Changes in Circulatory Antioxidant Status in Horses during Prolonged Exercise\textsuperscript{1,2}

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\textbf{ABSTRACT} Prolonged low-medium intensity exercise is associated with increased oxidative stress in humans. We hypothesized that competitive equine endurance racing would induce changes in circulatory antioxidants and produce systemic oxidative stress. Forty horses competing in a 140-km endurance race in warm conditions [shade temperature 15–19°C; 62–88% relative humidity (%RH)] were sampled before (Pre), immediately after exercise (End) and at approximately 16 h into recovery (+16 h). Plasma ascorbic acid concentration was not different between Pre [11.1 (median); 4.6–20.3 nmol/L (range)] and End [9.7; 3.0–38.9 (range) nmol/L] but was significantly decreased at +16 h (5.5; 2.8–15.5 nmol/L; \(P < 0.05\)). Total red cell hemolysate glutathione (TGSH) concentration was significantly reduced by exercise (Pre 1261; 883–1532 nmol/L; End 1065; 757–1334 nmol/L; \(P < 0.05\)) and at +16 h recovery (1032; 752–1362 nmol/L; \(P < 0.05\)). Glutathione redox ratio was unchanged by exercise but was significantly decreased at +16 h compared with that at both Pre and End (\(P < 0.05\)). The concentration of total barbituric acid reactive substances (TBARS) in plasma was increased compared with that at Pre (309; 66–1048 nmol/L), both at End (408; 170–1196 nmol/L; \(P < 0.05\)) and +16 h (380; 99–1161 nmol/L; \(P < 0.05\)). Total red cell hemolysate glutathione (TGSH) concentration was significantly reduced by exercise (Pre 1261; 883–1532 nmol/L; End 1065; 757–1334 nmol/L; \(P < 0.05\)) and at +16 h recovery (1032; 752–1362 nmol/L; \(P < 0.05\)). Glutathione redox ratio was unchanged by exercise but was significantly decreased at +16 h compared with that at both Pre and End (\(P < 0.05\)). The concentration of total barbituric acid reactive substances (TBARS) in plasma was increased compared with that at Pre (309; 66–1048 nmol/L), both at End (408; 170–1196 nmol/L; \(P < 0.05\)) and +16 h (380; 99–1161 nmol/L; \(P < 0.05\)). \(\alpha\)-Tocopherol was unchanged by exercise or recovery. Mean race speed was 16.5 ± 1.6 km/h and ranged from 13.9 to 19.7 km/h. Mean speed during competition in horses that completed the full 140 km (\(n = 28\)) was significantly correlated with end of exercise ascorbic acid (\(r = 0.622; P = 0.0004\)). Although there were increases in creatine phosphokinase (CK), aspartate aminotransferase (AST) and TBARS and a loss of TGSH, this study failed to demonstrate evidence of classical oxidative stress. J. Nutr. 132: 1622S–1627S, 2002.

\textbf{KEY WORDS:} • horse • reactive oxygen species • antioxidants • prolonged exercise

The role of free radicals, reactive oxygen species (ROS)\textsuperscript{4} and antioxidants in many aspects of health and disease has become of increasing interest. Free radicals are molecules or single atoms that have an unpaired electron in their outer orbit, which decreases stability and increases their reactivity with other molecules or atoms. Reactive oxygen species have been defined as oxygen-containing molecules that are more reactive than the triplet oxygen molecule present in air (1). Not all radicals are unstable and the tocopheroxyl and dehydroascorbate radical are examples of stable radicals.

The presence of ROS in the body is a natural occurrence and they have a number of beneficial physiological roles, including synthesis of biologically essential compounds, energy production and phagocytosis. However, it is also well documented that ROS can be detrimental and can be linked to several diseases such as diabetes, heart disease and cancer, aging and also to some of the deleterious effects of exercise (2,3). Exercise is a potent stimulator of ROS production and there is increasing evidence to suggest that ROS production may contribute to exercise-induced disturbances in muscle homeostasis as well as muscle fatigue and injury (4). The concentrations of ROS within the body are therefore normally well controlled and mechanisms exist to prevent or limit damage.

