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Short Communication

Biochemical and Antioxidant Changes in Plasma, Serum, and Erythrocytes of Horses before and after a Jumping Competition

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A B S T R A C T

We designed an experiment to determine the concentrations of a marker of lipid peroxidation in erythrocytes of horses submitted to jumping competitions. Erythrocytes of exercised horses showed a significant increase in the concentration of thiobarbituric acid-reactive species content immediately after exercise ($P < .001$), which returned to normal levels 24 hours after exercise. Nonprotein sulfhydryl groups and superoxide dismutase activity (EC 1.15.1.1) in erythrocytes were significantly higher 24 hours after exercise, as compared with the resting period and control group ($P < .001$). Immediately after exercise, horses had increased serum concentrations of uric acid ($P < .002$) and plasma lactate, as well as increased creatine kinase (EC 2.7.3.2) and lactate dehydrogenase (EC 1.1.1.28) activities ($P < .001$), as compared with resting period and control group. All parameters returned to normal values 24 hours after exercise, except for uric acid serum levels that remained increased ($P < .001$). We conclude that the oxidative stress in erythrocytes of exercised horses may contribute to tissue damage. In addition, our results showed that horses submitted to a jumping competition showed higher production of free radicals and as a consequence, lipid peroxidation.

1. Introduction

Under normal conditions, natural antioxidant defenses are sufficient to neutralize reactive oxygen species (ROS). However, excessive production of ROS may overwhelm the antioxidant defense, especially during prolonged aerobic exercise or in times when antioxidant status is compromised by nutritional deficiencies or disease, causing oxidative stress. Exercise markedly increases oxygen uptake by active muscles and consequently increases generation of ROS. During exercise, there is an increased consumption of oxygen by the mitochondria, 2% to 5% of which is not completely reduced to water, which generates univalent reduced reactive oxygen [1]. In skeletal muscle, an overproduction of ROS caused by exercise can induce oxidative damage of muscular fibers [2-4]. Much attention has been paid to the role of lipid peroxidation and antioxidant system in exercise and physical training. Many studies have reported that acute submaximal exercise increases exercise-induced lipid peroxidation [5,6], whereas regular physical training causes an increase in the antioxidant system and a reduction in lipid peroxidation [7-9].

Several studies have suggested that individuals who exercise regularly, placing a constant oxidative stress on the muscles and other cells, have an augmented antioxidant

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defense system to reduce exercise-induced oxidative risk [10-13]. Studies have been performed to evaluate changes in biochemical and lipid peroxidation parameters in exercised horses and concluded that dietary supplementation with antioxidants, especially vitamins E, selenium, and ascorbate, decreases lipid peroxidation [10,14]. Exercise is a potent stimulator of ROS production and evidence suggests that ROS production may contribute to muscular damage. Thus, the objective of the current study was to determine the concentrations of a marker of lipid peroxidation in erythrocytes, plasma, and serum as an index of damage caused by ROS before and after a jumping competition.

2. Materials and Methods

2.1. Animals

The experimental animals were exercised horses (*Equus caballus*), 12 males and 13 females belonging to three different breeds: Brasileiro de Hipismo (nine), Quarter Horses (seven), and Thoroughbreds (nine), whose mean age was 11.7 $\pm$ 1.56 years. For the control group, 10 horses were used, six male and four female belonging to three different breeds: Brasileiro de Hipismo (five), Quarter Horses (five), and Thoroughbreds (four). This experiment was conducted at room temperature of 27°C, typical temperature for the season. Diets were fed to all horses considering a daily consumption of 2% of body weight of animals, based on dry matter (kg feed/animal/d). The diets were composed of coastcross grass hay, fed three times per day in the proportion of one-third of the forage and two-thirds of pelleted concentrated diet. Commercial pelleted concentrate diet for high-performance horses was provided by Supra Brazil (Alisul, São Leopoldo RS, Brazil). We aimed to verify the effect of physical exercise on levels of lipoperoxidation in a control group not submitted to any type of exercise.

