Antioxidant Status of Horses during Two 80-km Endurance Races\(^1,2\)

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EXPANDED ABSTRACT

**KEY WORDS:** antioxidant status, muscle leakage, endurance race, horse

Oxidative stress is a detrimental imbalance in the oxidative–antioxidative system of cells. It may damage DNA and contribute to aging or damage cell membranes, especially in muscles during strenuous exercise. Increased oxidation during exercise may be implicated in oxidative injury to muscle cells by free radicals and other reactive oxygen species (ROS)\(^5\) produced by oxidative reactions (1,2). If antioxidant systems become depleted during a bout of exercise, the susceptibility of cells and tissues to ROS damage is enhanced (3).

We propose that the antioxidant defenses of endurance horses are severely tested during prolonged and strenuous endurance exercise, and that the degree of oxidative stress may be related to muscle leakage and microtrauma, hydration status and animal welfare. The objective of this study was to evaluate the antioxidative status of horses competing in two endurance races over the same distance but under different ambient conditions.

**MATERIALS AND METHODS**

Thirty horses were studied during either the No Frills (NF, \(n = 12\)) or the Old Dominion (OD, \(n = 18\)) 80-km endurance races, in the Blue Ridge Mountains near Front Royal, VA. The NF and OD races were held in April and June 2000, respectively, and each race had a time limit of 12 h. Both races were conducted over similar terrain but dissimilar ambient conditions. The mean ambient temperature during NF was 5.3°C (4°C during prerace sample collection), and during OD was 28°C (range 18 to 34°C). Information on the dietary intake of antioxidants was not uniformly reliable and represents a limitation of this study. However, most endurance horses are provided with abundant vitamin and mineral supplementation (4). Veterinarians examined horses before and after the race, and during three rest stops. The Institutional Animal Care and Use Committee of Virginia Tech approved this protocol.

Race times, minus hold-times at rest stops, and mean speeds were calculated. At NF, a plastic weight tape, placed behind the elbow, was used to measure heath girth and estimate prerace body weight (BW). At OD, pre- and postrace BWs were measured by portable weight scales. Blood samples were collected at 0, 80 km and after 60-min recovery (REC) at NF, and at 0, 40, 80 km and REC at OD. Blood samples were analyzed for packed cell volume (PCV), total plasma protein (TPP), plasma \(\alpha\)-tocopherol (VIT E), erythrocyte glutathione (GSH) and glutathione peroxidase (GPX) at both NF and OD; ascorbic acid (VIT C), aspartate aminotransferase (AST) and creatine kinase (CK) were also analyzed at OD. A portable laboratory was set up at the race and blood samples were analyzed for PCV (microhematocrit centrifugation) and TPP (refractometer) on site, and all other samples were prepared and frozen on dry ice for later analyses. Plasma \(\alpha\)-tocopherol (VIT E) concentrations (5,6) and VIT C concentrations (7) were determined by HPLC procedures. Erythrocyte concentrations of GSH and GPX were determined using BIOXYTECH GSH-420 and GPX-340 colorimetric assays, respectively (8). Plasma AST and CK activities were determined using a chemical analyzer.

Data are summarized as means ± SE. Changes with time were evaluated with ANOVA, and significance was inferred at \(P < 0.05\). A post hoc Fisher's protected LSD test was performed to test for differences between means. Logarithmic transformations were applied to data not normally distributed. Data from NF and OD were not statistically compared. Simple regressions \((y = a + bx)\) of indices of muscle leakage \((y\); CK and AST) on indices of antioxidant status \((x\); VIT C, GSH, GPX) were performed. Statistical tests were performed using SAS procedures (9).

