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Oxidative stress during exercise in racehorses: Relationships between nutrition, training and biochemical defences against free radicals

L. Avellini, E. Chiaradia, F. Rueca, A. Spaterna, A. Gaiti and *G. Fruganti*

Centro di Studio del Cavallo Sportivo. Università degli Studi di Perugia

Summery

It has been suggested that the pathogenesis of exercise-induced myopathies and hemolysis in horses may be related to oxidative stress caused by free radicals. As acute strenuous exercise and chronic exercise training increase the consumption of natural antioxidants, we decided to evaluate the suitability of dietary supplementation with antioxidants during training. Three year old Maremmana stallions were randomly selected from two groups while undergoing special training: one group was fed with a diet enriched with 20 μg/kg of body weight of selenium per day. The horses were followed by testing: 1) some enzymatic activities both in the erythrocytes and plasma; 2) plasma vitamin E content and 3) red blood cell membrane composition. From the results obtained one may deduce that the diet of the first group of animals studied was unable to support the increased antioxidant defense needs caused by the training. However, it seems that there was no direct relationship between antioxidant defences and muscle damage.

Keywords: racehorse, free-radical defences, selenium, nutrition, training

Oxidativer Streß bei Rennpferden während Belastung

Es wird angenommen, daß beim Pferd die Pathogenese belastungsindizierter Myopathien und Hämolysen in Zusammenhang mit oxidativem Streß, hervorgerufen durch freie Radikale, steht. Da einmalige, anstrengende Belastungen sowie wiederholte Dauerbelastungen den Verbrauch natürlicher Antioxidantien erhöhen, entschieden wir uns, in dieser Studie den Nutzen einer Supplementierung mit Antioxidantien während eines Trainings zu überprüfen. Vierzehn 3-jährige Maremmana-Hengste wurden in eine Kontroll- und Versuchsgruppe eingeteilt. Die tägliche Futterration beider Gruppe enthielt 3 mg Selen sowie 1.000 IU Vitamin E. Die Ration der Versuchgruppe wurde zudem mit 20 μg Selen pro Kilogramm Körpergewicht angereichert. Beide Gruppe absolvierten für eine Dauer von 60 Tagen an 6 Tagen der Woche eine 30 minütige Belastung. Nach der Belastung wurden Blutproben zur Bestimmung der folgenden Parameter entnommen: 1) Aktivität einiger Enzyme in Erythrozyten und Plasma; 2) Vitamin E-Gehalt im Plasma; 3) Zusammensetzung der Membran der Erythrozyten. Aus den in dieser Studie gewonnenen Ergebnissen läßt sich schließen, daß der Gehalt an Antioxidantien in der täglichen Futterration der Kontrollgruppe nicht ausreichte, um die durch das Training erhöhte Beanspruchung des antioxidativen Schutzes auszugleichen. Dennoch ergab sich in dieser Studie keine direkte Beziehung zwischen antioxidativem Schutz und Schädigungen der Muskulatur.

Schlüsselwörter: Rennpferde, Abwehr freier Radikale, Selen, Fütterung, Training

Introduction

The mechanism by which physical exercise induces muscle damage has not been established in detail, even if it is well known that O_2 free radicals play an important role (*De Quiroga,* 1992; *Ji,* 1995). Ideed, during intensive physical exercise, muscles need an enormous amount of oxygen. About 5% of this amount of O_2 is converted into free radicals which, in turn, cause a membrane damage mainly through the mechanism of lipid peroxidation.

The concentration of reactive oxygen species (ROS, i.e. H_2O_2, hydroxy radical, oxygen superoxide) in the cells during rest or light physical exercise is efficently lowered by the action of different scavengers (superoxide dismutase, glutathione peroxidase, glutathione, vitamin E, and so on). On the other hand, when free radical generation exceeds the antioxidant capacity of cells or extracellular fluids, an oxidative stress develops which in turn induces tissue damage (*Ji*, 1995).

As the muscle shows a lower ROS scavenger capacity as compared to liver cells and erythrocytes, it has been suggested that the pathogenesis of exercise-induced myopathies in horses may be related to exercise-induced oxidative stress. However, it is difficult to find a precise relationship between the exercise intensity and the onset of myopathy symptoms because it differs from one horse to another, suggesting that the cellular resistance to free radicals is strictly related to genetic heritage (*Valberg* et al, 1993).

On the other hand, it has been well established that the free radical scavenger capacity of the cells may be lowered by an inadequate diet (*Ji*, 1995) and increased by adequate training before exercise (*Hintz,* 1994).

In the light of the above findings, the purpose of this study is to verify the relationship between exercise-induced oxidative stress and myopathy onset as well as the effect of training and a diet enriched with Selenium. To do this we studied a group of horses while undergoing special training by testing: 1) some enzymatic activities in the blood correlated to muscle activity (CK and LDH) as well as vitamin E (Vit E) content; 2) the Se-dependent glutathione peroxidase in the erythrocytes, and 3) the erythrocyte cell membrane composition.

