EFFECT OF VITAMIN E ON OXIDANT AND ANTIOXIDANT CAPACITY IN FOOTBALL PLAYERS

Nazmi SARITAŞ[1]

Abdulkadir NAKAÇ[2]

Cevat YAZICI[3]

Serdar BÜYÜKİPEKÇİ[1]

Betül COŞKUN[4]

ABSTRACT

In this study,investigating the effect of vitamin E on oxidant and antioxidant capacity in football players was aimed. A total of 27 volunteer men composed of 15 trained football players as supplement group and 12 men as control group participated in the study.

Body weight and body mass index parameters were taken to determine physical characteristics. Systolic and diastolic blood pressure, pulse rate and oxygen saturation values were taken to determine the physiological characteristics. Total antioxidant capacity, thiobarbituric acid reactive substances, and creatine kinase and lactate dehydrogenase activities were evaluated.

In the intergroup comparison, while no difference was found in physical and physiological findings between pre- and post-exercise ($P>0.05$), there was a significant difference in pre-exercise pulse rate ($p<0.05$). In intra- and intergroup comparisons, no significant difference was found in pre- and post-exercise TAC, TBARS, CK and LDH values ($p>0.05$).

In conclusion, antioxidant supplementation in accordance with the type, duration and intensity of physical activity may be beneficial for athletes in order to decrease oxidative stress and increase antioxidant capacity. It is thought that supporting exercises with vitamins and the other antioxidant supplements may be effective to decrease oxidant capacity and increase the antioxidant level.

Key Words: Soccer, Oxidative Stress/Antioxidant, Alpha-tocopherol

FUTBOLCULARDA E VITAMINI KULLANIMININ OKSIDAN VE ANTIOKSIDAN KAPASITE ÜZERINE ETKISI

ÖZET

Bu çalışmada E vitamininin futbolcularda oksidan ve antioksidan kapasite üzerine etkisinin araştırılması amaçlandı. Çalışmada, 15 antrenmanlı futbolcudan oluşan deney grubu ve 12 erkekten oluşan kontrol grubu olmak üzere toplamda 27 erkek gönüllü olarak yer aldı.

Fiziksel özelliklerin belirlenmesi için vücut ağırlığı ve vücut kitle indeksi parametrelerine bakıldı. Fizyolojik özelliklerin belirlenmesi için sistolik ve diastolik kan basıncı, kalp atım hızı ve oksijen saturasyonu değerlerine bakıldı. Total antioksidan kapasite, tiyobarbitürik asit-reaktif substans, kreatin kinaz ve laktat dehidrojenaz aktiviteleri değerlendirildi. Gruplar arası karşılaştırmada, egzersiz öncesi ve sonrası fiziksel ve fizyolojik bulgularda herhangi bir farklılık bulunmazken ($P>0.05$), egzersiz öncesi kalp atım hızında anlamlı bir farklılık bulundu ($p<0.05$). Grup içi ve gruplar arası karşılaştırmalarda ise, egzersiz öncesi ve sonrası TAC, TBARS, CK ve LDH değerlerinde anlamlı bir farklılık bulunmadı ($p>0.05$).

Sonuç olarak, antioksidan takviye sporcularda fiziksel aktivitenin tipi, süresi ve şiddetine göre oksidatif stresi azaltmak ve antioksidan kapasiteyi artırmak için faydalı olabilir. Egzersizin vitamin ve diğer antioksidan takviyelerle desteklenmesinin, oksidan kapasiteyi azaltma ve antioksidan seviyeyi yükseltmede etkili olabileceği düşünülmektedir.

Anahtar kelimeler: Futbol, Oksidatif stress / Antioksidan, Alfa-tokoferol

[1] Erciyes University, Physical Education and Sports College, Kayseri, Turkey
[2] Provincial Directorate of Youth Services and Sports-Tokat, Turkey
[3] Department of Biochemistry, School of Medicine, Erciyes University, Kayseri, Turkey
[4] Faculty of Education, Department of Physical Education and Sports, METU, Ankara-Turkey

INTRODUCTION

Exercise raises oxygen consumption and causes a derangement in the pro-oxidant-antioxidant balance. Because athletes undergo acute and chronic stress which may cause an increase in generation of oxidative species, oxidative stress increases in them. The oxidative stress-induced cell damage may be decreased by taking an antioxidant like alpha-tocopherol (Patil et al., 2009).

