THE EFFECT OF USING VITAMIN E ON MUSCLE DAMAGE, OXIDANT AND ANTIOXIDANT LEVELS OF RUNNERS PERFORMING ENDURANCE TRAINING

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ABSTRACT

This study was made to research the effect of vitamin E on muscle damage, oxidant and antioxidant levels of runners. 28 students participated in the study as the experiment group at the age of 20,75±1,84 (n=16) and the control group (n=12) at the age of 20,42±1,78 who are physically active, have the similar physical features and education at Erciyes University. In this study, the same exercises were performed on both groups three times a week for thirty days. 400 IU (268 mg) vitamin E was given to the athletes in experiment group in every day for 30 days. Nothing was given to the control group. The body weights and the percentage of body fat were measured at the pre and post one month exercise. Total antioxidant capacity, malondialdehyde, creatine kinase and lactate dehydrogenase were measured by making biochemical analyses. While any significant difference on pre-exercise CK and LDH levels, pre and post exercise TAC and MDA levels of experiment and control groups was not found, there was a significant difference on post exercise CK and LDH levels as a result of the intergroup comparison. As for the intragroup comparison, there was a significant increase on post exercise MDA level of both experiment and control group. This result was thought that the antioxidant capacity remains incapable; despite usage of vitamin E counter to oxidative stress increasing connectedly exercise. The reason of this inability in the antioxidant defense system is thought that this is because of the amount of daily vitamin E.

Key words: Oxidative stress, ergogenic aid, alpha tocopheral, lactate dehydrogenase, creatine kinase.

DAYANIKLILIK ANTRENMANI YAPAN ATLETLERDE E VİTAMİNİ KULLANIMININ OKSİDAN VE ANTİOKSİDAN KAPASİTESİ VE KAS HASARI ÜZERİNE ETKİSİ

ÖZET

Bu çalışma, atletlerde E vitamini kullanımının kas hasarı, oksidan ve antioksidan kapasite üzerine etkisinin araştırılması amacıyla yapılmıştır.
Çalışmaya Erciyes Üniversitesi Beden Eğitimi ve Spor Yüksekokulunda okuyan aktif olarak spor yapan, benzer fiziksel özelliklere sahip, yaşları 20,75±1,84 olan (n=16) deney grubu ve yaşları 20,42±1,78 olan (n=12) kontrol grubu olmak üzere 28 kişi katıldı. Çalışmada her iki gruba da aynı egzersizler haftada 3 gün olmak üzere 30 gün süreyle uygulandı. Deney grubundaki atletlere 30 gün boyunca her gün 400 IU (268mg) E vitamini verildi. Kontrol grubundaki atletlere herhangi bir şey verilmedi. Bir aylık antrenman öncesi ve sonrası atletlerin vücut ağırlığı ve vücut yağ yüzdesi değerleri ölçüldü. Biyokimyasal analizler ile total antioksidan kapasite, malondialdehit, kreatin kinaz ve laktat dehidrojenaz parametrelerine bakıldı. Deney ve kontrol gruplarının gruplar arası karşılaştırmaları sonucunda egzersiz öncesi CK ve LDH değerlerinde, egzersiz öncesi ve sonrası TAC ve MDA değerlerinde anlamlı farklılık bulunamazken ($p>0,05$), egzersiz sonrası CK ve LDH değerlerinde anlamlı farklılık meydana geldi ($p\leq0.05$) Grup içi karşılaştırmada ise, hem deney grubunun hem de kontrol grubunun egzersiz sonrası MDA değerinde anlamlı derecede artış saptanmıştır. Bu sonuç, egzersize bağlı olarak artan oksidatif strese karşı, E vitamini kullanımına rağmen antioksidan kapasitenin yetersiz kaldığını düşündürmektedir. Antioksidan savunma sistemindeki bu yetersizliğin nedeni olarak günlük alınan E vitamini miktarı düşünülmektedir.

Anahtar Kelimeler: Oksidatif stres, ergojenik yardım, alfa tokoferol, laktat dehidrojenaz, kreatin kinaz.

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INTRODUCTION

Atoms or molecules which have one or more unpaired electrons are called free radicals. The imbalance between the increase of free radicals and antioxidant defense system is called oxidative stress (12). The level of free radical is balanced in low-stress conditions. However, it increases excessively in high-stress conditions such as radiation, eccentric exercise and illness (15).

Exercise raises the production of free radicals resulting in lipid peroxidation and oxidative stress (11). The rate of damage varies depending on the exercise intensity, the training status and the tissue. Antioxidant supplementation can decrease the level of oxidative damage, but it does not increase the aerobic capacity or the maximal exercise capacity. In addition, the effects of antioxidants on endurance are not clear. Although physical exercise has many positive effects, it increases the production of free radicals in muscle, liver, blood and some tissues and causes the oxidative stress (5).

