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Research Article

Investigation of the effect of vitamin E application on lipid peroxidation and antioxidants in exercised horses

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ABSTRACT

Objective: In this study, the effect of vitamin E plus selenium (Se) application on malondialdehyde (MDA), glutathione (GSH), vitamin E, C, A and β-carotene were aimed to investigate in exercised horses.

Material and Methods: For this purpose, 50 healthy Anatolian type local horse breed aged between 3-5 from Altındere Study Farm were used. The animals were divided into two equal groups. While 1st group received nothing, horses in second group were received vitamin E+Se intramuscularly. Then animals in both groups were exercised for 1500 meters. Blood samples were taken handily from all animals before and after exercise. This samples were analyzed for MDA, GSH, vitamin E, C, A and β-caroten spectrophotometrically.

Results: MDA and GSH concentration in 1st group were found to increase significantly ($p<0.001$) after exercise. On the other hand, serum vitamin E, C, A and β-carotene levels did not changed significantly. In the second group, serum vitamin E levels increased significantly ($p<0.001$) after vitamin E+selenium application. Furthermore, MDA ($p<0.05$) and GSH ($p<0.001$) levels increased significantly after exercise in the second group. Vitamin E levels decreased significantly ($p<0.01$) after exercise. However, vitamin A and C levels did not change significantly. In addition, in the second group, β-carotene levels were also changed significantly ($p<0.05$) when the values obtained before vitamin E application compared with the values obtained after vitamin E application. When comparison made between groups, while MDA and vitamin E values were statistically important ($p<0.05$), GSH, vitamin C, A and β-carotene values were not important statistically

Conclusion: As a result, acute exercise can increase free-radical production which, the results shows that increase in both MDA and GSH can be shown as the indicator of it. Furthermore, decrease in MDA level in vitamin E applied group the indicator of the rise in antioxidant defense and protective effect of vitamin E.

Key Words: Antioxidant, horse, exercise, lipid peroxidation, vitamin E

INTRODUCTION

The basis of sustainable aerobic life on earth is composed of chemical and thermal energy which is created by the burning of nutrients containing carbon and hydrogen with the help of oxygen taken from water or air. In some of the steps of these chemical

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reactions which have a great importance in sustaining life, oxygen is degraded, and intermediate products defined as reactive oxygen types are created. All these factors which cause the creation of these reactive metabolites are called "prooxidant or oxidant" (Dündar and Aslan, 2000).

A great number of defense mechanisms have been developed in the body to prevent the formation of reactive oxygen types and to prevent the damage caused by these. These are known as antioxidant defense systems or in short as "antioxidants" (Varsankari et al., 1997). They consist of enzymatic and non-enzymatic structures (Dündar and Aslan, 2000; Karlsson., 1997).

Free radicals are products which occur as a natural cause of physiological activity in humans and animals (Akkuş, 1995). However, there should be a balance in the body of living beings with the activities of oxidants-antioxidants. In addition to playing a big role in protecting this oxidant-antioxidant balance which causes the degradation of cell membrane, vitamins also show significant interactions among themselves.

Since enzymatic defense systems generally dissolve in water, they destroy harmful oxygen derivatives in cytoplasm and deactivate chemical radicals in cell membrane and Vitamin E, which is the most effective antioxidant in biological membranes (Dündar et al., 2000; Karlsson et al., 1997; İsbir, 1994; Tıp and Top, 1993; Aslan et al., 1995).

Over formation of free radicals in a way that they will exceed the protective effect of defense system will affect compound lipids the most. Unsaturated bonds in phospholipids in the membranes and cholesterol easily react with free radicals and create peroxidation products. Oxidative destruction of unsaturated fatty acids is called lipid peroxidation and it is very harmful. Lipid peroxidation continues in the form of chain reactions continuing on their won. The most famous peroxidation product is MDA. It is generally accepted as lipid peroxidation index (Poter, 1984; Yagi, 1994).

Antioxidant system becomes more important against oxidative stress that occurs suddenly during exercise. The effect of exercise on antioxidants and lipid peroxidation has been discussed recently in several studies. Many of these studies report that acute submaximal exercise causes increase in lipid peroxidation and regular exercise causes positive change in antioxidant status (Di Mascito et al., 1991; Demopoulus et al., 1986).

The major purpose in studies associated with the physiology of exercise is to better understand the changes occurring in metabolism during performance sports as well as exercise for health and the adaptation of organism to exercise. For that purpose, studies have concentrated on free radicals and antioxidants (Selçuk, 2003).

