Peripheral Lymphocyte DNA Damage and Oxidative Status in Football Players after a Three-day Football Tournament

Mustafa Atli¹, Mehmet Aslan², Mehmet Emin Kucukoglu², Haci Bayram Temur¹, Abdullah Taskin³ and Hakim Celik³

Abstract

Objective  Regular physical activity is well known to play a cardioprotective role. The objective of this study was to investigate peripheral lymphocyte DNA damage and oxidative status in adult football players a three-day football tournament.

Methods  Twenty-five adult male football players and 25 sedentary male subjects were enrolled in the present study. Plasma total antioxidant status (TAS), total oxidant status (TOS) and the oxidative stress index (OSI) were determined. Peripheral lymphocyte DNA damage was determined using an alkaline comet assay.

Results  Plasma TOS, OSI and peripheral lymphocyte DNA damage were significantly lower in the adult football players than in the sedentary subjects (all: p<0.001), while TAS was significantly higher in the football players (p<0.001). The plasma TAS levels were inversely correlated with TOS, OSI and peripheral lymphocyte DNA damage (r =-0.683, p<0.001; r =-0.909, p<0.001; r =-0.608, p<0.001; respectively) in the adult football players.

Conclusion  These results indicate that physical activity is associated with increased antioxidant capacity and decreased oxidative stress. Such conditions are important for a healthy life. Further studies are needed to clarify the mechanisms underlying this association.

Key words: football players, peripheral lymphocyte DNA damage, total antioxidant status, total oxidant status, oxidative stress index

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Introduction

The beneficial effects of exercise on health are well known. It is generally accepted that regular physical activity is an important factor for the prevention and treatment of cardiovascular diseases (1). Moreover, regular physical activity is associated with many positive health benefits, including reduced blood pressure, maintenance of ideal body weight, improvements in lipid profiles and decreases in the incidence of non-insulin dependent diabetes (2-4).

It has been reported that the number of reactive oxygen species (ROS) increases under aerobic endurance stress. The major source of ROS is thought to be the mitochondria of active muscles (5). Oxidative stress is caused by an imbalance between ROS production and antioxidant scavenging activities. It has been documented that ROS can be detrimental and are associated with several diseases, including diabetes and heart disease (6). Additionally, ROS can cause severe damage to biological macromolecules such as nucleic acids, proteins and lipids (7, 8). Regular training may enhance antioxidant defense mechanisms (9).

Genomic damage has been reported to be associated with many pathological conditions such as various cancers, neurodegenerative diseases and aging (10, 11). The comet assay (single-cell gel electrophoresis: SCG) is a well-established tool used to detect genotoxic damage, especially single and double strand breaks, and is easy to perform. It has the further advantages of speed, simplicity and the fact that observations are made at the level of single cells (12).

¹Physical Education and Sports, Faculty of Education, Yuzuncu Yil University, Turkey, ²Department of Internal Medicine, Medicine Faculty, Yuzuncu Yil University, Turkey and ³Department of Clinical Biochemistry, Medicine Faculty, Harran University, Turkey
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Correspondence to Dr. Mehmet Aslan, m.aslan301@mynet.com
This simple, rapid and sensitive technique is extremely useful. In addition, the comet assay is a potential tool for estimating DNA damage at the single-cell level and provides information regarding the presence of DNA damage among individual cells (13, 14).

Plasma total antioxidant status (TAS) is an accurate index of oxidative stress and provides a measure of total plasma defenses against oxidative stress (15). Some authors have reported that peripheral lymphocyte DNA damage is caused by oxidative stress (16, 17).

Several studies have investigated oxidant, antioxidant and DNA damage under many different conditions (18-23). Banfi et al. (18) assessed the plasma oxidative stress biomarkers, nitric oxide and heat shock protein 70, in trained elite soccer players. Moreover, the effects of multi-day relay trail running on muscle soreness and damage and systemic immune, inflammatory and oxidative responses have been evaluated in human studies (19). A previous study investigated the levels of seminal plasma 8-isoprostan, ROS, malondialdehyde, superoxide dismutase, catalase, total antioxidant capacity and sperm DNA fragmentation in elite athletes and recreationally active men (20). Recently, the impact of endurance and ultraendurance exercise on DNA damage has been investigated in a clinical study (21). In addition, Hamurcu et al. (22) investigated the effect of regular wrestling exercise on oxidative DNA damage, while Tanimura et al. (23) examined the effects of three consecutive days of exercise on lymphocyte DNA damage in both young untrained and endurance-trained men.

To our knowledge, peripheral lymphocyte DNA damage and oxidative status in football players have not yet been evaluated. Therefore, the objective of this study was to investigate peripheral lymphocyte DNA damage assayed using comet assays and oxidative status in adult football players after a three-day football tournament.

Materials and Methods

Subjects

In this prospective study, 25 adult male football players and 25 sedentary male controls were evaluated. The 25 adult male football players were selected among Yuzuncu Yil University, Physical Education and Sports students.

