

HORSE SPECIES SYMPOSIUM: The effect of oxidative stress during exercise in the horse¹

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ABSTRACT: Oxidative stress is an imbalance of the oxidant-to-antioxidant ratio in the body. Increases in oxidative stress and changes in antioxidant status have been shown during endurance and intense exercise and eventing competition in horses. Antioxidants include vitamins, minerals, enzymes, and proteins that must be synthesized in the body or obtained from the diet. Therefore, exercise level and diet are both factors that play a role in influencing the oxidative stress and antioxidant status of the equine athlete. Along with exercise intensity and duration, diet, age, and training program can also affect oxidative stress in the horse. Several studies using exogenous supplementation of vitamin E, vitamin C, and alpha-lipoic acid have shown positive results in decreasing the effects of exercise (endurance and intense exercise)-induced oxidative stress and increasing the antioxidant status based on the markers and antioxidants measured, whereas other studies using superoxide dismutase showed little effects on the exercise horse. The “free radical theory of aging” states that long-term effects of the degenerative changes associated with aging may

induce oxidative stress. However, in old horses (22 ± 2 yr), lipid peroxidation levels and blood antioxidant concentrations were similar to those found in younger but mature (12 ± 2 yr) horses both at rest and during exercise. Other studies found that yearlings (18 ± 2.4 mo) that are novel to forced exercise had less lipid peroxidation and greater antioxidant status than mature mares (13 ± 2.1 yr) prior to exercise training. Exercise training reduced oxidative stress markers and improved antioxidant status in mares, whereas few effects were seen in yearlings. This indicates that youth provided more defense against oxidative stress due to exercise than the exercise training program. Other studies during competition (endurance, jumping, eventing, and racing) have investigated the influence on oxidative stress with varying results. Despite the multitude of studies examining the levels of lipid peroxidation, antioxidant status, and other related metabolites in the horse during exercise, we still have a long way to go before we fully understand the large variation in results both with and without antioxidant supplementation.

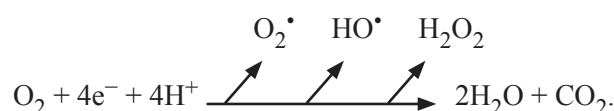
Key words: antioxidant, exercise, horse, oxidative stress

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doi:10.2527/jas2015-9988

INTRODUCTION

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 (Marlin et al., 2002; Williams et al., 2004a). Oxidation provides energy for maintenance of cellular integrity and function. 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 (ROS), as indicated in the equation



¹Based on a presentation at the Horse Species Symposium titled “Exercise physiology of the horse” held at the Joint Annual Meeting, July 12–16, 2015, Orlando FL, with publication sponsored by the Journal of Animal Science and the American Society of Animal Science.

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Received October 15, 2015.

Accepted December 17, 2015.

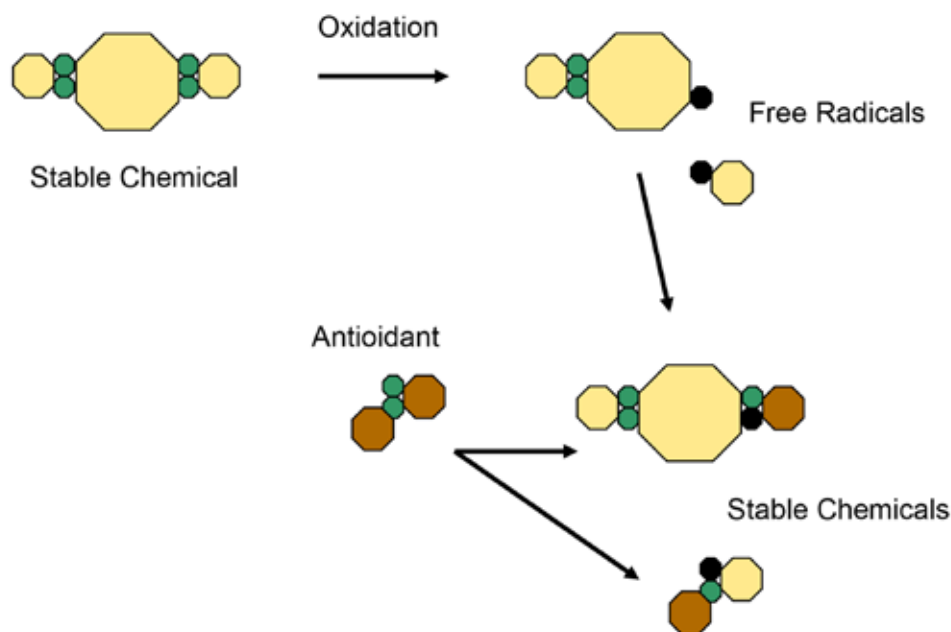


Figure 1. Illustration of how an antioxidant can scavenge a free radical or render them inactive by reforming 2 stable chemicals. Reprinted from Williams et al. (2007).