Enzymic and nonenzymic antioxidants form part of the body’s defense mechanisms to suppress the formation of ROS and also to scavenge radicals to reduce their damaging effects.
such as lipid peroxidation. Primary antioxidant enzymes in cells include superoxide dismutase, glutathione peroxidase and catalase, and each detoxifies a particular ROS. There are many nonenzymic antioxidants in cells and on cell membranes, including α-tocopherol (lipophilic) and ascorbic acid and glutathione (hydrophilic). Maintenance of the balance between ROS and antioxidants is important because oxidative stress occurs when ROS production exceeds antioxidant buffering capacity. This can result from increases in ROS production or depletion of antioxidants.

Oxidative stress has been shown to occur with specific modes of exercise, although the severity and level of tissue damage have not always been consistent between studies, even when using similar protocols. This may be related to variations between different studies in a number of factors, including age, diet, previous training history and analytical methods (2,3,5).

Prolonged low-medium intensity exercise has been the mode of exercise most frequently reported to induce marked oxidative stress in humans (6–8). As far as we are aware there are only limited reports in the literature of antioxidant status and markers of oxidative damage in horses competing in endurance rides, but these were covering 80 or 160 km in 12 and 24 h, respectively, at relatively slow mean speeds (9,10).

The present study was therefore undertaken to determine the concentrations of the major nonenzymic antioxidants in equine blood and plasma and the concentrations of a marker of lipid peroxidation as an index of damage from ROS before and after a 140-km competitive endurance race ride.

MATERIALS AND METHODS

Competition

The horses in the study were competing in the Tattersalls CEI (A) Newmarket 140-km ride held under Federation Equestre Internationale rules and regulations in association with the British Endurance Riding Association and British Equestrian Federation rules in July 2000. The course consisted predominantly of grass tracks with approximately 10 km of roads, the remainder being all-weather surfaces, farm tracks and bridleways with minimal changes in altitude. Environmental conditions during the period of the ride ranged from 15 to 19°C (shade temperature) and 62 to 88% relative humidity. The first blood sample was collected following the first inspection between 4 and 6 h before the ride (Pre). The second sample was collected when the horses had finished competing (End). This was either when the horse was withdrawn, eliminated following presentation at a mandatory veterinary examination ("vet gate") during the ride or after the final "vet gate" (end of ride). All samples were taken within 5–30 min of each horse reaching a "vet gate". The third sample was taken between 7 and 9 AM the day after the ride, approximately 16 h from the end of the ride (+16 h).

Animals

The total number of horses starting was 68, of which 42 (62%) completed, 24 were withdrawn or eliminated during the ride and 2 eliminated at the finish. Out of this total entry of 68 horses, blood samples were collected from 60 horses whose riders had agreed to participate at the rider briefing. Blood samples at all three time periods were collected from 40 horses. Of these 40 horses sampled, 28 finished the ride (70%), 10 were eliminated or withdrawn prior to the final vet gate and 2 were eliminated at the finish. The 40 horses sampled were predominantly Arab or part-bred Arab (33/40). The mean age was 11 y (SD ± 2.5; range 7–17). Diet could not be controlled in this study and, as a result, the animals sampled would have been fed according to each individual rider's preferences.

Sample collection and processing

Blood samples (20 mL) were collected by venipuncture using a syringe and needle (1.5 in. × 19 g). Samples were aliquoted into tubes containing lithium and heparin (3 × 5 mL) and potassium EDTA (5 mL) and placed on ice. After centrifugation at 400 g for 10 min, 5-mL aliquots of plasma were snap frozen and stored at −80°C for uric acid, iron (Fe) and α-tocopherol analysis. For ascorbic acid analysis, 0.5 mL of EDTA plasma was added to 0.5 mL of 10% metaphosphoric acid containing 1 mmol Na2EDTA (Sigma-Aldrich Company Ltd, Poole, Dorset, UK), vortexed, snap frozen in amber tubes and stored at −80°C. For hemolysate glutathione analysis, 0.5 mL of red blood cells was added to an equal volume of 0.9% NaCl containing 2 mmol Na2EDTA (Sigma-Aldrich Company Ltd), snap frozen and stored at −80°C. Lithium heparin plasma (1 mL) for total barbituric acid reactive substances (TBARS) analysis was added to the evaporated residue of 120 μL of butylated hydroxy toluene (BHT; 100 mg/mL in ethanol; ICN Biomedicals, Aurora, OH) and 120 μL deferal (100 mg/mL in water; Sigma-Aldrich Company Ltd), vortexed and stored at −80°C.

