

Vitamin E Intake and Oxidative Stress in Endurance Horses

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ABSTRACT: The objective was to compare the vitamin E content in the total diet of horses competing in the *Research Ride 2002* to the oxidative stress level throughout the race. The ride covered 80 km in northern Virginia. A few months before the ride riders completed a detailed nutritional survey. Blood samples, temperature and heart rate, were taken the day before the race, 27 and 48 km during the ride, at completion of the race (80 km) or wherever each horse was eliminated, and after 3 h of recovery. Plasma lipid hydroperoxides (LPO), α -tocopherol (α -TOC), creatine kinase (CK), aspartate aminotransferase (AST), albumin, and red and white blood cell total glutathione (GSHt), and glutathione peroxidase (GPx) were measured. Data were analyzed by Pearson's correlation in SAS. Twenty-four horses finished the race, where 16 did not finish for reasons including lameness, metabolic problems, and rider option. From the pre-ride survey it was calculated that the horses were consuming 2265 ± 114 IU/d of vitamin E (1150 to 4700 IU/d), which is higher than the recommended amounts of 80 IU/kg intake or about 1000 IU/d given by the NRC (1989). A negative correlation was found between the vitamin E intake and CK ($r = -0.23$; $P = 0.002$), and AST ($r = -0.22$; $P = 0.003$), and a positive correlation was found with intake and plasma α -TOC ($r = 0.21$; $P = 0.005$) at all sample times. These results show that a higher vitamin E intake is associated with less muscle leakage and oxidative stress, and higher antioxidant status in horses throughout an endurance ride. This indicates that horses undergoing heavy exercise will have an improved welfare and possibly performance if they are supplemented with a higher level of vitamin E.

Key Words: Antioxidants, Alpha-tocopherol, Endurance, Equine, Muscle enzymes, Nutritional survey

Introduction

Oxidative stress has been implicated in the pathogenesis of certain diseases (e.g. cancer, AIDS, Alzheimer's disease, and other neurodegenerative diseases) and has been studied dealing with aging and exercise. Oxidative stress occurs because of increased production and accumulation of reactive oxygen species (ROS) due to either increased exposure to oxidants from the environment, increased production within the body from an increase in oxygen metabolism during exercise for example, or an imbalance in antioxidants (McBride and Kraemer, 1999). If ROS production continues it can potentially lead to cell or tissue damage.

Cell damage by free radicals and ROS is opposed by the antioxidant defense system. An antioxidant is a substance present at low concentrations compared to an oxidized substrate, which can inhibit the oxidation of that substrate. Vitamin E is a powerful fat-soluble antioxidant that is perhaps the most commonly supplemented antioxidant for horses. A study by McMeniman and Hintz (1992) supplemented one group of horses with vitamin E 100 IU/d above the other group that received a basal diet level of 44 IU/kg intake (NRC, 1989; recommends 80 IU/kg intake for exercising horses). The plasma thiobarbituric acid reactive substances (TBARS; an indicator of lipid peroxidation and oxidative stress) were increased by exercise, and even more so in horses with low plasma vitamin E. Siciliano et al., (1996) also supplemented vitamin E, up to 300 IU/kg intake, and found that a single bout of submaximal exercise did not affect vitamin E status in the horse. They concluded, however, that when under intense conditioning horses might require a higher level of vitamin E supplementation than recommended by the NRC.

Plasma enzyme activity, specifically creatine kinase (CK) and aspartate aminotransferase (AST), is used as an indicator of muscle leakage during exercise. Enzyme activity can fluctuate for a number of reasons, including alteration in membrane permeability, cell necrosis, impaired enzyme clearance, and increased enzyme synthesis (Harris, 1998).

The objective of this study was to calculate and compare the vitamin E content in the total diet of horses' competing in the *Research Ride 2002* with oxidative stress measures and antioxidant status throughout the 80-km race.

