Status of biochemical and antioxidant variables in horses before and after long distance race

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SUMMARY

To study the effect of strenuous physical exercise on the levels of lipid peroxidation indices and some blood antioxidant activity in horses, blood of 60 horses subjected to different levels of a 120 km endurance race were collected. Blood was collected from each horse three times, before, at the end of the race (group 1 at 60km, group 2 at 90 km and group 3 at 120 km), and two weeks after the race. For each blood sample, the levels of lipid peroxides (LPO) and thiobarbituric acid reactive substances (TBARS), and the activities of glutathione peroxidase (GSH-Px) and creatine phosphokinase (CPK) were measured. The levels of LPO, TBARS and CPK were significantly (P < 0.01) increased in all horses at the end of the race. On the contrary, a significant (P < 0.05) reduction in the activity of GSH-Px was observed at the end of the race. The levels of LPO and TBARS, and the activities of GSH-Px and CPK returned to their normal levels 2 weeks after the race. The levels of LPO and TBARS in the blood of horses eliminated at the different check points were significantly higher (P < 0.05) than those of the horses able to run the 120 km race.

Keywords : horses - lipid peroxides - TBARS - glutathione peroxidase - creatine phosphokinase - endurance race.

Introduction

It has been suggested that changes in lipid peroxidation and eventually generation of free radicals may have an important role in the pathogenesis of exercise induced myopathies, hemolysis and fatigue in horse after strenuous exercise [2, 6, 30].

LOVLIN et al. [18] reported a decrease in plasma lipid peroxidation with exercise at 40 and 70 % of maximal oxygen uptake. Untrained horses are more likely to show high indices of lipid peroxidation after exercise [1]. In athletic man, the level of lipid peroxidation was minimal compared to non-athletes [28]. The levels of lipid peroxidation were found to be lower in trained horses and trained rats when compared to untrained ones [1, 17]. Thus, the activity of antioxidant enzymes increased significantly in trained rats [23].

This increase in lipid peroxidation during heavy exercise may suggest that the body’s defense system is unable to control the cascade of lipid peroxidation [16]. The failure of the body’s control of lipid peroxidation will result in the subsequent formation of lipid peroxides and free radicals, then in the accumulation of their final products as malondialdehyde in the different body tissues [7]. In addition to the role of the body defense mechanism, environmental factors such as humidity and temperature had been suggested to be correlated with the regulatory mechanism of lipid peroxidation [21].

A protective antioxidant mechanism is usually developed by the biological system in the body to overcome the various physical and chemical stresses. This antioxidant system including the enzymatic components (including catalase, glutathione peroxidase and superoxide dismutase) and the non-enzymatic components (including vitamins E, A and C), plays a vital role in the protection of biomembrane from oxidative damage [12]. In horses, supplementation of diets with antioxidants has been suggested to increase antioxidant defenses in extracellular fluids and blood cells [2, 9, 15, 22].

The aim of this study was to examine the changes in some biochemical and antioxidant variables of plasma and erythrocytes of horses participating at different levels in an endurance race.

Materials and Methods

STUDIED ANIMALS

Sixty healthy adult horses of different breeds (Arabian 14, Thoroughbred 13, Anglo-Arabian 10, Russian 1, and 22 horses of unknown breed) and comprising 29 geldings, 18 stallions, and 13 mares, all between 5 to 17 years old (mean age ± SD = 11.4 ± 2.7 year), which participated in The Emirates Airline Endurance Ride-120 km (Wadi-Rum, Jordan) were used in this study. The ride was held in early October, when ambient temperature ranged from 15 to 25 °C and relative humidity from 30 to 32 %. These meteorological values represent the actual values during the race and were obtained from the Jordanian Department of Meteorology. All horses were fed a diet consisting of barley, oat, corn, hay. Water was provided ad libitum. Owners were allowed to provide supplemental feed and salts to their horses. Information on supplementation of horse diets with antioxidant compounds, such as vitamin E and/or selenium, is not available. All horses had no medications during the two weeks prior to the ride. Information regarding levels of training and previous activities was not available.

