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# Antioxidant supplementation and subsequent oxidative stress of horses during an 80-km endurance race<sup>1</sup>

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**ABSTRACT:** This study tested the development of oxidative stress and the effects of antioxidant supplementation in an 80-km ride. A precompetition survey revealed that no competitor would participate without vitamin E supplementation; therefore, 46 horses were paired for past performances and randomly assigned to two groups of 23 each for 3 wk of supplementation before the ride. One group (E) was orally supplemented with 5,000 IU of vitamin E per day; the other group (E+C) received that dose of vitamin E plus 7 g/d of vitamin C. Blood samples, temperature, and heart rate were taken the day before the race, at 21 and 56 km during the ride, at completion, and after 20 min of recovery. Plasma was assayed for lipid hydroperoxides,  $\alpha$ -tocopherol, total ascorbate, albumin, creatine kinase (CK), and aspartate aminotransferase (AST). Total glutathione and glutathione peroxidase activity were de-

termined in red blood cells and white blood cells. Thirty-four horses completed the race, 12 horses (six in E and six in E+C) did not finish for reasons including lameness, metabolic problems, and rider option. Plasma ascorbate was higher ( $P = 0.045$ ) in the E+C group than in the E group. Other than ascorbate, neither antioxidant status nor CK and AST activities were affected by supplementation with E+C vs. E. Red blood cell glutathione peroxidase, white blood cell total glutathione, lipid hydroperoxides, CK, and AST increased, and red blood cell total glutathione and white blood cell glutathione peroxidase activity decreased with distance ( $P < 0.001$ ). Positive correlations were found for plasma lipid hydroperoxides on CK ( $r = 0.25$ ;  $P = 0.001$ ) and AST ( $r = 0.33$ ;  $P < 0.001$ ). These results establish an association between muscle leakage and a cumulative index of oxidative stress.

Key Words:  $\alpha$ -Tocopherol, Ascorbate, Endurance Exercise, Equine, Muscle Enzymes, Oxidative Stress

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## Introduction

The welfare of competing sport horses has attracted public attention following deaths at Olympic and other championships (Jeffcott and Kohn, 1999). Welfare can be partially assessed by objective indicators of stress (heart rate and various blood metabolites; Broom, 1991). Evidence of oxidative stress in horses has been

described in reports dealing with intense (Chiaradia et al., 1998; White et al., 2001) and endurance exercise (Frankiewicz-Jozko and Szarska, 2000; Marlin et al., 2002).

Oxidation provides energy for maintenance of cellular integrity and functions. Most of the consumed oxygen forms carbon dioxide and water; however, 1 to 2% of the oxygen is not completely reduced and forms reactive oxygen species (Clarkson and Thompson, 2000). When antioxidant systems are insufficient, oxidative processes may damage DNA, lipids, and contribute to degenerative changes, including aging and cancer (Harman and Piette, 1966). Lipids are protected directly by  $\alpha$ -tocopherol in the membranes and by other antioxidants, including ascorbic acid (Chan, 1993).

Vitamin E was supplemented to horses above and below current recommendations (McMeniman and Hintz, 1992). Plasma lipid peroxidation increased with

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exercise, especially in horses with low plasma  $\alpha$ -tocopherol. Another study found that a single bout of submaximal exercise did not affect plasma  $\alpha$ -tocopherol but suggested that horses in conditioning may require higher levels of vitamin E supplementation (Siciliano et al., 1996). Ascorbate potentiates the effects of  $\alpha$ -tocopherol by reducing its radicals and restoring its activity (Chan, 1993). However, at maintenance, horses have the ability to synthesize sufficient ascorbic acid, but increasing demands possibly create a dietary requirement.

This field study tested two hypotheses: 1) that muscle membrane leakage may be associated with oxidative stress during endurance exercise and 2) that these changes may be abated by supplementation with vitamins E and C compared to vitamin E alone.

## Materials and Methods

Forty-six trained endurance horses (35 Arabians, 9 part-Arabians, 1 Thoroughbred, and 1 grade type), 10.8  $\pm$  0.6 yr of age, participated in the Middleburg Research Ride, on April 1, 2001. The protocol was approved by the Institutional Animal Care and Use Committee and performed at the Virginia Tech Middleburg Agricultural Research and Extension Center.

