med) | Test |
|---|---|---|
| Energy (Kcal/day) | | |
| Preparatory period | 2808 ± 571 | $P > 0.05$ |
| Competition period | 2781 ± 708 | |
| Carbohydrates (g/day) | | |
| Preparatory period | 392 ± 71 | $P > 0.05$ |
| Competition period | 409 ± 91 | |
| Protein (g/day) | | |
| Preparatory period | 112 ± 29 | $P > 0.05$ |
| Competition period | 105 ± 32 | |
| Fat (g/day) | | |
| Preparatory period | 88 ± 19 | $P > 0.05$ |
| Competition period | 85 ± 24 | |
| β-Carotene (mg/day) | | |
| Preparatory period | 4.5 ± 2.2 | $P > 0.05$ |
| Competition period | 4.8 ± 2.5 | |
| Vitamin E (mg/day) | | |
| Preparatory period | 22.4 ± 9.8 | $P > 0.05$ |
| Competition period | 18.7 ± 10.4 | |
| Vitamin C (mg/day) | | |
| Preparatory period | 215 ± 79 | $P > 0.05$ |
| Competition period | 228 ± 82 | |

decreased (X ± SE: from 7.95 ± 0.13 U/g Hb × $10^3$ in the preparatory period to 6.65 ± 0.28 U/g Hb × $10^3$ in the competition stage; $P < 0.01$).

After a single karate training bout, there was statistically significant decrease of $O_2^-$ (X ± SE: from 32.7 ± 4.9 nmol/ml in the preparatory period to 24.5 ± 2.5 nmol/ml in the competition stage; $P < 0.05$) and significant increase of $H_2O_2$ (X ± SE: from 11.8 ± 1.0 nmol/ml in the preparatory period to 14.2 ± 0.9 nmol/ml in the competition stage; $P < 0.01$). There was also significant CAT increase (X ± SE: from 6.6 ± 0.6 U/g Hb × $10^3$ in the preparatory period to 8.5 ± 0.5 U/g Hb × $10^3$ in the competition stage; $P < 0.05$). Differences in pro/antioxidant responses to acute and chronic exercise are shown in Figs. 1-4.

## Discussion

Sports engagement includes upregulation of many cellular processes and physiological functions (15, 42) which leads to improvement of sports performance. The absence of significant changes in aerobic power of our subjects after three-month preparatory period can be explained by initially high aerobic power of the subjects, which does not allow for a significant increase unless highly stimulated (which was not the case in their preparatory program). A recent study on the effects of two different twelve-week preparatory periods on oxidative stress biomarker response in judo athletes reported that concurrently performed training for strength and endurance induced increased anaerobic power and maximal oxygen uptake, but also affected oxidative stress biomarkers. On the other hand, the usual training program pattern (strength training and perfecting of specific judo techniques, but without any endurance training) had no effects on morphofunctional characteristics nor on oxidative stress levels (35). A number of studies tried to assess the correlation between changes in functional and antioxidant properties of athletes after a certain period of programmed exercise (16, 28) and it seems that changes in antioxidant enzyme activity are not correlated with the increase of $Vo_{2max}$ observed during training process (18). Thus, the findings of our study related to changes in the athletes' redox status despite the absence of functional changes is in consent with previous investigations.

Regular training has been claimed to increase

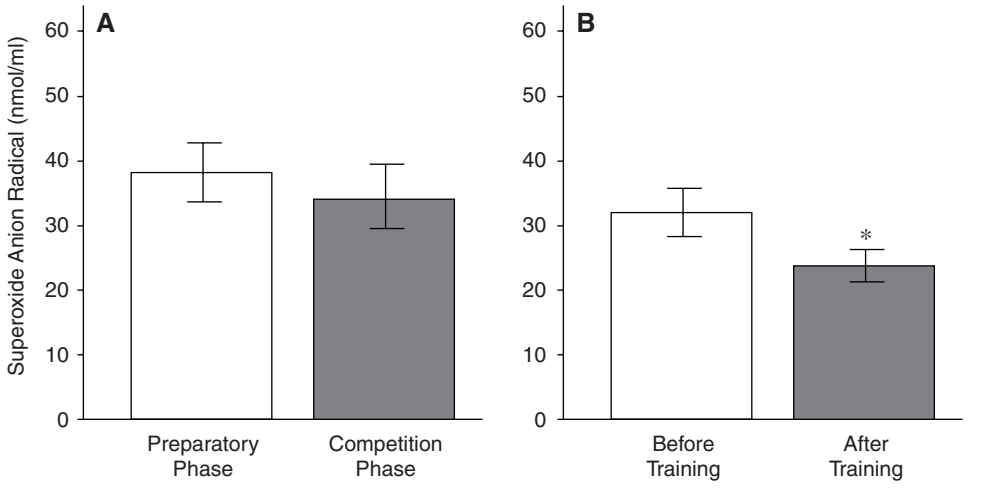


Fig. 1. $O_2^-$ levels (X ± SE nmol/ml) in different phases of the training process ($P > 0.05$) and before and after a single bout of exercise (*$P < 0.05$).

