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.
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 ± 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 sulphydryl (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 1,500g 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, 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 1,000g, 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 Sulphydryl 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
2.5. Statistical Analysis

Results are expressed as means ± SD. The results were evaluated by analysis of variance and Duncan’s test for contrast of mean (SPSS for Windows 8.0, SPSS 1998, Chicago, IL). Differences between groups were considered to be significant at P < .05.

3. Results

Biochemical parameters of oxidative stress in the erythrocytes of exercised horses showed a significant increase in the concentration of erythrocytes TBARS content immediately after exercise (P < .001), which returned to normal levels 24 hours after exercise, as compared with the resting period and control group (Table 1). Antioxidants defense represented for NPSH groups in erythrocytes of exercised horses did not increase immediately after exercise but was significantly increased 24 hours after exercise, as compared with the control group and at the resting period (P < .001). Similarly, CuZn-SOD activity did not increase immediately after exercise; however, it was significantly increased 24 hours after exercise, as compared with the control group and at the resting period (P < .001). Immediately after exercise, horses had increased serum concentrations of uric acid (P < .002), plasma lactate, as well as CK and LDH activities, as compared with control group and at the resting period (P < .001). Although all values of these parameters returned to initial values approximately 24 hours after exercise, the uric acid serum levels remained significantly increased (P < .001; Table 1).

Table 1

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Biochemical and antioxidant values in serum and RBC hemolysates from 25 horses submitted to a jumping competition at the rest period, immediately after the exercise, and 24 hours after exercise and from ten horses from the control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biochemical Assay</td>
<td>Exercised Group</td>
</tr>
<tr>
<td></td>
<td>Time 1</td>
</tr>
<tr>
<td>Serum</td>
<td></td>
</tr>
<tr>
<td>Total protein (g/L)</td>
<td>77 ± 10</td>
</tr>
<tr>
<td>Plasma lactate (mmol/L)</td>
<td>0.91 ± 0.05</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>120 ± 10</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>385 ± 38</td>
</tr>
<tr>
<td>Uric acid (mmol/L)</td>
<td>35 ± 16.12</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>302 ± 75.02</td>
</tr>
<tr>
<td>TBARS (nmol MDA/L)</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>NPSH (nmol/L whole blood)</td>
<td>28.01 ± 4.3</td>
</tr>
<tr>
<td>SOD (UI/mg Hb)</td>
<td>0.29 ± 0.03</td>
</tr>
</tbody>
</table>

AST, aspartate aminotransferase; CK, creatine kinase; LDH, lactate dehydrogenase; NPSH, nonprotein sulfhydryl groups; RBC, red blood cells; SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive species.

Values are expressed as mean ± SD. Units are given in parentheses.

*aCorresponds to P < .001, in comparison with before exercise.

*bCorresponds to P < .002, in comparison with before exercise.

4. Discussion

Plasma lactate concentration increased immediately after indicating an anaerobic component of exhaustive or strong acute exercise. The increase in erythrocyte MDA concentration, measured as TBARS, in horse after a jumping competition could be attributed to oxidative damage owing to free radicals being produced as a consequence of exercise. Simultaneously, an increase in erythrocyte antioxidant capacity was observed for both SOD activity and NPSH groups 24 hours after exercise. This can be attributed to a compensatory response to the increased amount of pro-oxidant species produced during strong exercise. Several studies showed the importance of the redox status maintained by NPSH groups [18-22]. Our results clearly indicated that short-term heavy exercise modulated the activity of two important antioxidant systems, which could in turn counteract the deleterious effect of hydroperoxide and other oxygen radicals, such as superoxide. In fact, previous data clearly indicate that exhaustive or strong acute exercise can result in the overproduction of superoxide [23-26]. Furthermore, contribution of endogenous antioxidant as SOD activity and NPSH groups seems to be adequate for controlling oxidative stress after exercise [27].

High-intensity exercise has been shown to cause a significant increase in uric acid content. Uric acid is an end product of purine metabolism and has been suggested to function as an antioxidant [28-30]. However, the available published data are controversial. According to Rasanen et al. [31] and McMeniman and Hintz [32], uric acid has antioxidant properties, whereas other authors, such as Patterson et al. [33], proposed a pro-oxidant property of uric acid.

However, all of them agree about the increase in uric acid levels after heavy exercise. According to Benzie
Strain [34], approximately 60% of the human plasma's ferric-reducing ability is determined by the uric acid content in the blood. Our results suggest that uric acid is the first line of antioxidant defense in horses submitted to a jumping competition.

The increase in activity of CK and LDH in the serum, immediately after exercise, could be mainly because of a selective increase of muscle membrane permeability [35]. This is produced by peroxidation phenomena occurring during heavy exercise, as determined by TBARS levels. Apparent muscle membrane alterations are transient, as shown by the decrease in serum CK activity 24 hours after exercise. Probably, the muscular damage attributed to oxidative damage was not too intense in view that the increase in activity of serum aspartate aminotransferase was not so elevated to a significant level.

The vulnerability of erythrocytes to osmotic shock is possibly caused by membrane properties linked to the membrane lipoperoxidation. However, the increase in antioxidant compensatory response by erythrocytes was detected by an increase in SOD activity, uric acid levels, and NPSH groups' concentration, suggesting a possible neutralization of the damage caused by lipoperoxidation in horses submitted to exercise.

5. Conclusion

Our results indicate that exercise increases induced lipid peroxidation in horse submitted to a jumping competition, but antioxidant defenses in extracellular fluids and blood cells protect against the damage caused by lipoperoxidation.

References

[34] Benzie IF, Strain JI. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 1996;239:70-6.