Materials and methods

Animals – 14 three-year old Maremmana stallions from different animal breeding farms were placed on a farm in Tuscany after a thorough clinical and genetic screening. The horses underwent physical training for 30 min a day, six days a week. During the first week exercise was without a rider. Exercise was then with a rider and its intensity was gradually increased. The animals were randomly divided into two groups: the control group horses were fed a diet which was, from the caloric point of view, considered adequate for light work (about 7 fodder units/ day per horses, French's system, INRA (*Martin-Rosset,* 1990) which also ensured at least 3 mg of Selenium and 1,000 International Units of Vit E per day; the second group received the same diet which was enriched with 20 μg/Kg of body weight per day of selenium.
Blood samples were collected at the beginning of the selection (t_0) and after 30 and 60 days, the end of the selection (t_{30}, t_{60}). Erythrocyte and erythrocyte ghosts were prepared as previously described (*Avellini* et al, 1995).

Chemical and enzymatic analyses
The serum enzymes CK and LDH were tested by using an authomatic analyzer (Super Z818, SCLAVO, Italy) and by using the SCLAVO kits CK-NAC and LDH-P (*Bergmeyer,* 1974).

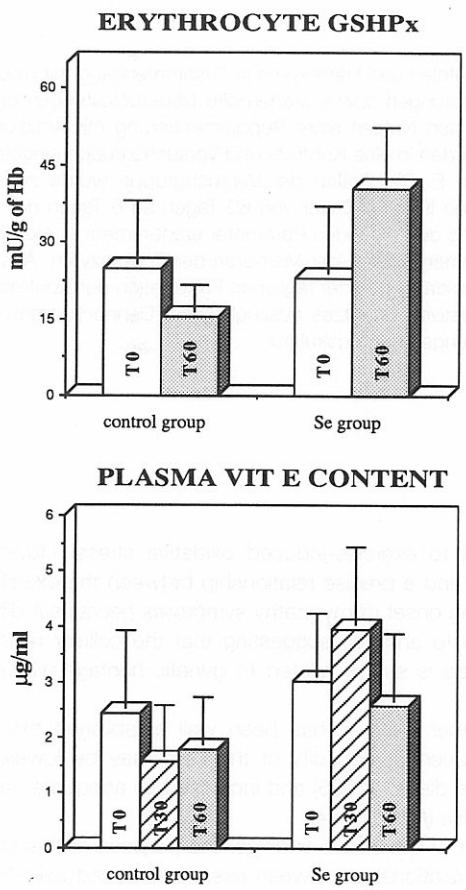

Fig 1: Erythrocyte GSH-Px content and plasma Vit E determined in both group of horses over the training period. The data of GSH-Px are expressed as enzymic units/mg of hemoglobin and the differences are statistically significant (t-test, $p<0.008$). Plasma Vit E content is reported as μg/ml and the differences are statistically significant (ANOVA repeated measures, $p<0.05$).

The serum Vit E content was determined and erythrocyte glutathione peroxidase (GSH-Px) assayed as previously described (*Avellini* et al, 1995).
The cholesterol/phospholipid P ratios were calculated on the basis of their content in erythrocyte ghosts. The lipids were extracted from erythrocyte ghosts (*Gatti* et al, 1986). An aliquot of the lipid extract was used to determine the P content according to the Fiske-Subbarow method, while another one was resuspended in 100 mM sodium phosphate buffer containing 0.3% Thesit as detergent. This aliquot was injected into a small column containing 320 mg (20 U/mg of protein) of cholesterol oxidase immobilized enzyme. The content of cholestenon in the eluate was determined in a Hewlett-Packard spectrophotometer 8452A at 240 nm by using a flux couvette. This value was referred to cholesterol content by using a calibration curve.

Statistical Analyses
The data were statistically manipulated with analysis of variance: ANOVA repeated measures or Student t-test.

Results

In Fig. 1 we report the results of the GSH-Px determined in the erythrocytes from both groups of horses at t_0 and t_{60}. While enzymatic activity appears significantly decreased at the end of test period in the control group, it is significantly increased in the group of animals that received Se supplementation (the data of T_{30} are not reported).

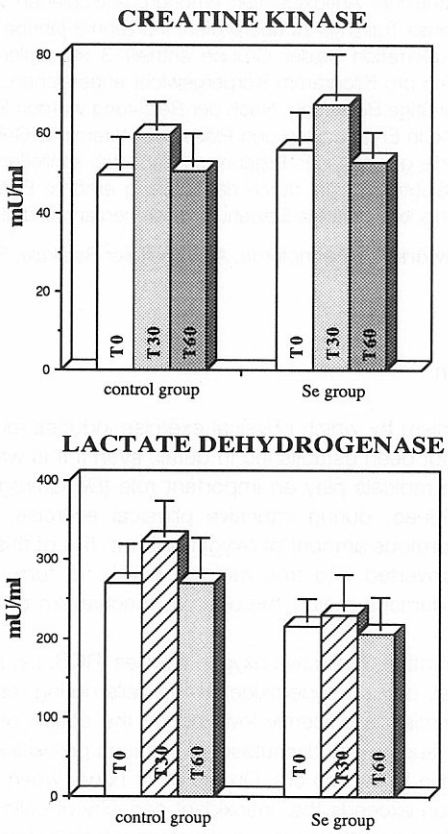

Fig 2: Creatine kinase and lactate dehydrogenase activities in serum from both horse groups. The results are expressed as mU/ml and the differences are statistically significant (ANOVA repeated measures, $p<0.001$).

