The Response of Oxidative Stress Indices of Soccer Players to Interval Activity

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ABSTRACT

Free radicals are chemical compounds produced naturally in human body. The immune system uses an antioxidant defense system to counter the negative effects of free radicals. An increase in the production of free radicals during intense exercise can cause serious inflammation and damage. The onset of the season of matches and intense exercise for the preparation of players as well as the lack of sufficient recovery during the exercises can lead to the weakening of the immune system in confronting the oxidants and their precursors, if not trauma. Here we studied the activity of glutathione reductase and catalase, which seem to prevent oxidative stress with their antioxidative action. This quasi-experimental study investigated the relationship between periodic activity and these two enzymes in a statistical population of 105 soccer players of the First Division League of Iran who volunteered to take part in this research. After they answered a special sports medicine questionnaire, 44 people were chosen via simple random sampling. The subjects were assigned to experimental and control groups randomly. 24 hours before the start of the research their personal characteristics were measured and recorded. Height, weight, and body mass index were calculated with the use of a medical scale and their percentages of fat were also measured with calipers. The subjects in the experimental group were evaluated with the Copenhagen Test. In order to determine the effects of periodic exercises on the concentration of catalase and glutathione reductase in the two groups, blood samples were taken from both groups before the start of the exercises. The experimental group underwent the research protocol while the control group had no activities. 3 and 24 hours after the test blood samples were taken again from the two groups. The collected data were analyzed descriptively and inferentially. Measures of central tendency were used to describe data. For inter-group comparison and analysis one-way analysis of variance (ANOVA) was used with frequent measurements. In order to determine the difference between means and the point of meaningfulness the Banferroni post-hoc test was used, which controls the general error with the error of each test into account. The level of meaningfulness was considered to be p<0.05, and all statistical tests were analyzed with SPSS version 19. Research findings showed that periodic exercise did not change the concentration of catalase, but the number and percentage of glutathione reductase cells changed significantly. As a result, periodic exercise increased the concentration of glutathione reductase in blood when athletes did sports activities compared to that before activities.

INTRODUCTION

Free radicals consisting of reactive oxygen and nitrogen species (RONS), are chemical compounds produced naturally in human body [1]. They can have both positive effects (e.g. their effect on the immune system and antioxidant defense) as well as negative ones (like the oxidation of DNA, proteins, and lipids of cells) [15, 27, 28]. These compounds are highly reactive and able to destroy cells by aiming at different cell parts especially the cell membrane and the internal organelle [4, 16]. To limit the negative effects of free radicals, the immune system uses antioxidant defense systems, including enzyme antioxidants (catalase, glutamine peroxidase, and superoxide dismutase) as well as non-enzyme ones (like vitamins A, C, and E) [26, 33]. Catalase (CAT) is an iron dependent enzyme found mainly in organelles called peroxisomes. It changes H$_2$O$_2$ to H$_2$O and acts like SOD. It is most active in the liver and least active in skeletal muscles [11]. In skeletal muscles its activity changes with the type of the fiber, with the most activity in type one fibers (I) and the least activity in type two fibers (IIa) [1, 11]. In the case of an imbalance between the production of free radicals and

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the antioxidant capacity, oxidative stress occurs [12, 19]. As a result of oxidation homeostasis cell revival is lost. This leads to cellular and muscular damage [15, 27]. An increase in the oxygen uptake during exercise is accompanied by an increase in the production of free radicals. A decrease in the negative effects of these compounds depends on the antioxidant system [19, 20]. Santo-silva et al. stated that intense and competitive exercise with the imposition of high proteolytic and oxidative stress can increase the liability of future cardiovascular diseases [34]. Some studies show that moderate-intensity exercise goes together with structural modifications in the cardiovascular system and also muscular system [36, 41], which can decrease diseases resulting from oxidative stress [17]. In their research, Falone et al. [16] analyzed the effect of exercise on systematic antioxidant capacity and resting levels of lipid peroxidation serum and damages of oxidative proteins in amateur runners in comparison with untrained people. Findings showed that long-term and moderate aerobic exercise can improve the antioxidant defense system and decrease protein oxidative processes in times of rest. Regulation of lipid oxidative stress decreases in response to a period of intense exercise [21]. Soccer is an intense periodic sport [2, 22] in which players undergo high body pressure in a rather long period (two 45-minute halves with a 15-minute interval). During a soccer match more than 90% of needs are satisfied via the aerobic system [9] and players run about 10 kilometers at an intensity level close to the anaerobic threshold [22]. Agullo et al. [9] did a research to prove the existence of oxidative stress during exhaustive exercise and to measure the antioxidative response. In this research 8 males did mountain biking for 171 km in 270±12 minutes. Blood samples were taken before the biking, immediately after the end, and in the morning the next day. Mountain biking significantly increased the activities of glutathione reductase and catalase, oxidative stress, GSSG, and serum uric acid concentration. Triglycerides and VLDL-cholesterol increased and remained high up to 3 hours after biking. Special use of alpha-tocopherol for oxidative stress was proved during the recovery. Much oxygen intake during a soccer match can be accompanied by an increase in the production of free radicals and the imposition of oxidative stress upon players [3, 30].