In general, physical activity is known with beneficial effects on the organism, but it causes an increase in the production of reactive oxygen species (ROS) and oxidative stress by exceeding antioxidant defence system. Oxidative damage is related to the features of exercise such as the intensity and time (Muñoz Marín et al., 2010).

Strenuous exercise causes an increase in oxygen consumption in the whole body (10-15 fold) and especially skeletal muscle (up to 100- fold) (Banerjee et al., 2003). Chronic exercise generates physiologic adaptations by developing antioxidant defences and reduces oxidative stress in the rest and following acute exercise (Selamoglu et al., 2000; Ørtenblad et al., 1997). Aerobic and anaerobic training increase the antioxidant enzyme activities, particularly superoxide dismutase (SOD) and glutathione-peroxidase (GPx) (Ørtenblad et al., 1997; Marzatico et al., 1997), so athletes usually improve an up-regulated antioxidant enzyme activity compared to non-trained subjects (Evelson et al., 2002; Robertson et al., 1991). The published data about the effects of training on non-enzymatic antioxidants has not agreed with some studies (Evelson et al., 2002; Brites et al., 1999; Cazzola et al., 2003) but not all (Balakrishnan & Anuradha, 1998) causing an increase in the plasmatic concentrations (Brites et al., 1999; Balakrishnan & Anuradha, 1998), so trained people may show a comprehensive development in antioxidant status and prevent oxidative stress compared to healthy sedentary subjects (Selamoğlu et al., 2000; Cazzola et al., 2003).

Regular exercise may produce both oxidants and antioxidant enzymes, so it may induce oxidative stress and also an increase in antioxidant synthesis because it has variable effects. Although physical activity has several benefits for health, heavy exercise may cause stress (Cooper et al., 2002), such as high performance football causing oxidative cellular damage.

Football training may cause oxidative stress that is an imbalance between RNOS and antioxidant defence system. Football is a popular sport which requires technical, tactical, and physical skills to succeed. It is described as an intermittent exercise which is predominantly aerobic and consists of high-intensity bursts periods (Escobar et al., 2009). Also, if there is an inability in antioxidant system, dietary supplements should be suggested, but there are many studies (Urso & Clarkson, 2003) that have not shown any benefit of antioxidant supplementation (vitamins E, C and Beta carotene) for athletes. However, a few studies related to biological status have demonstrated a low antioxidant vitamin status or a high basal lipoperoxidation index (Marzatico et al., 1997; Balakrishnan & Anuradha, 1998; Schröder et al., 2000). Despite all, it has been shown that there is a better antioxidant status in football and basketball players compared to sedentary subjects (Brites et al., 1999; Pincemail et al., 2000).

The reactive oxygen species occur in consequence of exercise which exceeds antioxidant defence system. Several endogenous antioxidants are determined in adult athletes, but there is not much data about antioxidant defence in adolescents (Carlsohn et al., 2008).

The aim of the present study is to compare intake of alpha tocopherol, total

antioxidant capacity, lipid peroxidation, and muscle damage and muscle damage indicators such as CK and LDH activities.

MATERIALS AND METHODS

Subjects

A total of 27 trained young football players from Kayseri Demirspor club supplement group (n=15) and control group (n=12) participated in the present study. Football players were randomly divided into two groups, supplemented and control. Participants in the supplement group received 400 IU (268 mg) α-tocopherol one time a day over 30 days during the investigation. Control group did not receive any supplement.

Both supplement group and control group participated in the same training programs of 90 minute per day, four days per week during one month. The training program consisted of mix of aerobic, endurance –type training. The training units consisted in a general warm up and stretching (about 15 min), a technical–tactical part (about 30 min), a heavy training load part including training of counterattacks and simulated full or half-court football games (about 40 min), and finally a cool down phase (about 15 min).

