Biology of Sport, Vol. 22 N°4, 2005

EFFECT OF A SINGLE DOSE OF VITAMIN E ON THE LEVEL OF OXIDATIVE DAMAGE MARKERS IN BLOOD OF THE SOCCER PLAYERS

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Abstract. The purpose of the study was to explain whether application of a single dose of α-tocopherol could reduce oxidative damage in proteins and lipids in blood of the soccer players who performed a submaximal physical exercise as well as whether there is a relationship between muscle damage and oxidative stress. The research was carried out on 22 students of the coaching studies who participated in a sparring soccer match. The athletes were divided into two groups: control (cod-liver oil) and a group supplemented with vitamin E (800 mg) 2 h before the exercise. In the blood plasma levels carbonyl groups, lipid peroxidation products (TBARS), β-glucuronidase (βG) and creatine kinase (CK) were estimated. The obtained data showed significant ($p<0.05$) increase in carbonyl groups concentration after the second half of the game; 30 min later it returned to the pre- exercise values. The TBARS concentration increased after the first and second halves of the game and maintained the high level till 30 min of rest. After 24 h rest the TBARS concentration in the supplemented group was significantly lower in comparison with the control group. The βG activity increased ($p<0.05$) after the second half of the game, whereas, the highest activity of the CK after 24 h rest was observed. Between the β-glucuronidase and creatine activities a positive correlation was recognised: control group $r=0.477$ ($p<0.001$), supplemented group $r=0.671$ ($p<0.001$). However, between the carbonyl groups and the TBARS concentrations and the activity of tested enzymes no relationship was recognized. The obtained results allow to conclude that protein and lipid oxidative damage facilitate the release of cellular enzymes from muscles and supplementation of a single dose of vitamin E does not prevent the process.

(Biol.Sport 22:363-374, 2005)

Key words: Exercise - Oxidative damages - Vitamin E - Soccer players

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Introduction

Oxidative stress has been defined as a state in which exposure on reactive oxygen species (ROS) or other oxidants results in structural damage in cells - oxidative damage [10]. The oxidative stress markers which are widely used include:

- lipid peroxidation products related to the ROS attack on chains of non-saturated fatty acids
- carbonyl groups formed due to fragmentation of α-amide polypeptide protein chain as well as during the direct ROS oxidation of lysine, arginine, proline and treonine [15].

According to some authors the protein damage appear independently and come first before lipid damage and make a more sensitive oxidative stress marker than lipid peroxidation [2,3,13]. However, during oxidative stress at high intensity both processes induce each other. Protein oxidation products7 (thiyl radicals) cause lipid peroxidation and lipid peroxidation products (aldehydes, alkenales) modify proteins. That causes cell damage and increase of intercellular enzymes activity in the plasma, e.g. β-glucuronidase (βG) and creatine kinase (CK) [11,27].

Numerous studies show that during a physical exercise at maximal intensity and at endurance exercise the ROS generation and cell oxidative damage appeared. This is why athletes were recommended to take higher amounts of antioxidants. The most often used was α-tocopherol (vitamin E) which athletes were supplemented with in amounts of 200 – 1200 mg/day within a week up to 7 weeks or as a single dose to 2400 mg. Such supplementary – ergogenic procedure brought a positive effect. That is, in the blood and muscle a decrease in lipid peroxidation products (malonyldialdehyde, lipid peroxides, conjugated dienes) concentration, a decrease in nucleic acid damage, creatine kinase activity and concentration of cytokines were recognised [14,19,25,29,18]. However, some investigation did not prove a positive effect of vitamin E supplementation in spite of its concentration increase in blood and muscles [8,31,23]. These authors suggested that the ROS generation during an exercise is not a main muscle damaging factor but it can elevate the cells susceptibility to mechanical damages. The reason for it is an increase in membrane rigidity and decrease in cellular deformability due to lipid and protein peroxidation induced by the ROS.

The purpose of the study was to explain whether a soccer game would cause proteins and lipids oxidative damage, lipid peroxidation would make cellular enzymes release and a single dose of vitamin E would prevent the process.

Materials and Methods

The study was carried out on 22 students of the coaching studies (most of whom were active soccer players), who took part in a sparring soccer match. The players of both groups entered and left the soccer field in pairs within the space of 3 min. Each player participated in the whole game (2 x 45 min).

Characteristics of the soccer players are presented in Table 1.

