Overall Discussion and Summary

The main objectives of these studies were to assess antioxidant status and oxidative stress in horses undergoing endurance exercise. Also tested were the effect of various antioxidant supplements. These data have been summarized and presented as the mean and 95 % confidence interval in **Table 1**.

Antioxidant status did not decrease with exercise, however slight numerical fluctuations were found for most variables. Measures of plasma α-tocopherol and ascorbate were adjusted for fluid shift using albumin, and remained similar between studies. Glutathione and glutathione peroxidase had a greater variation with exercise and was not consistent in the white blood cells due to storage time of the samples. Plasma lipid hydroperoxide, creatine kinase, and aspartate aminotransferase increased over the course of exercise. The range of the confidence intervals become much wider after exercise due to individual horse variation and exercise intensity.

The following paragraphs illustrate the similarities and differences between two previous equine endurance studies performed similarly to the present studies. Both of the equine endurance studies cited in the Journal of Nutrition supplement for the International WALHAM Research Symposium (Hargreaves et al., 2002; Marlin et al., 2002) dealt with horses performing in 80-km competitive endurance rides. Both of these studies were observational studies without treatment groups. These studies also had no dietary records for horses, which makes it hard to discern some of the results concerning antioxidant status.

Similarities between Studies. Both studies (Hargreaves et al., 2002; Marlin et al., 2002) found no change in plasma α-tocopherol concentrations throughout the rides. This is consistent with the present studies (Journal Paper 2, 3 and 5). Ascorbic acid concentrations decreased with distance in both studies. This is true in the present study Journal Paper 5, which decreased after the 47.5 km sample. However, Journal Paper 2 showed an increase in the group supplemented with the vitamin E and C combination. Both previous studies also reported that total glutathione decreased with distance. Marlin

et al. (2002) reported that oxidized glutathione concentrations decreased as well as reduced glutathione. Hargreaves et al. (2002) only measured total red blood cell glutathione, so no conclusions were made regarding the oxidized form. The present studies found varying results regarding red and white blood cell total glutathione concentrations, but found whole blood total glutathione to follow the same pattern of decrease as ascorbic acid in Journal Paper 5.

Plasma creatine kinase increased with distance both studies, however, Marlin et al. (2002) reported peak creatine kinase concentrations of 2,000 IU/L and Hargreaves et al. (2002) reported 600 IU/L. One hypothesis for this may be that Hargreaves only included finishers where Marlin included non-finishers as well, which may have shown clinical signs of exertional rhabdomyolysis. The present studies showed peaks of about 1400, 2300, and 900 IU/L for Journal Papers 2, 3, and 5 respectively. Journal Paper 3 incorporated the results from *Research Ride 2002*, which was determined to be the most challenging ride with the highest ambient temperature. The concentrations of creatine kinase as well as aspartate aminotransferase and lipid hydroperoxides were higher in this ride than in the other present studies.

Differences between Studies. In Hargreaves et al. (2002) the temperature was 10° C warmer than in the ride presented by Marlin et al. (2002). This would affect the results depending on the temperature the horses had been adapted to. For instance, in the present study ambient temperatures in Journal Papers 2 and 3 (reached 10 and 30° C, respectively) had an impact on the oxidative stress and muscle enzyme concentrations during the race, with Journal Paper 3 being much higher throughout. In Journal Paper 5 the horses that raced on the warmer days had an increase in white blood cell apoptosis. Temperatures across studies were difficult to directly compare because it depends on the accommodation of the horses to that particular temperature. For example, the horses in *Research Ride 2002* were not accustomed to the 30° C temperature because it was the first hot day in early April. Hargreaves et al. (2002) reached similar temperatures however, it was the middle of June when horses had more time to get accustomed to summer. The warmer temperatures in Journal Paper 5 only reached 20° C, however, the few weeks prior to this

were about 5° C and above 90 % humidity so the horses had a much more difficult time exercising on these warmer days.

In general, blood and plasma lipid hydroperoxides are a measure of cumulative cell membrane lipid peroxidation, similar to the more commonly measured TBARS or malondialdehyde. In both Journal Papers 2 and 3 lipid peroxidation increased with exercise and remained high through recovery, which suggests it is a cumulative response. Journal Paper 5 failed to find any increases in lipid hydroperoxides. Marlin et al. (2002) showed a large variation in TBARS pre-exercise (66 to 1048 nmol/L), which also increased post-exercise (150 to 1200 nmol/L). Unless comparing data from individual horses, it is difficult to demonstrate an increase with exercise. In the present studies, lipid hydroperoxides only had a coefficient of variation of 5 %, which eliminates the large overlap found in Marlin et al. (2002). Hargreaves et al. (2002) did not report any measure of lipid peroxidation or other indicator of oxidative stress.

