The effects of short-term exercise on the parameters of oxidant and antioxidant system in handball players

Recep Kurkcu

Harran University, School of Physical Education and Sports, Sanliurfa-Turkey. E-mail: kurkcu.recep@gmail.com.

Accepted 29 June, 2010

The aim of the study was to evaluate the effects of short-term exercise on total antioxidant capacity (TAC), lipid hydroperoxide (LOOHs), total oxidative status (TOS) and oxidative stress index (OSI) in handball players. A total of 20 amateur handball players participated in the study. Handball players were training regularly 3 days a week for 2 h. 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 TAC level in the plasma. Oxidative status was evaluated by measuring the total peroxide level. The percentage ratio of TAC 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 TAC levels (p < 0.05) 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: Handball players, antioxidants, oxidative stress, short-term exercise.

INTRODUCTION

Handball performance depends not only on running, jumping, sprinting, arm throwing, hitting, blocking, and pushing, but also technical and tactical skills as well as high levels of strength and muscle power (Gorostiaga et al., 2004). Proportion of energy systems in handball is estimated to be 20% alactic anaerobic, 30% lactic anaerobic and 50% aerobic. It requires significant anaerobic fitness, and operates within a moderate-level of aerobic system in exercise molecules in several cellular pathways, redox and recovery after exercise (Bompa, 2006). Exercise disturbs the balance between free radicals and antioxidants and the resultant state is known as oxidative stress (Alessio et al., 2000; Urso et al., 2003). 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 (Tsai et al., 2001). The increase in O\textsubscript{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 (Tsai et al., 2001). Despite ROS having a fundamental role as signaling changes induced by increased ROS production during exercise are...
negatively related to cellular homeostasis and might compromise cellular function (Close et al., 2005). Additionally, the emerging role of free radicals in delayed-onset muscle soreness and contraction-induced muscle injury has been reported (Close et al., 2005). 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 (Alessio, 1993; Finaud et al., 2006). There would be serious oxidative damage in biomolecules as a result of defects occurring in ROS during exercise. There have been many studies on the determination of oxidant stress in vivo (Finaud et al., 2006; Dernbach et al., 1993). 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 (Viña et al., 2000). 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 (Dernbach et al., 1993; Viña et al., 2000). In literature, there are many studies related to the effects of submaximal exercise on the athletes’ total antioxidant capacity, lipid hydroperoxide, total oxidative status and oxidative stress index in different sports (Finaud et al., 2006; Inal et al., 2001; Viña et al., 2000; Umegaki et al., 2000). There are the limited numbers of research on short term exercise on TAC, LOOHs, TOS and OSI. Therefore, the aim of this study was to evaluate the effects of short-term exercise on total antioxidant capacity (TAC), lipid hydroperoxide (LOOHs), total oxidative status (TOS) and oxidative stress index (OSI) in handball players.

MATERIALS AND METHODS

Subjects

Twenty male handball players with the mean age 15.3± 2.12 years; weight 55.9 ± 10.59 kg; height 167.4 ± 12.22 cm, BMI 20.0 ± 3.2 kg/m² from the regional league participated in the study. The players were training for three days a week and for two hours at each workout. The subjects were informed not to eat or drink for three hours before the tests. Any subjects who smoked or had any chronic illnesses or asthma were excluded from the study. None of the subjects was taking any drug known to affect lipid and lipoprotein metabolism. Attention was paid to exclude subjects who were taking anabolic drugs, vitamins or other antioxidants, or who were smokers.

None of the subjects was following a special diet. We conducted a face to face interview with every participant and administered a detailed food frequency questionnaire to obtain information about their dietary habits. The quality, quantity and frequency of consumption of red meat, chicken, fish, eggs, vegetables, fruits, milky products and soft drinks was similar in all of the subjects. The study protocol was approved by the local Ethical Committee. Informed consent was obtained from all participants’ parents.