All riders responding to a precompetition survey—which detailed nutritional management (grain, hay, pasture, supplements, etc.), training regimen (distance and time), performance (races and miles completed), and medical history—insisted on continuing supplementation with vitamin E at their customary dosages (1,500 to 4,800 IU/d in total diet). In effect, this requirement determined our positive control treatment. Horses were paired by grain intake, experience, and past performance and then randomly assigned to two groups of electrolyte supplementation (Hess et al., 2002) and two groups of vitamin supplementation in a 2  $\times$  2 factorial design. Three weeks before the competition, horses terminated all supplements and started their treatments. One group (**E**) was orally supplemented with 5,000 IU/d of DL- $\alpha$ -tocopheryl acetate (1 mg of synthetic DL- $\alpha$ -tocopheryl acetate is 1.00 IU; however, 1 mg of natural D- $\alpha$  tocopheryl acetate is 1.36 IU of vitamin E; Ames, 1979), and the other group (**E+C**) with the same  $\alpha$ -tocopherol dose plus 7 g/d of ascorbic acid (Loscher et al., 1984; Snow et al., 1987). The treatment groups did not differ in terms of grain and nutrient intake of the horses (Table 1).

The race covered 80 km in northern Virginia, with terrain ranging from 121 to 442 m of elevation. Ambient temperature ranged from 4.7°C in the morning to 10.7°C in the afternoon and evening, with 90 to 100% relative humidity. Horses were weighed without tack the day before the race, at the 56-km veterinary check, and after completion of the race on an electronic scale (Tyrel platform, TC-105, Alweights Hamilton Scale Corp, Richmond, VA). Veterinary checks were performed according to the American Endurance Ride Conference rules (Mackay-Smith et al., 1999) on the day

**Table 1.** Grain intake (as a percentage of total diet) and nutrient composition (feed manufacturer information) of the grains fed to horses in the vitamin E and vitamin E plus C groups combined<sup>a,b</sup>

Nutrient	Amount	SEM
Grain intake		
(% of total diet)	16.9	1.8
CP, %	11.9	0.38
Crude fat, %	7.6	1.3
Crude fiber, %	9.2	1.0
Ca:P	1.4	0.05
Copper, ppm	44.6	3.2
Zinc, ppm	143.3	11.4
Selenium, ppm	0.56	0.05
Vitamin E, IU/kg	203.3	43.4
Vitamin A, IU/kg	9721	635
Ascorbic acid, mg/kg	226.5	38.9

<sup>a</sup>All values are on an as-fed basis.

<sup>b</sup>The remainder of total diet consisted of free-choice pasture or hay.

before the race at 1300 to 1800 and at 21, 37, 56, 72, and 80 km during the race. Blood samples were collected in sodium heparin vacuum tubes (Becton Dickinson and Company, Franklin Lakes, NJ) via jugular venous puncture the day before the race (**PRE**), within 2 min after the horse entered a veterinary check and immediately before the physical examination at 21, 56, and 80 km, and after 20 to 30 min of recovery (**REC**). Rectal temperature and heart rate were also recorded at these stages. Blood samples were placed immediately in crushed ice and transported to the laboratory within 15 to 30 min to be processed into red blood cell (**RBC**), white blood cell (**WBC**), and plasma aliquots.

