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Oxidative predictors and lipoproteins in male soccer players

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Aim: To determine male soccer players' lipoprotein levels, and their total antioxidant capacity, total oxidant status, oxidative stress index, and lipid hydroperoxide and lipoprotein levels after exercise.

Materials and methods: The study group included 33 certificated male soccer players with an age range of 17-20 years and the control group included 53 healthy male volunteers with an age range of 18-20 years. Their total antioxidant capacity, total oxidant status, oxidative stress index, and lipid hydroperoxide and lipoprotein levels were analyzed and compared.

Results: In the baseline, plasma levels, total oxidant statuses, oxidative stress indices, and lipid hydroperoxide levels were found of both groups were found similar ($P > 0.05$). However, the mean total antioxidant capacity of the study group was higher compared to the control group ($P < 0.05$). The total antioxidant capacity and lipid hydroperoxide level of the study group decreased while their total oxidant status and oxidative stress index increased after exercise ($P < 0.05$). Triglycerides and VLDL-cholesterol levels of the male soccer players were lower compared to the control group ($P < 0.05$). No significant differences were found between the means of total cholesterol, HDL-cholesterol, LDL-cholesterol levels for the 2 groups ($P > 0.05$).

Conclusion: The total antioxidant capacity of the study group was higher than that of the control group, and decreased after exercise. An increased total oxidative status and oxidative stress index were found after exercise. Hence, an increase in oxidative state was observed after exercise in male soccer players.

Key words: Male soccer, antioxidant capacity, oxidant status, lipid hydroperoxides, lipoproteins.

Erkek futbolcularda lipoproteinler ve oksidatif belirleyiciler

Amaç: Bu çalışmanın amacı egzersiz sonrası erkek futbolcuların toplam antioksidan kapasitesini, toplam oksidant durumunu, oksidatif stres indeksini, lipid hidroperoksit seviyesini ve lipoprotein seviyelerini belirlemektir.

Yöntem ve gereç: Yaşları 17-20 arasında 33 tane amatör futbolcu ve 18-20 yaşları arasında gönüllü 53 sağlıklı erkek bu çalışma için kayıt edildi. Toplam antioksidan kapasite, toplam oksidan durum, oksidatif stres indeksi, lipid hidroperoksit seviyeleri ve lipoprotein seviyeleri analiz edildi ve karşılaştırıldı.

Bulgular: Kontrol grup ile erkek futbolcuların taban seviyede toplam antioksidan kapasite, toplam antioksidan durum, oksidatif stres indeksi, ve lipid hidroperoksit plazma seviyelerinde benzerlikler olduğu belirledi ($P > 0,05$).

Ancak erkek futbolcuların ortalama toplam oksidat kapasiteleri, kontrol grubundan yüksek bulundu ($P > 0,05$). Erkek futbolcularda toplam antioksidan kapasite ve lipit hidroperoksit seviyesi egzersizden sonra düşmüştür. Toplam oksidatif durum ve oksidatif stres indeksi egzersiz sonrası yükselmiştir ($P < 0,05$). Erkek futbolcuların trigliserit ve VDLD kolesterol seviyeleri kontrol grubundan daha düşüktür ($P < 0,05$). Gruplar arasında toplam kolesterol, HDL kolesterol ve LDL kolesterol bakımında anlamlı bir fark bulunamamıştır ($P > 0,05$).

Sonuç: Erkek futbolcuların toplam antioksidan kapasiteleri, control grubundan yüksekti ve egzersiz sonrası azaldığı belirlenmiştir. Egzersiz sonrası toplam oksidatif durumlarında ve oksidatif stres indeksinde artış belirlenmiştir. Sonuç olarak erkek futbolcuların egzersiz sonrası oksidatif durumları artmıştır.

Anahtar sözcükler: Erkek futbolcular, antioksidan kapasite, oksidat durumu, lipid hidroperoksit, lipoprotein

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Introduction

The oxidative/antioxidant balance shifts towards the oxidative status under some conditions lead to an increase in oxidants and a decrease in antioxidants. As a result, oxidative stress, which has been implicated in different disorders, develops (1, 2). Since antioxidant effects of antioxidant components of plasma are additive, the measurement of total antioxidant capacity reflects the antioxidant status of plasma. The total antioxidant status of plasma with a total antioxidant potential as well as the total oxidative status of plasma were evaluated through measurement of the total peroxide level (3 – 6).

