Evaluation of Systemic Oxidant and Antioxidant Status in Amateur Adolescent Athletes

Amatör Adölesan Sporcuların Sistemik Oksidan-Antioksidan Durumlarının Değerlendirilmesi

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ABSTRACT Objective: Physical exercise in athletes creates various changes in the oxidant-antioxidant balance. Regularly performed, moderate exercise has many beneficial effects, whereas intense exercise can produce damage in skeletal muscle and other tissues. **Material and Methods:** To investigate and compare the oxidative-antioxidative status and oxidative stress index in amateur adolescent athletes with those of a healthy control group of similar age and sex but with a normal lifestyle, and to determine any relationship between total oxidative status (TOS), total antioxidative capacity (TAC), oxidative stress index (OSI) and regular exercise. The study group consisted of 64 adolescent amateur athletes who for 2 years had regularly undertaken 2 hours of training per day at least 3 days per week. **Results:** Significantly high levels of total antioxidative capacity, total oxidative status, oxidative stress index and lipid hydroperoxide were found in the athlete group in comparison to the control group ($p< 0.0001$). **Conclusion:** We suggest that there is a dual effect associated with amateur adolescent athletes taking regular exercise over a long time in that oxidative stress appears with the development of oxidants, and at the same time induces antioxidant enzymes thus increasing antioxidant synthesis.

Key Words: Adolescent; exercise; oxidative stress

ÖZET Amaç: Sporcularda fiziksel egzersizin oksidan-antioksidan dengesinde çeşitli değişiklikler yaptığı gösterilmiştir. Bu çalışmada amatör adölesan sporcularda oksidan, antioksidan durum ile oksidatif stres indeksini aynı yaş ve cinsiyetteki sağlıklı kontrol grubu ile karşılaştırmak ve düzenli egzersiz yapmanın total oksidatif durum (TOD), total antioksidan kapasite (TAK) ve oksidatif stres indeksi (OSİ) üzerindeki etkisini değerlendirmek amaçlandı. **Gereç ve Yöntemler:** Çalışma grubu amatör olarak sporla uğraşan 64 adölesan sporcu ile benzer yaş ve cinsiyette 32 sağlıklı katılımcıdan oluştu. Amatör olarak sporla uğraşan 64 adölesan, en az haftada 3 gün, günde 2 saat düzenli olarak antrenman yapıyordu. Kontrol grubu sporla uğraşmıyor ve normal olarak hayatlarını devam ettiriyorlardı. **Bulgular:** Amatör adölesan sporcularda TAK, TOD, OSİ, lipid hidroperoksit (LOOHs) değerleri kontrol grubu ile karşılaştırıldığında ($p< 0.0001$) kontrol grubuna göre anlamlı derecede yüksek bulundu. **Sonuç:** Bu çalışmanın bulguları, amatör adölesan sporcularda uzun süre boyunca düzenli egzersiz yapmanın, bir yandan oksidan oluşumu ile oksidatif stresi ortaya çıkarırken, diğer yandan da antioksidan enzimleri indükleyip antioksidan sentezini artırarak çift etki gösterdiğini düşündürmektedir.

Anahtar Kelimeler: Adölesan; egzersiz; oksidatif stres

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Regular exercise has the dual effect of producing oxidants and thus oxidative stress, while at the same time inducing antioxidant enzymes thus increasing antioxidant synthesis.

Regular physical activity, associated with a balanced diet, is considered an important factor for health.[1] However, exhaustive and/or intense phys-

ical activity can induce diseases, injuries and chronic fatigue, which can lead to overtraining syndrome, partly because of free radical (FR) toxicity. FRs are produced extensively during physical exercise and although they exert positive effects on the immune system and essential metabolic functions, they are also involved in muscular fatigue, many diseases and the ageing process.[1-4] Antioxidants are components that suppress FR and their harmful effects. If the production of FR is larger than antioxidant activity, there is an oxidative stress state with cell damage.[5] Physical activity increases FR production and the antioxidant utilization. Nutrition provides an important source of antioxidants but it has often been reported that athletes had an insufficient micronutrient supply.[6,7] It has also been shown that oxidative stress could increase during periods of intensive training.[8,9]