Table 1
Characteristics of the soccer players under study (mean ± SD)

Age (years)	Body mass (kg)	Height (cm)	Period of training (years)
22.1±4.1	73.3±6.3	178.5±5.7	11.0±3.8

The soccer players were divided into two groups:
- control group – placebo (cod-liver oil gelatine capsules)
- supplemented group - vitamin E (800 mg of α-tocopherol acetate in gelatine capsules, 2 h before exercise).

All the athletes were informed about the aim of the studies and gave their consent for taking blood samples. The studies obtained the agreement of the Local Bioethical Committee in Gorzów Wlkp.

Blood samples were collected from cubital vein with an anticoagulant ($EDTAK_2$) before the exercise, after the first and second halves of the game, after 30 min and 24 h rest. Plasma was obtained by centrifugation at 2500 x g for 10 min, then frozen and stored at -20^oC. All samples were analysed within 7 days. In the plasma the thiobarbituric acid – reactive substances (TBARS) concentration was estimation according to the procedure described by Buege and Aust [7], carbonyl groups concentration by the method of Levine *et al.* [21], β-glucuronidase activity (βG, EC 3.2.1.31) using Sigma kit (USA) and creatine kinase activity (CK, EC 2.7.3.2) using HTL kit (Poland). The protein concentration was estimated by the Bradford's method [5].

The lactate (LA) concentration in the whole blood was assessed using Dr Lange kit (Germany).

Statistical analysis was carried out using Statistica program. Data were tested by two – way ANOVA to determine exercise or α-tocopherol related effects. Between the tested parameters Pearson correlation coefficient was analysed. The accepted level of significance was $p<0.05$.

Results

The data illustrated in Table 2 and Figs 1 - 2 show that the participation in the sparring soccer meeting at submaximal intensity (LA-E1 placebo group 3.82±1.95 $mmol \cdot l^{-1}$, LA-E1 vitamin E group 3.06±1.14 $mmol \cdot l^{-1}$) resulted in statistically significant changes in the tested parameters.

Table 2
Changes in the lipid peroxidation products (TBARS), carbonyl groups (carbonyls) concentrations and the β-glucuronidase (βG), creatine kinase (CK) activities in plasma of the soccer players. Placebo - control group, vitamin E - supplemented group (mean ± SD)

Placebo	P	E 1	E 2	R30	R24
TBARS $nmol \cdot ml^{-1}$	0.72±0.41	2.09±0.31*	2.72±0.31*	1.83±0.61*	0.99±0.40
Carbonyls $nmol \cdot mg^{-1}$	1.376±0.205	1.277±0.127	1.622±0.295*	1.386±0.274	1.703±0.515
βG U/ml^{-1}	19.52±4.55	20.86±4.27	26.11±2.41*	26.43±7.24*	22.87±6.22
CK $U \cdot l^{-1}$	170±65	167±71	228±87	275±101*	275±92*

Vitamin E	P	E 1	E 2	R30	R24
TBARS $nmol \cdot ml^{-1}$	0.88±0.38	1.84±0.38*	2.57±0.60*	1.67±0.21*	0.50±0.22
Carbonyls $nmol \cdot mg^{-1}$	1.492±0.254	1.530±0.348	1.745±0.239*	1.281±0.205	1.716±0.327
βG $U \cdot ml^{-1}$	19.67±4.67	20.89±5.29	27.49±7.68*	25.11±8.24*	22.93±8.01
CK $U \cdot l^{-1}$	154±106	197±125	263±128	271±100*	330±95*

*indicates significant ($p < 0.05$) differences from the pre – exercise value;
P- pre – exercise; E1 – after a 45 min exercise, E2 – after a 90 min exercise, R30- 30 min rest, R24- 24 h rest.