**RESULTS**

Mean race times were 7.44 h (range 6.02 to 9.12 h) at NF and 9.10 h (range 7.38 to 10.23 h) at OD. Mean speeds were 10.8 and 8.5 km/h at NF and OD, respectively. Mean BW of horses competing at NF was 420 ± 10 kg. Mean pre- and postrace BWs
of horses competing at OD were 444 ± 12 and 421 ± 12 kg, respectively. During NF, PCV at 0 km was 39 ± 1.7% and increased (P = 0.0004) to 52 ± 2.0% at 80 km. At OD, PCV at 0 km was 41 ± 1.1% and no changes (P = 0.901) were found at 40 km (41 ± 0.7%), 80 km (42 ± 0.8%) or REC (41 ± 0.8%). At NF, TPP concentration at 0 km was 5.8 ± 0.2 g/dL and tended to increase (P = 0.06) to 6.4 ± 0.2 g/dL at 80 km. At OD, TPP concentration was 5.7 ± 0.1 g/dL and no changes (P > 0.076) were found at 40 km (5.7 ± 0.2 g/dL), 80 km (6.1 ± 0.2 g/dL) or REC (6.1 ± 0.2 g/dL). At NF, α-tocopherol concentration was 5.8 ± 0.5 μg/mL at 0 km and no changes were found (P = 0.913) at 80 km or at REC (Fig. 1). At OD, α-tocopherol concentration was 5.0 ± 0.4 μg/mL at 0 km and no changes were found (P = 0.955) at 40, 80 km or REC (Fig. 1). At OD, mean VIT C concentration at 0 km was 46 ± 0.1 μg/mL and decreased (P = 0.002) by 15% at REC (Fig. 2). Erythrocyte GSH concentration at 0 km at NF was 223 ± 30 μmol/g and decreased (P = 0.031) by 36% at REC. At OD, erythrocyte concentration was 171 ± 29 μmol/g and decreased (P = 0.0001) by 59% at REC (Fig. 3). At NF, GPX activity at 0 km was 33 ± 5 mU/mg and no changes (P = 0.14) were found at 80 km (39 ± 7.0 mU/mg). At OD, GPX activity at 0 km was 7.5 ± 0.9 mU/mg and increased (P = 0.013) to 21.5 ± 4.2 mU/mg at 80 km. At OD, AST activity at 0 km was 280 ± 14 IU/L and increased (P = 0.010) to 352 ± 17 IU/L at 80 km. Plasma CK activity at 0 km was 277 ± 36 IU/L and increased (P = 0.011) to 611 ± 70 IU/L at 80 km (Fig. 4).

Plasma AST activities were correlated with erythrocyte GPX activities and GSH concentrations, but not with plasma VIT C concentrations. Plasma CK activities were correlated with erythrocyte GPX activities, erythrocyte GSH and plasma VIT C concentrations. The regressions are summarized in Table 1.

**FIGURE 1** Plasma vitamin E concentrations (α-tocopherol μg/mL) for horses that completed the 80-km No Frills (NF, n = 10, black bars) and Old Dominion (OD, n = 11, hatched bars) endurance races at 0, 80 km and after 60-min recovery (REC). Bars are means; flags are standard errors of the mean. Means with unlike letters are significantly different (NF, P = 0.913) or during OD (P = 0.955).

**FIGURE 2** Plasma vitamin C concentrations (ascorbic acid μg/mL) for horses that completed the 80-km Old Dominion (OD, n = 11) endurance race at 0, 40, 80 km and after 60-min recovery (REC). Bars are means; flags are standard errors of the mean. Means with unlike letters are significantly different (P = 0.002). Ascorbic acid concentrations decreased by 15% from 0 km to REC.

**FIGURE 3** Erythrocyte glutathione concentrations (μmol/g protein) for horses that completed the 80-km No Frills (NF, n = 10, black bars) and Old Dominion (OD, n = 11, hatched bars) endurance races at 0, 80 km and after 60-min recovery (REC) at NF, and at 0, 40, 80 km and REC at OD. Bars are means; flags are standard errors of the mean. Means within a race with unlike letters are significantly different (NF, P = 0.031; OD, P = 0.0001). Concentrations of GSH decreased by 36% from 0 km to REC in NF and by 59% in OD.