The adult football players were asymptomatic with unremarkable medical histories and normal physical examination findings. None of the adult football players had diabetes mellitus, hyperlipidemia, hypertension, coronary artery disease, a history of smoking or use of supplemental vitamins or a history of psychiatric, metabolic, hepatic or renal disease.

All subjects were informed about the study and provided their written informed consent prior to the beginning of the study. The protocol of the study was conducted in accordance with the Helsinki Declaration as revised in 2000 and was approved by the local ethics committee.

Measurement of total antioxidant status

Plasma TAS was determined using an automated measurement method developed by Erel (24). In this method, a hydroxyl radical, the most potent biological radical, is produced. In the assay, a ferrous ion solution present in Reagent 1 is mixed with hydrogen peroxide present in Reagent 2. The subsequently produced radicals, such as the brown-colored dianisidinyl radical cation produced by the hydroxyl radical, are potent radicals. Using this method, the antioxidantative effects of the sample on the potent free radical reactions that are initiated by the produced hydroxyl radicals are measured. The assay has precision values below 3%. The results are expressed as mmol Trolox Equiv./L.

Measurement of total oxidant status

Plasma total oxidant status (TOS) was determined using a novel automated measurement method developed by Erel (25). The oxidants present in the sample oxidize the ferrous ion-o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules that are abundantly present in the reaction medium. The ferric ion forms a colored complex with xylene orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated using hydrogen peroxide, and the results are expressed in terms of the micromolar hydrogen peroxide equivalent per liter (μmol H₂O₂; Equiv./L).

Determination of the oxidative stress index

The oxidative stress index (OSI) was defined as the percent ratio of the TOS level to the TAS level (26). OSI (Arbitrary Unit) = TOS (μmol H₂O₂; Equiv./L)/TAS (mmol Trolox Equiv./L).

DNA damage determination using the alkaline comet assay

After a three-day football tournament, 6-mL peripheral blood samples obtained from each subject were withdrawn into heparinized tubes and kept on ice. Lymphocyte isolation for the comet assay was performed within two hours, as described elsewhere (27).

Endogenous DNA damage in the lymphocytes was analyzed using an alkaline comet assay according to Singh et al. (12) with minor modifications. After electrophoresis, the
slides were stained with ethidium bromide (2 μ/mL in distilled H2O; 70 μL/slide), covered with a coverslip and analyzed using a fluorescence microscope (Nikon). For each subject, images of 50 randomly selected cells (25 cells from each of two replicate slides) were analyzed visually, as described elsewhere (27, 28). Each image was classified according to the intensity of the fluorescence in the comet tail and was given a value of either 0-3 or 4 (ranging from undamaged (Class 0) to maximally damaged (Class 4)). Therefore, the total slide score was between 0 and 200 arbitrary units.

**Blood samples**

After a three-day football tournament, blood samples were collected from the subjects in an overnight fasting state between 08:00 h and 10:00 h from an antecubital vein. Peripheral venous blood samples were taken into heparinized tubes in the fasting state after the three-day football tournament. The blood was centrifuged at 3,000 rpm for 10 minutes for plasma separation. The plasma samples were stored at -80°C until the analysis of the oxidative status parameters.

**Statistical analysis**

The results were presented as the mean and standard deviation (meana±SD). Nonparametric continuous variables were compared using the Mann-Whitney U-test. Parametric variables were compared using Student’s t-test. The correlation analyses were performed using Pearson’s correlation test. The results were considered to be statistically significant when the p value was less than 0.05. The data were analyzed using the SPSS® for Windows computing program (Version 11.0).

**Results**

There were no significant differences between the groups with respect to age and body mass index (p>0.05) (Table). Plasma TOS, OSI and peripheral lymphocyte DNA damage were significantly lower in the adult football players than in the sedentary subjects (all: p<0.001), while TAS was significantly higher in the adult football players (p<0.001) (Table).

The plasma TAS levels were inversely correlated with TOS, OSI and peripheral lymphocyte DNA damage (r = -0.683, p<0.001; r = -0.909, p<0.001; r = -0.608, p<0.001; respectively) in the adult football players.

The plasma TAS levels were inversely correlated with the OSI values (r =-0.861, p<0.001) in the control subjects. No correlations were observed between the plasma TAS levels, the plasma TOS levels and peripheral lymphocyte DNA damage (p>0.05) in the control subjects.

**Discussion**

We used the comet assay to identify DNA damage in this study. The comet assay has been proposed to be the most sensitive procedure for detecting DNA fragmentation. Furthermore, the comet assay has been found to be technically suitable for the routine measurement of DNA damage. Oxidative damage to DNA is of great importance due to the growing recognition that such damage can both initiate and promote carcinogenesis (29, 30). The single-cell gel electrophoresis (comet) assay is a useful method for quantifying DNA damage (31). Due to its simplicity and sensitivity, the comet assay has rapidly gained acceptance as a genotoxicity assay (31). Additionally, the comet assay has shown that strand breaks arise from DNA damage generated by oxidative stress (32).