Regularly performed, moderate exercise has many beneficial effects, whereas intense exercise can produce damage in skeletal muscle and other tissues.[10,11] Despite the growing amount of data, the relationship between physical activity and oxidative stress is far from being linear. Experimental evidence indicates that exhaustive exercise induces lipid peroxidation, DNA damage and alteration of the antioxidant defense system.[12-16] Conversely, the protective effects of training are usually associated with the up-regulation of endogenous antioxidant defense and repair systems, thus explaining why athletic individuals display less cell damage than sedentary ones.[17-19]

Comparatively, during endurance exercise there is a 10- to 20-fold increase in whole body oxygen consumption and oxygen uptake in the active skeletal muscle increases 100 to 200-fold.[20-22] The body has a complex antioxidant defence system to minimize the FR damage within the cells, to the cell membranes and in the extra-cellular fluid.[23-26,27] Within the strategy to maintain redox balance in oxidant conditions, the blood transporting antioxidants to every part of the body, plays a central role.

The aim of this study was to investigate and compare the oxidative-antioxidative status and OSI in amateur adolescent athletes with those of a healthy control group of similar age and sex but with a normal lifestyle, and to determine any relationship between total oxidative status (TOS), total antioxidative capacity (TAC), oxidative stress index (OSI) and regular exercise.

MATERIAL AND METHODS

SUBJECTS

The study group consisted of 64 adolescent amateur basketball players (57 boys, 7 girls) who for at least 2 years had regularly undertaken 2 hours of training per day at least 3 days per week. The control group was formed from 32 healthy adolescents of similar age (24 boys, 8 girls) who did no sports and led a normal life. They were selected among healthy adolescents who presented to our pediatric clinic. The girls in both the study and control groups were post-menarche.

Both groups were informed not to eat, drink or take any antioxidant medicine for 3 hours prior to sample collection.

All enrolled participants were healthy with no familial or personal history of diabetes or dyslipidaemia and with normal thyroid, hepatic and renal functions. Subjects were withdrawn from the study if there was any indication of cardiovascular disease, physical discomfort or chronic illness or if exercise was likely to trigger asthma. The questionnaire to be used was developed by researchers. A face-to-face interview was made with the participants and a detailed food frequency questionnaire was completed in order to obtain information about their dietary habits. A food consumption questionnaire was used to record the consumption frequency and daily consumption of different types of food of the participants. The types of food consumed by the subjects were recorded in the forms as portion or as weight for every meal, daily, 1-3 times a week, 3-5 times a week, once in two weeks, and once a month. These food types were categorized as dairy products, egg, meat, grains, dry beans, bread and cereals, fresh fruit and vegetables, oil, sugar, desserts, drinks and their subgroups. The daily energy amounts obtained from different

food groups of the study and the control groups were calculated and recorded by using the data on the food consumption questionnaire.

No subject was taking any drug known to affect lipid and lipoprotein metabolism. Special care was taken to exclude subjects who were taking anabolic drugs, vitamins or other antioxidants or who were smokers. No subject was following a special diet.

The amount of sport undertaken weekly, the number of years they had been doing that sport, dietary habits, personal and familial health history, socio-economic position, smoking habit or exposure to smoking, use of vitamins or medication were questioned.

The quality, quantity and frequency of consumption of red meat, chicken, fish, eggs, vegetables, fruits, milk products and soft drinks was similar for all subjects. The economic and socio-cultural status was similar in both the study and the control group. The dietary habits of both groups were similar.

Prior to the study, all subjects were medically examined. Their medical and sporting histories were recorded. The study protocol was approved by the Local Ethics Committee. The details of the study were explained to the participants prior to enrollment and all participants provided informed consent.