Horses, which are used in rural areas as working animals in agriculture, stand out with race horses becoming widespread. In addition to being a great number of people's hobby, horse races have become a big sector recently with the increase in jockey clubs.

The purpose of this study is to research the effect of vitamin E and selenium application on lipid peroxidation and antioxidant vitamins in horses which take exercise.

MATERIALS AND METHODS

Animal Material: For livestock material of the research, 50 native stock Anatolian type horses between the ages of 3 and 5 in Erciş Altındere Agricultural Enterprise were chosen. The horses which underwent all kinds of health controls were grouped in 2 including 25 horses in each group. While the first group did not receive any interventions, each of the horses in the second group were injected with vitamin E+selenium preparade 8 ml as IM (intramuscular) to *M. cleidotransversarius* (*M. omotransversarius*). 1.5 hours were given for the medication to be absorbed and to reach its peak level (Kovác and Vrzgula, 1978). The animals were made to exercise at full gallop for 1500 meters (White et al., 2001). Animals in each group completed the exercise in an average of 7 minutes by forming groups of 5. Blood samples were taken from the animals before and after exercise.

Taking the blood samples: While blood samples were taken before and after exercise from animals in Group 1 which were not given Vitamin E+selenium, blood samples were taken from Group 2 which was given Vitamin E+selenium before the Vitamin E+selenium was given. After Vitamin E+selenium was given, time to reach plasma concentration was waited and blood was taken from the animals again. Later, they were made to exercise again, and their blood samples were taken for the third time. Blood samples were taken from the *V. jugularis* of the animals duly in two plastic tubes, one of which had EDTA. One of the tubes was centrifuged immediately; its serum was taken out and put into serum tubes. The other tube was kept as whole blood, kept in a cooled thermos, brought to the laboratory in cold chain and studied there.

Biochemical Analysis: MDA (Gutteridge, 1995, Sushil et al., 1989) and GSH (Beutler et al., 1963, Rizzi et el., 1988) and vitamin E (Martinek, 1964) and Vitamin C (Omaye et al., 1979) and vitamin A and B Carotene (Suziki et al., 1990) activity were measured spectrophotometrically.

Statistical Analysis: All the data obtained were interpreted statistically by using unpaired t-test and Kruskal-Wallis method (Akgül A, 1997).

RESULTS

In the study, pre-exercise and post-exercise blood MDA, GSH, serum vitamin E, vitamin C, β carotene and vitamin A levels were measured in both groups.

Parameter	n	T1 X ± SEM	T2 X ± SEM
MDA (nmol/ml)	25	0.92±0.04 [a]	1.90±0.07
GSH (mg/dl)	25	9.48±0.53 [a]	19.33±0.57
Vitamin E (mg/dl)	25	0.30±0.01	0.28±0.01
Vitamin C (mg/dl)	25	0.75±0.03	0.77±0.04
Beta-Carotene (µg/dl)	25	23.76±2.86	17.92±3.51
Vitamin A (µg/dl)	25	64.5±4.33	58.4±4.88

Table 1. Pre-exercise (T1) and post-exercise (T2) MDA, GSH, vitamin E, A and β-carotene levels of animals in Group I (which were not given vitamin E+selenium). a: p<0.001

Parameter	n	T0 X ± SEM	T1 X ± SEM	T2 X SEM
MDA (nmol/ml)	25	1.46±0.09 [a]	0.83±0.09 [b]	1.21±0.13
GSH (mg/dl)	25	15.68±0.68 [b]	13.69±0.60	17.93±0.35 [a]
Vitamin E (mg/dl)	25	0.31±0.01 [a]	0.44±0.01 [c]	0.36±0.01
Vitamin C (mg/dl)	25	0.79±0.06	0.83±0.09	0.80±0.05
Beta-Carotene (µg/dl)	25	16.93±0.97[b]	19.87±1.31	22.02±1.35
Vitamin A (µg/dl)	25	55.12±3.59	59.02±2.80	63.25±4.89

Table2. Pre-exercise and pre- vitamin E+selenium (T0), vitamin E+selenium and pre-exercise (T1), post vitamin E+selenium and post-exercise (T2) MDA, GSH, vitamin E, C, A and β-carotene levels of animals in Group II (which were given vitamin E+selenium). a: is different from T1. (p<0.001) b: is different from T2. (p<0.05) c: is different from T2. (p<0.01)