The players' anthropometric characteristics were measured and are presented in Table 1. No subject was vegetarian or a smoker, nor did they use tobacco products, anti-inflammatory drugs, or antioxidant supplements before (for a minimum of 3 months).

Informed consent was obtained from all subjects and their family before the study. The study protocol and the procedures were approved by the Ethics Committee at the Medical Resarch Centre, Erciyes University. The study was conducted in accordance with the Declaration of Helsinki or local laws depending on whichever afforded greater protection to the subjects.

Anthropometric measures were taken standing height (cm) was measured with precision 0.1 cm using a stadiometer. Body mass (kg) with minimal clothing, no shoes, was recorded with a scale (Tanita) to nearest 0.01 kg. BMI was calculated according to the Formula body mass (kg) divided by height squared (m^2). Blood pressure, both systolic and diastolic, was determined with an electronic (Microlife) instrument, with which heart rate (beats/min) was simultaneously measured.

Blood Sample

Venous blood from each participant was collected from the antecubital vein using venous puncture with subjects in a sitting position before the first supplementation and at the end of the study. All sampling procedures were performed at the same time, place and in the same conditions. Blood samples for analysis were immediately centrifuged at 3000 rpm 15 min at room temperature. All serum samples were then stored in micro tubes at -80 ^{o}C until analyses

Measurements

Determination of TBARS levels

Serum TBARS was measured using ELİSA Kit (Catalogue; 10009055, Cayman Chemical, and Ann Arbor, USA) Serum TBARS levels were expressed in µM. Calibration, curve fitting and data analysis was done according to the instructions of the manufacturer.

Determination of plasma total antioxidant capacity

The total antioxidant capacity (TAC) of the serum was measured using a commercially available assay kit from Cayman Chemical Co (catalog no: 709001). Analysis was done in a micro titer plate.

Determination of plasma CK and LDH activities

Also the activities of CK and LDH were measured from the serum samples by using an auto analyzer and the commercial kits (Siemens Advia 1800 Chemistry System) in the biochemistry laboratory of university hospital.

Statistical analysis

Statistical analysis was conducted using SPSS version 13.0 for Windows. The data were tested for normal distribution with the Shapiro-Wilk test and for homogeneity variances with Levene's test. Biochemical data were analyzed by a two factor repeated measures analysis of variance (ANOVA) followed by bonferroni's post hoc intra group comparisons. All data were reported as means±standard error of mean (SEM). 0.05 was accepted as significant.

RESULTS

Table 1. Physical and physiological characteristics of subjects

Variable	Training+Supplement Group (n=15)		Training+ Control Group (n=12)		F Values		
	Pre-training	Post-training	Pre-training	Post-training	Time	Time x Group	Group
	Mean ± SEM	Mean ± SEM	Mean ± SEM	Mean ± SEM			
Age (years)	16.20 ± 0.46		15.58 ± 0.42				
Height (cm)	172.47 ± 1.28		168.08± 1.90		0.77	0.77	4.36‡
Body mass (kg)	60.10 ± 1.64	60.31 ± 1.58	58.46 ± 1.72	59.09 ± 1.66	7.58*	1.87	0.37
BMI (kg/m^2)	20.09 ± 0.46	19.58 ± 0.37	20.68 ± 0.54	20.46 ± 0.59	2.32	0.34	1.32
RSBP (mmHg)	121.07 ± 3.03	120.27 ± 3.04	122.08± 3.79	126.92 ± 3.44	0.69	0.26	0.91
RDBP (mmHg)	74.27 ± 2.43	81.47 ± 6.69	75.25 ± 3.65	76.42 ± 2.48	1.44	0.75	0.15
O_2 Saturation (%)	97.60 ± 0.32	97.13 ± 0.38	97.92 ± 0.36	97.08 ± 0.26	7.53*	0.60	0.10
RHR (beats/min)	65.07 ± 1.65#	68.87 ± 2.69	77.08 ±4.21#	74.58 ± 2.15	0.11	2.51	7.04‡