MEASUREMENTS

The ages of the participants were recorded; heights were measured to the 0.1 cm and weight were measured to the 0.1 kg in both groups. Respiratory function was measured by one flow tester screen mark spirometer and the systolic and the diastolic blood pressure was measured by stethoscope and sphygmomanometer. Heart rate was measured from the wrist radial artery by counting the pulse with the index and middle fingers over 15 seconds then multiplying by 4 to give the number of beats per minute.

SAMPLES

Blood samples were withdrawn into heparinized tubes from a cubital vein and were immediately stored in ice. Plasma was separated from cells by centrifugation at 3000 rpm for 10 min. Plasma samples were stored at -80°C until analysis.

MEASUREMENT OF TOTAL ANTIOXIDATIVE CAPACITY OF PLASMA

The total antioxidant status of the plasma was determined using a novel-automated measurement method, developed by Erel.[28] In this method, hydroxyl radical, which is the most potent biological radical, is produced. In this assay, the antioxidative effect of the sample against the potent free radical reactions, which are initiated by the produced hydroxyl radical, is measured. The assay has excellent precision values of lower than 3%. The results were expressed as mmol Trolox equivalent/l.

MEASUREMENT OF TOTAL PEROXIDE CONCENTRATION OF PLASMA (LOOHs)

The total peroxide concentrations of the plasma samples were determined using the FOX2 method with minor modifications.[29,30] The FOX2 test system is based on 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 absorbency can be measured. The FOX2 reagent was prepared by dissolving ammonium ferrous sulphate (9.8 mg) in 250 mmol H_2SO_4 (10 mL) to give a final concentration of 250 mmol 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 mmol ammonium ferrous sulphate, 100 mmol xylenol orange, 25 mmol H_2SO_4, and 4 mmol BHT in 90% vol/vol methanol in a final volume of 100 mL). The blank working reagent contained all components of the previous reagent except ferrous sulphate. Aliquots (200 Al) of plasma were mixed with 1800 Al FOX2 reagent. After incubation at room temperature for 30 min, the vials were centrifuged at 12.000 g for 10 minutes. 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 the test and the 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 TOTAL OXIDANT STATUS

TOS of serum was determined using a novel automated measurement method as previously described.[31] 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 total peroxide level to the TAC level was considered the OSI.[30] To perform the calculation, the result unit of TAC, mmol Trolox equivalent/l, was changed to mmol Trolox equivalent/l and the OSI value was calculated using the following formula; OSI=[(Total peroxide, mmol/l)/(TAC, mmol Trolox equivalent/l)/100].

MEASUREMENT OF LIPID PROFILES

Plasma triglyceride, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL) and very low-density lipoprotein (VLDL) levels were measured by automated chemistry analyser (Aeroset, Abbott, USA) using commercial kits (Abbott).

Statistical Analysis

Student's t-test and chi-square analyses were performed using SPSS for Windows, Release 11.5 computer program (SPSS). A $p< 0.05$ was considered significant.

RESULTS

The study consisted of 64 adolescent amateur athletes (57 boys, 7 girls) and a control group of 32 healthy adolescents of similar age (24 boys, 8 girls) who did no sports and led a normal life. The mean age was 15.2 ± 1.9 years for the athlete group and 14.8 ± 1.5 years for the control group. The mean height was 164.5 ± 11.5 cm and 162.2 ± 8.5 cm and the mean weight 54.2 ± 12.5 kg and 53.1 ± 10.4 kg for the athlete group and the control group respectively. In the athlete group body mass index (BMI) was (kg/m²) 19.8 ± 3.2 and in the control group, 20.0 ± 2.4. In terms of age, gender, height, weight and BMI, there was no statistically significant difference between the two groups (Table 1).

No difference was seen between the two groups in the pre-study measurements of systolic and diastolic blood pressure levels, pulse and respiratory rate.

There was no statistically significant difference between the two groups for respiratory function tests, arterial blood pressure, pulse and respiratory rate.