Oxidant damage can be prevented by antioxidants in several ways: scavenging of ROS, decreasing the conversion of less reactive to more reactive ROS, facilitating repair of damage, and providing an environment favorable for activity of other antioxidants (Clarkson and Thompson, 2000; Fig. 1). When antioxidant defense systems are insufficient or when accumulation of ROS becomes chronic, oxidative processes may damage DNA, lipids, and proteins, which is termed “oxidative stress.” This oxidative stress can contribute to degenerative changes associated with aging, cancer, and other neurodegenerative diseases. For example, lipids are directly protected by alpha-tocopherol in the membranes and by other antioxidants, including ascorbic acid, in the cytosol or intercellular spaces.

It is important to note that ROS in moderation do play an important role in normal physiological activities. Production of superoxide by phagocytosing cells to kill invaded bacteria, upregulation of endogenous defense systems to eliminate procarcinogens, and hydrogen peroxide regulating cell death pathways are a few examples (Franco et al., 1999). In addition, during exercise, ROS production may be required for normal force production in skeletal muscle, development of training-induced adaptation in endurance performance, and the induction of endogenous defense systems (Powers et al., 2010).

Despite these beneficial functions, there is still a role for the antioxidant system to regulate ROS production and accumulation. Antioxidants are interrelated and may prevent oxidant damage in several ways.

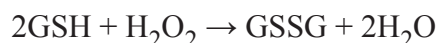
ANTIOXIDANT SUPPLEMENTATION TRIALS

Vitamins E and C

Lipid peroxidation occurs in tissues and parts of cells with a high concentration of PUFA, such as cell and organelle membranes, lipoproteins, adipose tissue, and the brain. Vitamin E is the most efficient antioxidant in preventing lipid oxidation in lipoproteins (Kagan et al., 1990) and is the most commonly supplemented antioxidant in horses (Williams, 2013). The NRC (2007) recommends 100 IU/d of vitamin E for every 100 kg of BW for the adult horse as maintenance, and this increases to 200 IU/d for every 100 kg of BW for horses in heavy to very heavy work and those in lactation. Membrane concentration of α -tocopherol is approximately 1 α -tocopherol molecule to 1,000 lipid molecules. The phytyl tail of the tocopherol molecule allows the positioning of the molecule within the membrane bilayer so that the active chroman ring lies close to the surface of the membrane (Esterbauer et al., 1991). Vitamin E status can be estimated by measurement of tocopherols and tocotrienols in plasma or other tissues. The most common measurement is plasma α -tocopherol by HPLC. The level of α -tocopherol in the plasma will depend on the level of consumption in the diet. Horses not supplemented with vitamin E had about 2 $\mu\text{g/mL}$ of plasma α -tocopherol, whereas after 30 d of supplementation with 300 IU/kg DMI, the plasma α -tocopherol increased to greater than 3 $\mu\text{g/mL}$ (Siciliano et al., 1996). The same study found that a single bout of submaximal exercise does not affect

plasma α -tocopherol concentration, but horses conditioned for several weeks may require greater levels of vitamin E supplementation than recommended by the NRC (2007; Siciliano et al., 1996), due to the possible increase in ROS.

It was found in various species that vitamin C potentiates the effects of vitamin E by reducing the tocopheroxyl radical and restoring its activity (Chan, 1993). Under maintenance conditions, horses have the ability to synthesize sufficient ascorbate, which is why no requirement has been determined (NRC, 2007), but demand increases as stress on the body is increased. Vitamin C is generally measured as ascorbate by HPLC; its antioxidant function is mainly to reduce α -tocopherol and peroxy radicals. The normal range of ascorbic acid for healthy horses not supplemented with vitamin C is 6 to 10 $\mu\text{g/mL}$. Normal supplementation of vitamin C to horses is 5 g/d, which increases plasma ascorbic acid concentration by 2 to 3 $\mu\text{g/mL}$ (Snow et al., 1987). One study examining the vitamin E and vitamin C interaction used 40 endurance horses competing in an 80-km race for the purpose of research (Williams et al., 2004b). Three weeks prior to the race, the horses were provided with vitamin E (5,000 IU/d α -tocopheryl acetate) or vitamin E plus vitamin C (same vitamin E dose plus 7 g ascorbic acid/d). Prior to the supplementation period, horses' diets included vitamin E in the range of 1,556 to 4,700 IU/d (Williams et al., 2005). Blood was sampled prior to the race, at the veterinary checks throughout the race, and after completion of the race. There was a 27% increase in erythrocyte (red blood cell [RBC]) glutathione peroxidase (GPx) observed in the last 2 stages of the race in both treatment groups, which likely reflects a response to utilizing reduced glutathione (GSH) during the radical scavenging process. Reduced glutathione donates an electron to reduce a wide variety of hydroperoxides using GPx as a catalyst. Glutathione peroxidase is found in the RBC and white blood cells (WBC) of mammals, which helps prevent oxidation of cell membranes by consuming free peroxide in the cell. It converts 2 GSH molecules plus hydrogen peroxide (H_2O_2) to an oxidized GSH (GSSG) and 2 water molecules (H_2O), as indicated in the equation