There are some complex internal protective mechanisms like enzymatic defenses including SOD, catalase, glutathione peroxidase, and non-enzymatic defenses such as vitamin C, vitamin E, ubiquinol co-enzyme Q-10 and reduced glutathione against the detrimental effects of free radicals. These protective mechanisms generate the antioxidant defense system (13). Antioxidants are the substances which decrease or prevent oxidative effects. Vitamin E has a strong influence and it is the main antioxidant substance which has a chain-breaking effect on the cell membrane. The effects of acute and chronic exercise on vitamin E in the skeletal muscles have been studied by various researchers, but there is no study indicating that exercise increases vitamin E activity (24). There are many studies supporting the fact that antioxidants increase during exercise and chronic exercise develops the antioxidant defense system. However, the changes between total antioxidant capacity during a short exercise period and oxidative stress index are not entirely known (8).

Serum CK and LDH are often used to determine the level of muscle damage. High levels of CK and LDH show the exercise-induced skeletal muscle damage. Some studies show that high CK and LDH levels are found 3-7 days after exercise bouts which include eccentric endurance training (9). Also, there are some studies suggesting that CK and LDH levels rise to peak level immediately after sustained running as a marathon race (23).

This study was made in order to observe the effects of vitamin E on muscle damage reflected by creatine kinase, lactate dehydrogenase serum activity and on oxidative stress reflected by thiobarbituric acid reactive substances and total antioxidant capacity.

METHOD

In total, 28 male volunteer students who perform the sports actively between the ages of 18-25 and have the similar physical characteristics participated in this study. The students were selected randomly from the people who are nonsmokers and do not take anything such as vitamin. Before starting the study, an ethics committee approval was received from The Local Ethical Committee of Erciyes University School of Medicine Deanery. After giving information about the study, a certification was taken from each person to take part in the study. Their daily diet was not hold in check, whereas they were warned to avoid nuts and vegetable oil. They were divided into two groups: a control group and a supplement group. Both of the two groups performed the same exercises three times a week for a month. The supplement group; composed of 16 athletes, received 400IU (268mg) vitamin E every day for a month. Nothing was given to the 12 athletes who were in the control group. Volunteers were told to give up any drug at least one week before the beginning of the study. The supplement group took vitamin E

in the custody of the researcher on the training days. On the other days, they took vitamin E compromisingly on a full stomach in the same hour of the days. At the end of the 30 days, some blood samples were taken from both of the groups in the same conditions.

Experimental Design

The measurements of the height was measured by using a height measuring mechanical weighing scale to the nearest 1 mm on a standing position with barefoot on the floor and in the situation that the heels are adjacent and the knees are strained. The body weight of participants was measured to the nearest 100g with minimal clothes. Body mass index (BMI) was calculated by dividing body weight (kg) to square of height (m^2). Biceps, triceps, sub scapula and subrailiac skinfold thickness was measured three times and their averages were noted to determine the body fat percentage by using the skinfold caliper (Holtain). Body fat percentage was detected by calculating the body densities.

Body Density = 1.1631 – 0.0632*Log (Biceps + Triceps + Subscapula + Subrailiac) (10). % Body Fat= (4.95/Body Density – 4.50)*100 (27).

Exercise programme

Twelve-minute jogging and then 20-minute stretching exercises were performed. A running programme composed of 2 sets x 3 serials x 800m was also applied. (In total: 4800m). 1:1 recovery or a break until the pulse rate decrease in the range of 120-130 rates was given between each two serials. Between the sets, 1-3 minutes of recovery was given. 800 meters distance was run with %90 of pulse. 5-minute jogging and then 5-10 minutes of stretching exercises were performed. Target pulse was determined according to the formula of below:

Target pulse = (220-age) x (90:100)

Blood sample collection

Blood samples of every participant was taken before the study and after a month (at the end of the supplementation and the training programme). 5 ml of blood was taken by the tubes containing anti-coagulant for blood plasma and 3-4 ml blood was taken by the tubes without anti-coagulant for blood serum. The blood samples were transported to the laboratory in a cold chain without waiting. After centrifuging the blood samples at +4^{o}C with 3000 turnovers for 15 minutes, the serum and the plasma were separated. These plasma and serum samples were preserved at -80 ^{o}C until the analyses were made.

Biochemical Analyses

LDH and CK activities of the samples were carried out with an auto analyzer and commercial kits (Siemens Advia 1800 Chemistry System). The kit of Cayman for TAC (catalog no: 709001) was used to determine TAC level and the kit of Cayman for MDA (catalog no: 10009055) was used to measure MDA level.