* Significant difference at the level of 0.05 as a result of repeated measures analysis of variance.
#Significant difference in groups pre-and post-training.
‡Significant difference between two groups pre-training.
RSBP: Resting systolic blood pressure, RDBP: Resting diastolic blood pressure, RHR: Resting heart rate, BMI: Body mass index

There was no significant difference in pre- and post-training levels of body mass, body mass index, resting systolic blood pressure, resting diastolic blood pressure, oxygen saturation and resting heart rate. In addition, the time- related changes of body mass (F= 7.58) and oxygen saturation (F=7.53) levels of supplement and control groups were significant. Also, RHR values were significantly difference in intergroup comparison (F=7.04; p<0.05). While post-training resting heart rate increased in supplement group, it decreased in control group (p<0.05).

Table 2: The level of muscle damage, oxidant and total antioxidant capacity of the groups

Variable	Training+Supplement Group (n=15)		Training+ Control Group (n=12)		F Values		
	Pre-training	Post-training	Pre-training	Post-training	Time	Time x Group	Group
	Mean ± SEM	Mean ± SEM	Mean± SEM	Mean± SEM			
TAC (mmol/L)	1.17±0.22	1.33± 0.26	1.50± 0.21	0.99± 0.44	0.29	1.11	0.01
TBARS (µmol/L)	0.11 ± 0.02	0.10 ± 0.01	0.09± 0.01	0.09± 0.01	0.01	0.01	0.87
CK (U/L)	247.53 ± 38.58	238.60 ± 37.74	262.08±51.40	206.67±22.96	1.11	0.58	0.04
LDH (U/L)	168.67 ± 14.32	172.80 ±9.08	183.67±13.35	189.50±10.00	0.34	0.01	1.15

No significant difference was observed in TAC (F=1.11) and TBARS (F=0.01) levels, and CK (F=0.58) and LDH (F=0.01) activities pre- and post-training program (p>0.05).

DISCUSSIONS

In this study, pre- and post-training resting blood pressure values of supplement and control group were not significantly different. In a study researching the effects of regular consumption of a flavanol-containing milk chocolate on vascular disease, oxidative stress and physical activity in young football players, whereas no significant difference was found in systolic blood pressure, there was a significant decrease in diastolic blood pressure pre- and post-supplementation (Fraga et al., 2005).

Pre- and post-training oxygen saturation values of supplement and control group showed a significant difference in the course of time. In a study carried out in order to analyze the effects of preseason regular exercise on blood antioxidant defence capacity and aerobic performance of the football players, preseason exercise program produced only a small increase in maxVO_2 values of both teams (Michalczyk et al., 2008). In another study examining how exercise intensity affects lymphocyte antioxidant response and cellular oxidative damage in football players, no significant difference was found in maxVO_2 values of the groups doing low-, moderate- and high-intensity exercise (Sureda et al., 2007). In another study in which the effects of vitamin C on blood antioxidants and oxidative stress parameters were investigated in basketball players following maximal exercise, 21-day vitamin C supplementation produced no significant effect on maxVO_2 (Cholewa et al., 2008).

There was a significant difference in RHR value in intergroup comparison. In another study in which the effects of two energy drinks on blood lactate level and maximal cardiorespiratory fitness were investigated in male athletes, no significant difference was found in heart rate between pre- and post- tests for both drinks (Rahmana et al., 2010).

In the study which was made by Kostaropoulos et al. to compare the blood redox status between long- and short-distance runners, any significant deference was not found in TAC between the groups (Kostaropoulos et al., 2006).

There was no significant difference in pre- and post- training TAC values. In a study investigating whether antioxidant supplement reduces post-exercise oxidative stress in young overweight adults, whereas no significant difference was found in TAC levels of normal-weight and overweight groups at rest, a statistically insignificant increase was found in TAC level of overweight antioxidant group during the 8-week exercise period (Vincent et al., 2006). In another study which was carried out in football players participating in regular exercise program in order to observe the lipoprotein profile and plasma antioxidant capacity, plasma total antioxidant capacity was found 25% higher in athletes than in control group (Brites et al., 1999). In another study in which the effects of a competitive soccer match on plasma levels of oxidative stress and muscle damage markers were investigated, plasma TAC level increased significantly 30 min after the game (Ascensao et al., 2008).