It also reflects the endogenous consumption of pro-oxidants generated during exercise. In contrast to the RBC changes, novel findings were observed in the changes to the WBC GSH system. Fluctuations of WBC GPx during exercise and the sharp 41% increase during recovery may reflect replenishment of reduced GSH. Compared with RBC, the greater concentration

of WBC GPx and less WBC total GSH (GSH-T) may affect phagocyte oxidative burst and other immune functions during prolonged exercise.

Plasma ascorbic acid concentrations were less in the horses supplemented with vitamin E alone than in those receiving vitamin E plus vitamin C at rest (Fig. 2). This difference progressively diminished during the race as ascorbic acid increased in the vitamin E-supplemented horses but remained unchanged in those also supplemented with vitamin C. This could be due to an increased mobilization of intracellular ascorbic acid stores when supplemented with only vitamin E, whereas when vitamin C was added, ascorbic acid levels were maintained using the exogenous source. These findings contrast with a previous study where a decrease in plasma ascorbic acid during a highly competitive and difficult 80-km race was found in unsupplemented horses (Hargreaves et al., 2003).

A study of polo ponies used similar vitamin E and vitamin C supplemented groups (Hoffman et al., 2001). Throughout the polo season, plasma concentrations of α -tocopherol and ascorbic acid were greater in hard-working ponies given vitamin E plus vitamin C than ponies given vitamin E alone. These results were not seen in ponies undergoing light work. These observations may reconcile the endurance findings where changes in antioxidant status were observed in the highly competitive midseason race (Hargreaves et al., 2003) but not in the lightly competitive early season race described in the previous paragraph (Williams et al., 2004b). In a survey taken after the race, riders ranked the exertion level of the endurance ride as easier than most of the rides later in the competition season.

Vitamin E Alone

Vitamin E intake was calculated in competitive endurance horses via a preride survey detailing intake 2 wk prior to the 80-km endurance race (Williams et al., 2005). Pasture intake was estimated by calculating 2.5% BW consumed per day and subtracting the amount of grain, hay, bran, and/or other supplements obtained from the surveys. Horses were estimated to consume 1,150 to 4,700 IU/d of vitamin E in their total diets during this time period. This level is 1.2 to 5 times greater than the recommended levels given by the NRC (2007), which average approximately 1,000 IU/d for each horse. The horses with less vitamin E intake generally were fed mostly pasture and minimal grain. A negative correlation was found between vitamin E intake and muscle enzymes (creatine kinase [CK] and aspartate aminotransferase [AST]), and a positive correlation was found with intake and plasma α -tocopherol at all sample times throughout the race.

