Biol Trace Elem Res (2008) 125:223–228
DOI 10.1007/s12011-008-8109-8

Effects of Vitamin E and Selenium on Serum Trace and Major Elements in Horses

Fatmagül Yur · Semiha Dede · Yeter Deger · D. Kilicalp

Received: 26 November 2007 / Accepted: 21 January 2008 /
Published online: 17 September 2008

Abstract The combined effects of vitamin E and selenium were studied in native Anatolian horses subject to strenuous exercise. The concentrations of copper, zinc, iron, calcium, potassium, and magnesium were determined in serum by atomic absorption spectrometry in two study groups (n=25 each), one of which served as untreated controls. After exercising the horses by running 1,500 m in about 7 min, only the copper level and the copper/zinc ratio significantly increased (p<0.05), but the concentrations of calcium, potassium, iron, and magnesium remained unchanged. In horses treated with vitamin E and selenium, the calcium and potassium levels decreased to levels lower than those of untreated controls before and after exercise. The iron levels were not changed by exercise or treatment alone but increased when the horses had been supplemented and exercised. The copper level and the copper/zinc ration increased as a result of exercise in both treated and untreated horses. These changes suggest that supplementation with vitamin E and selenium had an important effect on the serum concentrations of calcium, potassium, copper, iron, and the copper/zinc ratio.

Keywords Exercise · Horses · Major elements · Trace elements · Vitamin E · Selenium

Introduction

There are still many unanswered questions regarding body composition and performance of horses. The body composition is directly related to aerobic capacity, a major factor of athletic performance in running horses.

F. Yur · S. Dede (✉) · Y. Deger
Department of Biochemistry, Faculty of Veterinary Medicine,
University of Yuzuncu Yil, 65080 Van, Turkey
e-mail: ssdede@hotmail.com

D. Kilicalp
Departments of Physiology, Faculty of Veterinary Medicine,
University of Yuzuncu Yil, 65080 Van, Turkey

Strenuous physical activity is known to induce oxidative stress leading to lipid peroxidation and tissue damage [1, 2]. It has been shown that appropriate training promotes the antioxidant defense in horses, thereby increasing its resistance against pro-oxidants [3]. Several studies have shown that oxidative stress in horses can be prevented by means of adequate supplementation with antioxidants [3-7].

Vitamin E acts as an antioxidant reducing muscle damage and enhancing recovery from exercise. Thus, in recent years, the feed of performance horses is now supplemented with vitamin E, which plays an important role in maintaining the integrity of cell membranes [6, 8-11].

As part of proper nutrition, horses need certain trace minerals. Selenium is one of great importance because it is required for muscle and immune system integrity, among other things. Thoroughbred horses exhibiting poor performance had lower serum selenium levels than those that performed well. If selenium is supplied together with vitamin E, it increases its ability to decrease the negative effects of reactive oxygen species [10, 11].

Copper and zinc are essential for the function of superoxide dismutase as part of the antioxidant system [13]. The roles of magnesium, iron, zinc, and copper in strenuous exercise remain controversial. These elements have biochemical functions with the potential to affect physical performance. They serve as structural or catalytic components of enzymes and regulators of many physiologic functions. While all horses require major and trace minerals, their growth, reproduction, lactation and performance determine the specific requirements [9, 12].

The present study was conducted to investigate the effect that exercise and supplementation with vitamin E and selenium have on the serum element status of performance horses.

Materials and Methods

Animals

Fifty native Anatolian horses aged 3–5 years from the Ercis Altindere Agricultural Managing Unit were used for this study. All of the animals were in good health and had no physical limitations of any kind.

Experimental Design

The horses were divided into two groups each containing 25 horses. One of the groups served as untreated controls, while all of the animals in the other group received a single 8 ml intramuscular dose of a commercially available combination of 60 mg/ml vitamin E and 1 mg/ml selenium. To ensure peak effect of the supplements, the horses were exercised or tested 90 min after the injection.

Exercise Program

The horses were galloped a 1,500 m distance in an average 7 min. Blood samples were taken from the animals in both groups before and after exercising.

Sampling and Mineral Analysis

The blood samples were taken from the jugular vein and collected in plastic tubes. The serum was then separated by centrifugation at 3,000 rpm for 10 min, and the serum was then appropriately diluted with doubly distilled water and analyzed for mineral content using a Unicam 929 atomic absorption spectrophotometer (UK), following established procedures [14, 15].

Statistical Analysis

The results are presented as means±standard deviation, and the statistical analysis was done using Duncan's test. The significance level was set at $p<0.05$.

