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Antioxidant supplementation to the exercising horse

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ABSTRACT - Evidence of oxidative stress in horses has been described in reports dealing with intense and endurance exercise. Oxidative stress occurs when antioxidant systems are insufficient causing oxidation to potentially damage DNA, proteins, and lipids, and contribute to degenerative changes. Vitamin E is the most commonly supplemented antioxidant in horses and has been shown that horses in exercise conditioning may require higher intakes of vitamin E than recommended. Also in various species vitamin C potentiates the effects of vitamin E; however, under maintenance conditions horses have the ability to synthesize sufficient ascorbate, the demand increases as stress on the body is increased. Competitive endurance horses were estimated to consume 1.2 to 5-times higher levels of vitamin E than recommended intakes. In these horses a negative correlation was found between the vitamin E intake and creatine kinase, and aspartate aminotransferase. Similarly, three-day event horses have vitamin E average intakes about 50% over recommended levels, and it was also found that intake of vitamin E negatively correlated with inflammatory markers. However, large doses of vitamin E at about 10-times the recommended levels could potentially interfere with beta-carotene absorption. While some studies have shown benefits of lipoic acid supplementation in endurance trained horses similar to that of vitamin E, other studies failed to show any benefit of supplemental superoxide dismutase in intensely exercising horses. The implications from this broad scope of work show potential benefits for supplementing various antioxidants however, before assuming efficacy from other species horse specific studies should be performed.

Key Words: equine, lipoic acid, oxidative stress, vitamin E

Suplementação de antioxidantes para cavalos atletas

RESUMO - Evidência de estresse oxidativo em eqüinos tem sido descrita em artigos que lidam com exercícios intensos e de resistência. O estresse oxidativo ocorre quando sistemas antioxidantes são insuficientes, causando oxidação com potencial de danificar DNA, proteínas e lipídios, e contribui para mudanças degenerativas. A vitamina E é o antioxidante mais comumente suplementado em cavalos e tem sido demonstrado que cavalos em exercício condicionado podem exigir maior consumo dessa vitamina que o recomendado. A vitamina C também potencializa o efeito da vitamina E em várias espécies. No entanto, sob condições de mantença, os cavalos têm capacidade de sintetizar ácido ascórbico suficiente, a demanda aumenta enquanto o estresse sobre o corpo aumenta. Foi estimado para cavalos de competição (corrida) um consumo de 1,2 até 5 vezes os níveis de vitamina E recomendados para ingestão. Há correlação negativa entre a ingestão de vitamina E, creatinina quinase e aspartato aminotransferase nesses cavalos. Do mesmo modo, cavalos participantes de competições de três dias ingerem em média 50% a mais dos níveis recomendados de vitamina E. Apurou-se, também, que a ingestão de vitamina E é negativamente correlacionada com marcadores inflamatórios. No entanto, grandes doses de vitamina E, cerca de 10 vezes o recomendado, poderiam interferir na absorção de beta-caroteno. Embora alguns estudos têm mostrado que os benefícios da suplementação de ácido lipóico na resistência de cavalos treinados são semelhantes aos da vitamina E, outros estudos não mostraram nenhum benefício da suplementação com superóxido desmutase, em exercícios intensos de cavalos. As implicações deste vasto campo de trabalho mostram os benéficos potenciais para suplementação de vários antioxidantes, no entanto, antes de se comprovar a eficácia, devem ser realizados estudos específicos em outras espécies de cavalos.

Palavras-chave: ácido lipóico, equino, estresse oxidativo, vitamina E

Introduction

Ever tried looking up '*oxidative stress*' on the Internet? Google brings up just under 4 million results (as of April 2010); when adding '*horse*' to this search you get about 124,000. Try '*antioxidant*' and you get over 8 million results on Google. Either way you look at it, these are hot topics. A lengthy review has recently been published detailing oxidative stress and antioxidant supplementation in horses and points out the many conflicting results (Kirschvink et al., 2008). In the following article I will try to highlight studies from our laboratory along with other

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pertinent studies of antioxidant supplementation in exercising horses.

Oxidative stress background

The welfare of competing sport horses has attracted public attention following deaths at the Olympics and other championships. Welfare may be assessed partially by objective indicators of stress (heart rate, and various blood metabolites). 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 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. When antioxidant systems are insufficient, oxidative processes may damage DNA, protein and lipids, and contribute to degenerative changes, including aging and cancer. Lipids are protected directly by α-tocopherol in the membranes and by other antioxidants, including ascorbic acid in the cytisol or external spaces around cells.

Antioxidants are inter-related and may prevent oxidant damage in several ways: scavenging of ROS; decreasing the conversion of less reactive oxygen species to more reactive oxygen species; facilitating repair of damage caused by reactive oxygen species; and providing an environment favorable for activity of other antioxidants (Clarkson & Thompson, 2000). Lipid peroxidation occurs in tissues with a high concentration of poly-unsaturated fatty acids, such as cell and organelle membranes, lipoproteins, adipose tissue, and brain.

Antioxidant supplementation trials

Vitamin E is the most commonly supplemented antioxidant in horses. One study found that a single bout of submaximal exercise does not affect plasma α-tocopherol concentration, but horses conditioned for several weeks, may require higher levels of vitamin E supplementation than recommended (Siciliano et al., 1996).