For assays using RBC lysate, 500  $\mu$ L of whole blood was transferred to a microcentrifuge tube and centrifuged at 2,500  $\times$  g for 5 min at 4°C. The plasma was removed and discarded from the sample. The pellet was then frozen at  $-80^\circ\text{C}$  until analysis, when it was thawed and lysed by 1 mL of ice-cold deionized water. For the determinations using WBC, the buffy coat was removed after centrifugation of whole blood at 2,500  $\times$  g for 5 min at 4°C, and transferred to a tube containing 10 mL of lysis buffer (0.15 M  $\text{NH}_4\text{Cl}$ , 0.01 M  $\text{NaHCO}_3$ , 0.03 M EDTA free acid). White blood cells were washed once in the lysis buffer to lyse any residual RBC, and then washed twice in Hank's Balanced Salt Solution (**HBSS**; Life Technologies, Carlsbad, CA). The pellet was then reconstituted in 1 mL of HBSS and mixed thoroughly; next, 0.5 mL was transferred into microtubes and frozen at  $-80^\circ\text{C}$  until sample analysis. Plasma aliquots were prepared by centrifuging the Vacutainer tubes at 2,500  $\times$  g for 5 min at 4°C and then transferring the plasma supernatant to microtubes, which were frozen at  $-80^\circ\text{C}$  until sample analysis.

Red blood cell lysate and WBC were analyzed for total glutathione (**GSH-T**; Biotech GSH-420, kit No. 51023; Oxis Health Products, Inc., Portland, OR; interassay CV 7.0%, intraassay CV 5.6%) and glutathione peroxidase activity (**GPx**; Biotech GPx-340, kit #51017;

Oxis Health Products, Inc.; interassay CV 4.2%, intraassay CV 5.0%) using an OxyScan Automated Oxidative Stress Analyzer. Total plasma lipid hydroperoxides (LPO; Biotech LPO-560, kit #21025; Oxis Health Products, Inc.) were analyzed using a spectrophotometer (interassay CV 3.0%, intraassay CV 4.6%). Creatine kinase, ascorbic acid, and ALB were analyzed using spectrophotometric assays (Beckman Instruments Inc., Brea, California). Total ascorbic acid and  $\alpha$ -tocopherol were analyzed by high-pressure liquid chromatography (Schiiep et al., 1987; Hargreaves et al., 2002). Plasma ascorbic acid (ASC) and concentrations at successive stages were adjusted for changes in fluid redistribution during exercise using plasma albumin concentration of albumin (ALB), giving adjusted variables (ASCadj and for  $\alpha$ -tocopherol [TOCadj], respectively), as follows:

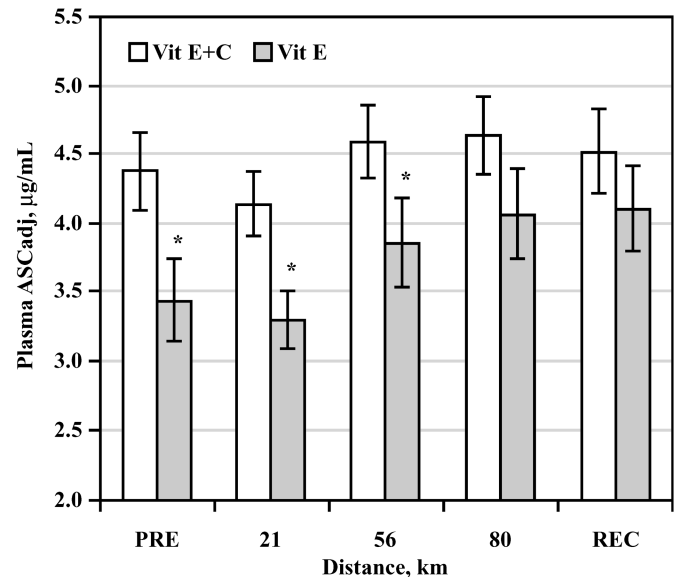
$$\text{ASCadj} = \text{ASC} \times (\text{PRE ALB}/\text{stage ALB})$$

The main data analysis was confined to horses that finished the 80-km race, and it was followed by certain comparisons of finishers and nonfinishers. Data were found to have normal distributions using the Shapiro-Wilk statistic. Outliers were determined as being greater than 2 SD from the mean and then dropped from the analysis using Fisher's normal deviant ( $z$ ). These data were summarized as least squares means  $\pm$  SEM. Treatments, stage of race (PRE through REC), and interaction were evaluated by ANOVA in a mixed model with repeated measures (SAS Institute Inc., Cary, NC). The data showed no interactions between the electrolyte and vitamin treatments, so the electrolyte treatment was removed from the statistical model. Significance was inferred when  $P < 0.05$ . The associations of muscle membrane leakage, oxidative stress, and antioxidant status were tested using Pearson's product-moment correlation. Horse was included in the model to test for significance, and, if insignificant, it was removed from the model.