Results

The results are summarized in Table 1.

The calcium concentration was decreased after exercise in both groups, but the treated horses had significantly lower levels than those of untreated controls before exercise ($p<0.05$).

After exercise, the potassium concentration in the treated group was lower than that of controls ($p<0.05$). There were no changes of potassium in the untreated animals before and after exercise. Vitamin E and Se treatment were increased serum K levels ($p<0.05$).

The magnesium concentration of the controls was unchanged after exercise. Treatment alone did not change these levels, but there was a significant increase after exercise ($p<0.05$).

In the case of iron, supplementation alone did not result in changes of its concentration, but exercise resulted in a significant increase in both groups ($p<0.05$).

The copper concentration in the controls increased after exercise. In the experimental group, the copper level increased after supplementation but significantly decreased after exercise until they reached the same level as that of non-exercised controls. All of the differences reported are significant ($p<0.05$).

Exercise did not change the zinc levels in the controls or experimental animals, but treatment with selenium and vitamin E resulted in decreased serum zinc levels. Consequently, the copper/zinc ratio of the controls was lower before than after exercise and after supplementation. Both the copper level and the copper/zinc level were increased in the controls after exercise.

Table 1 Major and Trace Elements in Horses Supplemented with Vitamin E and Selenium, Before and After Strenuous Exercise and Controls

	Controls		Subjects	
	BE	AE	AS	ASE
Ca (mg/dl)	24.87 ± 1.49^{a}	21.76 ± 2.24^{ab}	18.94 ± 1.63^{b}	19.71 ± 1.30^{b}
K (mg/dl)	33.37 ± 0.99^{a}	36.74 ± 1.09^{a}	28.93 ± 1.15^{b}	34.74 ± 1.33^{a}
Mg (mg/dl)	2.32 ± 0.06^{a}	2.34 ± 0.09^{a}	2.42 ± 0.12^{ab}	2.68 ± 0.15^{b}
Fe (mg/dl)	0.178 ± 0.012^{a}	0.254 ± 0.021^{ab}	0.216 ± 0.013^{a}	0.274 ± 0.012^{b}
Cu (mg/dl)	0.074 ± 0.003^{a}	0.191 ± 0.019^{b}	0.151 ± 0.023^{c}	0.110 ± 0.009^{ac}
Zn (mg/dl)	0.062 ± 0.002^{a}	0.063 ± 0.003^{a}	0.052 ± 0.003^{b}	0.053 ± 0.004^{b}
Cu/Zn ratio	1.195 ± 0.038^{a}	3.229 ± 0.422^{b}	2.958 ± 0.510^{b}	2.235 ± 0.175^{b}

BE Before exercise, *AE* after exercise, *AS* after supplementation, *ASE* after supplementation and exercise

$p<0.05$; significant differences between the two groups, before and after exercise. Differences between means with different small (a,b,c) letters in same line for each parameters are significant ($p<0.05$). The same letters in the same parameters means non-significance between groups

Discussion

Physical activity demands increasing amounts of vitamins and minerals [9]. Along with vitamin E, selenium is a key part of the antioxidant defense mechanism, acting as free radical scavengers. In particular, selenium has received a great deal of attention in horse nutrition in recent years. Positive and significant correlations were found between vitamin E and glutathione peroxidase, which has selenium as cofactor. There are several reports on the use of vitamin E and selenium as supplements to increase endurance in horses [2, 6, 7, 16]. Thoroughbred horses undergo significant changes of several antioxidant markers after training, which are at least partially counterbalanced by antioxidant supplements [4].

In performance horses, calcium is a mineral of primary importance. Physical activity is known to affect calcium homeostasis in horses of all ages. Increases in bone mineral density or cortical volume have been reported in young horses [17-19].

Vitamin E prevents bone calcium loss due to increases of oxygen consumption and production of free radicals [20]. Luo et al. [21] reported that administration of vitamin E in exercising rats protects the function of skeletal muscle mitochondria. Our observation that the calcium level significantly decreases after supplementation with vitamin E and selenium suggests that it prevents excessive bone and muscle calcium loss induced by exercise.

Increases in plasma potassium were seen in trained Arabian horses due to migration of intracellular ions into the plasma, required to maintain the blood pH during hard exercise. Electrolyte balance is restored as soon as exercise is stopped [22, 23].

We found significantly lower potassium in sera of the treated horses compared to non-supplemented controls before and after exercise. This means that the potassium level is changed by supplementation but not so by exercise, possibly due to changes in the anti- and pro-oxidant balance caused by selenium and vitamin E.