The TAC, TOS and OSI values of the amateur adolescent athletes were significantly high in comparison to the control group. In a statistical comparison of TAC, TOS, LOOHs and OSI values between the two groups, the athletes' values were significantly high in all parameters (Figure 1, Figure 2, Figure 3, Figure 4), (Table 2).

There was no statistically significant difference between the two groups for triglyceride, cholesterol, HDL-cholesterol, LDL-cholesterol, and VLDL-cholesterol levels (Table 3).

TABLE 1: Demographic and clinical characteristics of amateur adolescent athletes and control group. Values are expressed as mean ± SD.

Variables	Amateur Adolescent Athletes (n= 64)	Control Group (n= 32)	p
Sex (M/F)	57/7	24/8	p= 0.07, OR: 0.36 (0.12-1.13)
Age (year)	15.2 ± 1.9	14.8 ± 1.5	p= 0.34
Height (cm)	164.5 ± 11.5	162.2 ± 8.5	p= 0.30
Weight (kg)	54.2 ± 12.5	53.1 ± 10.4	p= 0.67
BMI (kg/ m²)	19.8 ± 3.2	20.0±2.4	p= 0.75

BMI; body mass index.

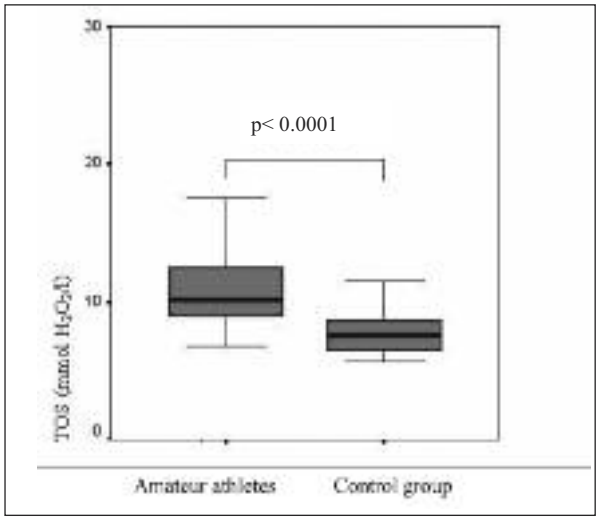

FIGURE 1: Mean values ± SD of the total oxidative status (TOS) according to groups.
The TOS level was significantly higher in the athlete group in comparison to the control group (p< 0.0001).

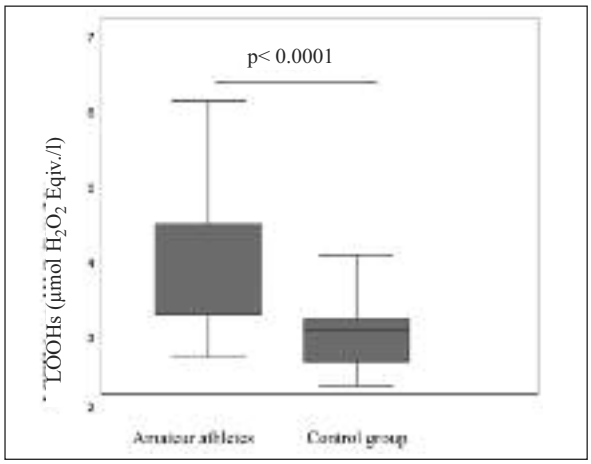

FIGURE 2: Mean values ± SD of lipid hydroperoxide (LOOHs) according to groups.
The LOOHs value was significantly higher in the athlete group in comparison to the control group (p< 0.0001).

DISCUSSION

In our study, the athlete group had significantly higher values of total antioxidant capacity, total oxidative status, oxidative stress index and amount of lipid hydroperoxide than the control group.