TBARS levels of supplement and control groups showed no significant difference pre- and post-training program. In a study in which the effects of vitamin C on blood antioxidants and oxidative stress parameters were investigated in basketball players following the maximal exercise, there was no significant effect of 21-day vitamin C supplementation on MDA level (Cholewa et al., 2008). In another study in which the effects of vitamin and mineral supplementation including vitamin C, E and beta carotene were observed pre-event and during an extreme running competition, whereas there was no significant difference in

TBARS activities of experimental- and control-group at the beginning of the study, TBARS level of supplement group remained stable during the competition, TBARS activity of placebo group increased significantly on the third day of the competition. TBARS level was found significantly lower in supplement group than in placebo group on the third day (Machefer et al., 2007). In another study observing the effects of 3-month antioxidant supplementation including coenzyme Q-10 on oxidative stress and antioxidant response after a 60-minute soccer match, plasma MDA level was significantly higher in placebo group than in training group after the match (Tauler et al., 2008). In another study investigating how the exercise intensity affects lymphocyte antioxidant response and cellular oxidative damage, a significant increase was found in MDA activity in the high-intensity group in comparison with the low- and medium-intensity groups. There was a significant increase in post-exercise ROS production in just high-intensity group. While this increase was comparing to the post-exercise values of low-intensity group, there was a significant difference (Sureda et al., 2009). In another study carried out in soccer players in premier league and 4. League teams to observe the effects of preseason regular exercise on antioxidant defence capacity and aerobic performance, there was a significant difference in MDA levels between the teams at the end of the preseason exercise program. Plasma MDA activity was found higher in the 4. League team (Michalczyk et al., 2008). In another study observing the effects of vitamin C and E in professional soccer players, plasma TBARS concentration was found 66% higher in placebo group than in supplement group (Zoppi et al., 2006).

In this study, there was no significant difference in pre- and post-exercise CK and LDH values of supplement and control groups. In another study carried out in young soccer players to investigate the effects of regular consumption of a flavanol-rich chocolate on vascular disease, oxidative stress and physical activity, a significant difference was found in LDH activity pre-and post-supplementation; however, no significant difference was found in CK (Fraga et al., 2005). In another study which was made on 25 college rugby players in order to determine the effects of training on physical condition, LDH and CK levels increased significantly during the 20-day camp (Mashiko et al, 2004). In another study in which the effects of a soccer match on plasma levels of oxidative stress and muscle damage markers were observed, plasma CK level showed a significant increase 72 hours later the match in the recovery period compared to the previous values (Ascensao et al., 2008). Ehlers et al. investigated the level of blood serum creatine kinase in football players and they took CK measurements 4 times over 10 days of preseason. The measurements were taken before beginning practices and on the mornings of the 4th, 7th, and 10th days. The measurements of 4th and 7th days were found higher than the first measurement (Ehlers et al., 2002). In the study observing the effects of vitamin C and E in professional soccer players, plasma CK activity was found 50% higher in placebo group than in supplement group (Zoppi et al., 2006).

In the study in which the effects of vitamin and mineral supplementation including vitamin C, E and beta carotene were observed pre-event and during an extreme running competition, plasma CK and LDH activities significantly increased on the third day of the competition and they were found higher at the end of the competition compared with their pre-race values (Machefer et al., 2007). Like this, in the study which was made on 23 elite male rugby players, there was a significant

increase (p<0, 01) in interstitial creatine kinase concentration between pre- and post-competition (Gill & Beaven, 2006).

In conclusion, antioxidant supplementation in accordance with the type, duration and intensity of physical activity may be beneficial for athletes in order to decrease oxidative stress and increase antioxidant capacity. It is thought that supporting exercises with vitamins and the other antioxidant supplements may be effective to decrease oxidant capacity and increase the antioxidant level.

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