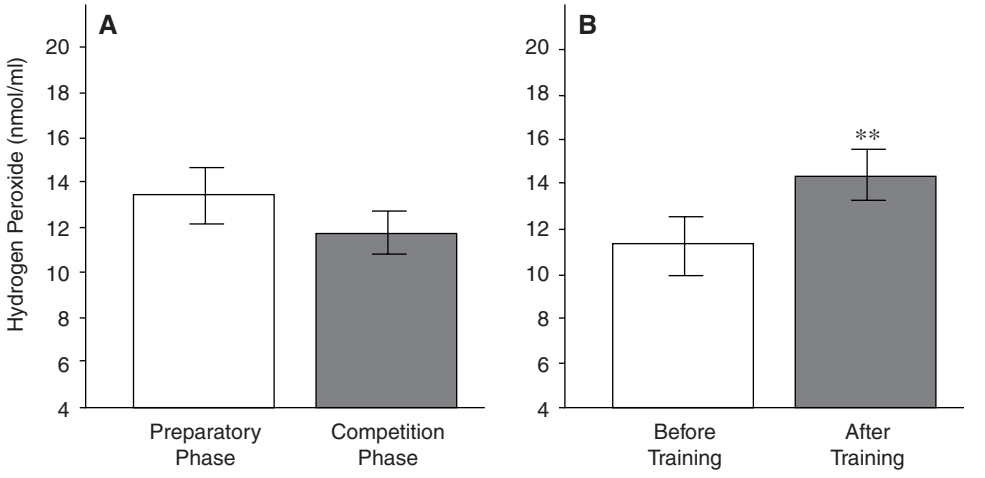


Fig. 2. $H_2O_2$ levels (X ± SE nmol/ml) in different phases of the training process ($P > 0.05$) and before and after a single bout of exercise (**$P < 0.01$).

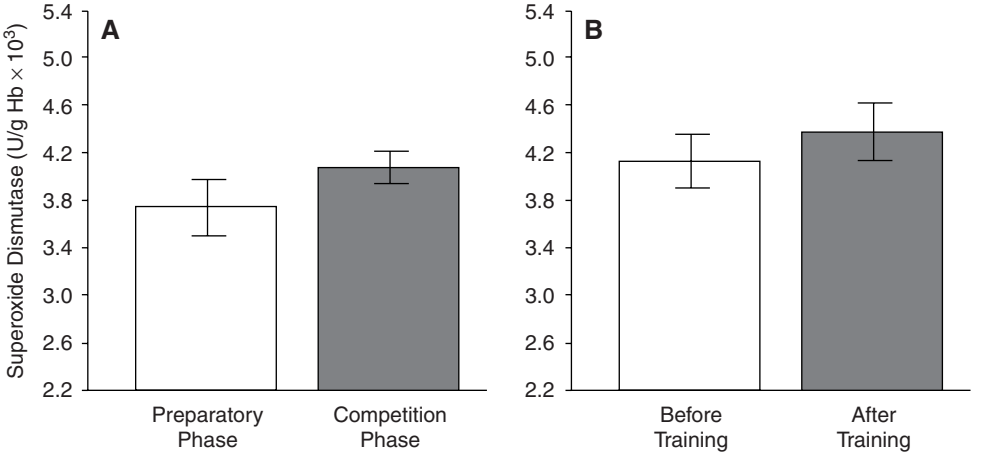


Fig. 3. SOD activity (X ± SE U/g Hb × $10^3$) in different phases of the training process ($P > 0.05$) and before and after a single bout of exercise ($P > 0.05$).

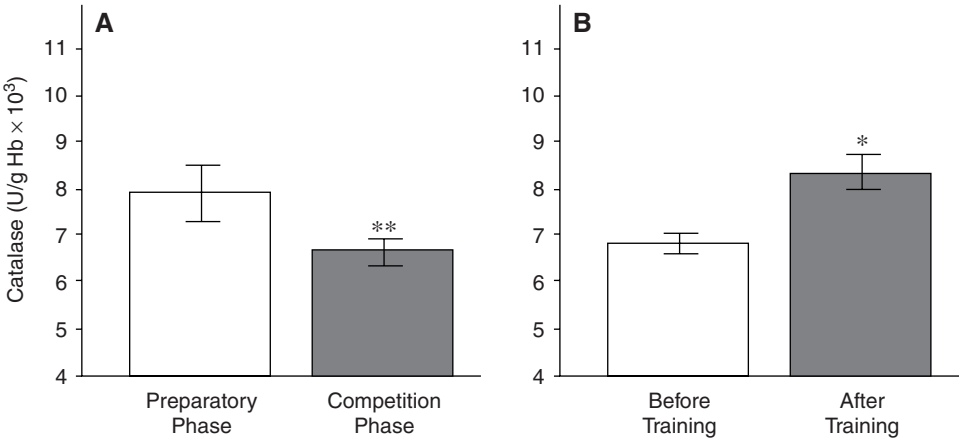


Fig. 4. CAT activity (X ± SE U/g Hb × $10^3$) in different phases of the training process (**$P < 0.01$) and before and after a single bout of exercise (*$P < 0.05$).