All horses were subjected to a comprehensive physical examination. This physical examination includes monitoring horses’ vital clinical signs such as rectal temperature, heart rhythm, rate and sounds, capillary refill time, as well as respiratory rhythm, rate and depth, examination of the mucous membrane, skin recoil and gut sounds. In addition, the hydration status, gait of the animal, and presence of any injuries, especially in the legs, girth, withers and back were monitored. Only horses that had normal clinical parameters were allowed to participate in the endurance race. During the race, horses were physically examined at 5 check points placed at different places along the 120 km racetrack. At each check point, only qualified healthy horses with no abnormal cardiac, respiratory, gastrointestinal and musculoskeletal problems and with a pulse rate below 64 beat/minute and good hydration status were permitted to continue after a rest period of 30 minutes. Horses that failed to show normal parameters and/or with heart rate over 64 after a 30-minute rest were eliminated from the race.

Animals included in this study were grouped based on the distance that they passed before being eliminated from the race due to unfitness. Group 1 included animals that were eliminated at the 60 km check point. Group 2 are those that were eliminated at the 90 km check point. Group 3 are those that were able to finish the whole race (120 km). Blood samples were collected from horses at three different times: before the start of the race, during the first physical exam \( t_0 \); at the end of the 120 km race or at the time of elimination for groups 1, and 2 \( t_{\text{end}} \) and two weeks after the end of the race \( t_{\text{2w}} \). During the 2 weeks period that followed the race, horses were kept at minimal exercise level. Horses were bled from the jugular vein using polyethylene syringes with a 20 gauge needle and immediately transferred into two sealed vacutainer glass tubes, the first tube containing acid citrate dextrose anticoagulant, for the measurement of erythrocyte glutathione peroxidase (GSH-Px) and plasma creatine phosphokinase (CPK), the second tube containing lithium heparin anticoagulant for the measurement of plasma thiobarbituric acid reactive substances (TBARS), lipid peroxides (LPO), and hematological findings. All samples were placed on ice after collection and transferred to the laboratory without delay for analysis to avoid the in vitro oxidation (distance between the race site and the laboratory is 10 Km).

PARAMETERS MEASURED

Plasma levels of TBARS were analyzed spectrophotometrically after extraction with n-butanol according to the optimized method of Yagi [30]. Lipid peroxides in plasma were estimated with a test kit (K-ASSAY, LPO-CC; Kamia Biomedical Company, Seattle, WA, USA). In this kit, lipid peroxides are quantitated by colorimetrically measuring methylene blue (at 675nm) produced by cleavage of the methylcarbamoyl dimethylamin phenothiazine chromogen as a result of oxidation. To ensure reproducibility and minimal inter and intra batch variations the procedure was repeated on 20% blood samples. Glutathione peroxidase activities were measured by the oxidation of glutathione using tert-butyl hydro peroxide in red blood cells (RBC) hemolysates [25]. Creatine phosphokinase activity was estimated by a spectrophotometric test kit (Sigma, Chemical, St. Louis, MO 63178 USA). Packed cell volume was determined by the microhematocrit method while total hemoglobin, erythrocyte and leukocyte counts were measured according to previously reported procedure [26].

STATISTICAL ANALYSIS

The collected data were initially assessed for their normal distribution before doing further statistical analysis. The parameters from the 3 different horse groups were compared using a single-way ANOVA followed by a stepwise analysis (Bonferroni correction). All statistical analysis was performed using SPSS-v9® software.

Results

Erythrocyte counts, leukocyte counts, hematocrit and hemoglobin values of horses at the end of the race had significantly increased \( p < 0.05 \) when compared with the pre-race values. These values returned to their normal physiological levels within two weeks from the end of the race. However, the intrinsic characteristics of erythrocyte such as mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV) remained unchanged (Table 1).