**FIGURE 4** Plasma creatine kinase activity (IU/L) for horses that completed the 80-km Old Dominion (OD, n = 11) endurance race at 0, 40, 80 km and after 60-min recovery (REC). Bars are means; flags are standard errors of the mean. Means with unlike letters are significantly different (P = 0.011). Plasma CK activities increased by 121% from 0 to 80 km.

**DISCUSSION**

These new findings of changes in antioxidant status and in indicators of muscle leakage during endurance exercise were associated with previously demonstrated markers of muscle damage and hypohydration. An unexpected finding was the maintenance of plasma α-tocopherol concentration. Similar results were
found in both races, despite differences in ambient conditions and speed.

Vitamin E is a radical scavenging antioxidant that inhibits the chain initiation and propagation of lipid peroxidation in cell membranes and attenuates oxidative damage. Vitamin E concentrations were maintained during NF and OD, and similar results were found in a 160-km race conducted concurrently with OD (10). Endurance horses in the UK competing in a 140-km Competitive Endurance Ride had no changes in α-tocopherol concentrations during exercise and after 16 h of recovery (11). Human plasma tocopherol concentrations increased during intense exercise (12), but exercise-induced changes in plasma volume were not accounted for. Sled dogs supplemented with vitamin E and vitamin C, or a placebo, had decreased plasma tocopherol and increased plasma ascorbate concentrations during 3 d of endurance exercise (13). Humans running a half-marathon had unchanged plasma tocopherol, increased plasma ascorbate and decreased GSH concentrations in samples taken immediately after running the race and 120 h postrace (14). Human plasma ascorbate concentrations increased immediately after a 21-km race and decreased at 24 h postrace to 20% below preexercise values for 48 h (15). Dissimilar responses in exercise-induced concentrations of plasma vitamins E and C, and GSH concentrations may reflect differences in species, exercise mode, intensity and duration, or whether values were adjusted for exercise-induced changes in plasma volume.

In this study, the maintenance of circulating α-tocopherol concentrations may be explained by the concomitant mobilization of α-tocopherol with fatty acids from adipose tissue stores (16), especially because fat is a major energy source during endurance exercise. Additionally, ascorbic acid regenerates α-tocopherol by reducing tocopheryl radicals produced by ROS reactions (17). Ascorbic acid concentrations decreased during OD, and may reflect radical scavenging and the support of circulating α-tocopherol concentrations. Similarly, erythrocyte GSH concentrations decreased during NF and OD, most likely through the regeneration of ascorbic acid by GSH (18,19); consequently, concurrent decreases in VIT C and OSH during racing may serve to sustain circulating α-tocopherol concentrations. Furthermore, this "sparking effect" of VIT E may have attenuated muscle CK leakage during the race, as reported in humans after intense endurance training (20,21). Muscle CK leakage was not reduced in sled dogs supplemented with vitamin E (13); however, plasma vitamin E concentrations of the sled dogs decreased during exercise, unlike the horses in this study. Large variances in muscle CK leakage between horses perhaps reflected this protective effect of VIT E, given that many horses had minimal changes in circulating CK levels during the race. Mean GPX activities at 0 km were fourfold higher at NF than at OD, indicating that horses were experiencing oxidative stress before the race, possibly because of lower ambient temperatures (shivering observed).

Evaluating the antioxidant status of endurance horses, by measuring concentrations of circulatory antioxidants, provides valuable information on the ability of the horse to cope with oxidative stress. Increased activities of muscle cell enzymes (plasma AST and CK) and enzymatic antioxidants (erythrocyte GPX) during endurance exercise are indicative of muscle cell leakage and may reflect oxidative damage. Associations between indicators of muscle cell leakage and of antioxidant depletion demonstrate the demands on the balance of the oxidative–antioxidative system of cells. These results suggest the testing of antioxidant supplements administered before and during a race to improve the performance and welfare of endurance horses.

ACKNOWLEDGMENT

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LITERATURE CITED

8. BIOXYTECH, Oxis International, Inc, Portland, OR.

TABLE 1

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1 Data for CK are logarithmically transformed.