## Results

The horses averaged  $421 \pm 5$  kg BW before the race,  $400 \pm 5$  kg at the 56-km veterinary checkpoint, and  $406 \pm 5$  kg at the 80-km completion of the race. Thirty-four horses completed the race (E = 17, E+C = 17), with none appearing heavily exerted. The reasons for 12 horses not finishing included rider option (5), lameness (3), exertional rhabdomyolysis (2), insufficient heart rate recovery index (1), and lack of gut sounds (1). The mean time including veterinary checks was 9 h 15 min, and the range was from 7 h 22 min to 11 h 22 min. During exercise (21 and 56 km), heart rate and rectal temperature ranged from 104 to 52 beats/min and 40.6 to 37.3°C, respectively; at REC, heart rate ranged from 66 to 44 beats/min and rectal temperature ranged from 38.9 to 37.6°C.

Plasma ascorbic acid concentration was  $3.8 \pm 0.1$   $\mu\text{g}/\text{mL}$  in the E group and 18% higher ( $P = 0.045$ ) in the



**Figure 1.** Plasma ascorbate adjusted for albumin (ASCadj) for 34 horses completing an 80-km endurance ride in the vitamin E-supplemented group (E;  $n = 17$ ) and the vitamin E and C supplemented group (E+C;  $n = 17$ ) before (PRE); 21, 56, and 80 km during; and after recovery (REC) of an 80-km endurance race. \*Treatments are different at  $P < 0.05$  for first three stages, and  $P = 0.045$  overall.

E+C group (Figure 1). Overall plasma  $\alpha$ -tocopherol concentration was  $5.4 \pm 0.16$   $\mu\text{g}/\text{mL}$  with no difference found between supplements ( $P = 0.86$ ). Two horses had extremely high  $\alpha$ -tocopherol values, and two had low values (one high and one low from each E and E+C group,  $11.4 \pm 0.1$  and  $1.9 \pm 0.4$   $\mu\text{g}/\text{mL}$ , respectively), which contributed to the large variation.

Heart rate, temperature, plasma LPO,  $\alpha$ -tocopherol (up to 56 km), CK and AST, and WBC GSH-T increased ( $P < 0.001$ ), whereas RBC GPx (up to 56 km), RBC GSH-T, and WBC-GPx decreased ( $P < 0.0001$ ) with distance and WBC GPx increased during recovery (Table 2). Patterns of change with stage were similar for RBC GSH-T and WBC GPx, and for RBC GPx and WBC GSH-T.

Plasma CK and AST activities increased ( $P < 0.001$ ) during the overall ride and remained high at REC (Figure 2). High outlier values were found for plasma CK in three horses and AST in one horse at 56 km, 80 km, and REC, without clinical signs of exertional rhabdomyolysis (stiffness of muscle, abnormality of gait, myoglobinuria, etc.). Positive correlations were found for plasma LPO on CK ( $r = 0.22$ ;  $P = 0.007$ ) and AST ( $r = 0.32$ ;  $P < 0.0001$ ).

Plasma LPO was lower ( $P < 0.05$ ) at 21 and 80 km in the finishers than at 21 km and the last stage before withdrawal of nonfinishers ( $n = 4$ ; Figure 3). Red blood cell GSH-T was higher ( $P = 0.005$ ) in finishers at 80-km than in nonfinishers ( $n = 12$ ). Plasma CK and AST were also higher ( $P = 0.001$ ) in nonfinishers eliminated for metabolic reasons ( $n = 4$ ) than in finishers (CK >

**Table 2.** Least squares means  $\pm$  standard errors for plasma lipid hydroperoxides (LPO),  $\alpha$ -tocopherol (TOC),  $\alpha$ -tocopherol adjusted for albumin (TOCadj), and red blood cell and white blood cell (RBC and WBC, respectively) total glutathione (GSH-T), and glutathione peroxidase (GPx) in 34 horses before (PRE); 21, 56, and 80 km during; and after recovery (REC) of an 80-km endurance race

