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Original Research

Oxidative Stress Biomarkers and Erythrocytes Hemolysis in Well-Trained Equine Athletes Before and After Exercise

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ABSTRACT

The chronic exposure to regular exercise training seems to increase resistance to oxidative stress and improves the antioxidant defense system. The purpose of the present study was to investigate the effect of an exercise test of moderate intensity on oxidative stress biomarkers, antioxidant enzymes activity, and osmotic resistance of erythrocytes in well-trained equine athletes. Eighteen middle-aged horses of Ukrainian warmblood (8.3 ± 1.6 years) and Holsteiner (7.4 ± 1.9 years) breeds were used in this study. All horses have been in regular training for several years. The exercise test induced a significant increase of erythrocyte values, hemoglobin concentration, and hematocrit in horses of both breeds. Regular training induces activation the antioxidant enzymes and thereby can reduce oxidative stress in athletic horses. Our results suggest that the exercise test in horses of both breeds attenuates oxidative stress and accompanied with a significant decrease of lipid peroxidation and oxidatively modified proteins in erythrocytes after exercise. The findings of the present study demonstrated the elevated level of erythrocytes' catalase and glutathione reductase in Ukrainian warmblood horses, as well as decreased level of superoxide dismutase and glutathione reductase in Holsteiner horses reporting changes in levels of exercise-induced oxidative stress biomarkers in horses of both breeds. Statistically significant differences in the percentage of hemolyzed erythrocytes between pre-exercise and postexercise tests were observed and thereby signifying an oxidative stress-dependent impairment of erythrocyte stability. Our data suggest that oxidative stress and enzymatic antioxidant defense biomarkers can be used for the monitoring of fitness level, health benefits, and performance of equine athletes.

1. Introduction

Regular physical exercises, is believed, lead to increase the mean life span. They have benefits, are well known for improved cardiovascular system function, enhanced energy metabolism and antioxidant defense, provide greater muscle strength and endurance, and attenuate pathologic states [1,2]. However, exhausting physical exercise may induce oxidative stress because of excessive oxygen uptake and elevated generation of reactive oxygen species (ROS) in the mitochondrial electron transport system, as well as reactions catalyzed by enzymes such as xanthine oxidase and NADPH dehydrogenase [3–5].

Among athletic/sports animals, the horse has a unique ability to increase its oxygen uptake by a factor of 60 during heavy exercise, which may in turn increase the ROS release during the mitochondrial electron transfer (2%–5% of the mitochondrial oxygen consumption) [3,4]. As a result of massive ROS generation, proteins, nucleic acids, and membrane phospholipids may be more oxidatively modified in exercised tissues, possibly leading to deleterious consequences of oxidative stress [1–4]. There is increasing

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evidence that horses are exposed to exercise-induced changes of oxidant and antioxidant balance, depending on exercise type, intensity and duration, training status, ambient conditions, and presence of diseases [4–7]. Antioxidant supplementation including vitamins, microelements, macroelements, and antioxidants might have beneficial effect on the oxidant/antioxidant equilibrium [8–10].

It has been suggested that both strenuous long-duration exercise and exhaustive sprint training may overwhelm capacity to detoxify ROS action within blood cells [11]. Erythrocytes appear much more vulnerable to oxidative damage during intense exercise because of their continuous exposure to high oxygen fluxes and their high concentrations of polyunsaturated fatty acids and heme iron [11,12]. On the other hand, the erythrocytes contain cellular defense mechanisms against free radical–induced lipid peroxidation including both enzymatic and nonenzymatic antioxidants [3,11]. Recent evidence indicates that endurance training reduces erythrocyte susceptibility to oxidative stress and increases erythrocyte antioxidant defense against oxidative stress [2,11,13]. Numerous studies strongly indicate that ROS generation during exercise is associated with the adaptation processes involving redox-sensitive transcription, upregulation of antioxidant enzymes, and more effective repair of ROS-related molecular attacks [2,14]. Recent studies of equine exercise physiology have focused mainly on determining the usefulness of biochemical parameters for evaluating physiological capacity and adaptation to increasing loads [6–8,13,15]. Therefore, biochemical blood determinations give a more complete picture of the horse's response to loads and its adaptation to the training program. The aim of the present study was to analyze the oxidative stress and antioxidant defense biomarkers, as well as osmotic resistance of erythrocytes in well-trained Ukrainian warmblood (UWB) and Holsteiner horses before and after a moderate exercise test. It was also tested whether breed differences of blood oxidant/antioxidant status could be evidenced.