Currently there is little known about the oxidative stress of a soccer match upon players. Therefore, the current research aims to determine the response of indices of oxidative stress of soccer players. An increase in the production of free radicals during intense exercise can cause serious damage and inflammation to athletes. The onset of the season of matches and intense exercise for the preparation of players as well as the lack of sufficient recovery during the exercises can lead to the weakening of the immune system in confronting the oxidants and their precursors, if not trauma. There are different causes to such a decline, one of them being the intensity of exercise in a session. If coaches and athletes are aware of the stress imposed on the body in a training session they can set the duration and the intensity of the training session and the recovery period. Soccer is among combined activities, defined to include aerobic and anaerobic metabolism [15]. Therefore, players undergo significant physiologic pressures during both preparation exercises and matches. Coaches’ and players’ awareness of the levels of these pressures can be an effective step in designing optimum exercises so that the performance of players improves.

What add to the necessity of this research are the biological duties and actions of glutathione reductase (GR) and catalase (CAT) which are necessary in many bodily physiological reactions to sustain life. Lack of such a research and ambiguity in the state of glutathione reductase and catalase after different exercise methods heighten the necessity of addressing this subject. Due to the antioxidant and biological duties of glutathione reductase and catalase in the body and the therapeutic effect of exercises, especially periodic ones, shown for different diseases and due to the fact that so far few researches have studied the effect of periodic exercise on the concentration of glutathione reductase and catalase in soccer players, the current research was designed to study and compare the effect of one session of periodic exercise on glutathione reductase and catalase in soccer players in the First Division League. Hence the current research aims mainly to determine the response of oxidative stress indices of soccer players to periodic activity. Especially, it wants to determine the effect of periodic activity on glutathione reductase and catalase of soccer players. Here researchers want to know if a session of periodic activity affects the concentration of glutathione reductase and catalase of soccer players.

**Research Methodology:**

**Samples:**

First the staffs of teams taking part in the Isfahan First Division League matches were made aware of the subject, aim, and the methodology of the research. 67 players of this league volunteered to take part in the test. After a special sports medicine questionnaire was filled, 44 players were randomly assigned to experimental and control groups. 24 hours before the start of the research their personal characteristics were measured and recorded. Height, weight, and body mass index were calculated with the use of a medical scale and their percentages of fat were also measured with calipers.

**Research Protocol:**

It was the time for preseason tournaments and the Copenhagen Test was given after 8 weeks of continuous exercise and when the players were fully ready. The control group did their usual activity. Before the execution
of the research protocol blood samples were taken from both groups. Then the experimental group underwent the research protocol while the control group had no activities. Immediately after the end of the protocol and also 3 and 24 hours after the end blood samples were taken from both groups. The Copenhagen Soccer Test (CST) was used to simulate the soccer match. This test includes two 45-minute activity periods with a 15-minute interval, designed according to the activity patterns of soccer matches [4].

**Blood Sampling Methodology:**

First 9 cc blood were taken from the right anti-cubital vein of subjects in sitting position. 3 ml of blood went into tubes with sodium citrate for measuring the activity of catalase, 3 ml went into vials containing EDTA for measuring hemoglobin and glutathione reductase, and 3 ml went into tubes containing an anticoagulant for measuring blood lipids. Then the serum of blood samples was separated with a centrifuge from the erythrocytes and kept at -80°C. Glutathione reductase and catalase were measured with human kits through the ELISA procedure in a laboratory. Glutathione reductase was measured with a special laboratory kit, ELISA (R&D) System, Minneapolis, MN tool, an OD scale, or a photometer made by Medsystem Bender in Austria on the basis of the kit protocol. The kit sensitivity was 2.3 pg/ml.