Materials and Methods

Forty-six trained endurance horses (35 Arabians, 9 part Arabians, 1 Thoroughbred, and 1 grade type), 10.8 ± 0.6 years of age, participated in the *Research Ride*, on April 1st, 2002. The protocol was approved by the Institutional Animal Care and Use Committee and performed at the Virginia Tech Middleburg Agricultural Research and Extension Center.

The ride covered 80 km in northern Virginia with terrain ranging from about 100 to 400 m elevation. Ambient temperature ranged from 25° C in the morning to 30° C in the afternoon and evening. A few months before the race, riders responded to a pre-competition survey that detailed nutritional management, training regime, performance and medical history.

Veterinary checks were performed according to the American Endurance Ride Conference (AERC) rules the day before the race, at 27, 48, 72 and 80 km during the race. Blood samples were collected in sodium heparin vacutainer tubes via jugular venous puncture within 2 minutes after the horse entered the veterinary check the day before the race (PRE), at veterinary check 27, 48 and 80 km during the ride, or at the veterinary check where the horse was eliminated, and after 170 to 190 min of recovery (REC). Horse weights without tack, heart rate, and temperature were also measured. Blood samples were placed immediately in ice-water and transported to the laboratory within 15 to 30 min to be processed into red blood cell, white blood cell, and plasma aliquots then stored at -80° C until further analysis. Plasma lipid hydroperoxides, α -tocopherol (TOC), CK, AST, albumin (ALB), and red and white blood cell total glutathione, and glutathione peroxidase were analyzed by methods previously described (Williams et al., 2003).

Data were summarized as means \pm SE. Outliers were determined as being > 2 SD's from the mean and then dropped from the analysis using Fisher's normal deviant (z). Data were tested for normality by the Shapiro-Wilk statistic. Pearson's product-moment

and Spearman's rank order correlations were used to test for an association with vitamin E intake and other variables measured (SAS Institute Inc., Cary, NC). Horse was included in the model to test for significance, if insignificant then it was removed from the model.

Results and Discussion

Twenty-four horses finished the race with an average time of 10 h 8 min, including veterinary check times. The speed of the first three loops of the race was 9.3, 11.1, and 9.7 km/h (loops were 27, 21, and 24 km, respectively); the last 8 km was completed in about 1 h. The 16 eliminated horses did not finish for reasons including lameness ($n = 6$), metabolic problems (5), rider option (2), and other (3). Horses weighed 442 ± 7.3 kg at the start of the race and lost 5 % of their body weight by the finish.

From the pre-ride survey dietary vitamin E intake two weeks prior to the race was calculated by subtracting amount of grain, hay, bran and/or other supplements from the estimated total consumption (using 2.5 % body weight eaten per day) to get an estimate of pasture consumption. Using NRC (1989) or manufacture information, amount of vitamin E for each component in the diet was calculated. Horses were consuming 2265 ± 114 IU/d of vitamin E (**Figure 1**; 1150 to 4700 IU/d) in their total diet during this time period. This level is 1.2 to 5-times higher than the recommended levels given by the NRC (1989); which, at this intake, averages 1000 IU/d. The horses with the lower vitamin E intake generally were the horses receiving mostly pasture and minimal grain to supplement their diet. The higher diets consisted of around 40 to 50 % grain and included supplements with at least 1000 IU vitamin E.

A negative correlation was found between the vitamin E intake and CK ($r = -0.23$; $P = 0.002$), and AST ($r = -0.22$; $P = 0.003$), and a positive correlation was found with intake and plasma α -TOC adjusted for ALB ($r = 0.21$; $P = 0.005$) at all sample times (**Figure 2**). Enzyme activity in plasma is used as an indicator of muscle leakage during exercise. Enzymes most useful in evaluating muscular leakage include creatine kinase

(CK) and aspartate aminotransferase (AST). They can fluctuate for a number of reasons, including alteration of the membrane permeability, cell necrosis, impaired enzyme clearance, and increased enzyme synthesis (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). Factors including age, gender, physical fitness, season of year and training can contribute to increased fluctuations in plasma CK and AST activity. As apparent in the correlations found in the present study dietary intake of vitamin E is also a contributing factor in muscle enzyme plasma concentrations during exercise.