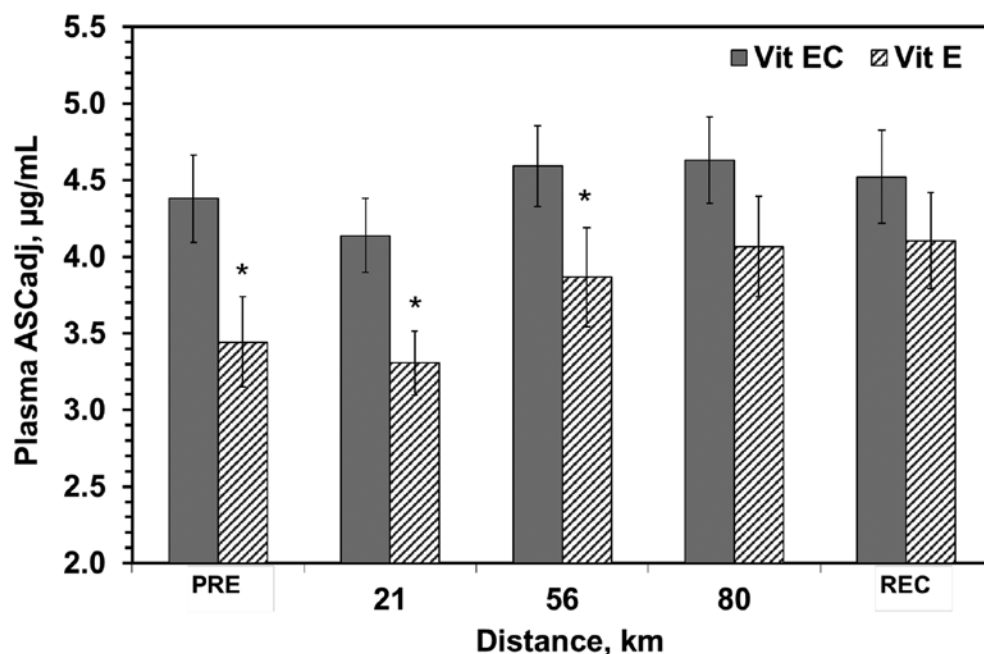


Figure 2. Plasma ascorbate adjusted for albumin (ASCadj) for 34 horses completing an 80-km endurance ride in the vitamin E-supplemented group (Vit E) and the vitamin E and vitamin C supplemented group (Vit EC) before (PRE); 21, 56, and 80 km during; and after recovery from an 80-km endurance race (REC). Asterisks indicate treatments are different ($P < 0.05$). Reprinted from Williams et al. (2004b).

Plasma increases in CK and AST activity, especially during the end of exercise and during recovery, reflect leakage of proteins and presumably other substances through muscle membranes (Harris, 1998). As illustrated by the correlations found in the present study, dietary intake of vitamin E is also a contributing factor in plasma muscle enzyme activity during exercise.

A negative correlation was found between finishing time of the race and vitamin E intake for the 24 horses that finished the race (Williams et al., 2005). One hypothesis for this finding is that the higher-placed horses were working at a greater intensity during the race and/or being trained harder prior to the race and, therefore, had more grain or additional supplements in the diet that could contain more antioxidants, in particular, vitamin E. A greater level of training also may have allowed these horses to work harder with lower plasma muscle enzyme activities, which also could have been the reason for horses with faster race times having lower levels of plasma enzyme activities.

However, caution needs to be taken when supplementing with high levels of vitamin E. Other studies have investigated the impact of pharmaceutical levels of vitamin E on oxidative stress, muscle enzymes, and antioxidant status (Williams and Carlucci, 2006). Horses supplemented with vitamin E at nearly 10 times the NRC (2007) recommended level (10,000 IU/d for 6 wk) did not experience lower concentrations of oxidative stress markers compared with control horses, which were receiving NRC-recommended amounts (Williams and Carlucci, 2006) when undergoing an

interval exercise test with two 2-min bouts of maximal exercise. Additionally, lower plasma β -carotene concentrations were observed in this group compared with control horses or a moderately supplemented group (receiving 5,000 IU/d), which may indicate that vitamin E has an inhibitory effect on β -carotene metabolism (Fig. 3). It is theorized that the exogenous high levels of vitamin E overwhelmed the carriers that are in common with these nutrients. Vitamin A is absorbed very similarly to vitamin E, with 78 to 87% carried through the blood by low-density lipoproteins, which could be the potential reason for the competition with α -tocopherol and β -carotene in the present study. This study failed to show that supplementation above recommended levels provides more protection from oxidative stress and antioxidant status in intensely exercising horses. However, this research has proven that supplementing vitamin E at excessive levels may be detrimental to β -carotene and should be avoided (Williams and Carlucci, 2006).

Vitamin E and Lipoic Acid

Alpha-lipoic acid and its reduced form, dihydroliipoic acid (DHLA), have received widespread attention as antioxidants with both preventative and therapeutic implications to benefit humans and experimental laboratory animals (Reed et al., 1951). They also have potential value in production and companion animals, which warrants investigation. Alpha-lipoic acid is an 8-carbon structure that contains a disulfide bond as a