Exercise program

After 15 min warm up period, a circuit training of 30 min was performed with three sets interspersed 5 min active rests. Circuit training consist of 8 exercises including push up, double leg vertical jumps, sit ups, 5 m shuttle sprint, abdominal crunch, vertical jump from prone lying position and back rise in prone lying position. Each exercise was executed with possible maximal effort in 20 s with 30 min rest between exercise intervals.

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 (TAC), total oxidant status (TOS), total peroxide concentration (LOOHs) and lipid profiles.

Measurement of plasma TAC levels

Plasma TAC levels were determined using a novel-automated measurement method developed by Erel (2004). This method is based on the bleaching of the characteristic color of a more stable 2,2’-azino-bis (3-ethylbenz-thiazoline-6-sulfonic acid) (ABTS) radical cation by antioxidants. The assay has got 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 FOX₂ method (Miyazawa, 1989) with minor modifications (Subudhi, 2001). The FOX₂ 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-xylene orange complex whose absorbance can be measured. The FOX₂ reagent was prepared by dissolving ammonium ferrous sulphate (9.8 mg) in 250 mM H₂SO₄ (10 ml) to give a final
Measurement of plasma TOS levels

Plasma TOS levels were determined using a novel automated measurement method as previously described (Erel, 2005). Oxidants present in the sample oxidized the ferrous ion-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 xylene 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 H₂O₂ equivalent/L).

Oxidative stress index

The percentage ratio of TOS level to TAC level was accepted as OSI (Subudhi AW., 2001). For calculation, the resulting unit of TAC was changed to mmol/L, and the OSI value was calculated using the formula below:

\[ \text{OSI (arbitrary unit)} = \frac{\text{TOS (mmol H₂O₂ equiv/L)}}{\text{TAC (mmol Trolox equivalent/L)}} \times 100 \]

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.

RESULTS

Twenty male handball players with the mean age 15.3 ± 2.12 years; weight 55.9 ± 10.59 kg; height 167.4 ± 12.22 cm, BMI 20.0 ± 3.2 kg/m² from the regional league participated in the study (Table 1).

Oxidative stress and antioxidant defense markers before and after the short-term exercise are given in Table 2. Plasma TOS (before exercise 1.68 ± 0.24 mmol H₂O₂/L, after exercise 1.57 ± 0.19 mmol H₂O₂/L) and OSI (before exercise 4.22 ± 0.93 AU, after exercise 3.76 ± 0.82 AU) levels were significantly increased (P < 0.05 and P<0.01, respectively) and TAC levels (before exercise 1.68 ± 0.24 mmol Trolox equiv./l, after exercise 1.57 ± 0.19 mmol Trolox equiv./l), were significantly decreased (P < 0.05) after the exercise test compared to before. There were no significant changes in plasma LOOHs levels, (before exercise 4.22 ± 0.93 Eqiv./L, after exercise 3.76 ± 0.82 Eqiv./L).

DISCUSSION

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

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. Free oxygen radicals produced in metabolic and physiological processes in exercise may damage to cellular components in tissues (Powers et al., 1999). The result of the present study was supported by Powers et al. (1999).

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 (Somani et al., 1995). 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 (Umegaki et al., 2000; Koyama et al., 1999). 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 (Koyama et al., 1999; Maughan et al., 1989). In several studies (Poulsen et al., 1996; Poulsen et al., 1999), investigators have failed to observe any signs of exercise-induced oxidative stress immediately.
Table 1. Anthropometric characteristics of the subjects.

<table>
<thead>
<tr>
<th>Parameters (n = 20)</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>15.3 ± 2.12</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>167.4 ± 12.22</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>55.9 ± 10.59</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>20.0 ± 3.2</td>
</tr>
</tbody>
</table>

Data are given as mean ± SD. Age (years), Height (cm), Body weight (kg), BMI (kg/m²).

Table 2. Comparison of plasma oxidative stress and antioxidant defense markers before and after short-term exercise in handball players.