The limited number of studies in the field include 2 experimental studies in which physiological stimulus (physical exercise) was used in men and it was reported that intensive physical exercise resulted in a decrease in antioxidant levels and an increase in the lipid peroxidation markers in target tissues and blood (6-9). Brites et al., (10) determined that trained young athletes who followed a regular training program exhibited increased total antioxidant capacity, ascorbic acid, uric acid, α-tocopherol levels, and superoxide dismutase activity in response to the oxidative stress imposed by physical activity. A significant increase in HDL-c levels was found in response to exercise (10). The observed data are in agreement with the findings in studies mainly focusing on aerobic types of physical activity (1, 6-9).

The aim of this study was to examine the total oxidant status (TOS, the total amount of oxidant molecules), oxidative stress index (OSI, the ratio of the total peroxide to the total antioxidant potential), lipid hydroperoxides (LOOHs), TAC, which includes the radical hydroxyl before and after training sessions, and lipoprotein levels in male soccer players, and to compare these with the data obtained from the healthy men in the control group.

Material and methods

The experimental group consisted of 33 certificated male soccer players with an age range of 17-20 years, and the control group included 53 healthy-male volunteers with an age range of 18-20. The annual and weekly training session schedules of the soccer players were obtained before the study. Soccer players in the regional league trained at least 3 days a week, 2 h daily. The control group members were selected from healthy and non-exercising males. Both groups were requested not to eat, drink, or use antioxidant drugs or materials 8 h before the collection of blood samples. All subjects underwent a medical examination before participating in this study, and their medical history and athletic experience were also investigated. Subjects who smoke and/or drink, have a chronic illness and exercise induced asthma were excluded from this study. All subjects were requested to follow a similar diet, and gave their informed consent. This study received the approval of the local ethics commission.

Mean age of the male soccer players and the men in the control group were recorded in years. Their height was measured with metal scales with 0.1 cm sensitivity and body weight was measured with a digital scale with 0.1 kg sensitivity.

Blood samples were withdrawn into heparinized tubes from a cubital vein after overnight fasting before and after the training course. Plasma was separated from cells through centrifugation at 3000 rpm for 10 min. Plasma samples were stored at -80 °C until the day of analysis.

The total antioxidant status of plasma was determined using a novel automated measurement method developed by Erel (11). In this method, hydroxyl, which is the most potent biological radical, is produced. In this assay, the antioxidant effect of the sample against the potent free radical reactions, which is initiated by the produced hydroxyl radical, is measured. The assay yields excellent precision values of lower than 3%. The results are expressed in mmol Trolox equivalent/L.

The total peroxide concentrations of the plasma samples were determined using the FOX2 method (12) with minor modifications (13). The FOX2 test system is based on oxidation of ferrous ions to ferric ions by various types of peroxides in the plasma samples to produce a colored ferric-xylenol orange complex whose absorbance can be measured. The FOX2 reagent was prepared by dissolving ammonium ferrous sulphate (9.8 mg) in 250 μM H_2SO_4 (10 mL) to obtain a final concentration of 250 μM 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 stirred into the mixture to produce the final working reagent (250 μM ammonium ferrous sulphate, 100 AM xylenol orange, 25 mM H_2SO_4, and 4 mM BHT in 90% v/v methanol in a final volume of 100 mL). The blank working reagent contained all components of the previous reagent except for 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 min. Absorbance of the supernatant was then determined at 560 nm. The total peroxide content of 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 variation coefficient for individual plasma samples was less than 5%.

The total oxidant status of serum was determined using a novel automated measurement method as previously described (14). Oxidants 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 in the sample. The assay was calibrated with hydrogen peroxide and the results were expressed in micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/L).

The oxidative stress index was taken as the percentage ratio of the total peroxide level to the total antioxidant capacity level (13). To perform the calculation, the result unit of the total antioxidant capacity, mmol Trolox equivalent/L, was changed to mmol Trolox equivalent/L and the oxidative stress index value was calculated with the following formula:

OSI = [(Total peroxide, mmol/L) / (TAC, mmol Trolox equivalent/L) × 100].