Levels of LPO and TBARS as well as the activities of CPK and GSH-Px in the different horse groups are shown in Figure 1. The levels of lipid peroxides in the blood of the different horse groups were significantly \( p < 0.01 \) increased immediately at the end of the race (approximately 2-folds higher than that before or two weeks after the race). Results of re-testing of 20% of the blood samples to insure the LPO kit reproducibility revealed absence of inter- and intra-batch variations.

Similarly, the concentrations of TBARS in the plasma of horses at the end of the race were significantly ($p < 0.01$) higher than that before race. A significant reduction ($p < 0.05$) in the activities of glutathione peroxidase was noticed in all horses at the end of the race. These activities were regained two weeks after. On the other hand, the activities of CPK in the plasma of horses at the end of the race were significantly higher than that before or 2 weeks after the end of the race. The activity of CPK in the plasma of horses at the end of the race was approximately 5-folds higher than that before or two weeks after the end of the race.

The levels of lipid peroxides in the plasma of horses that were able to run the 120 km race at the three different sample points ($t_0$, $t_{end}$ and $t_{2weeks}$) were significantly ($p < 0.05$) lower statistically different ($p \leq 0.05$) within the different sampling times ($t_0$, $t_{end}$ and $t_{2weeks}$).

1 $t_0$: time before the start of the race; $t_{end}$: time at the end of the race for each group; and $t_{2weeks}$: time after 2 weeks from the end of the race.

2 MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration.

### TABLE I. - Hematological findings (Means ± SD) of horses at check points 60, 90, and 120 km.

<table>
<thead>
<tr>
<th>Horse Groups</th>
<th>LPO$^2$ (nmol/l)</th>
<th>TBARS$^2$ (nmol/l)</th>
<th>GSH-Px$^2$ (IU/gHb)</th>
<th>CPK$^2$ (IU/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1$^1$</td>
<td>3.8 ± 0.5</td>
<td>2.8 ± 0.1</td>
<td>73 ± 2.7</td>
<td>61 ± 2.8</td>
</tr>
<tr>
<td>Group 2$^1$</td>
<td>4.4 ± 0.5</td>
<td>3.6 ± 0.2</td>
<td>79 ± 2.0</td>
<td>62 ± 5.0</td>
</tr>
<tr>
<td>Group 3$^1$</td>
<td>2.3 ± 0.4*</td>
<td>1.8 ± 0.2*</td>
<td>75 ± 2.8</td>
<td>64 ± 3.0</td>
</tr>
</tbody>
</table>

* Statistically different ($p \leq 0.05$) from other mean values in the same column.

1 Group 1: horses eliminated at check point 60km; Group 2: horses eliminated at check point 90km; Group 3: horses ran the whole race (120km).

2 LPO: lipid peroxides; TBARS: thiobarbituric acid reactive substances; GSH-Px: Glutathione peroxidase; CPK: Creatine phosphokinase.
than that in the group of horses that were eliminated at check points 60 km and 90 km. A lower level of TBARS was detected in the plasma of horses that were able to finish the whole 120 km ($p < 0.05$). However, no significant differences were detected between the levels of CPK or glutathione peroxidase in the blood of horses that were able to finish the 120 km and those that were eliminated at check points 60 and 90 km (Table II).

**Discussion**

Increased oxygen consumption during exercise causes mitochondrial reactive oxygen species production and any resulting enhanced leakage of oxygen could prompt lipid peroxidation and tissue damage [14]. In this study, we investigated the effect of long distance race on the levels of LPO, TBARS, GSH-Px, and CPK in blood and plasma of horses. Changes in hemoglobin and packed cell volume observed in this study may not be due to significant alterations in the size or hemoglobin content of the red blood cell, but might be due to significant changes in plasma volume as a result of dehydration or splenic release of red blood cells with exercise [4].

