EVALUATION OF OXIDATIVE STATUS IN SHORT-TERM EXERCISES OF ADOLESCENT ATHLETES

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ABSTRACT: The aim of the study was to evaluate the effects of short-term exercise on total antioxidant status (TAS), lipid hydroperoxide (LOOHs), total oxidative status (TOS) and oxidative stress index (OSI) in adolescent athletes. A total of 62 adolescent participated in the study. Athletes were trained regularly 3 days a week for 2 hours. All subjects followed a circuit exercise program. Blood samples were collected just before and immediately after the exercise program. Antioxidant status was evaluated by measuring the TAS level in the plasma. Oxidative status was evaluated by measuring the total peroxide level. The percentage ratio of TAS to total peroxide level was accepted as the OSI. Plasma triglyceride, total cholesterol, LDL, HDL and VLDL were measured by automated chemical analyzer using commercially available kits.There was a significant increase in TOS ($p<0.05$) and OSI ($p<0.01$) levels and a significant decrease in TAS levels ($p<0.01$) compared to the resting state. There were no significant changes in LOOHs levels before and after the short-term exercise. After short-term exercise, the balance between oxidative stress and antioxidant status moves towards oxidative stress as a result of increasing oxidants and decreasing antioxidants.

KEY WORDS: adolescent, athlete, antioxidants, oxidative stress, short-term exercise

INTRODUCTION

Exercise disturbs the balance between free radicals and antioxidants and the resultant state is known as oxidative stress [1,9]. During exercise, oxygen consumption is 10-15 times higher compared to a resting state and therefore the free radical production capacity of mitochondria increases temporarily [4,31]. The increase in O_2 uptake concomitant with physical exercise is related to a rise in the production of reactive oxygen species (ROS) by cells and tissues. It is known that the production of oxidants increases with an elevating metabolic rate as a result of contractions of skeletal muscles [8,27]. In fact, despite ROS having a fundamental role as signaling molecules in several cellular pathways, redox changes induced by increased ROS production during exercise are negatively related to cellular homeostasis and might compromise cellular function. Additionally, the emerging role of free radicals in delayed-onset muscle soreness and contraction-induced muscle injury has been reported [11,13]. Oxygen usage increases with increasing metabolic activity and this results in ROS production. Oxygen usage and electron leakage from the mitochondrial electron transport chain increases with metabolic activity, therefore many reactive oxygen forms emerge, such as superoxide, hydrogen peroxide and hydroxyl radicals [16,38].

There would be serious oxidative damage in biomolecules as a result of defects occurring in these oxidants during exercise. There have been many studies on the determination of oxidant stress *in vivo*. In this way, measurement of antioxidants in plasma and other body fluids and changes in target molecules and determination of final products could be useful for the possible use of antioxidants as treatment [38]. Moderate-intensity exercises are healthy activities but exhaustive exercises cause an increase in free radical production. Especially in untrained people, intense loading triggers the signals of oxidative stress in blood and muscle. This may be related with increasing lipid peroxidation, glutathione oxidation, cellular lipids, proteins and DNA damage. The degrees of oxidative stress and muscle damage are related to the exhaustion level of the athlete rather than the total intensity of exercise [7,23].

The aim of this study was to determine the effects of short-term exercise on oxidative stress and antioxidant defence systems in adolescent amateur basketball players.

MATERIALS AND METHODS

The ethical consent to study on human subjects was provided by The Ethical Committee of Zonguldak Karaelmas University according to The Declaration of Helsinki.

Subjects. Sixty-two (7 girls and 55 boys) amateur basketball players (age 15.3±1.8 years; weight 53.9±12.0 kg; height 164.3±36.4 cm) from the regional league participated in the study. The players were training for three days a week and for two hours at each session. Required medical examinations were applied before the study and written informed consent was obtained from the players and their parents.

*Exercise Program.*The subjects were informed not to eat or drink for three hours before the tests. They had not been taking any vitamins, antioxidants or smoking for at least three months before the study. Any subjects who smoked or had any chronic illnesses or asthma were excluded from the study. A standard diet was suggested to all subjects one week before the study. A circuit training program consisting of eight stations was applied. Subjects trained for three sets for each session, loading for 20 seconds, and resting for 30 seconds between loads, and rested for eight minutes between sets. A warm-up session of 15 minutes before the exercise session was applied.

Blood Sampling. Fasting venous blood samples were withdrawn into heparinized tubes from a cubital vein just before and immediately after the exercise test, then centrifuged at 3000 rpm for 10 min to separate the plasma. The plasma samples were stored at −80°C until the analysis of total antioxidant status (TAS), total oxidant status (TOS), total peroxide concentration (LOOHs) and lipid profiles.