The samples collected at the first inspection were processed within 60 min of collection, whereas the samples collected following competition and at 16 h post were processed within 30 min.

Sample analysis

HPLC with electrochemical detection was used to measure reduced (GSH) and oxidized glutathione (GSSG) in red blood cell hemolysates (11). Total glutathione (TGSH) = GSH + GSSG and glutathione redox ratio (GRR) = GSSG/TGSH × 100. Plasma uric acid was determined using a standard kit (Sigma kit 685-10). Plasma Fe was analyzed according to the method of Pepper et al. (12). Plasma TBARS were analyzed using a fluorometric assay. Samples were defrosted, spun (5000 g/5 min), and 0.2 mL plasma was added to 1 mL 0.4% thiobarbituric acid and 0.15 mL 0.2% BHT. The samples were heated to 95°C for 45 min, cooled on ice, extracted into butanol and the fluorescence was read using an excitation wavelength of 532 nm and an emission wavelength of 550 nm. A standard curve was prepared using standards of 0, 0.25, 0.5, 1, 1.5, 2 and 3 μmol/L tetraethyloxepropane. Plasma ascorbic acid was measured using HPLC with uv detection (245 nm). The mobile phase consisted of ammonium phosphate/metaphosphoric acid buffer (pH 2.5) flowing through a 25-cm 5-μm Hypersil column. Defrosted samples were spun (5000 g/5 min) and the supernatant diluted 1:1 in the mobile phase and filtered before injection. The concentration of α-tocopherol in plasma was measured by HPLC according to the method described by Kędzierska et al. (13). Chemicals were purchased from Sigma-Aldrich Company Ltd or Rathburn Chemicals (Walkerburn, Scotland, UK). The concentrations of glucose and total protein and the activities of creatine phosphokinase (CK) and aspartate aminotransferase (AST) in plasma were measured using routine clinical chemistry procedures on an autoanalyzer.

Statistical analysis

Data were analyzed for normality of distribution using the Shapiro–Wilks test. The majority of data sets were found not to be normally distributed. In each case, a simple transformation using either the natural logarithm or square was applied, depending on the direction of the skew. The Shapiro–Wilks test was then reapplied on the transformed data. This approach resulted in some but not all of the data sets approximating normality of distribution. Therefore, further analysis was undertaken using nonparametric tests. To investigate differences over time (Pre vs. End vs. +16 h), the Kruskal–Wallis test was used. When significance was reached (P < 0.01), further investigation to determine differences between means was undertaken using the Wilcoxon signed-rank test. Data from horses that completed the full distance (Finishers) and those that did not (Nonfinishers) were compared using the Mann–Whitney U-test. Data are presented as median, 10th, 25th, 75th and 90th percentiles and range, unless otherwise indicated. The relationship between mean speed during competition of those horses that completed 140 km and all measured variables at the end of exercise or at +16 h.
recovery were investigated using Spearman’s rank correlation coefficient, as was the relationship between biochemical variables at Pre and End exercise and between all biochemical variables at End exercise. All statistical analysis was performed using Statmost V3.0 (Datamost Corp., South Sandy, UT).

RESULTS

The mean speed of the horses sampled that completed the total 140 km (n = 28) was 16.5 ± 1.6 km/h and ranged from 13.9 to 19.7 km/h.