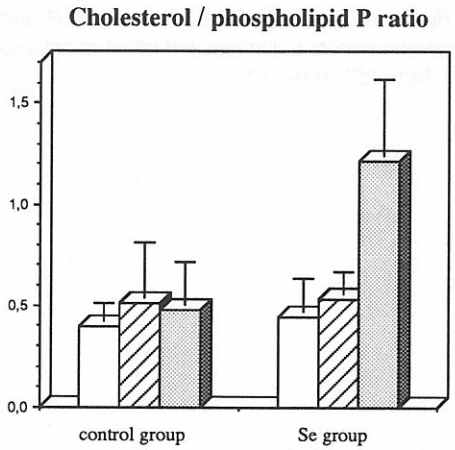

Fig 3: Cholesterol / phospholipid ratio determined in erythrocite membranes. The differences are statistically significant (ANOVA repeated measures, $p<0.005$).

The serum Vit E content significantly decreased ($p<0.05$, t-test) in the control group. On the contrary, in the group of horses that received Se supplementation we observed a significant increase between t_0 and t_{30}, while the value determined at t_{60} is identical to the t_0 (Fig. 1).

The contents of both serum CK and LDH were significantly increased in both the animal groups during the first 30 days, while the value came back to the t_0 value during the last 30 days of the test (Fig 2).

The cholesterol/phospholipid P ratio of erythrocyte membranes significantly increased in both control group horses and treated animals and this increase is due to an increase in cholesterol (Fig. 3).

In Tab. 1 we reporte some haematological parameters tested over the test period. It is possible to note an increasing trend in all three parameters. The differences were significant ($p<0.05$) for hemoglobin and hematocrit.

Discussion

The working hypotheses were: 1) to verify if the necessary physical exercise during horse training, which is a chronic repetition of the same exercises, is able to cause oxidative stress and, as a consequence, muscle damage; 2) as tissues seem to increase their antioxidant defences under chronic activation, to study whether the special training we adopted may be able to do this (i.e. increase antioxidant defences); 3) to verify if the diet of the animals studied was able to support the increased need for antioxidant defenses caused by the training.

Tab. 1: Some haematological changes during training period.

	t_0	t_{30}	t_{60}
Red blood cells (n°/mm³)	8,09±1,2	8,68±1,32	8,54±1,4
Hemoglobin (g%)	11,9±1,3	12,8±1,4	13,0±1,6
Hematocrit (%)	32,9±4,1	35,9±4,3	39,6±4,9

First of all, our data indicate that our training is effective (*Ono* et al, 1990), as confirmed by the time dependent increase of hemoglobin content, hematocrit and red cell number (Tab. 1).

In the control group we found a decrease in both chemical and biochemical defences against the free radicals tested during the time interval of the training in accordance with what we previously observed (*Avellini* et al, 1995). Since cellular protection against lipid peroxidation and other types of oxidative damage is accomplished by diverse enzymatic and non-enzymatic means, GSH-Px activity and Vit E levels appear to be strictly correlated, as demonstrated by the possibility of preventing an increase in lipid peroxidation caused by a decrease in GSH-Px activity with adequate Vit E in the diet (*Haenen* et al, 1987; *Ono* et al, 1990; *Witt* et al, 1992). Our data confirm that the diet of the control group of animals was unable to support the increased need for antioxidant defenses caused by our training.

The above finding is also confirmed by the results obtained in the group of animals which received Selenium supplementation. Indeed, a significant increase in their GSH-Px activity was observed over the entire time interval of the training. Interestingly, Vit E content increased between t_0 and t_{30} confirming the correlation between GSH-Px activity and Vit E content.

The decrease in vitamin content observed between t_{30} and t_{60} may indicate that by increasing the intensity of the physical exercise an increased consumption of the vitamin takes place. In any case, these data confirmed that more Selenium and Vit E are necessary for animals even undergoing light exercise to balance the increased need of free radical scavengers, necessary to counteract the oxidative stress caused by training.

The similar time dependent variation of CK and LDH contents in the serum from the animals of both groups indicate that there was a progressive adaptation of the muscles to physical exercise during the time interval of the training. Furthermore, these results suggest that there was no relationship between the decrease in the antioxidant defences we observed and muscle damage, at least in our experimental model.

Finally, the increased cholesterol content, together with the increased body weight of the horses of both groups (about 2%), clearly indicate that the diet of the animals studied was probably unbalanced and inadequate for the special training that we used.

In conclusion, the results obtained indicate that it is necessary to test different diets during training, at least from a caloric point of view. Such diets should also be enriched with substances which can increase defences against free radicals. To verify whether this supplementation is adequate, animals must undergo a trial of strength at the end of the training period.

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A. Gaiti
G. Fruganti

Centro di Studio del Cavallo Sportivo
Università degli Studi di Perugia
Italy