Variable	PRE	21	56	80	REC	SEM
LPO, $\mu\text{M}$	8.19 <sup>a</sup>	7.48 <sup>a</sup>	9.55 <sup>a</sup>	14.5 <sup>b</sup>	16.6 <sup>b</sup>	1.3
RBC GSH-T, $\mu\text{mol/g}$ protein	129 <sup>a</sup>	173 <sup>b</sup>	144 <sup>ac</sup>	150 <sup>c</sup>	93.8 <sup>d</sup>	4.9
WBC GSH-T, $\mu\text{mol/g}$ protein	18.0 <sup>a</sup>	19.4 <sup>a</sup>	20.8 <sup>a</sup>	30.1 <sup>b</sup>	30.6 <sup>b</sup>	1.3
RBC GPx, mU/mg protein	46.9 <sup>ac</sup>	41.9 <sup>b</sup>	44.5 <sup>bc</sup>	49.1 <sup>ad</sup>	52.7 <sup>d</sup>	1.3
WBC GPx, mU/mg protein	114 <sup>ab</sup>	130 <sup>a</sup>	104 <sup>b</sup>	117 <sup>ab</sup>	165 <sup>c</sup>	5.4
TOC, mg/dL	5.18 <sup>a</sup>	5.47 <sup>ab</sup>	5.81 <sup>b</sup>	5.42 <sup>ac</sup>	5.36 <sup>ac</sup>	0.4
TOCadj, mg/dL	4.92	5.17	5.50	5.13	5.01	0.4

<sup>a,b,c,d</sup>Means within a row with different subscripts differ ( $P < 0.05$ ).

5,000 IU/L and AST > 3,000; out of measurement range).

## Discussion

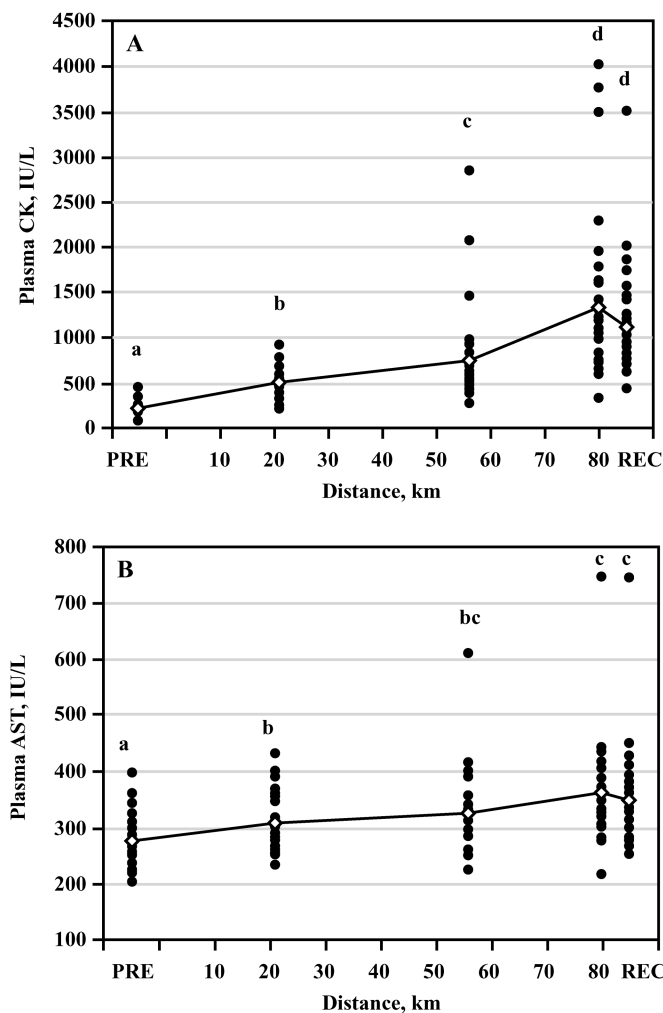
Our results confirm previous reports of increased muscle leakage during endurance exercise in horses (Frankiewicz-Jozko and Szarska, 2000; Hargreaves et al., 2002; Marlin et al., 2002). New findings concerning LPO and WBC antioxidants demonstrate possible oxidative stress. Differences between present results and other studies relate to ascorbic acid correlations with muscle enzymes and improvements in antioxidant status with ascorbic acid supplementation.