Supplementation of diet with antioxidants such as vitamin E and selenium may influence the level of lipid peroxides and TBARS, since oxidative damage depend on the capacity of the scavenging mechanism, which is increased by high level of antioxidants in the body [9, 15]. Unfortunately, informations regarding supplementation of diet with antioxidants was not available in this study.

It was reported that, in untrained horses, exercise causes marked elevation in the LPO levels [1, 11, 29]. Our results showed that the levels of LPO were significantly lower before race ($t_0$) in horses that were able to complete the whole race (120 km) than in horses eliminated at check points 60 and 90 km.

The levels of TBARS were estimated using a spectrophotometric assay. Previous reports suggested that this assay is not specific for malondialdehyde (MDA) determination because amino acids, carbohydrate and bile pigments in the serum could give positive TBARS reaction. To improve TBARS specificity, additional HPLC separation step has been recommended [10]. Unfortunately, because of lacking of fund and equipments, we were not able to perform the HPLC separation step in this study.

Similarly, TBARS levels were significantly lower at $t_0$ in horses which completed the 120 km race, compared with those eliminated at check points 60 km and 90 km (Table II). TBARS is considered a marker for endogenous lipid peroxi-
dation and is usually used to express the magnitude of oxidative damage caused by the imbalance in antioxidant activities. Two weeks after the ride, the levels of TBARS were lower than those after the end of the ride, but they were higher than those before the race, which indicates the slow process of elimination of lipid peroxidation end products [6]. These results suggest that LPO and TBARS levels at resting could be used as markers for endurance capabilities during long distance races. In addition, LPO and TBARS at resting may reflect the adequacy of pre-race training and antioxidant diet supplementation. In addition, levels of LPO and TBARS can be influenced by stresses such as transportation of horses before the race. LPO and TBARS levels were lower in horses that completed the 120 km race, which indicate that the overall antioxidant capacity of tissues were greater in those horses allowing their muscles to withstand the oxidative stress caused by the strenuous exercise of long distance race. Similar findings were suggested previously in humans [8].

The significant increase of plasma CPK activity after completing the race in our study was similar to that reported following any physical exercise in man and animals [11]. Interestingly, the levels of CPK at t_end for the horses that completed the whole 120 km race were lower than the CPK level at the t_end for horses eliminated from the race at 60 and 90 km check points. The majority of horses eliminated at check points 60 and 90 km might have suffered from muscle soreness, exertional myositis, tying-up syndrome and articular or skeletal lameness which is usually associated with extensive muscle damage manifested by high level of CPK. This significant change in CPK following 60, 90, and 120 km exercise was accompanied by a significant change in the concentration of TBARS and LPO (Figure 1). A positive correlation was seen between these parameters, possibly indicating that the oxidative stress during long distance race is associated with some degree of muscle damage by a mechanism related to lipid peroxidation. Similar findings were reported previously [19]. Elevation of CPK activity could result also from mechanical compression of muscle during exercise [27]. However muscle blood perfusion could be controlled by the mechanical compression of the active muscles through training, which offers the advantage of better oxygen availability and byproduct removal [20].

CHAIVAT et al. [5] reported an up regulation in the activity of glutathione peroxidase enzyme in human after short and long distance exercise. On the contrary, our study suggested that the activity of glutathione peroxidase is significantly decreased immediately after race (Figure 1). Our results agree with those reported by BALOGH et al. [3] and ONO et al. [24]. The depletion in the activity of GSH-Px as a result of oxidative stress indicates that the antioxidant system in some of these horses worked effectively. These results were similar to those reported previously by HAR-GREAVES et al. [13].

In conclusion, we confirm that long distance endurance race in horses causes detectable biochemical and lipid peroxidation changes in the blood. In addition, results of this study suggest that LPO and TBARS levels in plasma of rested and exercised horses could be used as prognostic markers for selecting horses that can withstand long distance endurance rides. An additional work targeting the evaluation of antioxidant defense system through determination of antioxidant scavengers in horses subjected to heavy exercise is necessary to establish more reliable prognostic markers for selecting horses that can withstand heavy exercises.

References