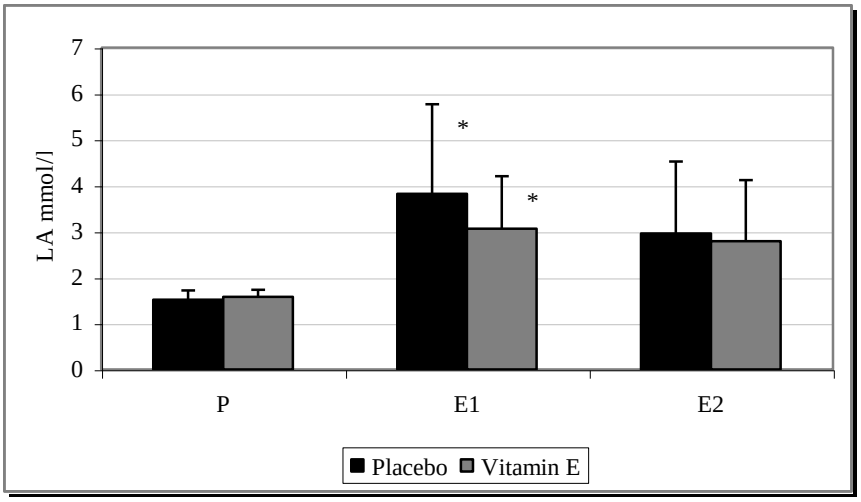

Fig. 1
Changes in blood lactate concentration (LA) in the soccer players
P-pre-exercise; E1-after a 45 min exercise; E2-after a 90 min exercise

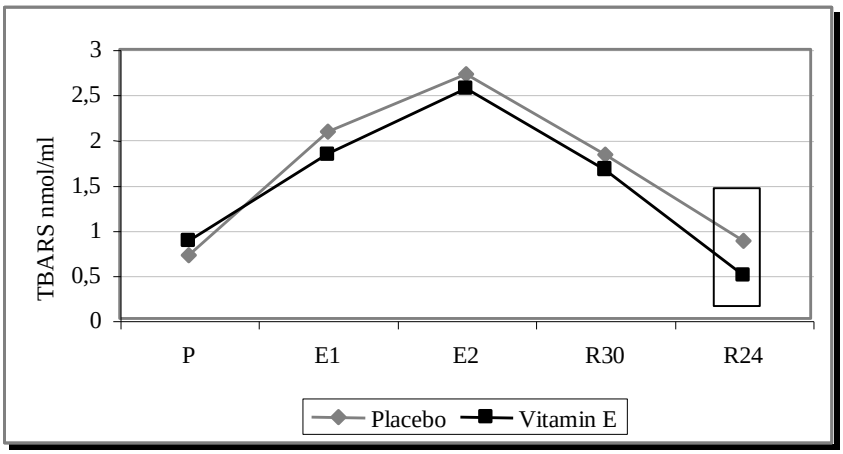

Fig. 2
Changes of lipid peroxidation products (TBARS) concentration in plasma of the soccer players
P-pre-exercise; E1-after a 45 min exercise; E2-after a 90 min exercise; R30-30 min rest; R24-24 h rest; □-indicates significant ($p<0.05$) differences between the placebo and vitamin E groups