Blood samples prepared on EDTA were washed in the above procedure and with the same volume of deionized distilled water and hemolysin %50 was made. 500 microliters of this hemolysin were kept for measuring the activity of glutathione reductase at -80°C. Blood samples taken on sodium citrate were washed in the above manner and hemolysin was produced. To these was added phosphate buffer to get a dilution ratio of 1/500. They were then quickly Duplicate tested. The activity of glutathione reductase in blood cells was measured in the same way as David M. Goldberg did it. In this method the catalytic activity of glutathione reductase is estimated with a decrease in light attraction with a wave length of 340 nanometers because of the oxidation of NADPH for the conversion of each 1 oxidated glutathione molecule (GSSG) to 2 restored glutathione molecules (G-S), and the activity of glutathione reductase is measured and reported with the unit of U/gHb.

Catalase activity in red cells was measured in the same way Hugo Aebi measured it. In this method the decomposition rate of enzyme substrate, i.e. hydrogen peroxide, is measured with a wave length of 240 nanometers for 30 seconds with 5- or 10- second intervals. The activity of catalase was calculated through measuring the constant of primary reaction speed of hydrogen peroxide decomposition and with the unit of k/gHb. Glutathione reductase and catalase activities were measured with the double beam spectrophotometer model Censil-9000.

Other measuring tools included a preliminary questionnaire to determine the general health conditions of participants, a medical scale model Seca made in Germany, Yagami calipers made in Japan, to determine the fat percentage in the method Jackson and Pollock did it, and a centrifuge to separate serum from plasma.

**Statistical Method:**

This is an applied, quasi-experimental research. Collected data were analyzed descriptively and inferentially. In descriptive statistics part data are described with measures of central tendency. For inferential analysis and comparison a one-way analysis of variance (ANOVA) test was used with frequent measurements. In order to determine the difference of means and the point of meaningfulness, Banferroni post-hoc test was used. Banferroni post-hoc test controls the general error taking into account the error of each of tests. The level of meaningfulness was considered to be p<0.05 and all statistical tests were analyzed with SPSS software version 19. The resulting data were turned into tables and diagrams with MS Excel.

**Research Findings:**

Tables 1 and 2 show personal characteristics of subjects in the pre-test and post-test (before the start and at the end of the research in the experimental group).

**Table 1:** Demographic characteristics of control and experimental group (mean±standard deviation).

<table>
<thead>
<tr>
<th>Item</th>
<th>Experimental Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21.60±1.71</td>
<td>24.60±1.71</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>181.46±2.73</td>
<td>179.46±4.73</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>79.27±7.01</td>
<td>76.27±3.01</td>
</tr>
<tr>
<td>Fat (percent)</td>
<td>14.99±0.8</td>
<td>12.76±0.48</td>
</tr>
<tr>
<td>Maximum Oxygen intake (ml/kg/min)</td>
<td>33.98±2.76</td>
<td>36.98±3.76</td>
</tr>
<tr>
<td>Body Mass Index (kg/square of height)</td>
<td>22.01±2.49</td>
<td>21.41±1.29</td>
</tr>
<tr>
<td>Maximum Heart Rate in Periodic Activity (No./min)</td>
<td>181±12</td>
<td>186±5</td>
</tr>
</tbody>
</table>

The results of measuring the activity of catalase in red cells showed that there was a meaningful reduction in the experimental group compared to the control group (*253.13±44.34) (Table 2). The study of glutathione reductase showed a meaningful difference in red cells of the experimental group compared to that of control
group (**23.86±5.96) and it was observed that the concentration of glutathione reductase during periodic activity and after activity increased significantly (Table 3).

### Table 2: Comparison of catalase and glutathione reductase activities of red cells in experimental and control groups.

<table>
<thead>
<tr>
<th>Item</th>
<th>Experimental Group</th>
<th>Control Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase (K/gHb)</td>
<td>**253.13±44.34</td>
<td>278.82±50.44</td>
</tr>
<tr>
<td>Glutathione Reductase U/gHb</td>
<td>**23.86±5.96</td>
<td>11.92±1.56</td>
</tr>
<tr>
<td>N</td>
<td>22</td>
<td>22</td>
</tr>
</tbody>
</table>

### Table 3: Statistical comparison of variables of personal characteristics of subjects.

<table>
<thead>
<tr>
<th>Item</th>
<th>Experimental</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>0.001</td>
<td>1.000</td>
</tr>
<tr>
<td>Fat (percent)</td>
<td>-1.065</td>
<td>0.351</td>
</tr>
<tr>
<td>Maximum Oxygen Intake (ml/kg/min)</td>
<td>-6.662</td>
<td>0.001**</td>
</tr>
<tr>
<td>Body Mass Index (weight/square of height)</td>
<td>0.200</td>
<td>0.984</td>
</tr>
<tr>
<td>Heart Beat (No./min)</td>
<td>3.034</td>
<td>0.014*</td>
</tr>
</tbody>
</table>

* p≤0.05 is meaningful.
** p≤0.001 is meaningful.