In general, blood and plasma GSH-T reflect recent changes in muscle cells (Noakes, 1987; Harris, 1998). The usual pattern during exercise is a progressive increase that continues for some minutes during recovery and then declines over 18 h, which was observed here and in previous studies of intense and endurance exercise in horses (Mills et al., 1996; Chiaradia et al., 1998; Hargreaves et al., 2002; Marlin et al., 2002). The 27% increase in RBC GPx observed in the last two stages of this race likely reflects a response to utilize reduced glutathione during the radical-scavenging process (reduced glutathione donates an electron to reduce a wide variety of hydroperoxides using GPx as a catalyst). It also reflects the consumption of pro-oxidants generated during exercise. In various breeds of horses racing 160 km, another study revealed that whole-blood GPx increased four- to fivefold about halfway through the race and returned to baseline at 12 h of recovery (Frankiewicz-Jozko and Szarska, 2000). In well-conditioned racing sled dogs, however, RBC GPx did not change during three 1-d stages of 54-km exercise (Hinchcliff et al., 2000). A likely explanation of the above results is that training enhances the capacity of the antioxidant system, as manifested by lesser perturbation of the RBC glutathione/glutathione peroxidase system, even dur-

ing harder competition. It has been previously shown that regular endurance exercise training results in increased GPx activity in active skeletal muscle (for review; Powers et al., 1999). This up-regulation is limited to the oxidative skeletal muscle fibers.

In contrast to the RBC changes, novel findings here were the changes in the WBC glutathione system. Fluctuations of WBC GPx during exercise and the sharp 41% increase during recovery (REC different from other samples at  $P < 0.0001$ ) may reflect replenishment of reduced glutathione; however, the reduced:oxidized ratio was not measured for technical reasons during this field study. Compared to RBC, the higher concentration of WBC GPx and lower WBC GSH-T may affect phagocyte oxidative burst and other immune functions during prolonged exercise. Although moderate endurance training in athletes may enhance the immune system, it was found that exhaustive endurance exercise might be detrimental (Vider et al., 2001). It was determined that human endurance athletes undergoing exhaustive endurance exercise have increased lipid peroxidation (thiobarbituric acid-reactive substances, or TBARS) and increased antioxidant status (whole-blood GSH-T). Positive correlations between GSH-T and lymphocyte mitogenic response (concanavalin A and phytohemagglutinin) after exercise have been reported in humans (Vider et al., 2001). Combining the WBC results from the present study with the whole blood and lymphocyte results from Vider et al. (2001) suggests that there could be a connection between oxidative stress and immune function.

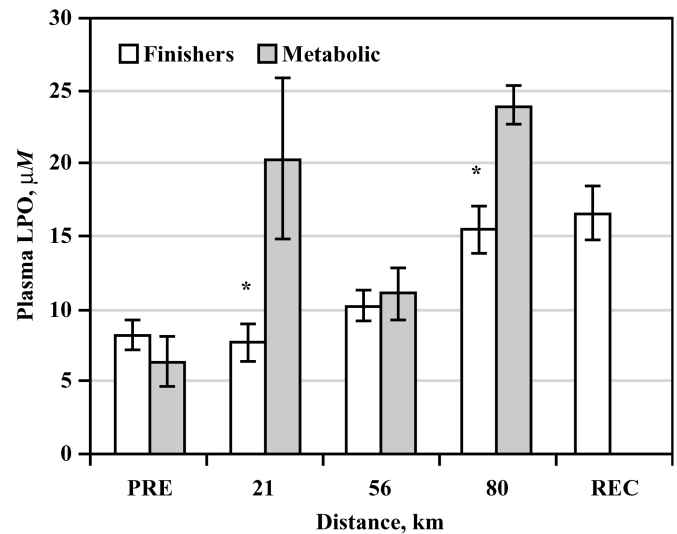
The plasma LPO increase during the race and at 30 min of recovery (Figure 3) may be taken to reflect cumulative cell membrane lipid peroxidation. In a previous simulation of the endurance phase of a 3-d event during intense heat and humidity, an increase in lipid hydroperoxides continued for 30 min of recovery and then decreased to baseline by 24 h (Mills et al., 1996). In other equine studies (Chiaradia et al., 1998; Frankie-