CK, AST, Fe, total protein and glucose in plasma the day before competition (Pre), at the end of competition (End) and the day after competition (+16 h) are shown in Table 1. Plasma Fe concentration was significantly decreased by exercise and did not return to normal in the recovery period. Plasma CK increased significantly from a median of 222 IU/L (Pre) to 2154 IU/L at the end of exercise (End). Although there was a marked decrease in plasma CK from End to +16 h, it was still significantly elevated compared with Pre at this time. There was a significant increase in the median concentration of AST after exercise of 133 IU/L from a Pre median of 368 IU/L. Between End and +16 h the median AST activity showed no further change (P > 0.05). Plasma total protein concentration was significantly increased by 6.8% as a result of exercise and at 16 h recovery was significantly lower than that at Pre (69.8 vs. +16 h 73.2 g/L, respectively, P < 0.01). Plasma glucose concentration decreased significantly from Pre (median 5.3 mmol/L) to End competition (4.3 mmol/L; P < 0.01) and was significantly elevated compared to that at both Pre and End at +16 h recovery (6.2 mmol/L; P < 0.01).

Concentrations of GSH, GSSG and TGS in red blood cells and glutathione redox ratio and concentrations of ascorbic acid, α-tocopherol, TBARS and uric acid in plasma are shown in Figure 1. GSH, GSSG and TGSH all decreased significantly from Pre to End exercise. GSH and TGSH showed no further change from End to +16 h, but GSSG was further decreased at +16 h (P < 0.001). Glutathione redox ratio did not change significantly with exercise but did decrease following recovery compared to that at both Pre and End. Plasma ascorbic acid concentration in individual horses either increased, decreased or remained unchanged from Pre to the end of exercise and overall was not significantly different. However, plasma ascorbic acid concentration was significantly decreased at +16 h compared to that at both Pre (P < 0.00001) and End (P < 0.0001). Plasma α-tocopherol concentrations were not significantly altered by exercise or at recovery. There was a relatively narrow range in plasma uric acid concentrations before exercise (7.3 to 17.4 μmol/L and a significant increase in uric acid at the end of exercise (P < 0.0001). By +16 h the median uric acid concentration (9.8 μmol/L) had fallen to below that at Pre (11.8 μmol/L).

Figure 1 Reduced (GSH), oxidized (GSSG) and total glutathione (TGSH) and glutathione redox ratio (GRR) in red blood cells and plasma ascorbic acid, α-tocopherol, total barbituric acid reactive substances (TBARS) and uric acid in 40 horses before (Pre), at the end of (End) and at approximately 16 h (+16 h) after a 140-km endurance competition. Data are presented as 10th, 25th, 50th (median), 75th and 90th percentiles and range (n = 40). Time points with different letters differ by at least P < 0.05.

<p>| TABLE 1 |
| Plasma iron (Fe), creatine kinase (CK), aspartate aminotransferase (AST), total protein and glucose in 40 horses before (Pre), at the end of (End) and at approximately 16 h (+16 h) after a 140-km endurance competition1 |</p>
<table>
<thead>
<tr>
<th>Pre</th>
<th>End</th>
<th>+16 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe, μmol/L</td>
<td>15.6A</td>
<td>9.3B</td>
</tr>
<tr>
<td>CK, IU/L</td>
<td>10.1–24.1</td>
<td>4.8–19.7</td>
</tr>
<tr>
<td>AST, IU/L</td>
<td>6.3–27.3</td>
<td>2.2–33.2</td>
</tr>
<tr>
<td>Total protein, g/L</td>
<td>222A</td>
<td>515B</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>157–538</td>
<td>217–2.849</td>
</tr>
<tr>
<td>TGSH, g/L</td>
<td>130–1,241</td>
<td>241–114,720</td>
</tr>
<tr>
<td>GSH, μmol/L</td>
<td>368A</td>
<td>501B</td>
</tr>
<tr>
<td>GSSG, μmol/L</td>
<td>284–563</td>
<td>381–1,409</td>
</tr>
<tr>
<td>TBARS, μmol/L</td>
<td>251–624</td>
<td>312–5,000</td>
</tr>
</tbody>
</table>