According to the above results and the resulting numerical t value, the maximum oxygen intake and heart beat of the experimental group showed a meaningful difference (P=0.001 and P=0.014 respectively).

### Discussion and Conclusion:

There are many factors that might determine if exercise can increase the damaging effects of free radicals. The most important factor is absolute intensity of exercise. Other factors which determine the extent of oxidative stress (the damaging effects of free radicals) are the degree of athlete’s preparedness, the fatigue of the one doing exercises, and finally the diet of the athlete [4, 10]. The results of studies on human and animals have shown that many of cells get adapted when facing oxidants to decrease the danger of damage to tissues. For example lymphocytes increase the activities of SOD, catalase, and GPX in response to endogenous oxidants [16] and an intense exercise can increase the activities of SOD, GPX, CAT, and glutathione reductase in skeletal muscles [16]. The 8-week fast exercise has useful adaptive effects on the activity of anti-oxidation enzyme superoxide dismutase and a decrease in peroxidation of fat malondialdehyde [16]. Here the activities of anti-oxidative enzymes catalase and glutathione reductase in red cells of soccer players showed different results. The activity of catalase of the experimental group increased significantly compared to the control group (P<0.05). But the activity of glutathione reductase of the control group showed a meaningful difference compared to the control group. The activity of glutathione of the experimental group increased significantly compared to the control group. Andersen et al. showed that there were no relationships between the activities of anti-oxidative enzymes catalase and glutathione reductase on the one hand and the age of people on the other [11]. The decrease in the activity of catalase in people is bigger with more intense periodic activity. To counter the superoxide which is sometimes biologically needed, higher cells require designing and developing advanced enzyme (superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase) as well as non-enzyme (glutathione) protective systems during their evolution to prevent the appearance of more dangerous radicals [31]. The two enzymes glutathione reductase and catalase are involved in the omission of hydrogen peroxide, the former being preferred [23, 13] because it is proven that catalase is effective in omitting hydrogen reductase when the latter is produced more than glutathione reductase can accommodate. Most researches show an increase in the activity of glutathione reductase after sports activity [32, 24], while some show an increase [35, 37], some no change [29, 38, 39] and some even a decrease in the activity of catalase after the sports activity [40, 34] which can show the role of an increase in glutathione resources as glutathione oxidase coenzyme. During the activity of glutathione reductase, glutathione is restored and changed into glutathione oxide which is itself changed from NADPH to GHS again by glutathione reductase [41, 42].

In a research Shemshaki et al. [4] studied the effect of intense alpine skiing exercise on antioxidant conditions of male skiers. In this research 12 male alpine skiers engaged in intense alpine skiing for 6 weeks. Before the start of exercise and immediately after its end sample plasma and red cells were taken to measure glutathione, uric acid, and total antioxidant capacity. Findings showed that the amount of plasma uric acid and its antioxidant capacity and the amount of glutathione in red cells before and after intense 6-week exercise increased, which was in line with the results of the current research. Therefore, the balance between the increase in oxidative stress and the induction of anti-oxidative pathways in intense alpine skiing activity after 6 weeks is to the benefit of an increase in total antioxidant capacity of plasma and red cells. Therefore, this activity would not increase free radicals.