Both studies found correlations relating muscle enzymes to antioxidant status, however, the similar correlations found in the present studies are in opposite directions. Of particular interest is the negative correlation of creatine kinase with ascorbate found in Hargreaves et al. (2002). In Journal Paper 5 there was found to be a positive correlation. This is also true with muscle enzyme correlations with glutathione peroxidase found in Journal Paper 3. Explanation for this phenomenon may be due to the differences in horse's fitness level during each study. Hargreaves et al. (2002) used horse subjects at a championship race, where most all of the horse were experienced and highly competitive. Horses participating in the present endurance races were not as athletically fit.

Implications

Overall these present studies have shown that oxidative stress was observed during competitive endurance races and treadmill endurance exercise. The extent of the oxidative stress and muscle enzyme leakage was dependent on the ambient temperature, conditioning level of the horse, and the intensity of work. Supplementing antioxidants vitamin E and lipoic acid is beneficial to endurance horses by decreasing the oxidative stress and muscle enzyme leakage, and increasing antioxidant status. Thus, we can provide better health and welfare to our equine athletes by supplementing with antioxidants, specifically vitamin E or lipoic acid, before they are asked to perform under intense conditions.

Future Studies

Fatigue during exercise is associated with increases in plasma hydrogen ion and lactate concentration (Mainwood and Renaud, 1985). The work level at which the blood lactate increases sharply during exercise is called the lactate threshold (or anaerobic threshold; Davis, 1985). During high intensity exercise glycolysis is needed to compensate for the low power provided by the fatty acid oxidation. An increase in acetyl-CoA and NADH/NAD+ ratio will inhibit the pyruvate dehydrogenase enzyme complex, as a consequence pyruvate is converted to lactate (Randle, 1986). Acetyl-CoA accumulation is diminished by transfer of the acetyl-group to carnitine. High rates of fatty acid transport in horses may reduce the carnitine available for storage of acetyl-groups, thereby contributing to the inhibition of pyruvate dehydrogenase and accumulation of lactate.

Lipoic acid is a co-factor in the pyruvate dehydrogenase enzyme complex. It has been shown that lipoic acid treatment to cells decreases the ratio of free NADH/NAD+ and increases the pyruvate to lactate ratio (Roy et al., 1997). By increasing the lactate threshold in horses we will decrease lactic acid accumulation and delay fatigue. The hypothesis is that horses orally supplemented with lipoic acid would have decreased blood lactate concentrations and therefore a lower lactate threshold when performing a 14-step incremental lactate breakpoint test (Kronfeld et al., 1995).

Table 1. Reference ranges for oxidative stress measures, and antioxidant status for horses competing in two 80-km endurance rides (Ride 2001 and 2002) and a 55-km treadmill study (Treadmill). Number of observations used is in parentheses after the variable.

Variable	Mean	95 % CI	Variable		Mean	95 % CI
Plasma α-tocopherol			Plasma LPO			
Ride 2001 (n = 216)	5.36	2.20-9.95	Ride 2001	PRE (40)	7.32	2.1-17.1
Ride 2002 (176)	6.45	3.64-9.88		80 km (33)	14.6	4.7-26.6
Treadmill (132)	5.03	3.11-7.08	Ride 2002	PRE (40)	16.6	11.4-25.5
Plasma ascorbate				80 km (20)	39.7	5.93-181.7
Ride 2001 (210)	4.12	2.39-6.54	Treadmill	PRE (12)	10.3	4.16-19.7
Ride 2002	NA[a]	NA		55 km (12)	12.7	5.80-20.5
Treadmill (143)	5.98	3.26-9.50				
RBC glutathione			Plasma CK			
Ride 2001 (211)	137.2	80.7-216.3	Ride 2001	PRE (46)	243.6	136.6-458.6
Ride 2002 (175)	267.7	192.8-362.8		80 km (33)	1350	597-3777
Treadmill (143)[b]	367.0	219.2-538.9	Ride 2002	PRE (40)	380.5	147.8-711.0
RBC glutathione peroxidase				80 km (24)	2267	326-1478
Ride 2001 (211)	46.02	28.5-59.3	Treadmill	PRE (11)	213.4	137.4-347.9
Ride 2002 (175)	89.7	21.5-130.0		55 km (11)	565.0	218-1651
Treadmill (144)	48.5	35.3-59.6				
WBC glutathione			Plasma AST			
Ride 2001 (205)[c]	23.4	8.19-38.2	Ride 2001	PRE (46)	304.8	207.5-346.5
Ride 2002 (175)	115.0	88.6-159.1		80 km (34)	364.7	276.5-440.5
Treadmill	NA	NA	Ride 2002	PRE (40)	277.8	204.3-329.3
WBC glutathione peroxidase				80 km (24)	254.0	230.0-378.5
Ride 2001 (205)[c]	124.0	72.6-192.6	Treadmill	PRE (11)	220.1	173-284.5
Ride 2002 (174)	59.3	30.2-86.2		55 km (11)	255.6	194.5-336.5
Treadmill (143)	39.6	26.8-49.2				

[a]NA = Not available

[b]Treadmill glutathione was analyzed using whole blood

[c]WBC samples from Ride 2001 were stored at –80 ° C for over 12 months before analysis

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