**Figure 2.** Plasma activities of creatine kinase (CK; upper) and aspartate aminotransferase (AST; lower) for 34 horses completing an 80-km endurance race in the vitamin E-supplemented group and the vitamin E and C supplemented group combined before (PRE); 21, 56, and 80 km during; and after recovery (REC). Individual observations are shown by the closed circles, and the mean is indicated by the open diamonds connected by a line. <sup>a,b,c</sup>Different subscripts indicate differences between the means of the distance ( $P < 0.001$ ).

wiez-Jozko and Szarska, 2000; White et al., 2001; Marlin et al., 2002), plasma TBARS, which are another index of lipid peroxidation, increased at the end of exercise and remained high for hours after recovery. A study on Thoroughbreds showed an increase in TBARS (1.7 to 2.2 nmol/L) after a race (White et al., 2001). In another report (Marlin et al., 2002), TBARS had a wide variation before exercise (66 to 1,048 nmol/L) and increased after exercise (150 to 1,200 nmol/L). Apparent CV of >40% for TBARS (Marlin et al., 2002) are greater than the CV of 5% for LPO in the present study.

Plasma ascorbic acid concentrations were lower in the E group than in the E+C group at rest. This difference progressively diminished during the race as



**Figure 3.** Plasma lipid hydroperoxides (LPO) for the horses that finished an 80-km endurance race (treatment groups combined;  $n = 34$ ), and horses that were removed because of metabolic problems ( $n = 4$  at each sample). Samples were taken before (PRE); 21, 56, and 80 km during; and after recovery (REC). Distance "80" represents 80 km for the finishers and the point in the race where the nonfinishers were eliminated. The ANOVA main effect of LPO increasing with distance was significant for the finishers at  $P = 0.001$ . \*Finished vs. metabolic differ,  $P < 0.05$ .

ascorbic acid increased in the E group but remained unchanged in the E+C group. This could be due to an increased mobilization of intracellular ascorbic acid stores in the E group, whereas the E+C group was able to maintain ascorbic acid levels using the exogenous source for its antioxidant capacity. These findings contrast with a previous result from our laboratory, in which a decrease in plasma ascorbic acid during a highly competitive and difficult 80-km race was found (Hargreaves et al., 2002). Plasma ascorbic acid also decreased during a race and through the racing season in sled dogs (Donoghue et al., 1993; Hinchcliff et al., 2000), and the season decline was prevented by vitamin C supplementation, 1 g/Mcal ME (Donoghue et al., 1993), compared to about 0.3 g/Mcal ME in the present study.

Plasma ascorbic acid ranges were similar to other reports from our laboratory (Hargreaves et al., 2002). The range of the current study was almost twice as wide as in one previous endurance study (Marlin et al., 2002), and one-half that found in a 1,000-m run to maximum velocity (White et al., 2001). These differences in plasma ascorbic acid ranges between studies could be attributable to dietary vitamin C presence and dosage, assay variation, lack of adjustment for water shift due to dehydration, conditioning of horses, or intensity of effort.