Magnesium is required in a wide variety of fundamental cellular activities that support diverse physiologic systems [24]. Gallagher and Stowe [25] found that the serum magnesium levels were significantly increased after exercise, $p<0.01$. In the present study, the magnesium concentration of the controls did not change following exercise. Supplementation with selenium and vitamin E also resulted in no changes of magnesium at rest, but the increase following exercise was significant.

Iron is a component of many enzymes involved in the delivery of oxygen to tissues and the use of oxygen at the cellular and sub-cellular levels. It was reported that strenuous physical exercise could increase absorption of iron. Exercise increases erythropoiesis and hence iron turnover [9, 26, 27]. The physiologic changes induced by exercise can mimic iron deficiency and decrease hemoglobin and ferritin concentrations [28]. In this study, the iron levels of horses in both study and control groups had changes of their serum iron only after exercise, suggesting that the element is transported into blood as a result of increased physical activity.

The copper levels were also changed by exercise. They increased in the untreated controls as a result of exercise and in the study group as a result of supplementation. After exercising the supplemented horses, however, the copper level returned to its normal value. This is consistent with literature reports about significant increases of copper in racing horses [25, 29, 30]. Also, a positive correlation was found between vitamin E and copper, possibly due to increases of some copper-dependent enzymes [16].

The importance of zinc is reflected by the numerous functions and activities over which it exerts a regulatory role. Zinc is also involved in mineralization of cartilage, the process by which cartilage is replaced by bone [31]. There were marked differences in the mean serum zinc concentrations in the blood of thoroughbreds housed in different stables [30].

Some researchers reported significant decreases in the levels of zinc as a result of exercise [9, 32, 33]. After training, the total plasma zinc had decreased significantly, suggesting a relative reduction of the circulating exchangeable zinc [32].

In this study, no changes of the zinc levels were seen as a result of exercise in both groups, but a significant decrease was observed after supplementation with vitamin E and selenium. The decrease of zinc influences the copper/zinc ratio, which is lower in controls before exercise when compared to the after-exercise treated horses. Such increases of the copper/zinc ratio as a result of lower zinc values have been reported in other studies [34, 35].

It can be concluded that supplementation of horses subject to strenuous exercise with selenium and vitamin E results in relevant changes of their serum levels of major and trace elements. The results presented here may be used for the adequate nutrition and supplementation of racing or working horses and, in general, in the search for adequate dietary vitamin and mineral intakes among physically active individuals.

References

1. Jenkins RR (1988) Free radical chemistry: relation to exercise. Sports Med 5(3):156–170
2. Reddy KV, Kumar TC, Prasad M, Reddanna P (1998) Pulmonary lipid peroxidation and antioxidant defenses during exhaustive physical exercise: the role of vitamin E and selenium. Nutrition 14(5):448–451
3. de Moffarts B, Kirschvink N, Art T et al (2004) Impact of training and exercise intensity on blood antioxidant markers in healthy Standardbred horses. Equine Comp Ex Physiol 1:211–220
4. de Moffarts B, Kirschvink N, Art T, Pincemail J, Lekeux P (2005) Effect of oral antioxidant supplementation on blood antioxidant status in trained thoroughbred horses. Vet J 169(1):65–74
5. Williams CA, Kronfeldt DS, Hess TM et al (2004) Antioxidant supplementation and subsequent oxidative stress of horses during an 80-km endurance race. J Anim Sci 82(2):588–594
6. Avellini L, Chiaradia E, Gaiti A (1999) Effect of exercise training, selenium and vitamin E on some free radical scavengers in horses (Equus caballus). Comp Biochem Physiol Part B Biochem Mol Biol 123 (2):147–154
7. Ormancı N (2003) Investigation of the effect of vitamin E application on lipid peroxidation and antioxidants in exercised horses. PhD Thesis in Biochemistry, Health Sciences Institute, University of Yuzuncu Yil, Van
8. Packer L, Almada AL, Rothfuss LM, Wilson DS (1989) Modulation of tissue vitamin E levels by physical exercise. Ann N Y Acad Sci 570:311–321
9. Lukaski HC (2004) Vitamin and mineral status: effects on physical performance. Nutrition 20(7–8):632–644
10. Bendich A (1991) Exercise and free radicals; effects of antioxidant vitamins. Med Sport Sci 32:59–65
11. Packer L (1991) Protective role of vitamin E in biological systems. Am J Clin Nutr 53:1050–1055
12. http://www.utextension.utk.edu/newsevents/newsletters/HEwinter06.pdf
13. Fridovich I (1995) Superoxide dismutases. Ann Rev Biochem 64:97–112
14. Greenberg WE, Trusell RR, Clescer LS (1996) Standart methods for the examination of water and wastewater, 6th edn. American Public Health Association, Washington, DC, USA, pp 143–179
15. Longbottom JE, Martin TD, Edgell KW, Long SE, Plantz MR, Warden BE (1994) Determination of trace elements in water by inductively coupled plasma-mass spectrometry: collaborative study. J AOAC Int 77 (4):1004–1023
16. Kirschvink N, de Moffarts B, Farnir F, Pincemail J, Lekeux P (2006) Investigation of blood oxidant/antioxidant markers in healthy competition horses of different breeds. Equine Vet J 36:239–244 (Suppl)
17. Buchholz-Bryant MA, Baker LA, Pipkin JL, Mansell BJ, Haliburton JC, Bachman RC (2001) The effect of calcium and phosphorus supplementation, inactivity, and subsequent aerobic training on the mineral balance in young, mature, and aged horses. J Equine Vet Sci 21(2):71–77
18. Vervuert I, Coenen M, Wedemeyer U, Chrobok C, Harmeyer J, Sporleder HP (2002) Calcium homeostasis and intact plasma parathyroid hormone during exercise and training in young Standardbred horses. Equine Vet J 34(7):713–718
19. Mc Carthy RN, Jeffcott LB (1991) Treadmill exercise intensity and its effects on cortical bone in horses of various ages. Equine Exercise Physiol 3:419–428