1 Data are presented as median, 10th to 90th percentiles and range. Means with different superscripts differ by at least P < 0.01.
h (median 5.9 μmol/L; P = 0.0086) compared with those of Nonfinishers (n = 12) (median 15.5 and 8.6 μmol/L, respectively). Plasma Fe was also significantly lower at End (median 9.1 μmol/L; P = 0.0008) and +16 h (median 9.7 μmol/L; P = 0.02) in Finishers compared with that of Nonfinishers (median 19.6 and 14.1 μmol/L, respectively). In contrast, GSH (median 1015 μmol/L; P = 0.018) and TGSH (1052 μmol/L; P = 0.02) were higher at +16 h in Finishers than in Non-Finishers (median 917 and 953 μmol/L, respectively).

There were significant correlations between mean speed during competition in horses that completed the full 140 km (n = 28) and end of exercise ascorbic acid (r = 0.622; P = 0.0004). All significant correlations found are shown in Table 2.

**DISCUSSION**

In the present study, approximately 8.5 h of competitive endurance exercise in cool-warm conditions at an estimated mean intensity of 30–40%VO2 max produced a significant reduction in red blood cell TGSH (because of a decrease in both GSH and GSSG) and an increase in plasma TBARS. This was in the absence of any significant changes in plasma ascorbic acid, α-tocopherol or GRR. At 16 h after the end of exercise TBARS still remained elevated and TGSH was still reduced and, in addition, plasma ascorbic acid and GRR were both lower than that before competition. Thus, in this group of horses as a whole, endurance exercise produced evidence of lipid peroxidation and changes in some antioxidants, but not marked oxidative stress. However, individual horses did demonstrate marked evidence of oxidative stress.

A limitation of the present study is that feeding could not be controlled or standardized because of the large number of horses studied. Therefore it is conceivable that some changes may be related to differences in diet. In addition, as samples were collected in the evening (Pre), morning (+16 h) and at various times of the day (End), an effect of diurnal variation cannot be excluded. As far as we are aware, significant diurnal variation of antioxidants or markers of oxidative stress measured in the present study have not been described in the horse.

The red blood cell GSH, GSSG and TGSH concentrations and the GRR before competition in the present study are similar to those reported previously in healthy horses by Mills et al., Art et al. and Kirshvink et al. (14–16). Plasma ascorbic acid concentrations are similar to those reported for racehorses in training by Snow et al. (17) but approximately half that reported by Hargreaves et al. (9). Unlike humans, but like most other vertebrates, the horse has no dietary requirement for ascorbic acid.

Concentrations of α-tocopherol in the present study are approximately twice those found in untrained 3-y-old stallions (18), untrained ponies and trained polo ponies (19), but similar to those reported elsewhere for endurance horses (9). Plasma Fe concentrations before the competition are considerably lower than those reported for trained elite 3-d event horses (20), or trained Thoroughbred horses (14). Mean uric acid concentration in plasma at rest has been reported to be between 7 and 14 μmol/L (14,21) and is similar to that in the present study. A wide range of values for TBARS in horses at rest have been reported, including as low as 2 nmol/L (22) to 7000 nmol/L (19). The mean concentration of plasma MDA (which in the horse accounts for over 90% of the concentration of TBARS; Smith and Marlin, unpublished observation), in untrained horses reported by Avellini et al. (18) of approximately 120 nmol/L appears to be the closest to the concentrations measured in the present study, although the range was from approximately 100 to 1000 nmol/L even before competition.

Following a short duration (<10 min) incremental exercise test to fatigue, Mills et al. (14) reported no significant changes in GSH, GSSG, TGSH, GRR or Fe at 5 min postexercise compared with preexercise, and no changes between 5 min and 24-h recovery. However uric acid was significantly increased by approximately sevenfold following exercise. When the same horses were exercised on a separate occasion for 60 min at intensities of 10, 30, 75 and 90%VO2 max (20°C/40%RH) there was a significant increase in GSSG and uric acid but no change in GRR. However, when the same exercise protocol was repeated in thermally stressful conditions (30°C/80%RH), there were significant increases in both GSSG and GRR, and a greater increase in uric acid. Increases in uric acid can be explained on the basis of adenine nucleotide loss through AMP degradation to IMP and eventually uric acid (23). In the present study an increase in speed at the finish, as was observed in some horses, could have accounted for increases in plasma uric acid. However, glycogen depletion and subsequent ATP depletion could also have contributed to uric acid production. Kirshvink et al. (16) reported no significant changes in GSH, GSSG, TGSH or GRR, but a small increase in uric acid, in trained healthy horses following an intermittent, incremental exercise test consisting of three, 2-min gallops at an estimated exercise intensity of approximately 80, 90 and 100%VO2 max.