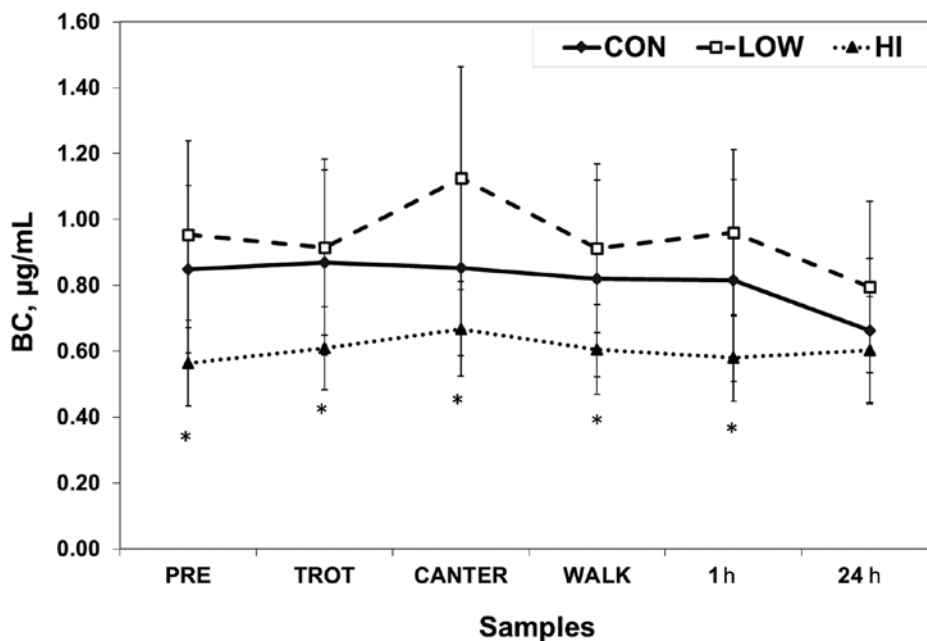


Figure 3. Plasma concentrations of β -carotene (BC) in horses supplemented with a HI (10,000 IU/d) or LOW (5,000 IU/d) dose of vitamin E or control (CON; no supplemental vitamin E). *Difference ($P < 0.05$) between HI and LOW. Blood samples were collected from venous catheters before (PRE), during (during the last 30 sec of the first trot [TROT], the 2nd 100 % heart rate max bout [CANTER], the 5 min of treadmill walk [WALK]), and during recovery, 1 h and 24 h post-exercise. Reprinted from Williams and Carlucci (2006).

part of a dithiolane ring with a 5-carbon tail. It is a co-factor in the conversion of pyruvate to acetyl CoA as part of the pyruvate dehydrogenase complex and also in α -ketoglutarate dehydrogenase (Reed et al., 1951). Both α -lipoic acid and DHLA protect the integrity of cell membranes by interacting with antioxidants GSH and vitamins E and C (Packer et al., 1995). Lipoic acid and DHLA are able to regenerate antioxidants including GSH, ascorbic acid, and (indirectly) α -tocopherol. Lipoic acid has a more negative redox potential ($E_0 = -0.32$ V) than GSH and cysteine couples ($E_0 = -0.24$ and -0.22 V, respectively), so it can reduce GSSG to GSH and oxidized cysteine to reduced cysteine but not vice versa (Navari-Izzo et al., 2002).

Lipoic acid approaches the “ideal” antioxidant because it fulfills the proper criteria including free radical scavenging, interacting with other antioxidants, and having metal-chelating activity as well as its location in aqueous and/or membrane domains repairing oxidative damage and affecting gene expression (Packer et al., 1995). For example, after scavenging ROS, an antioxidant forms a radical of itself (i.e., GSH forms GSSG); in order for it to regain its antioxidant capacity, it needs to be scavenged. Lipoic acid and DHLA are able to regenerate antioxidants including GSH, ascorbic acid, and (indirectly) α -tocopherol.

Arabian horses trained to run on an equine treadmill were supplemented with vitamin E, α -lipoic acid, or nothing as a control before they underwent a simulated endurance exercise test of 3 exercise bouts total-

ing 55 km, with 20-min veterinary checks in between (Williams et al., 2004a), for a total test time of approximately 5 h. The results showed that apoptosis occurs in WBC (measured by flow cytometry) during exercise and it can be moderated by supplementation with vitamin E or α -lipoic acid (Fig. 4). The vitamin E group had 50% less apoptosis and the α -lipoic acid group had 40% less apoptosis compared with the control group. The increase in antioxidant status in the vitamin E and α -lipoic acid groups preserved the WBC by scavenging the ROS that triggered the apoptosis in these cells. The ROS, along with radiation and inflammation activation factors, and activated macrophages can trigger apoptosis in healthy WBC (Chandra et al., 2000).

Antioxidants are linked in various ways through enzymatic pathways and other molecules; this explains the increase in antioxidant status with supplementation of vitamin E and α -lipoic acid. In the present study, α -lipoic acid and vitamin E increased the GSH-T concentrations in whole blood compared with control horses (Williams et al., 2004a). Both the vitamin E and α -lipoic acid groups had about 40% more whole blood GSH-T, 30% more plasma α -tocopherol, and 15% more plasma ascorbic acid than the control group. This illustrates recycling and scavenging of antioxidant radicals using exogenous sources of vitamin E and α -lipoic acid.