ROS may induce oxidative modification of macromolecular damage in proteins, lipids, carbohydrates and DNA (32). ROS have been identified as crucial factors in the pathogenesis and development of a variety of chronic and degenerative diseases, including cancer and immune dysfunction (33). ROS have also been shown to cause extensive DNA damage, including single strand breaks, formation of modified bases, chromosomal damage and mutations in mammalian cells (16). Moreover, ROS induce formation of oxidized bases, single-strand breaks and crosslinking of DNA (16).

In the present study, a novel automated colorimetric measurement method to assess oxidative status (TAS, TOS and OSI) developed by Erel was used. This method has advantages over other methods. It is simple, cost-effective, reliable and sensitive. It does not interact with normally available serum components such as serum lipids, bilirubin and anticoagulants (24, 25).

In the present study, we measured peripheral lymphocyte DNA damage and the levels of plasma TAS, TOS and OSI in adult football players after a three-day football tournament. We observed that the football players had lower levels of plasma TOS, OSI and peripheral lymphocyte DNA damage compared to those observed in the sedentary subjects. In addition, the plasma TAS levels were higher in the adult football players compared to those observed in the sedentary subjects. In our study, we showed that the plasma TAS levels were inversely correlated with TOS, OSI and peripheral lymphocyte DNA damage in adult football players.

It is generally accepted that a sedentary lifestyle is related

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**Table. The Demographic Characteristics, Peripheral DNA Damage and Oxidative Status of the Two Groups**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Football players</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male)</td>
<td>25</td>
<td>25</td>
<td>ns</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>20.8±0.4</td>
<td>20.1±0.2</td>
<td>ns</td>
</tr>
<tr>
<td>DNA damage (arbitrary unit)</td>
<td>32 (0-81)</td>
<td>74.81 (18-162)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total antioxidant status (mmol Trolox equivalent/L)</td>
<td>1.22 (1.01-1.92)</td>
<td>0.90 (0.56-1.09)</td>
<td>0.001</td>
</tr>
<tr>
<td>Total oxidant status (μmol H2O2 Equiv./L)</td>
<td>7.23 (4.58-11.74)</td>
<td>9.84 (7.65-12.65)</td>
<td>0.001</td>
</tr>
<tr>
<td>Oxidative stress index (Arbitrary Unit)</td>
<td>0.59 (0.42-1.16)</td>
<td>1.14 (0.79-2.28)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

The values are expressed as the mean ± SD.
to an increased risk of coronary heart disease. In contrast, regular physical activity has been identified as a protective factor against the occurrence and progression of coronary heart disease (34). In addition, regular physical exercise is a protective factor against cardiovascular diseases and enhances antioxidant systems, whereas acute exercise appears to be a major source of increased oxidative stress and lipid peroxidation (35).

Previous studies have assessed oxidant, antioxidant and DNA damage (18-21, 36, 37). In a study by Banfi et al. (18), higher plasma glutathione reductase activity and nitric oxide and Hst 70 levels were observed in soccer players than in sedentary controls. Moreover, Rowlands et al. (19) described oxidative stress, inflammation and muscle soreness after an 894-km relay trail run. They reported increased total oxidative stress, inflammation and muscle soreness than in sedentary controls. Moreover, Rowlands et al. (19) described oxidative stress, inflammation and muscle soreness after an 894-km relay trail run. They reported increased total antioxidant capacity and decreased urinary 8-OHdG levels after the relay trail run. Recently, Tartibian et al. (20) investigated the levels of seminal plasma 8-isoprostane, ROS, malondialdehyde (MDA), superoxide dismutase (SOD), catalase, total antioxidant capacity (TAC) and sperm DNA fragmentation in elite athletes and recreationally active men and found that the seminal SOD, TAC and catalase levels were significantly higher, while those of seminal ROS, MDA and 8-isoprostane were significantly lower in recreationally active men than in elite athletes. In another study, Wagner et al. (21) evaluated the impact of endurance and ultraendurance exercise on DNA damage. Meanwhile, Peters et al. (36) reported that no significant changes were found in DNA strand breaks in endurance-trained athletes during prolonged, submaximal exercise. On the other hand, Stefanie et al. (37) suggested that Ironman triathlons do not cause long-lasting DNA damage in well-trained athletes.

Several studies have investigated the effects of exercise on lymphocyte oxidative DNA damage (22, 23). Hamurcu et al. (22) evaluated the effects of regular wrestling exercise on oxidative DNA damage. They reported decreased 8-OHdG levels in adolescent boys compared to sedentary subjects. In addition, Hamurcu et al. (22) reported no significant differences in the levels of 8-OHdG either before or after exercise. Furthermore, Tanimura et al. (23) examined the effects of three consecutive days of exercise on lymphocyte DNA damage in both young, untrained and endurance-trained men. They reported that lymphocyte oxidative DNA damage increased concomitantly with exercise sessions in both groups.

These results indicate that physical activity is associated with increased antioxidant capacity and decreased oxidative stress. Such conditions are important for a healthy life. Further studies are needed to clarify the mechanisms underlying this association.

The authors state that they have no Conflict of Interest (COI).

References