Hargreaves et al. (9) reported that in endurance horses covering a distance of 160 km in 24 hours, plasma ascorbic acid was significantly decreased at the end of the ride compared with prerace concentrations, which is in contrast to the present study. However, the prerace concentrations of ascorbic acid were approximately twice those in the present study. Hargreaves et al. (9) also reported no change in α-tocopherol, in agreement with our study. These authors also reported increases in CK and AST but these were not as marked as in the present study. Direct comparison of the two studies is difficult because the study by Hargreaves et al. (9) was over 24 hours at a lower mean speed, (8-9 km/h) and in more thermally stressful conditions.

Whereas there are a limited number of publications describing changes in antioxidants and markers of oxidant damage with exercise in the horse, there are a considerable number of publications examining the impact of exercise in humans.
Concentrations of red blood cell GSH, GSSG and TGSH at rest in trained and untrained males are similar to those reported in the present study (7, 24, 25). Resting Fe concentrations in humans are also similar to those seen in the present study (26, 27).

Plasma concentrations of ascorbic acid, α-tocopherol and uric acid appear to be considerably higher in man compared with the horse. For example, plasma ascorbic acid concentrations from 43 μmol/L (28) to 176 μmol/L (29) have been reported, which are considerably higher than those seen in horses. Also α-tocopherol concentrations in male athletes are approximately three times higher and uric acid concentrations are approximately 10–20 times higher (7, 28) than those reported in horses in the present study and by others (14, 18, 19).

In humans, as in the horse, there is a wide range in reported values for plasma TBARS concentrations, from a low of 860 nmol/L (25) to a high of 9280 nmol/L (28). There does seem to be some agreement, however, in the values reported by Itoh et al. (30), Balakrishnan and Anuradha (31), Child et al. (32) and Sanchez-Quesada et al. (32), all of which are about 3000 nmol/L. Thus, in general, resting plasma TBARS concentrations appear to be much greater in human than in the horse.

Different responses to exercise in red blood cell glutathione have been reported in humans. For example, Laaksonen et al. (25) reported a significant decrease in GSH following 40 min exercise at 60% VO_{2 max}, which is similar to the response seen in the present study, although Sastre et al. (24) found no change following an incremental exercise test to exhaustion. In the same studies a significant increase in GSSG with exercise was demonstrated and Laaksonen et al. (25) also found a trend toward a decrease in TGSH, which is also in agreement with our study. However, following 35 min running at 60% VO_{2 max}, Camus et al. (29) reported no significant changes in GSH, GSSG, TGSH or GRR. In marathon competition, only GSH appears to have been measured and did not change (7).

Following a marathon, concentrations of α-tocopherol and ascorbic acid in male athletes have been reported to either be unchanged (6) (which is the same as in the present study), for ascorbic acid to be increased but α-tocopherol to be unchanged (7) or for both α-tocopherol and ascorbic acid to be increased (28). Although in the present study five individual horses showed marked increases in ascorbic acid with exercise, this was not accompanied by any change in α-tocopherol. Overall ascorbic acid did not increase significantly with exercise and both increases and decreases were seen. However, end exercise ascorbic acid concentration was significantly correlated with mean speed. It has been suggested that ascorbic acid concentration in plasma may increase as a result of efflux of ascorbate from the adrenal glands mediated by increases in cortisol (34). Endurance exercise of the type used in the present study has been shown to produce a threefold increase in plasma cortisol (35) and the magnitude of the increase might reasonably be expected to relate to exercise intensity (i.e., mean speed).