A negative correlation was found between finish time and vitamin E intake (**Figure 3**; $r = -0.31$; $P = 0.0006$) for the 24 horses that finished the race. One hypothesis for this finding could be that the higher placed horses were working at a greater intensity and/or being trained harder, thus having more sweet feed or supplements in the diet. Their higher level of conditioning may also have allowed these horses to work harder with lower muscle enzyme activities. Careful consideration needs to be taken when reporting these results because of the possibility of confounding the data due to competitiveness of the rider. Many riders be training harder and adding more supplements to their horses' feed to ensure they have a competitive advantage. It is also important to note that this study failed to determine an optimal level of supplementation. Therefore feeding even higher amounts that given in the present study is not recommended. Previous research has shown possible detrimental effects in various species fed with high doses of vitamin E including a decreased feed and protein efficiency (Dysmsza and Park, 1975), increased liver weights, decreased hematocrit and hemoglobin (Abdo et al., 1986), decreased prothrombin and blood coagulation time (Corrigan and Marcus, 1974), and an increased demand for vitamins D and K (Kusin et al., 1974).

Implications

These correlations suggest that a higher vitamin E intake may be associated with less muscle leakage and higher antioxidant status in horses throughout an endurance ride. From these results we conclude that horses undergoing heavy exercise will have an improved welfare and possibly performance if they are supplemented with a vitamin E level greater than recommended in NRC. However, more research needs to be done to find the upper limit on the level of supplementation needed to give the horse the optimum level of performance.

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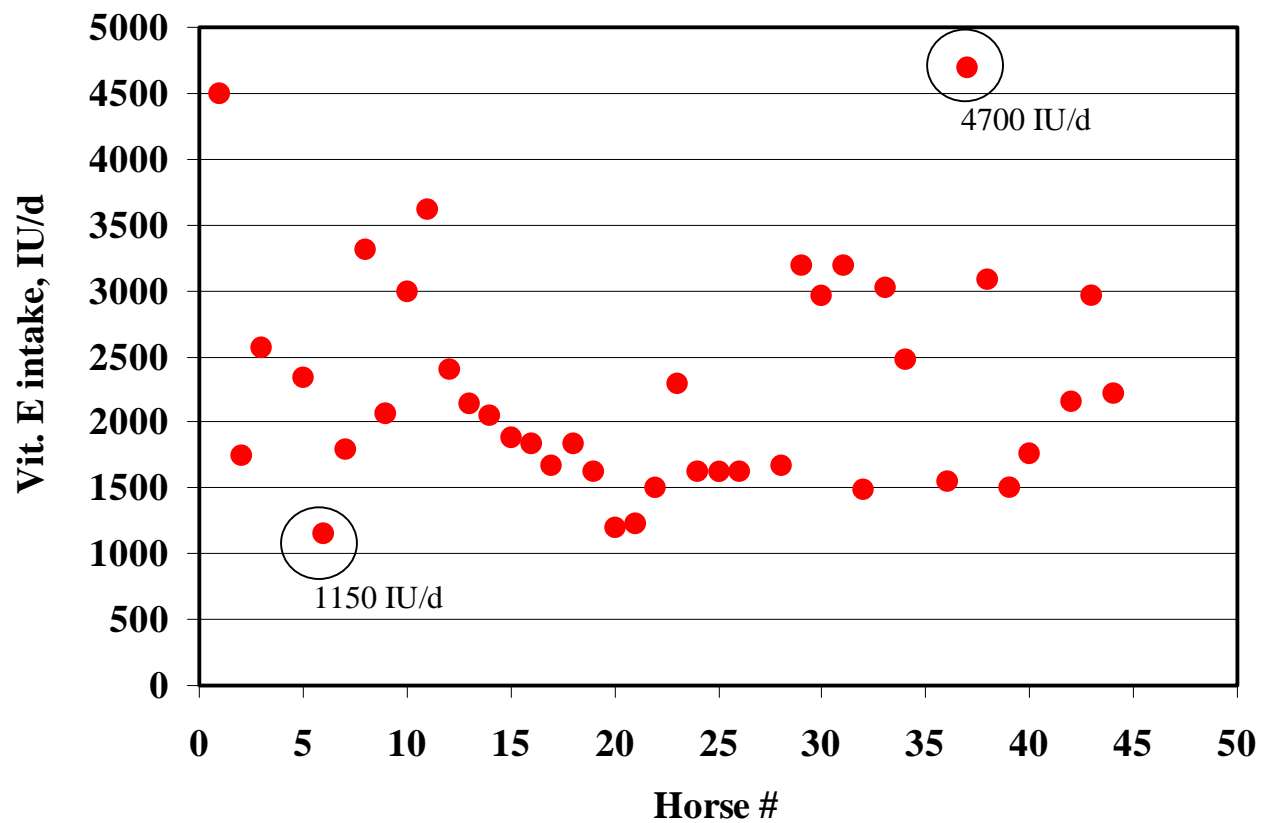