In a research Akkus [10] studied the effect of intense exhaustive exercise and long-term exercise on the activities of TBARs, PC, GSH, and total SOD. 32 young healthy men and women who volunteered to take part in this research underwent endurance exercises for two months. The training program consisted of biking for 60 minutes 4 days a week. Before and after the training period they did intense exhaustive exercises. Blood samples
were taken exactly before, immediately after, and 30 minutes after the exercise. The exercise increased the oxygen intake significantly. TBARs, PC, and GSH levels were significantly affected by intense exercise in men and women. Intense exercise increased the activity of SOD. There were no interactive effects among time, intense exercise, and group in the activities of TBARs, PC, GSH, and SOD in men and women. These results showed that protein and fat damages do not change in response to intense exercise via aerobic training. In a research Miyazaki et al. [18] studied whether intense endurance exercise decreased oxidative stress or not. 9 untrained men ranging 19-21 in age did exhaustive exercise on ergometer bicycles (before and after training) in a 12-week program and an intense period. The exercise program consisted of running with an intensity of %80 HRmax for 60 minutes per day, 5 days a week, for 12 weeks. Blood samples were taken in rest, immediately after exhaustive stress, to measure signs of oxidative stress and enzyme antioxidant activity (SOD, GPX, and CAT) in erythrocytes. After the exercise with an increase in VO2max the aerobic capacity improved. Results showed that a period of exhaustive exercise increased the ability to produce neutrophil superoxide anion before and after the exercise but the amount was smaller after the exercise. There was seen a significant increase in lipid peroxidation in erythrocyte membrane after the exhaustive exercise, but in any case exercise decreased this effect. During the rest after the exercise the activities of SOD and GPX increased. The activity of catalase did not change with exhaustive activity and exercise, which is in line with the results of this research. As a result, intense endurance exercise can increase antioxidant enzyme activity and exhaustive exercise can decrease the production of neutrophil superoxide. Also, regulation of antioxidant defenses goes together with a decrease in lipid peroxidation in erythrocyte membrane resulting from exercise. Bloomer et al. [14] compared the oxidative changes of blood proteins, lipids, DNA, and glutamine in the 24 hours after aerobic and anaerobic exercise in similar muscle groups. In this research 10 randomly selected trained men with a mean age of 24.3±3.8 did continuous cycling for 30 minutes with an intensity of %70 VO2max and periodic dumbbell squats with an intensity of %70 of a maximum repetition during 1-2 weeks in a cross-sectional study. The findings showed that 30 minutes of aerobic and anaerobic exercise can have different effects on blood biomarkers of oxidative stress. GSSG increased slightly and GSH showed transient decreases immediately after each of the two exercises. There were no mutual relationships between the main effects of MDA or 8-OHdG. PC was more after squat exercise than biking. In a research Feairheller et al. [17] measured the racial differences in oxidative stress responses of time period on intense exercise. In this research 9 African-Americans and 9 whites with a mean age of 21±0.4 did the submaximal treadmill test. Blood samples were taken frequently before and 30, 60, and 120 minutes after the exercise and SOD, TAC, PC, and TBARs were measured. The African-Americans had significantly higher SOD, TAC, and PC than the whites. Rowlands et al. [33] studied multi-day two-hoc effects on muscle contusion and damage, systemic safety, inflammation, and oxidative response. In this research 16 male and 4 female athletes ran 894 kilometers in 47 stations in 95 hours. Before and after the running there were observed increases in creatine kinase, isoprostane/8-creatine in urine, TNF-a, the amount of leukocytes and destruction of neutrophils, and decreases in hemoglobin, hematocrit, and destruction of lymphocytes. During the run there was observed contusion of quadriceps muscle of thigh. The muscle pain threshold in lateral vastus and gastrocnemius was lower after the run. A moderate speed of running was related to pain and the amount of leukocytes. The results showed that multi-day stationary running race increase inflammation and made lipid peroxidation, muscle damage, contusion, and oxidative stress of DNA go up. An increase in the level of TAC was related to a decrease in lipid peroxidation but was not related to safely response or muscle damage [25].

In general, based on the findings of the current research it can be concluded that periodic sports exercises increase blood glutathione level. According to the findings of the current research it can be said that after sports exercises we usually face two types of temporary and permanent increases in blood glutathione. Temporary increase is transient and occurs only after an intense sports training session or competition and returns to the level before the competition only after a period of return to the primary multi-day status for tissue repair and attraction of probable radicals like TBARS and MAD. Permanent increase gets on a compatibility nature and occurs at least after a multi-month training period. The sustainability of its increase depends on continuation of regular intense sports exercise and it was proved in this research that (aerobic) periodic exercises are effective. To the extent that intense sport exercises are done with a higher percentage of maximum oxygen intake, glutathione resources increase. As it was said before, blood glutathione level of professional marathon runners is almost 4 times higher than that of people who engage regularly in non-professional sport exercises. The increase in glutathione reductase resources relates more to H2O2 among ROS factors. As mentioned, among the enzymes of the antioxidant system the two antioxidant enzymes catalase and glutathione reductase are responsible for the omission of H2O2. A comparison between H2O2 of catalase and glutathione reductase shows that given the bigger affinity between glutathione reductase and H2O2, the amount of catalase increases when there is not enough glutathione reductase available to the cell to collect H2O2. Glutathione reductase mostly plays a role in cell cytosol and changes H2O2 to regular mineral water with the help of glutathione, without the production of molecule oxygen. More H2O2 is possibly produced during periodic sports activity, which makes the amount of glutathione resulting from periodic exercise increase in cell compatibility with this poisonous substance in comparison with resting time. Based on the findings of this research it can be explicitly stated that periodic
activity has a positive and direct influence on the increase in concentration of glutathione reductase, but, depending on different conditions, can make the concentration of catalase decrease or increase, or leave it unchanged.

REFERENCES