Parameter	n	Group I T2 X ± SEM	Group II T2 X ± SEM
MDA (nmol/ml)	25	1.90±0.07[a]	1.21±0.13
GSH (mg/dl)	25	19.33±0.57	17.93±0.35
Vitamin E (mg/dl)	25	0.28±0.01[a]	0.36±0.01
Vitamin C (mg/dl)	25	0.77±0.04	0.80±0.05
Beta-Carotene (µg/dl)	25	17.92±3.51	22.02±1.35
Vitamin A (µg/dl)	25	58.4±4.88	63.25±4.89

Table 3. Post-exercise MDA, GSH, vitamin E, C, A and β-carotene levels of animals in Group I and II.

DISCUSSION

During exercise, in parallel with the increase in oxygen consumption, oxygen use in muscles increases and the resulting oxidative stress accelerates the production of free radicals (Demopoulus et al., 1986; Holloszy, 1996).

Excess free radical production in skeletal muscles results from excessive use of energy by muscles in physical exercise (Demopoulus et al., 1986).

It has been reported that in horses, in the exercise pathogenesis caused by hemolysis and myopathy, lipid peroxidation occurs as a result of the increase in free radical production during both chronic and acute exercise (Chiaradia et al., 1998).

In their study conducted on the effects of physical exercise on horses, Chiaradia et al. (1998) reported that MDA level increased significantly (p<0.0001) after exercise; similarly, on their study where they applied vitamin E and selenium in the diets of horses, Avellini et al (1999) reported significant increase in MDA level during exercise period (p<0.05). They reported that the increase in MDA levels of the group to which only Vitamin E was given was found to be significantly lower than those of the group which was not given Vitamin E.

In their study, Ono et al. (1990) reported that in horses they injected vitamin E and selenium, pre-exercise MDA level was found to be decreased, in addition they reported that vitamin E and selenium application were effective in preventing post-exercise lipid peroxidation.

The results in the present study are in line with the literature. As can be seen in Table 1 and 2, post-exercise MDA level was found to increase significantly in Groups I and II. In Group II, a significant decrease was found in post vitamin E+selenium MDA level ($p<0.001$). This decrease got higher after exercise ($p<0.001$); however, it was still lower than the initial value ($p<0.05$). When the post-exercise levels of both groups were compared, significant difference was found between two groups ($p<0.05$). In both groups, less increase was observed in Group II which was given vitamin E+selenium although MDA level increased. Vitamin E+ selenium application can be said to cause a significant decrease in lipid peroxidation level.

In studies about antioxidants, it can be seen that generally organism related antioxidant components such as glutathione have been studied more (Şaşmaz, 1997). This results from the fact that they are 80% effective in primary antioxidant defence (Clarkson, 1995). Generally, glutathione is thought to be a multifunctional antioxidant which binds different harmful systems. Blood glutathione content is used as one of the indicators of body total antioxidant capacity (Avellini et al; 1999).

Chiaradia et al. (1998) reported a significant increase in post-exercise GSH level ($p<0.0001$). Brady et al. (1977) reported that in horses made to run until exhaustion, reduced glutathione did not increase; however, total and active reduced glutathione was found to increase immediately following exercise. Mills et al. (1996) observed increase in intense post-exercise blood GSH level in horses. Evola et al. (1992) and Ohno et al. (1986) reported a significant increase in post-exercise GSH level.

In the present study, post-exercise GSH level was found to have increased significantly in line with the literature in Group I ($p<0.001$). In Group II, it was found to decrease insignificantly following vitamin E+selenium injection ($p>0.05$). It was found to increase again following exercise ($p<0.001$). When the post-exercise GSH levels of both groups were examined, although GSH level of Group II was found to be lower, the result was not found to be significant ($p>0.05$).

In line with the studies conducted, a significant increase was found in GSH of the group which was not given post-exercise vitamin E+selenium. Following acute exercise, GSH found in tissues like muscles is quickly given to blood. As a result of this, blood GSH level increases and GSH level in muscle and other tissues decrease (Mills et al., 1990; Nemeth and Boda., (1994). In the group which was given Vitamin E+selenium, while decrease was found in glutathione level following vitamin E+selenium injection, an increase was observed following exercise. Since the need for antioxidant is met from Vitamin E as a result of Vitamin E+selenium injection, release of glutathione from tissues decreases and its level in blood decreases (Avellini et al., 1999).