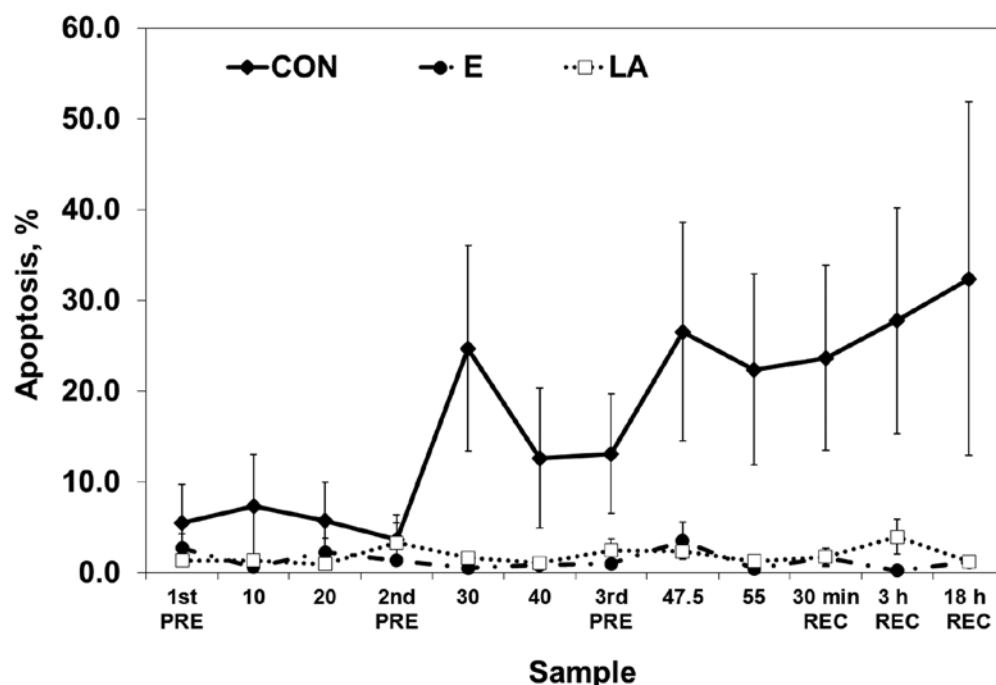


Figure 4. White blood cell apoptosis for the control (CON; $n = 3$), vitamin E (E; $n = 3$), and lipoic acid (LA; $n = 3$) groups. Blood samples were collected from the jugular vein before the start of the test (1st PRE), and at the start of each 'loop' (2nd and 3rd PRE), halfway through (10, 30, and 47.5 km), and near the end of completing each 'loop' (20, 40, and 55 km), and 0.5, 3, and 18 h of recovery (REC). Reprinted from Williams et al. (2004a).

Superoxide Dismutase

Superoxide dismutases (SOD) are enzymatic antioxidant defenses along with GPx and catalase. Superoxide dismutases can be categorized as metalloenzymes, which exert their action on a specific substrate by catalyzing the same chemical reaction despite different AA sequences and several distinct components (Staninger, 2009). Superoxide dismutases catalyze the dismutation of superoxide ions into O_2 and H_2O_2 , thereby preventing the formation of the hydroxyl radical (Fridovich, 1975). In both chronic (during exercise conditioning or illness) and acute inflammatory states (after 1 bout of exercise or acute trauma), the increased production of the superoxide anion surpasses the endogenous SOD scavenging capacity.

Evidence in the literature indicates that oral formulations of SOD are bioavailable as well as effective in reducing markers of inflammation and oxidative stress in several species. Mice orally supplemented with SOD for 28 d showed a rise in circulating antioxidant enzyme activity that was correlated with an increased erythrocyte resistance to oxidative stress-induced hemolysis (Vouldoukis et al., 2004). Previous studies with rats (Radak et al., 1995) and humans (Arent et al., 2009, 2010) have shown beneficial results; however, studies with horses failed to show similar benefits. Horses supplemented with 3,000 IU of SOD for 6 wk did not have a decrease in oxidative stress markers or an increase in antioxidant status (including

SOD activity) compared with unsupplemented horses (Lamprecht and Williams, 2012). However, a study has tested a SOD derivative on exhaustively exercising rats and found that it provided effective protection against oxidative stress in the liver, kidney, and skeletal muscle (Radak et al., 1995). In humans, an oral SOD supplement given to preseason collegiate soccer players (Arent et al., 2010) and football players (Arent et al., 2009) improved athletic performance. Football players had greater improvements in peak power and less muscle breakdown, measured by CK, along with lower levels of 8-isoprostane (an indicator of lipid peroxidation). It was theorized that the SOD may not have been effectively delivered to the target tissue or the enzyme activity may have been compromised in the digestive and/or absorption processes of the horse. Fit human athletes were also used in the human studies where unfit horses were used here. Another possibility could have been an insufficient dosing rate that could have allowed for the SOD supplementation to be non-beneficial to the horse.