The plasma levels of triglycerides, total cholesterol, LDL-c, HDL-c, and VLDL-c were measured with an automated chemistry analyzer (Aeroset, Abbott, USA) and commercial kits (Abbott).

Statistical analysis

The rates and the proportions for the categorical data and the means ± standard deviations for the continuous data were calculated. Total oxidant status (TOS, the total amount of oxidant molecules), oxidative stress index (OSI, the ratio of the total peroxide to the total antioxidant potential), lipid hydroperoxides, and the total antioxidant capacity, which includes the radical hydroxyl before and after training sessions, and lipoprotein levels were analyzed in the male soccer players, and compared with those of the control group. Statistical analyses were performed using the t-test. The correlation of total antioxidant capacity, lipid hydroperoxides, total oxidative status, and oxidative stress index with lipoproteins were analyzed using the Spearman correlation. Statistical significance was set at $P < 0.05$.

Results

In terms of the physical characteristics of the male soccer players and the control group, there were no significant differences in mean age, height, body weight, and body mass indices ($P > 0.05$). The age, weight, height, and body mass index were matched. Similar results in the means of total cholesterol, HDL-cholesterol, and LDL-cholesterol were found between the groups ($P > 0.05$). On the other hand, the mean triglyceride and VLDL-cholesterol levels of the soccer players were lower than those of the control group ($P < 0.05$) (Table 1).

No significant differences were found in the baseline lipid hydroperoxides, total oxidant status, and oxidative stress index of the soccer players and the control group ($P > 0.05$). However, the mean total antioxidant capacity was higher for male soccer players compared to the control group ($P < 0.05$) (Table 3).

In male soccer players, a significantly decreased total antioxidant status and lipid hydroperoxides (5.4% and 12.3%, $P < 0.05$, respectively), and increased total oxidant status and oxidative stress index were found after exercise (18.5% and 26.7%, $P < 0.05$, respectively) as shown in the Figure.

Correlation analyses of total antioxidant status, lipid hydroperoxides, total oxidant status, and

Table 1. Baseline characteristics and lipid parameters of the male soccer players and controls.

Baseline characteristics	Male Soccer Players (n = 33)	Control group (n = 53)	P
Age (years)	19.3 ± 0.7 (17 – 20)	19.3 ± 0.8 (18-20)	0.959
Weight (kg)	57.2 ± 9.2 (34 – 70)	56.9 ± 6.7 (40 – 68)	0.644
Height (cm)	1.66 ± 0.13 (140 – 182)	1.64 ± 0.12 (140 – 187)	0.580
Body mass index (kg/m^2)	20.9 ± 4.0 (15.1 – 32.7)	21.5 ± 3.8 (14.9 – 29.6)	0.581
Training Experience (years)	2.5 ± 1.3 (1 – 6)	-	-
Weekly Training Time (hours)	7.8 ± 2.6 (1 – 14)	-	-
Total cholesterol (mg/dL)	177.4 ± 46.7	168.9 ± 27.6	0.294
HDL-cholesterol (mg/dL)	48.7 ± 11.5	52.2 ± 13.8	0.231
LDL-cholesterol (mg/dL)	82.3 ± 27.2	89.5 ± 18.2	0.144
VLDL-cholesterol (mg/dL)	38.2 ± 16.1	24.4 ± 10.2	0.001
Triglycerides (mg/dL)	194.3 ± 80.3	122.4 ± 49.4	0.001

Table 2. Comparison of the oxidative and the antioxidant parameters between male soccer players and controls.