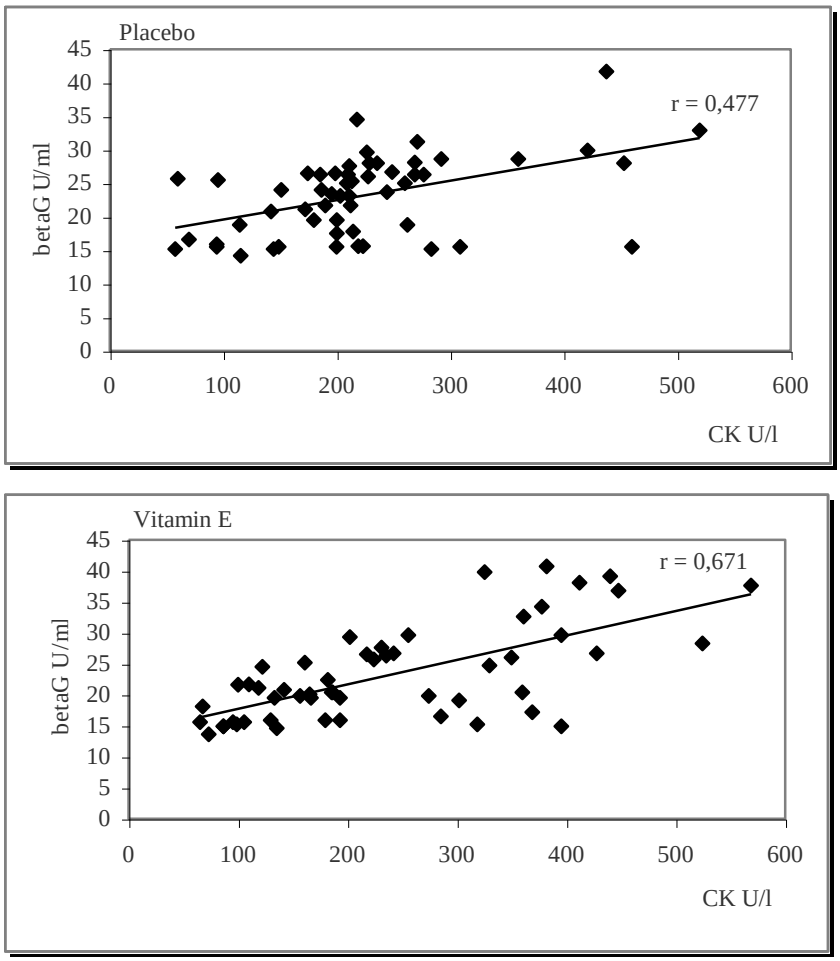

Fig. 3
Relationship between creatine kinase (CK) and β-glucuronidase (βG) activities in placebo group and vitamin E group

The plasma TBARS concentration increased significantly after the first and second halves of the game and after 30 min rest an attenuation of peroxidation process was observed. After 24 h rest the TBARS concentration returned to the pre - exercise values. In the vitamin E group after 24 h rest the TBARS concentration was significantly lower compared with the placebo. Between the LA and TBARS

concentrations positive correlations in placebo group r=0.467 ($p<0.001$) and vitamin E group r=0.361 ($p<0.001$) were recognised.

The carbonyl groups concentration increased significantly after the second half of the game, and after 30 min rest returned to the initial values in both groups. No significant differences were observed between the vitamin E and placebo groups.

The βG activity increased by about 40% ($p<0.05$) after the second half of the game in both groups. After 24 h rest the βG decreased to the pre – exercise value, whereas, the CK activity reached the highest activity. Between the β-glucuronidase and creatine kinase activities positive correlations in placebo group r=0.477 ($p<0.001$) and vitamin E group r=0.671 ($p<0.001$) were recognised (Fig. 3). No effect of α-tocopherol application on the βG and CK activities was observed.

Discussion

A soccer game is compared to a long-distance run at variable speed (run, walk, sprint) so it is an aerobic – anaerobic exercise with the predominance of aerobic processes [26]. In such conditions the respiratory chain and the oxidative phosphorylation processes are the main source of the ROS. The second – rate sources are xanthine oxidase reactions related to temporary hypoxemia and reperfusion periods, autooxidation reactions of respiratory proteins and catecholamines and oxidase NADPH reactions in activated phagocytes responsible for removing parts of a damaged tissue.

In the present study a slight increase in lactate (LA) concentration in the soccer players' blood was observed, what indicates the predominance of aerobic processes.

After the first half of the game the LA concentration did not exceed 4 $mmol \cdot l^{-1}$ and after the second half it decreased <3 $mmol \cdot l^{-1}$. These values are considerably lower than those observed during a soccer game played in standard conditions. Quanz [26] reported the LA concentration during a game can rise up to 9.5 $mmol \cdot l^{-1}$

Despite a slight increase in the LA concentration during a simulated soccer game, lipid peroxidation and protein damage were intensified. According to Sen [28] skeletal muscles possess high oxidative capacity unbalanced by antioxidative capacity, which makes the tissue susceptible to oxidative damages. For this reason the observed increase in the TBARS and carbonyl groups concentration can have resulted from oxidative damage of active skeletal muscles and the release of peroxidation products into the blood. The elevation of peroxidation lipid products level appeared with an increase in blood lactate concentration. Between the LA and

the TBARS concentration positive correlation was recognised in both groups - control and supplemented with vitamin E.

The comparison of exercise intensity with peroxidation intensity had been already carried out by Lovlin *et al.* [22] and Kretzschmar *et al.* [20], but the results were discrepant. In case of the first ones, the authors obtained a positive correlation between the plasma LA and malonyldialdehyde concentration (r=0.51) in the subjects performing an incremental exercise to exhaustion. In the second study the authors obtained a negative correlation (r=-0.69) between the LA and lipid peroxides concentration in the blood of marathon runners. Furthermore, Vina *et al.* [30] claimed that the degree of oxidative stress and muscle damage are not determined by exercise intensity but the degree of exhaustion of the subject who performs an exercise. Training and treatment with antioxidants such as vitamin E partially protect against oxidative damage [1,30].