OTHER EXERCISE TRIALS

Oxidative Stress in Old Horses

Older horses may be good candidates for antioxidant supplementation, especially in combination with exercise. Developments in human molecular biology have supported the role of ROS production as a contributor to aging. The "free radical theory of ag-

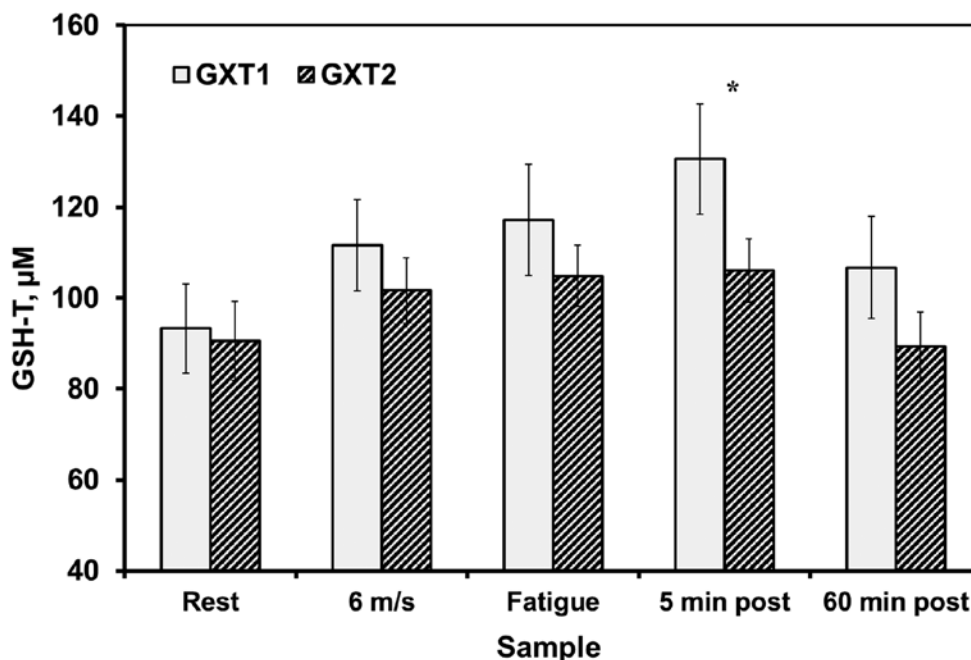


Figure 5. Total red blood cell glutathione (GSH-T) concentrations for before and after training (graded exercise test 1 [GXT1] and graded exercise test 2 [GXT2], respectively). The data from the mature and older horses have been combined due to lack of age group differences. *A difference ($P < 0.05$) between pre- and post-training tests. Blood samples were taken at rest, 6 m/s, fatigue, and at 5 and 60 min post-fatigue (5 min post and 60 min post). Reprinted from Williams et al. (2008).

ing” states that long-term effects of the degenerative changes associated with aging may create an accumulation of ROS and subsequent cellular damage and apoptosis (Harmon, 1956). In contrast to work with humans, studies in older horses have not found similar evidence of an oxidant imbalance during exercise and aging (Williams et al., 2008). In old horses (22 ± 2 yr), lipid peroxidation levels and blood antioxidant concentrations were similar to those found in mature but younger (12 ± 2 yr) horses, both at rest and during exercise. Neither group had lipid peroxidation changes with acute exercise, performed as a graded exercise test (GXT) to exhaustion before and after 8 wk of exercise training; however, there was a greater concentration of GSH-T in the pre- vs. post-training GXT in both age groups (Fig. 5). The observation that more GSH-T was needed during the pretraining GXT for both old and younger groups of horses indicates that training helped the horses prime their antioxidant systems for the intense post-training exercise tests. The study also found that apoptosis of WBC (measured by flow cytometry) was significantly less in the younger than in the older horses, signifying that age may have a greater impact on the immune system than on the oxidant-antioxidant system.