Statistical Analyses

A statistical package programmed called “SPSS 13.0 for Windows” was used for the evaluations. The Shapiro-Wilk test was used to control the normal distribution. 2 (2x2) Factor Variance Analysis was used for the repeated measures. In the event of significant interaction between time x group, independent t test and paired t-test were performed in order to determine reasons of the differences. All results were expressed with the mean and the mean of standard error. P< 0.05 was accepted as statistically significant.

RESULTS

The mean of body weight of the participants in control group is significantly higher than supplement group in both pre- and post-exercise comparisons (F=13.02, (p<0.001).). In addition, the time-related changes in both groups are not meaningful (F=041). The changes of body weight (F=0.41), BMI (F=0.91) and body fat percentage (F=0.40) of the supplement and control groups are similar in pre- and post- exercise (Table 1)

Table 1. Comparing some physical parameters of supplement and control group.

	Control Group (n=12)		Supplement Group (n=16)		F values		
	Pre-experiment	Post-experiment	Pre-experiment	Post-experiment	Time	Time x Group	Group
Variables	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**			
Age (years)	20.42 ± 0.51	------	20.75 ± 0.46	-----			
Height (cm)	177.00 ± 2.18	------	172.44 ± 1.50	------			
Weight (kg)	**69.69 ± 1.72**	**69.59 ± 1.82**	**61.89 ± 1.31**‡	**61.81 ± 1.43**†	0.41	0.01	**13.02*****
BMI (kg/m^2)	22.20 ± 0.54	**22.07 ± 0.50**	21.21 ± 0.62	**20.62 ± 0.44**†	0.91	0.37	3.32
Body Fat (%)	13.58 ± 0.96	13.20 ± 0.94	11.77 ± 0.57	10.98 ± 0.74	3.02	0.40	3.54

Significance level in the comparisons of; §,intragroup, ‡intergroup pre-exercise, †intergroup post-exercise, ***p<0.001

The time- related changes of CK level are significant (F=7.00). Also, CK levels between the groups are not significantly different (F=0.01). The changes of CK levels of supplement and control group in the study period are similar (F=3.20). LDH levels are significantly different in intergroup comparison (F=5.26) and the time-related changes in both group are not significant. The changes of LDH levels of supplement and control group in pre- and post- exercise are similar (F=1.15). The changes of TAC levels in supplement and control group are similar (F=0.01). The time-related effect on MDA levels of the groups is significant (F=11.43). However, MDA levels of the groups are not significantly different according to intergroup comparison (F=0.60). Also, time and group interaction effect on MDA levels is not significant (F=0.04) (Table 2).

Table 2. Comparing some blood samples of supplement and control group.

	Control Group (n=12)		Supplement Group (n=16)		F values		
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise	Time	Time x Group	Group
Variables	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**	**Mean ± SEM**			
CK (U/L)	155.83 ± 15.23	**135.75 ± 16.50**	**196.94 ± 38.27**	**93.06 ± 8.13**$^{†, §}$	**7.00***	3.20	0.01
LDH (U/L)	138.00 ± 8.67	**156.33 ± 8.57**	129.50 ± 6.56	**131.94 ± 5.78**†	1.97	1.15	**5.26 ***
TAC (mmol/L)	1.59 ± 0.11	1.69 ± 0.09	1.76 ± 0.13	1.86 ± 0.10	1.01	0.01	2.03
MDA (µmol/L)	**0.06 ± 0.00**	**0.10 ± 0.02**§	**0.06 ± 0.01**	**0.09 ± 0.01**§	**11.43****	0.04	0.60

Significance level in the comparisons of; §,intragroup, ‡intergroup pre-exercise, †intergroup post-exercise
*p<0.05, **p<0.01

DISCUSSION

In pre- and post-exercise comparisons, a significant difference was found in the body weight of supplement and control group in only intergroup comparisons while there was not any significant difference in body fat percentage. Similarly, Nieman et al. (2004) studied middle age athletes who participated in Triathlon World Championship in order to determine the effects of vitamin E on oxidative stress and immune changes, and found no significant difference in body fat percentage between vitamin E and placebo group (21). Also, in another study which was made on adolescent athletes to evaluate the effects of exercise on antioxidant status and protein alterations, no significant difference was found in body fat percentage of control and experimental group (7).