20. White A, Estrada M, Walker K et al (2001) Role of exercise and ascorbate on plasma antioxidant capacity in thoroughbred race horses. Comp Biochem Physiol A Mol Integr Physiol 128(1):99–104
21. Luo JW, Yu B, Qin CH et al (2003) Ultrastructural changes of the skeletal muscle cells of rats subjected to exhausting exercises and the protective effect of vitamin E. Di Yi Jun Yi Da Xue Xue Bao 23 (12):1326–1328
22. Hess TM, Kronfeld DS, Does RA (2006) usefulness of potassium supplementation depend on speed? Equine Vet J Suppl 36:74–79
23. Bayly WM, Kingston JK, Brown JA, Keegan RD, Greene SA, Sides RH (2006) Changes in arterial, mixed venous and intraerythrocytic concentrations of ions in supramaximally exercising horses. Equine Vet J Suppl 36:294–297
24. Shils ME (1998) Magnesium. In: O'Dell BL, Sunde RA (eds) Handbook of nutritionally essential mineral elements. Marcel Dekker, New York, p 117
25. Gallagher K, Stowe HD (1980) Influence of exercise on serum selenium and peroxide reduction system of racing Standardbreds. Am J Vet Res 41(8):1333–1335
26. Schobersberger W, Tschann M, Hasibeder W, Steidl M, Nachbauer W, Koller A (1990) Consequences of 6 weeks of strenuous training on red cell oxygen transport and iron status. Eur J Appl Physiol 60:163–168
27. Weight LM, Byrne MJ, Jacobs P (1991) Haemolytic effects of exercise. Clin Sci 81:147–152
28. Zoller H, Vogel W (2004) Iron supplementation in athletes—first do no harm. Nutrition 20(7–8):615–619
29. Lewis RD, Johnson MA, Dengal DR, Jones MT, Norton KI (1993) The effects of maternal training on plasma copper, magnesium and zinc in rats. Nutr Res 13(7):771–778
30. Stubley D, Campbell C, Dant C, Blackmore DJ, Pierce A (1983) Copper and zinc levels in the blood of thoroughbreds in training in the United Kingdom. Equine Vet J 15(3):253–256
31. Vallee BL, Falchuk KH (1993) The biochemical basis of zinc physiology. Physiol Rev 73:79–118
32. Lukaski HC, Hoverson BS, Gallagher SK, Bolonchuk WW (1990) Physical training and copper, iron and zinc status of swimmers. Am J Clin Nutr 51:1093–1099
33. Ohno H, Sato Y, Ishikawa M et al (1990) Training effects on blood zinc levels in humans. J Sports Med Phys Fitness 30(3):247–253
34. Lin CC, Huang JF, Tsai LY, Huang YL (2006) Selenium, iron, copper, and zinc levels and copper-to-zinc ratios in serum of patients at different stages of viral hepatic diseases. Biol Trace Elem Res 109:15–24
35. Fota-Markowska H, Rolla-Szczepanska R, Modrzewska R, Lis-Tonder J, Borowicz I (2003) Copper/zinc ratio in measles patients. Ann Univ Mariae Curie Sklodowska 58(1):291–296