Oxidative Stress in Growing Horses

Just as oxidative stress has been theorized to have greater impact later in an animal’s life, the effect of

oxidative stress in young growing horses that have just started exercise training could be problematic as well. Most studies of this nature use already-trained horses or 3- and 4-yr-old horses. However, recent studies used a group of Standardbred yearling fillies that had performed only voluntary exercise (pasture turnout) since birth (Smarsh, 2013). These studies showed that yearlings did not begin exercise training with greater levels of oxidative stress markers in muscle or blood than mature mares, as the authors originally hypothesized. The mares had greater lipid peroxidation (measured by malondialdehyde and lower antioxidant status (measured by GSH and GPx) in the middle gluteal muscle prior to the same exercise training as the yearlings, implying that the mares had greater levels of oxidative stress overall. However, the older horses’ antioxidant status and oxidative stress levels improved at the completion of 8 wk of exercise training, resulting in levels similar to the yearlings (Smarsh, 2013). This indicates that youth alone helped the young horses during acute exercise and after exercise training and that extra antioxidant supplementation over a well-balanced diet could be unnecessary for young horses early in training; however, more research would be necessary to directly determine this.

Oxidative Stress and Three-Day Event Competition

Elite 3-d event horses competing internationally at a CCI** or CCI*** level (CCI**** is Olympic level competition or Concours Complet International) were the subjects for 3 studies comparing nutritional status, inflammation, oxidative stress, and antioxidant status before and after different phases of the competition. Detailed pre-event nutritional surveys were collected to determine the intake level of antioxidants and other nutrients that would affect the level of stress during competition (Burk and Williams, 2008; Williams and Burk, 2010). Through these surveys, feed was weighed and estimated daily intakes of vitamin E, Ca, P, K, and Mg were calculated based on manufacturer nutrient composition information as well as estimates from the NRC feed tables and found to be 2 to 4 times greater than daily levels recommended by the NRC (2007; Williams and Burk, 2010). In response to competition, tumor necrosis factor- α , nitric oxide, and β -carotene decreased in a blood sample taken 30 min after the cross-country phase; the muscle enzymes CK and AST increased whereas α -tocopherol and retinol remained unchanged throughout the study. The authors theorized that the decrease and lack of change in these variables was due to the horses' training and conditioning. Additionally, the elevated antioxidant and mineral intakes may have enhanced the horses' ability to modulate the inflammatory response and potential oxidative stress that is normally associated with rigorous bouts of acute exercise.

In another study, horses displayed no differences between the CCI** or CCI*** divisions for cortisol, α -tocopherol, retinol, β -carotene, AST, and GPx (Williams and Burk, 2012). Total GSH, however, was greater in the horses competing in the lower CCI** level than horses at the CCI*** level. Total GSH also peaked immediately after the cross-country phase of the competition. The cross-country phase at this level of competition requires horses to jump over 35 to 40 obstacles covering 5 to 7 km of terrain traveling at about 9 to 10 m/s. The GSH-T then returned to baseline levels after 18 to 24 h of recovery at both competition levels, indicating that the GSH was needed to help combat the increased ROS and oxidative stress after the high intensity exercise of the cross-country phase. Other measures, including CK, AST, GPx, β -carotene, retinol, cortisol, and lactate, also peaked immediately after the cross-country phase and were less before the competition started compared with 24 h after the cross-country phase. Overall, these results provided the first report of antioxidant status of horses competing in either a CCI** or a CCI*** 3-d event (Williams and Burk, 2010, 2012).

SUMMARY AND CONCLUSIONS

Overall, these exercise studies have observed oxidative stress during endurance exercise, intense competition, and treadmill exercise. The extent of oxidative stress and muscle enzyme leakage was dependent on the conditioning level and the age of the horse and the intensity of work. Supplementation of antioxidants such as vitamin E, vitamin C, and lipoic acid is beneficial to horses by decreasing the markers of oxidative stress and muscle enzyme leakage and by increasing antioxidant status. Therefore, we can provide better health and welfare to our equine athletes by supplementing them with antioxidants before they are asked to perform under intense conditions. However, caution needs to be used if supplementing above the recommended levels due to possible interference with the absorption of other nutrients. Different age groups of horses respond to exercise training as an oxidative stress reductant and improved antioxidant status differently. Mature mares showed a decrease in the markers associated with oxidative stress and an increase in antioxidants, whereas few effects were seen in yearlings. This indicates that youth provided more defense against oxidative stress due to exercise than the exercise training program. Other studies during competition have investigated the influence on oxidative stress with varying results. Even though there have been many studies examining the levels of lipid peroxidation, antioxidant status, and other related metabolites or markers in the horse during exercise, we still have a long way to go before we fully understand the large variation in results, both with and without antioxidant supplementation.

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