Oxidation Parameters	Soccer Players		P	Control Group	P*
	Before Exercise	After exercise			
Total Antioxidant Capacity (TAC, mmol Trolox equiv./L)	1.698 ± 0.201	1.607 ± 0.206	0.030	1.057 ± 0.412	0.001
Lipid hydroperoxides (LOOH, μmol H_2O_2 Eqiv./L)	4.172 ± 0.757	3.660 ± 0.643	0.006	3.962 ± 0.979	0.298
Total oxidant status (TOS, mmol H_2O_2/L)	10.420 ± 1.543	12.347 ± 2.789	0.001	10.736 ± 2.726	0.136
Oxidative stress index [OSI, AU (Arbitrary unit)]	10.325 ± 1.946	13.078 ± 4.671	0.004	10.887 ± 3.274	0.321

*P values; pre-training values of the male soccer players vs. controls

oxidative stress index with lipoproteins revealed a positive correlation only between LDL-cholesterol and lipid hydroperoxides (r = 0.361, P = 0.039) (Table 3).

Discussion

Acute physical exercise results in oxidative stress, but a regular training program could regulate the antioxidant status (15-17). Adult athletes are reported to have a better antioxidant status and increased oxidative stress than non-athletes while child swimmers are found to have higher oxidative stress at basal state than their untrained counterparts (15-17). Harmful oxidative reactions may occur in organisms, which remove reactive oxygen species via enzymatic antioxidant mechanisms in metabolic and physiological processes (15-17). An acute bout of exercise increases the production of reactive oxygen species through mechanisms involving the NADPH

Table 3. The relationship between lipoprotein and oxidative stress.

Parameters		Triglycerides	Total cholesterol	HDL-cholesterol	LDL-cholesterol	VLDL-cholesterol
TAC, pre-training	Correlation	0.000	-0.085	-0.137	-0.097	0.115
	P	0.998	0.639	0.449	0.591	0.524
LOOH, pre-training	Correlation	0.128	0.306	-0.099	0.361(*)	0.081
	P	0.477	0.083	0.583	0.039	0.653
TOS, before exercises	Correlation	0.323	0.049	0.182	-0.196	0.331
	P	0.067	0.789	0.311	0.274	0.060
OSI, before exercises	Correlation	0.155	-0.222	-0.124	-0.136	0.168
	P	0.389	0.215	0.492	0.450	0.350
TAC, before exercises	Correlation	0.110	0.116	-0.150	0.046	0.022
	P	0.543	0.521	0.406	0.797	0.904
LOOH, after exercises	Correlation	0.013	-0.026	0.281	-0.132	-0.006
	P	0.944	0.887	0.113	0.463	0.974
TOS, after exercises	Correlation	-0.041	0.090	-0.199	0.122	-0.042
	P	0.820	0.619	0.266	0.497	0.815
OSI, after exercises	Correlation	0.014	0.341	-0.169	0.139	-0.026
	P	0.939	0.052	0.346	0.440	0.886

* Correlation is significant at the 0.05 level (2-tailed).

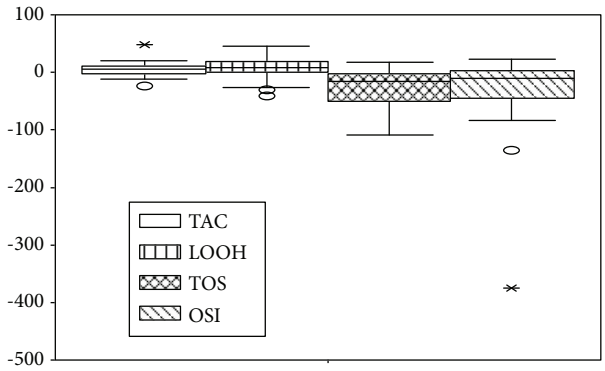

Figure. The percentages of total antioxidant capacity, lipid hydroperoxides, total oxidant status, and oxidative stress index after training sessions.
(TAC, total antioxidant capacity; LOOH, lipid hydroperoxides; TOS, total oxidant status; OSI, oxidative stress index)

oxidase system (18-20). During adaptation to acute exercise-induced oxidative stress, the increased level of reactive oxygen species induces antioxidant enzyme gene expression via an intracellular signaling pathway, which involves the redox regulated transcription factor, NF-κB (21). Several large trials in humans have shown that systemic oxidative stress level is correlated with cardiovascular disease and its various risk factors (22, 23).