Measurement of plasma TAS levels: Plasma TAS levels were determined using a novel-automated measurement method developed by Erel [36]. This method is based on the bleaching of the characteristic color of a more stable 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation by antioxidants. The assay has excellent precision values, which are lower than 3%. The results were expressed as mmol Trolox equivalent/l.

Measurement of plasma LOOHs levels: Plasma LOOHs levels were determined using the FOX_2 method [29] with minor modifications [34]. The FOX_2 test system is based on the oxidation of ferrous ion to ferric ion by various types of peroxides contained within the plasma samples, to produce a colored ferric-xylenol orange complex whose absorbance can be measured. The FOX_2 reagent was prepared by dissolving ammonium ferrous sulphate (9.8 mg) in 250 mM H_2SO_4 (10 ml) to give a final concentration of 250 AM ferrous ion in acid. This solution was then added to 90 ml of HPLC-grade methanol containing 79.2 mg butylated hydroxytoluene (BHT). Finally, 7.6 mg xylenol orange was added and stirred to make the final working reagent (250 AM ammonium ferrous sulphate, 100 AM xylenol orange, 25 mm H_2SO_4, and 4 mM BHT in 90% vol/vol methanol in a final volume of100 ml). The blank working reagent contained all the components of the previous reagent except ferrous sulphate. Aliquots (200 μl) of plasma were mixed with 1800 Al FOX_2 reagent. After incubation at room temperature for 30 min, the vials were centrifuged at 12000 g for 10 min. Absorbance of the supernatant was then determined at 560 nm. The total peroxide content of the plasma samples was determined as a function of the absorbance difference between test and blank tubes using a solution of H_2O_2 as standard. The coefficient of variation for individual plasma samples was less than 5%.

Measurement of plasma TOS levels: Plasma TOS levels were determined using a novel automated measurement method as previously described [20]. Oxidants present in the sample oxidized the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction was enhanced by glycerol molecules abundantly present in the reaction medium. The ferric ion produced a colored complex with xylenol orange in an acidic medium. The color intensity, which could be measured spectrophotometrically, was related to the total amount of oxidant molecules present in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in terms of micromolar hydrogen peroxide equivalent per liter (mmol H_2O_2 equivalent/L).

Oxidative stress index: The percentage ratio of TOS level to TAS level was accepted as OSI [15]. (11). For calculation, the resulting unit of TAS was changed to mmol/L, and the OSI value was calculated using the formula below;

OSI (arbitrary unit) = [TOS (mmol H_2O_2 equiv/L)/TAS (mmol Trolox equivalent/L)/100].

Measurement of Lipid profiles: Plasma triglyceride, total cholesterol, LDL, HDL, VLDL were measured by automated chemistry analyzer (Aeroset, Abbott, USA) using commercial kits.

Statistical Analysis. Data were analyzed using SPSS 11.5 for Windows (Chicago, IL). Results are presented as mean ± SD. The Student's *t* test for paired samples was used to compare the blood samples before and after exercise, assuming a 95% confidence interval.

TABLE I. ANTHROPOMETRIC CHARACTERISTICS AND BLOOD LIPID PROFILES OF THE SUBJECT (N=62)

	Mean±SD
Age (years)	15.3±1.8
Height (cm)	164.3±36.4
Body weight (kg)	54.9±12.0
BMI (kg/m^2)	19.8±3.2
Triglyceride(mmol/L)	1.5±0.9
Cholesterol(mmol/L)	4.62±1.06
HDL (mmol/L)	1.37±0.34
LDL (mmol/L)	2.5±0.6
VLDL (mmol/L)	0.67±0.4

Legend: Data are given as mean±SD
HDL - high-density lipoprotein, LDL - low-density lipoprotein, VLDL - very low-density lipoprotein

TABLE 2. COMPARISON OF PLASMA OXIDATIVE STRESS AND ANTIOXIDANT DEFENSE MARKERS BEFORE AND AFTER SHORT-TERM EXERCISE IN BASKETBALL PLAYERS

Variables	Before Exercise	After Exercise	p
TAS (mmol Trolox equiv./l)	1.03 ± 0.13	0.96 ± 0.14	p<0.01
LOOHs (mmol H_2O_2 Eqiv./L)	3.84 ± 0.73	4.03 ± 0.86	p>0.05
TOS (mmol H_2O_2/L)	10.88 ± 2.71	12.19 ± 3.21	p<0.05
OSI (AU)	10.69 ± 3.07	12.84 ± 3.96	p<0.01

Legend: Data are given as mean±SD, TAS - total antioxidant status, LOOHs - lipid hydroperoxide, TOS - total oxidative status, OSI - oxidative stress index

RESULTS

Anthropometric characteristics and blood lipid profiles of the subjects are given in Table 1. Oxidative stress and antioxidant defence markers before and after the short-term exercise are given in Table 2. Plasma TOS and OSI levels were significantly increased ($p<0.05$ and $p<0.01$, respectively) and TAS levels were significantly decreased ($p<0.01$) after the exercise test compared to rest. There were no significant changes in plasma LOOHs levels.