the activity of antioxidant enzymes (16, 28) and, consequently, augments the resistance to oxidative stress (33, 44). Enrolment in moderate aerobic physical activity training appears to be beneficial for previously sedentary individuals, but intense physical training may result in a decline in circulating antioxidants in moderate- and well-trained athletes (6, 41, 44). It has been shown that athletes have higher antioxidant capacity than non-athletes (9, 11, 12, 17, 43, 46), but also that there are differences in its activity in different phases of meso and macrocycle training process (19, 28, 39, 44). Large volume of training performed by elite sportsmen could lead to a chronic oxidative stress state, so redox markers should be monitored throughout the season to timely detect those athletes at increased risk and to take steps to avoid consequences to athletes' health and sports performance (44).

Having considered all the above, we assessed the redox status of our subjects before and after the three-month preparatory training process, *i.e.* in the preparatory and in the competition phases. Unlike SOD, whose rise in activity after the preparatory period did not reach statistical significance, the fall in CAT activity was confirmed as statistically significant. These effects of chronic exercise on redox status of athletes are opposite to the effects of acute exercise that was found in the part of investigation that was related to changes in redox status after a single bout of karate training (30). After specific karate training, levels of $O_2^-$ were statistically decreased, and levels of $H_2O_2$ and CAT activity were statistically increased (30). The rise of SOD activity after specific karate training was again statistically insignificant (30). One of the possible explanations of statistically significant decrease of the level of $O_2^-$ and increase of the level of $H_2O_2$ after the acute load is in the chemical reaction rapidity in which these free radicals were decompounded - $O_2^-$ decompounding is much faster than $H_2O_2$ decompounding (21). The decrease of $O_2^-$ concentration after the load is followed by paradoxically irrelative changes in SOD activity. The explanation is probably that the $O_2^-$ quantity before and after the load was not high enough to significantly stimulate SOD. Generally, there were only slight differences in SOD activity in different stages of our research, which corresponds to findings of other researchers that SOD activity significantly changes only when ROS is produced in large quantities (40). Also, there is the probability that other antioxidants that were not measured in our study, like ascorbic acid and dihydroascorbic acid, scavenged enough $O_2^-$ released due to physical load (30). Furthermore, significantly increased $H_2O_2$ levels after the acute load induced the increase in CAT activity, which is expected since CAT affinity for $H_2O_2$ rises as the concentration of $H_2O_2$ rises (32). The opposite happened after exposure to chronic physical loads. The decrease in CAT activity observed after the three-month training period corresponds to findings of some studies on other athletes (3, 25), but there are also a number of studies that reported no change in CAT activity as a consequence of exercise training (2, 24, 28). It has previously been confirmed that there is selenium (Se) defficiency in Serbia (13). Consequently, the antioxidant defence system for $H_2O_2$ neutralization in erythrocytes of Serbian population is altered (reduced glutathione peroxidase, GSH-Px, activity (29)). Hence, it could be expected that CAT activity would increase in order to alleviate the consequences of decreased GSH-Px activity, especially in athletes bacause they experience additional Se spending due to intensive exercise training. On the contrary, CAT activity was decreased in our athletes after the intensive training process, which might indicate that our athletes need antioxidant supplementation with

increases in Fe, Se and other minerals.

Previous investigations on the correlation between blood levels of lactates and oxidative stress have suggested that, on the one hand, lactate ion is a scavenger of free radicals (hydroxyl radical and superoxide anion) *in vitro* (22), but on the other hand, the formation of lactate *in vivo* cannot be dissociated from subsequent metabolic acidosis, which may have a pro-oxidant effect (8). Bloomer and Cole (8) have concluded that there is not sufficient evidence to assume that either blood or intramuscular lactate at exercise-induced concentrations is associated with oxidative stress in healthy, trained men. Thus, we hypothesize that the finding of our study regarding statistically lower lactate levels of our athletes in the competition period compared to the preparatory period is not in relation with CAT activity decrease after the preparatory period, especially because the lactate levels in basal state are not the indicators of training status, but rather the consequence of exercise bouts in previous days (and our athletes did not have intensive training two days before the research). Although the three-month training process induced, at the first sight, negative changes in redox state (the decrease in CAT activity), the adequate response of the antioxidant system of our athletes to acute exercise remained preserved.

The limitations of our study are that we have not measured GSH-Px activity and nitric oxide (NO) levels, because both decreased Se levels and increased NO levels can inhibit CAT activity (4, 10, 36). Correlation of all these parameters would help understanding the observed changes in redox state.

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