Creatine kinase is an important enzyme which is determined by way of periodical fluctuations in the tissues requiring energy such as skeletal muscle, myocardium and brain (22). Intensive and heavy exercise can cause muscle damage and increase the level of muscle proteins like LDH and CK in blood (18). Changes in serum levels of muscle enzymes occur in both normal subjects and athletes after a

strenuous exercise. The time of CK clearance from plasma differs according to the level, type, intensity and duration of exercise. Serum CK reaches up to the peak level of about 2-fold above baseline 8 hours later strength trainings and increases between 2 and 7 days after particularly eccentric exercise. There is a decrease in CK level between 4 and 10 days by providing an adaptation (6). However, there are some studies suggesting that CK increased 24h after exercise and returned to the baseline level in 48h (2). In this study, a significant decrease was detected in the level of CK of supplement group. Although an increase was expected in CK level because of the exercise, a significant decrease was found in CK level of supplement group and it made us think that this decrease resulted from vitamin E supplementation, because vitamin E is one of the most important antioxidative molecules located specifically in the cell membranes (20). Vitamin E is a fat-soluble antioxidant that has a chain-breaking effect and a major protective effect against to the oxidative stress and prevents the formation of lipid peroxidation by inhibiting free radicals in biological membranes (25). In a study which investigated the effects of vitamin E on the exercise-induced oxidative stress, while any significant change was not found in CK levels of supplement and control groups before supplementation with vitamins, a significant increase was detected in CK after the first and 24th hour of the exercise (14). In another study which observed the effects of vitamin E supplementation on the exercise-induced muscle damage, pre-exercise CK level significantly increased (3). A study was carried out in twenty elite athletes to determine the effects of a grape extract consumption on antioxidant status and physical performance. In that study, a statistically insignificant decrease was determined in CK at the end of thirty days (17). Also, Schröder et al. (2001) observed the effect of an antioxidant vitamin supplementation on the symptoms of oxidative, hormonal and enzymatic exercise in thirteen male professional basketball players and did not find a significant difference in CK levels of vitamin and placebo groups (26).

One of the substances which were used to determine skeletal muscle and myocardial damage is LDH (lactate dehydrogenase) (18). In this study, a significant increase was found in post-exercise LDH activities of supplement and control groups in intergroup comparison. In control group, LDH significantly increased because of the muscle damage connected to exercise, whereas an exercise-induced increase was prevented in supplement group by means of vitamin E. Like our study, in the research carried out in male professional basketball players, a significant difference was found in post-exercise LDH activity of placebo group and in the blood samples taken from vitamin group 24 hours later the exercise (26). In another study which vitamin E and C were given to the ultra marathon runners for 6 weeks, compared to pre-competition levels, LDH increased during the competition and reached the maximum level after the competition (19).

In this study, any significant change was not found in TAC concentration of the groups according to the comparisons of supplement and control group. Similarly, any meaningful difference was not found in TAC according to the intergroup comparison in a study in which Keong et al. (2006) investigated the effects of vitamin E on the exercise-induced oxidative stress (14). In another study in which 22 male wrestlers and 12 sedentary men participated in order to be researched on the effects of regular exercise on oxidative stress and antioxidant capacity, post-season TAC level of wrestlers group was significantly found higher than sedentary group (16).

A significant increase was found in MDA concentration of the supplement and control group in intragroup comparison. Because both of the groups participated in the same exercise programme during a month, we thought that the increase in MDA level in intragroup comparison resulted from

exercise. Bader et al. (2007) made a research on the healthy men to determine the effects of oxygen in high pressure and vitamin E and C supplementation on oxidative stress markers and found no significant difference in pre- and post-supplementation MDA level in intergroup comparison (1). In another study in which 50 elite cyclists participated, vitamin E and C were taken during a period of 2 months, a significant decrease was found in MDA level at the end of the second month. This result indicated vitamin E and C improved an antioxidant system which made the performance of the cyclists better (13). In a study which was made by Keong et al. (2006), plasma MDA concentration in the recovery period was found significantly lower in the vitamin E group, whereas post-exercise MDA level of both group was found higher than the recovery (14). In another study in which the relation between "oxidative stress markers" and "exercise status and diet" was researched, MDA level was detected significantly lower in the trained women compared to the trained men and found lower in the trained athletes compared to the untrained athletes (4).

The present study has some limitations. The participants were not on a special diet during the study and daily energy intake could not be determined and monitored which may have an effect on the results of the study. Also, this study was limited in that it lasted 30 days. Longer exercise programs may lead to different findings.

CONCLUSION

The results of our study present that vitamin E supplementation affected positively the exercise-induced muscle damage indicators, but could not improve a sufficient antioxidant defense system against the exercise-induced oxidative stress. We thought that this inability of the antioxidant defense resulted from the amount of daily vitamin E. (400 IU/268 mg).

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