Figure 1. Distribution of vitamin E intake by horse's race number. Mean \pm SE = 2265 \pm 114 IU/d.

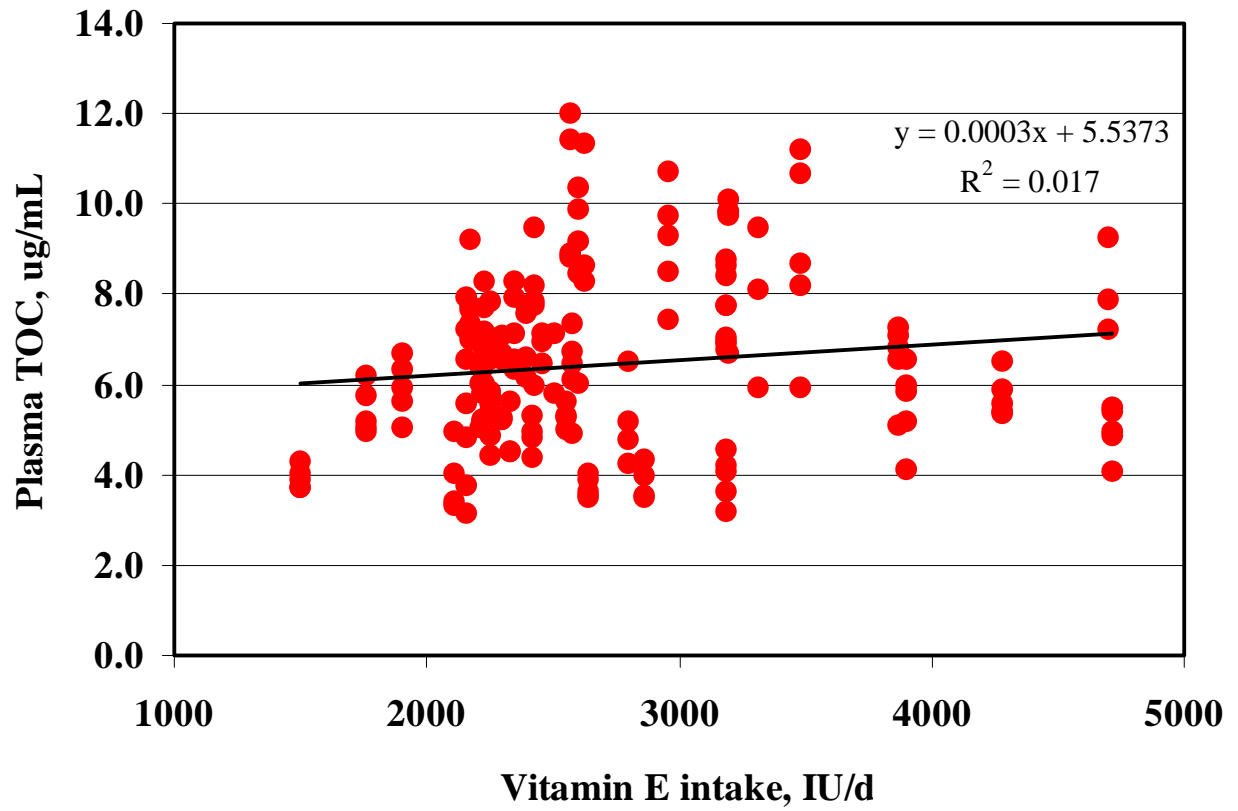


Figure 2. Vitamin E intake vs. plasma alpha-tocopherol (TOC) adjusted for fluid shifts during exercise using albumin. Pearson's correlation was used to determine $r = 0.21$ ($P = 0.005$).