First, the health condition of the horses was evaluated by clinical checkups of the animals during the resting period. Before the competition, the treatment horses were submitted to a warm-up period which consisted of different types of physical exercises with increased intensity. First, the horses were stimulated to walk with increased speed until they started trotting, followed by a series of 25 jumps, starting with a height of 80 cm and reaching up to 120 cm. After the warm-up session, the horses had 2 minutes of rest until the initiation of the competition, which consisted of 15 jumps of 110 cm of height and a total distance of 400 m. The average speed was estimated to be 350 m/min.

2.2. Blood Sample Collection

Blood samples from the experimental group and control group were collected at the same moment in three different time intervals: (1) during the clinical checkup with the animals in absolute rest (e.g., 07:00 AM hours), (2) immediately after the jumping competition (e.g., 07:00 AM hours), and (3) 24 hours after of the jumping competition. Samples from the control group were collected three times to minimize the interassay error. In total, 10-mL samples were collected from the jugular vein. In all, 5 mL of blood was collected in polypropylene tubes containing heparin and was gently mixed to avoid cell lysis. The heparinized blood was used to analyze the erythrocyte biochemistry, including thiobarbituric acid-reactive substances (TBARS), copper−zinc superoxide dismutase (CuZn-SOD) activity, and nonprotein sulfhydryl (NPSH) groups. Another 5 mL of blood was collected in regular polypropylene tubes to use for serum biochemistry assays.

Cell osmotic fragility test is based on the resistance of red blood cells to lysis as a function of decreasing sodium chloride (NaCl) concentration. Erythrocytes were washed twice in isotonic saline and incubated for 30 minutes at 37°C at decreasing NaCl concentrations (50, 25, and 0 mmol/L). After incubation, erythrocytes were centrifuged for 10 minutes at 1,500*g* and hemoglobin level was determined in the supernatants using commercial kits (Labtest, Belo Horizonte, Minas Gerais, Brazil). Hemolysis in each tube was expressed as a percentage in relation to the maximal release of hemoglobin obtained using distilled water (0% concentration of NaCl).

2.3. Biochemical Assays

Aspartate aminotransferase (EC 2.6.1.1), total protein, uric acid, lactate dehydrogenase (LDH, EC 1.1.1.28), and creatine kinase (CK, EC 2.7.3.2) were measured with commercial kits using enzymatic methods (Labtest, Belo Horizonte). Measurements of plasma lactate concentration were made on an Accusport (Boehringer, Mannheim, Germany).

2.4. Determination of Oxidative Stress

2.4.1. Thiobarbituric Acid-Reactive Species

TBARS were determined in erythrocytes according to the method described by Ohkawa et al in 1979 [15]. The blood samples were centrifuged for 10 minutes at 1,000*g*, and erythrocytes were washed three times with 0.9% NaCl (weight:volume). Next, the erythrocytes were diluted with 0.9% NaCl and adjusted to 50% hematocrit (1:1). This solution was precipitated with two volumes of 40% trichloroacetic acid. After centrifugation, the supernatant was removed and kept on ice for 30 minutes. TBARS were quantified by the addition of 1 mL of the supernatant fractions to the color reaction medium. The amount of TBARS produced was measured spectrophotometrically at 532 nm using malondialdehyde (MDA) for construction of the standard curve. Results were expressed as nmol MDA/L of erythrocytes diluted in hematocrit of 50%.

2.4.2. Superoxide Dismutase Activity in Erythrocytes

The CuZn-SOD (EC 1.15.1.1) assay method was based on the capacity of the enzyme in inhibiting the epinephrine autoxidation at alkaline pH. The reaction was observed at 480 nm, in accordance with the method described by Sun and Zigman in 1978 [16]. Results were expressed as IU/mg Hb.

2.4.3. Determination of Nonprotein Sulfhydryl Groups in Erythrocytes

The concentration of NPSH groups in the erythrocytes was determined using Ellman's reagent, 5,5′-dithiobis-2-nitrobenzoate. Blood samples were precipitated with two