In modern medicine, regular physical exercise is an important tool in the prevention and treatment of diseases. Although intense exhaustive exercise increases oxidative stress, exercise training was shown to up-regulate antioxidant protection.[32]

Recently it has been suggested that the production of free radicals depended upon the increase of oxygen consumption in the human body, with a clear relationship with exercise. Exercise leads to more free radical production and increased metabolic processes by increasing the oxygen consump-

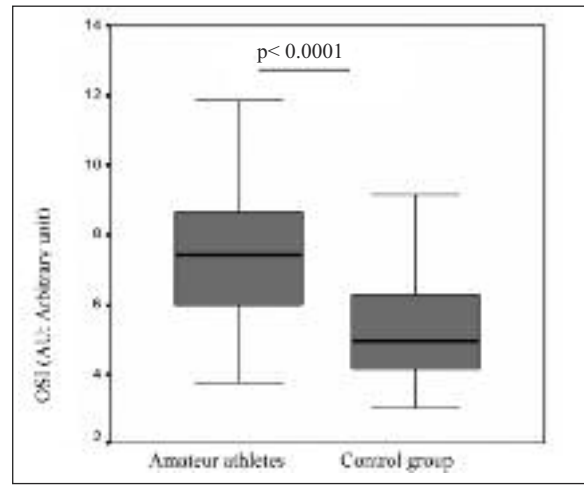

FIGURE 3: Mean values ± SD of the oxidative stress index (OSI) according to groups
The OSI level was significantly higher in the athlete group in comparison to the control group (p< 0.0001).

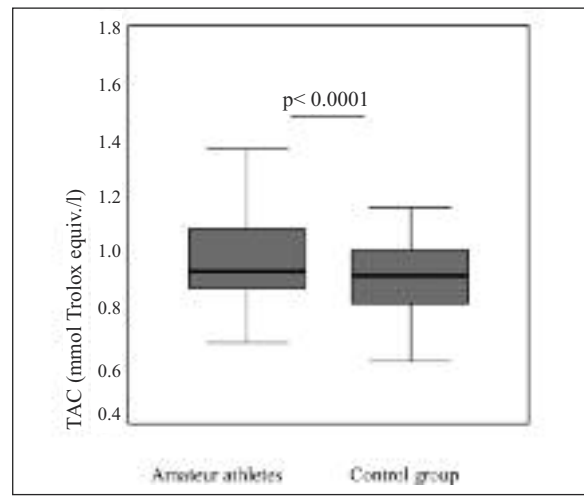

FIGURE 4: Mean values ± SD of the total antioxidative capacity (TAC) according to groups.
The TAC level was significantly higher in the athlete group in comparison to the control group (p< 0.0001).

TABLE 2: Oxidative and antioxidative parameters of the amateur adolescent athletes and the control group. Values are expressed as mean ± SD.

	Amateur adolescent athletes (n= 64)	Control group (n= 32)	p
TAC (mmol Trolox equiv./l)	1.07 ± 0.14	0.92 ± 0.12	< 0.0001
LOOH (μmol H_2O_2 Eqiv./L)	3.95 ± 0.9	3.05 ± 0.43	< 0.0001
TOS (mmol H_2O_2/l)	11.15 ± 3.20	8.11 ± 2.27	< 0.0001
OSI (AU)	10.67 ± 3.63	9.0 ± 2.93	0.027

TAC, total antioxidative capacity; LOOH, lipid hydroperoxidase; TOS, total oxidative stress; OSI, oxidative stress index; AU, Arbitrary Unit.

TABLE 3: Lipid profiles of the amateur adolescent athletes and the control group. Values are expressed as mean ± SD.

	Amateur adolescent athletes (n= 64)	Control group (n= 32)	p
Triglyceride (mmol/L)	1.47 ± 0.87	1.66 ± 1.29	> 0.39
Cholesterol (mmol/L)	4.62 ± 1.06	4.54 ± 1.01	> 0.22
HDL-cholesterol (mmol/L)	1.37 ± 0.34	1.39 ± 0.39	> 0.65
LDL-cholesterol (mmol/L)	2.5 ± 0.58	2.37 ± 0.71	> 0.55
VLDL-cholesterol (mmol/L)	0.67 ± 0.40	0.76 ± 0.59	> 0.39

HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

tion according to the strenuousness and duration of the exercise.[33]

Recent reports suggested that mild and regular exercise could increase the antioxidant capacity.[34,35] From this point of view, the cells are protected from the injury caused by free radical production because antioxidant levels increase in those who exercise regularly. The general opinion is that physical activity plays a beneficial role in the prevention of disease.[36]

The assimilation force of the total antioxidant capacity is weakened by the individual measurements of the likely effects of different antioxidants on each other in vivo. TAC, the antioxidants present in the biological fluid, seems to have the capacity to protect membranes and other cell components from oxidative damage.[27]

Therefore, reports suggest that to determine the plasma TAC and TOS, biological measuring of all antioxidants known to be present in plasma (or serum) and their cumulative effect will yield information that is more valid. Thus, in our study we used a test showing the total oxidant-antioxidant state. This is the first study on this subject with amateur adolescent athletes using the method developed by Erel.[5,28] This method enabled the study to be simple, economical, time saving and accurate. Moreover, this method can also show the total body oxidant-antioxidant values reliably, easily and cheaply.[28,31]

Chronic exercise was shown to increase the antioxidant defense and reduce the basal level of oxidative stress in adult humans, despite other studies yielding no such effect.[37,38]

In a study of elite skiers after 2 days of vigorous exercise, there was an increase in plasma total antioxidant capacity and no sign of an increase in the level of oxidative stress.[39] Another study of women wrestlers reported an increase in oxidative stress with exercise and a fall in the antioxidant capacity.[40] The antioxidant level increased within 40 minutes after swimming when compared to baseline in 800-metre swimmers.[41]

In addition to the studies suggesting that different forms of exercise lead to different levels of oxidative stress, there are others showing that long-term training improves the antioxidant defenses.[23,25,42,43]

Rather than exercise increasing oxidative stress, long-term habitual exercise always creates oxidative stress while improving the antioxidant system. While our findings of a significant increase in TAC in the athlete group in comparison to the control group is in accordance with the results of some studies, they contradict with others.[44-49]

Even though the subjects in our study group were amateur, they had been exercising regularly for several years and took part in competitive sport. We suggest that this may account for the increased TAC in the amateur athlete group.

LOOHs values had significantly increased concurrently with TOS in the athlete group. Reactive oxygen derivatives brought about by lipid peroxidation are one of the best indicators of the level of

molecular damage.[50] Several studies have shown that lipid peroxidation produced by exercise was higher in relation to the control group.[1,51] However, another study has found no difference in lipid peroxidation.[52]

The high values of TOS and LOOHs in the athlete group were parallel to the findings in other studies, whereas studies with conflicting data also exist. However, since amateur athletes were training regularly and were actively taking part in sports during the study period, there was thought to be a relationship between the extra training done at times of frequent sporting competitions and the significantly high level of oxidative stress. Nevertheless, associated with this increase in the oxidant-antioxidant capacity, the athletes' oxidative stress index increased. The results on this subject in the literature are various, of which some are in accordance with our results and some are not.[45-50] The main reason for the variable findings 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.[53]

The next step to get detailed information on the effect of the redox balance will be to compare a control group against a group of amateur adolescent athletes on a diet rich in antioxidants. Thus, the damaging effect of the redox balance on the DNA of amateur adolescent athletes on an antoxidant-rich diet or with the addition of supplements may be investigated.

CONCLUSION

The increase in total antioxidants, oxidants, oxidative stress and lipid hyperperoxidation in amateur adolescent athletes is connected to long-term regular exercise and has the dual effect of oxidant development leading to oxidative stress and at the same time inducing antioxidant enzymes giving rise to antioxidant synthesis. Even in amateurs, the sporting activity strengthened the antioxidant system, but in comparison with the control group, there was a higher level of oxidative stress. To reduce this high level of oxidative stress in amateur adolescent athletes and to protect against harmful effects it may be useful to have a diet rich in antioxidants or to take antioxidant supplements.

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