In addition to endogenous physiological defence systems such as glutathione, exogen antioxidants also create a second defence line in the organism (Selçuk, 2003). The most studied antioxidants are vitamins. Vitamin E creates the first defence line against oxidative stress in biological environments it exists (Van-Der-Meulen et al., 1997)

In their study they conducted on horses which had vitamin E + selenium in their diets and regular exercise, Avellini et al. (1999) found significant increase in Vitamin E levels of the group which was given Vitamin E and this level was found to decrease significantly following exercise. In their study, Sürmen Gür et al. (1999) reported that Vitamin E supplement increased serum Vitamin E level and that this value decreased following exercise. Kumar et al. (1992) and Lang et al. (1987) reported that plasma Vitamin E concentration decreased following exercise in rats.

In the present study, serum Vitamin E level of Group I decreased following exercise, which was in line with literature ($p>0.05$). In Group II, serum Vitamin E level was found to increase significantly ($p<0.001$). Vitamin E level of this group was found to decrease significantly following regular exercise ($p<0.01$).

It is said that acute exercise increases free oxygen radicals and Vitamin E supplement improves free radical scavenger systems, prevents and therefore decreases lipid peroxidation regularly (Kumar et al., 1992; Avellini et al., 1999).

Antioxidant capacity in plasma occurs in uric acid, especially Vitamin C and activities of some big molecule proteins (Dündar et al., 2000). It is reported that Vitamin C can show its antioxidant effect by scavenging free radicals (Ashton et al., 1999; Wilson et al., 2001). In the presence of glutathione, Vitamin C plays a role in the reactivation of Vitamin E which has become tocopherol radical and lost its antioxidant property (Jocob et al., 1987; Selçuk, 2003). White et al. (2001) and Evans, (2000) reported that following exercise, Vitamin C level increased mildly.

In the present study, post-exercise serum vitamin C level increased insignificantly in Group I ($p>0.05$). Duthie et al. (1990) stated that vitamin C increased as a response to increase in cortisol level. Serum vitamin C level in Group II increased mildly following vitamin E+selenium injection ($p>0.05$). Following exercise, vitamin C level decreased insignificantly ($p>0.05$). When low molecule weight antioxidants are insufficient or in the presence of systems which scavenge free radicals such as vitamin E, it is not obligatory to release molecules such as vitamin C, uric acid, etc. from tissues (Avellini et al., 1999). In the present study, the decrease in Vitamin C level following exercise can be resulting from insufficient Vitamin E.

Carotenoids are antioxidants which dissolve in fat and which are found in primary tissue membranes. Antioxidant property of carotenoids result from their structural organization which consists of long chains bound with double bond. Beta-Carotene shows its antioxidant activity by preventing the formation of free radicals and collecting the radicals in the environment. This antioxidant activity of is directly related with the oxygen concentration of the environment it is in (Selçuk, 2003; Byung, 1994).

While it is reported that Vitamin A is not influenced by exercise, it is also reported that Vitamin A supplement may be dangerous. Although β-carotene is the pre-material of Vitamin A, it is not toxic and it serves as antioxidant (Clarkson 1995).

In the present study, insignificant decrease was found in the post-exercise serum vitamin A and β-carotene levels of the animals in Group I ($p>0.05$). However, $p<0.05$ significance was found in β-carotene level and between T0 and T2 times.

Vitamin E prevents the decomposition of Vitamin A, which is an important vitamin sensitive to oxygen and helps the body to meet its Vitamin A level at a high degree (Yaşar, 2008). In this study, the result that post-exercise Vitamin A and β-carotene increased regularly in the group given vitamin E+selenium can be explained with the effect of Vitamin E on Vitamin A. It can be said that this caused a decrease in post-exercise Vitamin A and β-carotene in the group which was not given Vitamin E+selenium and an increase in the need for antioxidant in exercise and the production of free oxygen radicals.

As a conclusion, the increase in the amount of MDA and glutathione in horses which were given intense exercise in this study is an indication of the increase in free radical production.

Vitamin E is an important antioxidant. It is in cell membrane and is effective in catching the free radicals resulting from especially mitochondria inner membrane and other biomembranes. In this study, the decrease in MDA level in the group given vitamin E+selenium can be explained as vitamin E+selenium's positive effect of strengthening the body's defense mechanism. In exercised animals, vitamin E+selenium can be said to be beneficial in preventing muscle harms which can occur during exercise.

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