A single dose of vitamin E did not cause expected results in the soccer players, such as a considerable attenuation of lipid peroxidation and protein damages. In both groups of the players changes in the TBARS and carbonyl groups concentrations were similar, except from significantly lower concentration of the TBARS in the supplemented group 24 h after completing the game.

There were probably a few reasons for the absence of a positive effect of vitamin E supplementation in the soccer players. Firstly, low exercise loading of the players during the game resulted in oxidative stress but its intensity was low. Besides, the intra- and extra-cellular antioxidants reduced the ROS fast and effectively. Therefore 30 min after the game a decrease in the TBARS and carbonyl groups concentrations was recognised.

Secondly, a high level of endogenous antioxidative defence in blood might protect the subjected players. It was supported by Brite *et al.* [6] who compared the plasma antioxidant status in group of the soccer players training regularly with a group of non-training subjects. The authors observed an increased concentration of such hydrophilic antioxidants as ascorbic and uric acids and α-tocopherol as well as an increased activity of superoxide dismutase in the players. Previously a similar comparative investigation was carried out by Balakrishnan *et al.* [4] who recognised a high level of ascorbic acid, ceruloplasmin, superoxide dismutase and glutathione peroxidase activities in the blood of athletes including soccer players. A higher resting activity of superoxide dismutase in soccer players' blood in comparison with non-training subjects was also recognised by Woźniak *et al.* [32].
Another reason for the absence of influence of vitamin E supplementation on the level of tested parameters might have been a release of α-tocopherol from fat tissue (adipocytes) due to lypolysis induced by exercise. The α-tocopherol concentration

increase in blood can have speeded up reduction of the ROS and probably prevented the tissue damage [17].

In exercise tests the measurement of plasma creatine kinase activity (CK) is regarded as a marker of cellular damage and the measurement of plasma β-glucuronidase activity (βG) is a marker of an increase in lysosome membranes permeability – cellular organelles responsible for removing fragments of damaged tissues [9,16]. The enzyme βG can be released from some other cells than muscle ones such as activated phagocytes (e.g. neutrophiles) migrating to a damaged places.

In plasma blood of the subjected players a positive correlation between the CK and βG activities was observed. It seems that both enzymes were released from the same tissue, that is, skeletal muscles. What is more, during active phases of the game a contact with another player causes muscle microdamages and emerging the enzymes from cells.

Because of the carried out physical exercise the CK and βG activities increased in blood of the soccer players. The βG activity increased after the second half and the CK activity 30 min after completing the game. The increase in the TBARS and carbonyl groups concentrations appeared at the same time, and in case of the TBARS it even came first before the CK and βG activities increase. It suggests oxidative damage of lipids and membrane proteins might have facilitated the release of these enzymes from muscle cells.

The observed changes in creatine kinase and β-glucuronidase activities as well as in concentration of lipid peroxidation products are comparable to the results of Child *et al.* [9]. These authors did not recognise any correlation between the enzymes (CK, βG) activities and peroxidation. However, it was stressed that appearing of a larger number of the CK and βG molecules in blood was the result of an increase in lysosome and sarkolemma permeabilty induced by the ROS during the exercise. A positive correlation between the CK activity and the TBARS concentration was observed by Drewa *et al.* [12] in weight-lifters performing 4 h training.

No effect of a single vitamin E supplementation on plasma creatine kinase and β-glucuronidase activities was recognised, just as in case of the TBARS and carbonyl groups concentrations. It is opposite to the investigation where a many weeks' vitamin E treatment was applied. For example, Itoh *et al.* [18] noticed that 4-week vitamin E supplementation in amount of 900 IU/day prevented the release of cellular enzymes and diminished lipid peroxidation during training. McBride *et al.* [24] recognised a reduce in the CK activity and malonyldialdehyde concentration in the plasma of the athletes received 900 IU/ day of vitamin E for a period of two weeks.

In summary, the simulated soccer game resulted in oxidative stress which was proved by the recognised increase of lipid peroxidation products (TBARS) and carbonyl groups concentrations. The intensification of lipid peroxidation enabled muscles damage and release of cellular enzymes. A single vitamin E supplementation did not affect the changes in the tested parameters, which probably related to low intensity of oxidative stress and effective endogenous antioxidative defence.

Conclusion

A single α-tocopherol application in soccer players does not attenuate oxidative stress intensity and does not prevent muscles damage, so it seems unnecessary to supplement the athletes performing a submaximal exercise with antioxidants.

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Accepted for publication 29.09.2003