Regular physical activity has been reported to be the most effective nonpharmacological intervention to enhance endogenous antioxidant capacity and alleviate oxidative stress-induced tissue damage (24-27). Moreover, the effect of exercise on the autonomic balance and the reduction in oxidative stress, which appears to play a major role in the sympathetic

activation of the central nervous system, was demonstrated (27). Regular exercise can strengthen the antioxidant defense and may reduce oxidative stress (28). In this study, the total antioxidant capacity of soccer players was determined to be significantly higher than that of the men in the control group. Total antioxidant status of male soccer players decreased after exercise. On the other hand, the baseline levels of total oxidative status and oxidative stress indices of male soccer players and the control group exhibited no difference. The decrease in lipid hydroperoxides, increase in total oxidative status, and oxidative stress index of male soccer players were found to be significant after exercise.

Increased reactive oxygen species and decreased antioxidant defense systems may also lead to oxidative stress after exercise; however, this is not reflected in triglycerides levels. It is known that senescent organisms are more susceptible to oxidative stress during exercise because of the age-related ultrastructural and biochemical changes that facilitate the formation of reactive oxygen species. Furthermore, muscle repair and regeneration capacity is reduced at old age, which could potentially enhance the accrual of cellular oxidative damage (14). It is reported that regular exercise increases selected components of the antioxidant system in trained tissue (29-31). The primary defense reactions against reactive oxygen species generation during exercise are superoxide dismutase, catalase, and glutathione peroxidase. These enzymes increase in response to exercise in in vitro and in vivo studies (32-25).

Soccer players have a regular aerobic and anaerobic training status. Gokhan et al. examined plasma malondialdehyde levels, which are markers of lipid peroxidation, and erythrocyte superoxide dismutase activity. They revealed that regular exercise in young male footballers is beneficial in cases of oxidative damage as it reduces the amount of lipid peroxidation and increases the antioxidant enzyme superoxide dismutase activity (36). Marzatico et al. report that oxidant stress can develop during exercise, and raise the antioxidant capacity during higher physical efforts of athletes (37).

The changes in the lipoprotein profile and plasma antioxidant levels were studied by Brites et al. They measured total plasma antioxidant capacity, hydrosoluble antioxidants (ascorbic acid, uric acid, and bilirubin levels), liposoluble antioxidants (α-tocopherol levels), and superoxide dismutase activity. They suggest that the elevation in plasma activities of antioxidant enzymes and the higher levels of free radical scavengers of low molecular mass may compensate the oxidative stress caused by physical activity. Additionally, high levels of high-density lipoprotein in plasma may offer additional protection by inhibiting low-density lipoprotein oxidation and thus liposoluble antioxidant consumption. Therefore, soccer players under regular training show an improved plasma antioxidant status in comparison to sedentary controls (10). Our study determined that serum levels of triglycerides and VLDL-c are significantly lower in male soccer players than in control subjects, and the mean serum levels of total cholesterol, HDL-c, and LDL-c do not change between groups. In addition, the serum levels of triglycerides and VLDL-c decreased in male soccer players through regular exercise.

This study has some limitations. It evaluates oxidative stress as reactive oxygen species in the blood samples including hydroxyl. This species is quite unstable, which implies that it may not be possible to state that reactive oxygen species produced in the skeletal muscles might reach the blood stream. Contracting skeletal muscles are an important site of reactive oxygen species generation. However, no control was described for soccer players to exercise different positions in exercise programs to overcome this problem. The idea that high levels of HDL-C may offer additional antioxidant protection was not supported by the present results. In addition, for technical reasons, the analyses of lipoprotein levels in soccer players after exercise failed.

In conclusion, although the total antioxidant capacity of male soccer players increased in the long term, a decreased total antioxidant capacity and increased total oxidant status and oxidative stress index were observed after the training session. While the antioxidant capacity was high and oxidative state was similar to that of the control group in the long term, oxidation state increased immediately after exercise in soccer players. Although physical activity has many well-established health benefits, strenuous exercise increases muscle oxygen flux and elicits

intracellular events that can lead to increased oxidative injury. Total oxidative status and oxidative stress index increased following training, despite a decrease in lipid hydroperoxides. Hence, the present study reveals the fact that soccer exercise had a beneficial effect on lipid peroxidation levels.

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