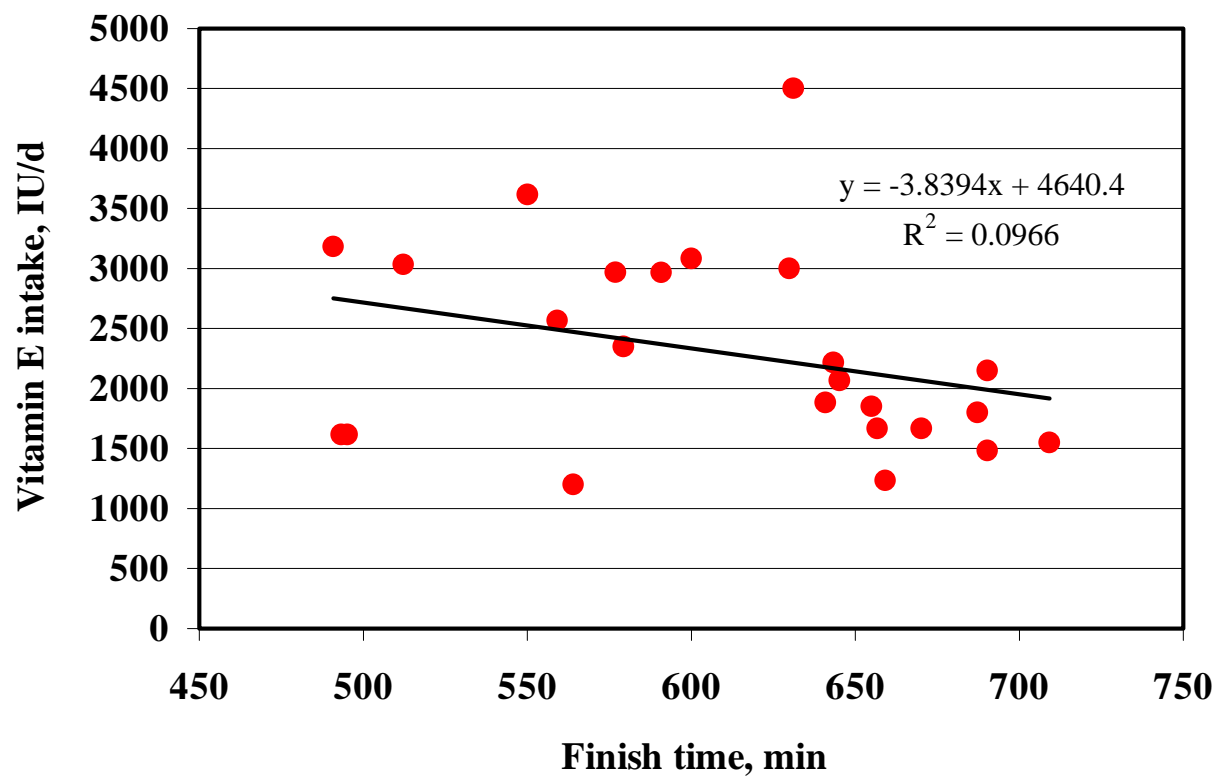


Figure 3. Correlation between vitamin E intake and finish time to complete the 80 km race. Only the 24 finishers were used in this correlation. Pearson's correlation was used to determine $r = -0.31$ ($P = 0.0006$).