DISCUSSION

The present study investigated whether there is any alteration in plasma TAS, TOS, LOOH and OSI levels after short-term exercise in adolescent basketball players. We found that plasma TAS levels decreased and plasma TOS and OSI levels increased after short-term exercise compared to a resting state in amateur adolescent basketball players. In addition, LOOHs levels were no different before and after short-term exercise.

Free radicals such as superoxide radical anion, hydroxyl radical and hydrogen peroxide are produced in metabolic and physiological processes. The oxidative effects of free radicals are controlled by exogenous and endogenous antioxidants such as scavenger enzymes - superoxide dismutase, glutathione peroxidase and catalase, and non-enzymetic antioxidants (vitamin C, vitamin E, bilirubin and uric acid). Under some conditions such as exercise, increases in oxidants and decreases in antioxidants cannot be prevented, and the oxidative/antioxidative balance shifts towards oxidative stress [35].

Exercise appears to increase free radical and ROS production and these are interacting with lipids, DNA and proteins. These interactions degrade proteins and damage DNA-strand breakage and other genomic structures [26]. It is well known that differences in exercise protocols, training status, age and gender could play a role in oxidant/antioxidant parameters and DNA damage [30,39]. For standardization of exercise, age and gender matched athletes were chosen for the study. Some parameters of oxidative stress may not change after exercise, and may reach their maximum levels only hours or even days after the end of exercise [21,30]. In several studies, investigators have failed to observe any signs of exercise-induced oxidative stress immediately after exercise [14,22,25]. Although it has been suggested that exercise training enhances antioxidant capacity, the causal mechanisms are not yet clearly known [24,25].

Previous studies have used different markers of antioxidant status and different training levels in subjects. In the current study, TOS and OSI levels increased after short-term exercise in amateur adolescent basketball players. The increase in oxygen consumption with short time high intensity exercise is thought to be related to high oxidative stress and this increase in oxidative stress index in athletes after short-term exercise might be the cause of a decrease in plasma TAS levels.

In previous studies, it has been found that TAS levels increased and some antioxidants decreased reduced immediately after exercise [17,28]. Some studies have shown a decrease in glutathione and an increase in glutathione peroxidase activity after exercise, which returned to baseline levels 1 hour after exercise [6,32]. It is widely assumed that oxidative stress is detrimental to exercise performance, but there is little experimental evidence to support this data. Although antioxidant supplementation has been shown to decrease exercise-induced oxidative stress in humans [5,37], there is no convincing experimental evidence that this is accompanied by an increase in exercise performance in healthy human subjects [15,18,33]. One limitation of the present study was that calculation of VO2max during the training period was not controlled.

Different exercise forms lead to different levels of oxidative stress [16,38]. Although, long time regular exercises ameliorate the antioxidant defence system [3,6], high intensity exhaustive exercises increases free radical production and causes oxidative stress by increasing oxygen consumption 10-15 fold [11,19,34].

In literature there are different results about the redox balance of athletes. Regular endurance and resistance training increases the antioxidant defence [23,24]. It has been demonstrated that, two days of intense exercise increased the plasma total antioxidant capacity in elite skiers [34]. In many studies, it has been shown that oxidative stress parameters did not change [2,12] or increased [21,30] and even decreased [23] after exercise.

The TOS values increased with short-time exercise in amateur basketball players and this result is similar to some of the literature results and not to others. The increase in oxygen consumption with short time high intensity exercise is thought to be related to high oxidative stress and related to this oxidative stress index increased in athletes with increase in oxidant-antioxidant capacity. There are some different results in literature. Some of these are similar to our results and some are not. In some studies, antioxidant supplements increased performance of athletes [10].

CONCLUSIONS

In conclusions, although short term intense exercises increases oxidative stress and decreases antioxidant status in amateur adolescent basketball players, physical activity can favorably affect antioxidant potential and prevent lipid peroxidation.

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