<table>
<thead>
<tr>
<th>Parameters (n=20)</th>
<th>Before exercise Mean ± SD</th>
<th>After exercise Mean ± SD</th>
<th>t</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC (mmol Trolox equiv./l)</td>
<td>1.68 ± 0.24</td>
<td>1.57 ± 0.19</td>
<td>2.27**</td>
</tr>
<tr>
<td>LOOHs (mmol H₂O₂ Eqv./L)</td>
<td>4.22 ± 0.93</td>
<td>3.76 ± 0.82</td>
<td>1.88</td>
</tr>
<tr>
<td>TOS (mmol H₂O₂/L)</td>
<td>10.41 ± 2.00</td>
<td>13.05 ± 3.35</td>
<td>-2.79**</td>
</tr>
<tr>
<td>OSI (AU)</td>
<td>10.43 ± 2.34</td>
<td>13.05 ± 3.35</td>
<td>-3.27*</td>
</tr>
</tbody>
</table>

*P< 0.01   **P < 0.05. Data are given as Mean ± SD. TAC, Total antioxidant capacity; LOOHs, lipid hydroperoxide; TOS, Total oxidative status; OSI, Oxidative stress index.

After exercise. Although it has been suggested that exercise training enhances antioxidant capacity, the causal mechanisms are not yet clearly known (Hartmann et al., 1994; Niess et al., 1996). The findings of many studies (Somani et al., 1995; Umegaki et al., 2000; Koyama et al., 1999; Maughan et al., 1989) were in agreement of this research. 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 handball 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 TAC levels.

In previous studies, it has been found that TAC levels increased and some antioxidants decreased reduced immediately after exercise (Liu et al., 1999; Child et al., 1999). Some studies have shown a decrease in glutathione and an increase in glutathione peroxidase activity after exercise, which returned to baseline levels 1 h after exercise (Powers and Lennon, 1999; Inal et al., 2005). 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 (Margaritis and Rousseau, 2008; Ashton et al., 1999), there is no convincing experimental evidence that this is accompanied by an increase in exercise performance in healthy human subjects (Rokitzki et al., 1994).

Different exercise forms lead to different levels of oxidative stress (Alessio, 1993; Finaud et al., 2006). Although, long time regular exercises ameliorate the antioxidant defense system (Powers and Lennon, 1999), high intensity exhaustive exercises increases free radical production and causes oxidative stress by increasing oxygen consumption 10 - 15 fold (Subudhi et al., 2001).

In literature there are different results about the redox balance of athletes. Regular endurance and resistance training increases the antioxidant defense (Dernbach et al., 1993; Niess et al., 1996). It has been demonstrated that, two days of intense exercise increased the plasma total antioxidant capacity in elite skiers (Subudhi et al., 2001). In many studies, it has been shown that oxidative stress parameters did not change (Stejnborn and Szyszka, 2001) or increased (Koyama et al., 1999; Maughan et al., 1989) and even...
decreased (Dernbach et al., 1993) after exercise. The results of this study was in agreement with many studies (Finaud et al., 2006; Viña et al., 2000; Somani et al., 1999; Umegaki et al., 2000; Koyama et al., 1999) and disagreement with others (Hartmann et al., 1994; Niess et al., 1996; Stejnborn and Szyszka K. 2001).

The main reason for the different findings from different studies may be that each study comprised participants of different socio-economic levels, different age groups and with different sporting activities. Some reports indicated that supplementing antioxidants would increase exercise performance.

**Conclusion**

The increase in total antioxidants, oxidants and oxidative stress in handball players is connected to short-term acute exercise and has the dual effect of oxidant development leading to oxidative stress and on the other hand antioxidant enzymes inducing an increase in antioxidant synthesis. To reduce this high level of oxidative stress in handball players and to protect against harmful effects it may be useful to have a diet rich in antioxidants or to take antioxidant supplements. However there is need for more detailed studies in order to assess possible relationships in oxidative stress produced with handball.

**REFERENCES**