2. Materials and Methods

2.1. Animals

A total of 18 equine athletes in regular training were used in this study. All horses performed regularly in national jumping competitions. Among them, nine were well-trained UWB horses (three mares, four geldings, two stallions): age, 8.3 ± 1.6 years; body weight, 484.10 ± 27.96 kg; mean withers height, 166.44 ± 4.27 cm; mean chest volume, 188.55 ± 3.57 cm; mean body length, 161.88 ± 2.60 cm; mean body condition score, 3.5. The remaining nine were sport horses of Holsteiner breed (three mares, six stallions): age 7.4 ± 1.9 years, body weight, 551.08 ± 34.73 kg; mean withers height, 170 ± 1.80 cm; mean chest volume, 196.88 ± 5.15 cm; mean body length, 169 ± 3.27 cm; mean body condition score, 4.0. All horses had been in regular training for several years. All horses were involved in training for show jumping. The animals were selected on the basis of clinical examinations performed by qualified veterinarians and hematologic analysis to exclude pathologic conditions. The females were nonpregnant. All horses were dewormed and vaccinated at similar time. All animals underwent fitness training for several years, 6 days per week with a rest day on Sundays. Training started at 9 AM every day and lasted 1 to 1.5 hours. All horses were participated in the same daily training program and had three times per week a higher-level exercise bout consisted of warmup (10-minute walk, 30-minutes trot, and 10-minutes gallop) and a show jumping course consisting of 10 fences with an average height of 110 ± 10 cm. The riders and trainers provided information that each horse was trained according to its individual's abilities, as assessed by the trainer, to achieve each horse's optimal fitness. A subjective appreciation of the level of performance of each horse was made by the trainer. During the observation period, none of the horses participated in official competitions.

2.2. Diet

All horses were housed in the same environment in individual boxes at natural indoor temperature (18°C–20°C). All horses had the same diet. This diet was composed of grass meadow hay (6 kg), oats (6 kg), carrot (1 kg), and wheat bran (2 kg), distributed three times a day (digestible energy, 11.4 MJ; 6.71% digestible protein; 3.16% crude fat; 14.7% crude fiber), and provided an average intake of 157-mg β-carotene ; 320.46-mg vitamin E; 0.0225-mg vitamin D; 63.5-g lysine; 57.1-g calcium; 172.4-g phosphorus; 86.1-mg Cu; 426.4-mg Zn; 15.2-mg Na; 51.3-mg Cl (based on analysis of hay, oats, carrot, and wheat bran). Salt and water were available *ad libitum*.

2.3. Exercise Test

The study design consisted of one exercise test according to the training program. This test consists of a series of physical exercise of average intensity and is shown in Table 1. The exercise test was performed in an indoor riding hall with sandy cove. The exercise test and general animal care were carried out by professional staff not associated with the research team. The exercise test was made by

Table 1
Exercise test for all horses.

Gait	Duration (min)	Speed (m/min)
Ukrainian warmblood horses		
Walk	5	50
Trot	10	100
Walk	5	50
Trot	10	100
Walk	10	50
Gallop	10	350
Walk	10	50
Holsteiner horses		
Walk	5	70
Trot	10	120
Walk	5	70
Trot	10	120
Walk	10	70
Gallop	10	400
Walk	10	70

Duration of training was 1 hour.