The plasma  $\alpha$ -tocopherol range was wide in the present study (1.75 to 12.88  $\mu\text{g}/\text{mL}$ ) and in previous reports concerning endurance horses (Frankiewicz-Jozko and Szarska, 2000; Hargreaves et al., 2002; Marlin et al., 2002). Vitamin E intakes of horses in this study were about 5 times the recommended minimum when on the treatments (NRC, 1989), compared with 1.2 to 5 times in the 46 horses reported in the pre-race survey before the treatments were administered. Vitamin E intakes were not reported in previous studies (Frankiewicz-Jozko and Szarska, 2000; Hargreaves et al., 2002; Marlin et al., 2002). The previous plasma  $\alpha$ -tocopherol ranges were similar to the present results, which suggest comparably high vitamin E intakes of endurance horses in Spain (Frankiewicz-Jozko and Szarska, 2000) and England (Marlin et al., 2002) as in the United States (Hargreaves et al., 2002). A beneficial effect of high intakes of vitamin E on oxidative stress may partially account for the universal acceptance of this supplementation.

A study of polo ponies used similar E and E+C groups (Hoffman et al., 2001). Throughout the polo season, plasma  $\alpha$ -tocopherol and ascorbic acid were higher in the E+C group than the E group in hard-working ponies, but not those in light work. These observations may reconcile the endurance findings in our laboratory where changes were observed in the highly competitive, mid-season races (Hargreaves et al., 2002), but not in this lightly competitive, early-season race. In a survey taken after the race, riders ranked the exertion level of the endurance ride as being easier than most of the rides later in the competition season. Also, ambient temperature was cooler in this race than in the summer, when the majority of endurance competitions are held.

Plasma increases in CK and AST, especially during the last two stages of the race and recovery, reflect the leakage of proteins and presumably other substances through muscle membranes (Harris, 1998). Factors including age, gender, physical fitness, season of year, and training can contribute to increased fluctuations in plasma CK and AST activity. Along with these, hypoxia from the intense aerobic exercise may contribute to increased membrane permeability, making the increased permeability more likely in endurance horses (Harris, 1998). Plasma CK and AST activities may increase during exercise without observation of clinical signs or histological detection of changes in muscle cell structure (Valberg et al., 1993). Three finishers developed high values of plasma CK (>3,000 IU/L) without showing noticeable clinical signs of exertional rhabdomyolysis or dehydration. Interestingly, two of these three horses finished in the top 10, which leads us to believe these two horses' values may have simply reflected more muscle effort (Harris, 1998). On the other hand, four metabolic withdrawals had high plasma CK and AST activities, which may indicate that muscle damage contributed to not finishing (Noakes, 1987).

The correlation of increasing cumulative lipid peroxidation and increasing muscle leakage found in the pres-

ent study is an indication of oxidative stress. A positive correlation between increasing TBARS and CK was found in a previous endurance study (Frankiewicz-Jozko and Szarska, 2000), and a negative correlation between ascorbic acid and CK in another (Hargreaves et al., 2002). A positive correlation between plasma isoprostanes and log plasma CK was found in racing sled dogs over repeated bouts of endurance exercise (Hinchcliff et al., 2000). Other equine endurance exercise studies failed to find the same relationship; however, less-sensitive measures of lipid peroxidation were used (Marlin et al., 2002).

Positive correlations of plasma CK and AST with various measures of antioxidant status, especially LPO, are consistent with the hypothesis that free radicals produced during exercise change membrane permeability of muscle cells (McBride and Kraemer, 1999). Exaggeration of oxidative stress associated with increased muscle membrane leakage during endurance exercise in certain horses could contribute to a form of oxidative fiber rhabdomyolysis, in contrast to previous reports of damage exclusively in glycolytic fibers (Valberg et al., 1993). Oxidative stress has also been suggested to contribute to several equine diseases, including the cartilage defect in osteochondrosis (Dimock et al., 2001), the membrane damage in recurrent exertional rhabdomyolysis (Valberg et al., 1993), the vascular defect in exercise-induced pulmonary hemorrhage, and degenerative motor neuron disease (Divers et al., 1997).

## Implications

This field study confirms oxidative stress during endurance exercise as represented by an association between increased lipid peroxidation and increase leakage of the lipoprotein membranes of muscle cells. Oxidative stress may have immediate or cumulative deleterious effects. All horses received vitamin E at five times the recommended level at the insistence of their owners, and the addition of vitamin C had no effect on the variables measured other than an increased ascorbate status. The effect of vitamin C in addition to vitamin E may depend on the dosage levels of both vitamins and the severity of the exercise challenge.

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