A number of studies have reported significant increases in uric acid in male athletes following both full and half marathons (6, 7, 28, 32), which compares with the present study, although Sanchez-Quesada et al. (33) reported no change. Following a 142-km run as part of an ultramarathon race, Fallon (26) reported a significant decrease in plasma Fe similar to that in the present study; however, following an incremental treadmill exercise test to fatigue, Kaczmarski et al. (36) found no significant changes.

An increase in plasma TBARS concentration of approximately 200 nmol/L has been reported following a half marathon (32) and an increase of approximately 600 nmol/L after 40 min exercise at 60% VO_{2 max} (25), which is similar to the mean increase of 174 nmol/L found in the present study. However, following full marathons either no significant change in the concentration of TBARS (33) or even a significant decrease has been reported (28).

The median increase in CK following exercise in the present study of 1932 IU/L is comparable to that previously reported by Rose et al. (35) of approximately 1500 IU/L for a 160-km ride but at a slower mean speed of 14 km/h (compared with 16.5 km/h in the present study). These increases are approximately 20 to 40 times greater than reported for human marathon runners after covering 42 km during competition (7, 28) and almost double the increase reported for sled dogs covering a distance of 77 km at a mean speed of 16.9 km/h (37).

In human marathon runners, antioxidant supplementation has been shown to both reduce (7) or have no effect on exercise-induced increases in CK (28). Two studies in horses have failed to demonstrate any reduction in CK following supplementation with either ascorbic acid (22) or α-tocopherol (38). Similarly, Piery et al. (37) found no attenuation in the CK increase in exercising sled dogs as a result of being fed an antioxidant supplement containing vitamins E, C and β-carotene. The results of the present study would seem to be in agreement, given that despite marked variation between horses in individual antioxidant concentrations precompetition, there was no clear relationship with CK or AST changes. It may be that this approach is too simplified and that it may be necessary to look at the sum or combinations of different antioxidants or an index of total antioxidant capacity in relation to changes in CK and or AST.

Despite the fact that horses have relatively low concentrations of systemic nonenzymic antioxidants, at least compared with humans, both the degree of oxidative stress observed in the present study and the degree of oxidative damage, as determined by changes in TBARS as a marker of lipid peroxidation, were both much lower than anticipated, given the intensity and duration of exercise. A possible limitation of the present study is that assessment of the enzymic antioxidant pathways were not included and therefore their contribution cannot be determined. It should also be considered that circulating antioxidants and markers of oxidative damage may not necessarily reflect those within the muscles that are the major site of ROS production during exercise. It is also possible that TBARS are not a sensitive enough marker of ROS damage in the horse during exercise, although there were significant negative correlations between Pre competition TGSH and α-tocopherol and end competition TBARS. This suggests that horses with low antioxidant reserves may be more prone to lipid peroxidation by ROS produced during prolonged exercise.

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LITERATURE CITED

ment. Int. J. Sport Nutr. 4: 205–220.
1033.
7. Kaikkonen, J., Kosonen, L., Nyyssonen, K., Pirkkala-Sarataho, E., Sa-
lonen, R., Korpeila, H. & Salonen, J. T. (1998) Effect of combined coenzyme Q10 and a-tocopherol acetate supplementation on exercise-induced lipid per-
12. Pepper, J. R., Mumby, S. & Gutteridge, J. M. (1994) Transient iron-
18. Avelidis, L., Chiaradia, E. & Gatti, A. (1999) Effect of exercise training, sodium oxide and vitamin E on some free radical scavengers in horses (Equus caball-
33. Sanchez-Quesada, J. L., Jobra, O., Payes, A., OtaI, C., Serra-Grina, R., Gonzalez-Sastre, F. & Ordonez-Llanos, J. (1998) Ascorbic acid inhibits the increase in low-density lipoprotein (LDL) susceptibility to oxidation and the pro-
portion of electronegative LDL induced by intense aerobic exercise. Coron. Artery Dis. 9: 249–255.