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OXIDATIVE STRESS BIOMARKERS IN THE BLOOD OF WELL-TRAINED HORSES UNDER EXERCISE

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There is both direct and indirect evidence that heavy physical exercise enhances reactive oxygen species (ROS) production in skeletal muscle and other tissues. The elevated metabolic rate associated with physical exercises can increase mitochondrial O₂ consumption in muscle tissue and, consequently, mitochondrial ROS generation (Deaton and Marlin, 2003). There are numerous reports that provide reasonable support to the notion that exercise increases the ROS production and that mitochondria are important sources of these oxidants (Deaton and Marlin, 2003; Kirschvink et al., 2008). ROS lead to lipid peroxidation (LPO), which induces adverse effects on the health status and performance of horses. Intense or moderate exercise in horses may increase ROS production exceeding the capacity of antioxidant defenses (Deaton and Marlin, 2003; Kirschvink et al., 2008). However, the cell conserves highly specific (e.g., superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase etc.) and less specific (e.g., vitamins C and E, glutathione etc.) antioxidant mechanisms to counteract the effects of free radicals and/or oxidants. The main goal of the present study was to investigate the effect of regular exercise on oxidative stress biomarkers and antioxidant enzymes activity in well-trained Ukrainian warmblood horses (UWB).

Twenty three UWB horses (9 females and 14 males), involved in jumping, eventing and dressage were used. Since all horses used in various kinds of equestrian sport, to assess the effect of training on oxidative stress biomarkers level and antioxidant defense, was proposed common to all horses the exercises of average intensity: walk – 5 min; trot – 10 min; walk – 5 min; trot – 10 min; walk – 10 min; gallop – 10 min; walk – 10 min. Duration of training was 1 hour. Blood samples were collected from the jugular vein of each horse into ethylenediaminetetraacetic acid (EDTA) tubes at two points: baseline at rest (in the morning 90 minutes after feeding) and immediately after exercises. Oxidative stress biomarkers (2-thiobarbituric acid reactive substrates level, the carbonyl derivatives content of protein oxidative modification) and antioxidant defenses (superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase, ceruloplasmin activity and total antioxidant capacity) were assessed. All statistical analyses were performed using Statistica 8.0 software (StatSoft, Poland).

Our results suggest that exercise training session caused different consequences of oxidative stress biomarkers in the blood, plasma, and erythrocytes of horses. Training session results in decrease level of Thiobarbituric acid reactive substances (TBARS) byproduct of lipid peroxidation in erythrocytes by 14,3 % ($p<0.05$), while in blood and plasma does not. This difference in TBARS level between rest and training periods most likely is a consequence of differing levels of oxidative stress occurring in the tissues and erythrocytes. Reduced of aldehyde (by 42%, $p>0.05$) and ketonic derivatives level (by 27%, $p>0.05$) of proteins destruction in the erythrocytes after exercise indicates about exercise-induced adaptation. Exercise can induce the activity of the proteasome complex involved in the degradation of oxidatively modified proteins (Radak et al., 2008). The results of present study also showed an increase of superoxide dismutase by 34.4% ($p<0.05$) and glutathione reductase by 60.4% ($p<0.05$) in the blood of UWB horses after exercise. Increased level of antioxidant defenses after exercise session may indicate about protective response of antioxidant enzymes against exercise-induced oxidative stress in sport horses.

The positive correlation between aldehyde derivatives of protein oxidation in plasma and ceruloplasmin content ($R=0.543$, $p<0.05$) in

the blood of UWB horses at the rest period was noted. There was a negative correlation between erythrocytes' TBARS level and ceruloplasmin content ($R=-0.518$, $p<0.05$), in the blood of horses at the rest period. At the same time, erythrocytes' TBARS level correlated negatively with aldehyde derivatives of protein oxidation in plasma ($R=-0.761$, $p<0.05$) after training session. Blood TBARS level correlated positively with aldehyde derivatives of protein oxidation in erythrocytes' suspension ($R=0.602$, $p<0.05$) in the UWB horses. Significant correlation between glutathione reductase activity and total antioxidant capacity ($R=-0.534$, $p<0.05$) as well as plasma TBARS level ($R=-0.523$, $p<0.05$) after training was noted.

In conclusion, the results of present study showed an increase of antioxidant enzyme activity (superoxide dismutase and glutathione reductase) and attenuate of exercise-induced oxidative stress in the blood of horses after training session. In the present study the significant decrease in erythrocytes' TBARS level in horses after training session could be attributed as adaptation to training which accompanied activation the antioxidant defenses and changes in antioxidant enzyme activity in erythrocytes. The exercise-induced adaptation involves the process of antioxidant system activation, interferes with the oxidative damage repair/eliminating systems. The stimulating effect during exercise is ROS formation, which evokes specific adaptation, such as increased antioxidant/oxidative damage-repairing enzyme activity, increased resistance to oxidative stress, and lower levels of oxidative damage. The correlation analysis between markers of lipid peroxidation (blood and erythrocytes TBARS levels) and carbonyl derivatives contents of protein oxidative modification indicate about close relationship between lipid and protein oxidation under the influence of physical activity in sport horses. Moreover, correlative analysis between the oxidative stress biomarkers and antioxidant defenses in the horses after training session may indicate a protective response of glutathione reductase and ceruloplasmin activities against exercise-induced oxidative stress.

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