It has been found in various species that vitamin C potentiates the effects of vitamin E by reducing the tocopheroxyl radicals and restoring its activity (Chan, 1993). Under maintenance conditions horses have the ability to synthesize sufficient ascorbate, but the demand increases as stress on the body is increased. One study looking at the vitamin E and 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 alpha-tocopheryl acetate) or vitamin E plus vitamin C (same vitamin E dose, plus 7 g ascorbic acid/d). The 27% increase in erythrocyte glutathione peroxidase observed in the last two stages of this race in both treatment groups 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 glutathione peroxidase as a catalyst). It also reflects the consumption of pro-oxidants generated during exercise. In contrast to the RBC changes, novel findings were the changes in the white blood cell glutathione system. Fluctuations of white blood cell glutathione peroxidase during exercise and the sharp 41% increase during recovery may reflect replenishment of reduced glutathione. Compared to erythrocyte, the higher concentration of white blood cell glutathione peroxidase and lower white blood cell total glutathione may affect phagocyte oxidative burst and other immune functions during prolonged exercise.

Plasma ascorbic acid concentrations were lower in the horses supplemented with vitamin E alone than those receiving the vitamin E plus C at rest (Figure 1). 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 adding C they were able to maintain ascorbic acid levels using the exogenous source for its antioxidant capacity. 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 (Hargreaves et al., 2003).

A study of polo ponies used similar vitamin E and C supplemented groups (Hoffman et al., 2001). Throughout the polo season plasma α-tocopherol and ascorbic acid were higher in those also given vitamin C in hard-working ponies, but not those in only light work. These observations may reconcile the endurance findings where changes were observed in the highly competitive, mid-season race (Hargreaves et al., 2003), but not in this lightly competitive, early season race (Williams et al., 2004b). In a survey taken after the race, riders ranked the exertion level of the endurance ride 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.

Vitamin E intake was calculated in competitive endurance horses via a pre-ride survey detailing intake two weeks prior to the 80-km endurance race (Williams et al., 2005). Pasture intake was estimated using 2.5% body weight eaten per day and subtracting amount of grain, hay, bran

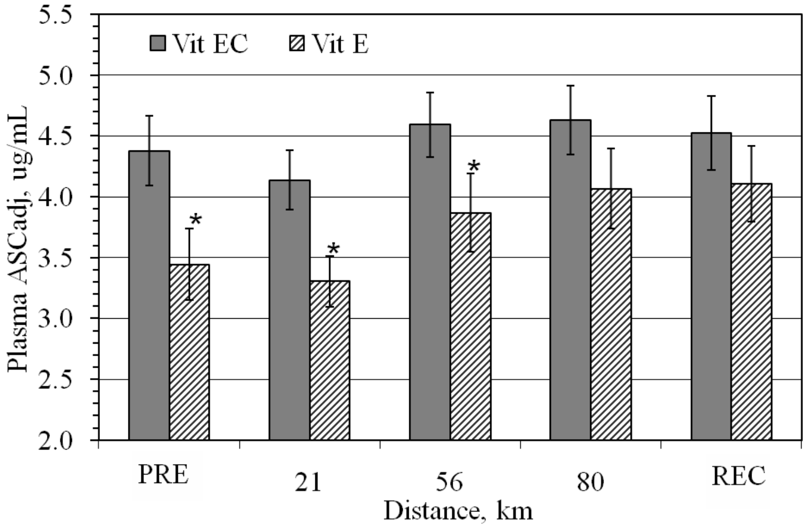

Figure 1 - Plasma ascorbate adjusted for albumin (ASCadj) for 34 horses completing an 80-km endurance ride in the vitamin E supplemented group (E) and the vitamin E and C supplemented group (EC) before (PRE), 21, 56, and 80 km during, and after recovery (REC) of an 80-km endurance race.
*Treatments are significantly different at P<0.05. Reprinted from Williams et al. (2004b).

and/or other supplements obtained from the surveys. Horses were estimated to consume 1150 to 4700 IU/d of vitamin E 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 (2007); 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. A negative correlation was found between the vitamin E intake and creatine kinase, and aspartate aminotransferase, and a positive correlation was found with intake and plasma α-tocopherol adjusted for albumin at all sample times. Enzyme activity in plasma is used as an indicator of muscle leakage during exercise. 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 finishing time and vitamin E intake for the 24 horses that finished the race (Williams et al., 2005). 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.

However, caution needs to be taken when supplementing with high levels of vitamin E. Other studies in the same laboratory have investigated pharmaceutical levels of vitamin E on its impact of oxidative stress, muscle enzymes and antioxidant status (Williams & Carlucci, 2006). Horses supplemented with vitamin E at nearly 10-times the NRC (2007) recommended level did not experience lower oxidative stress compared to control horses (Williams & Carlucci, 2006). Additionally, there was found to be lower plasma β-carotene levels observed in this group compared to control or a moderately supplemented group, which may indicate that vitamin E, has an inhibitory effect on β-carotene metabolism (Figure 2). This study failed to show that supplementation above control levels is more beneficial to oxidative stress and antioxidant status in intensely exercising horses. However, this research has proven that supplementing with levels in 10-times excess may be detrimental to β-carotene and should be avoided.

Arabians trained to run on an equine treadmill were supplemented with vitamin E, lipoic acid, or nothing as the control group, before they underwent a simulated endurance exercise test of 3 exercise bouts totaling 55 km, with 2-20 min vet checks in-between (Williams et al., 2004a). These results showed that apoptosis occurs in white blood cells during exercise and it can be moderated by supplementation with vitamin E or lipoic acid. The vitamin E fed group had 50% lower and the lipoic acid fed group had 40% lower apoptosis compared to the control group (Figure 3). The increase in antioxidant status in the vitamin E and lipoic acid groups

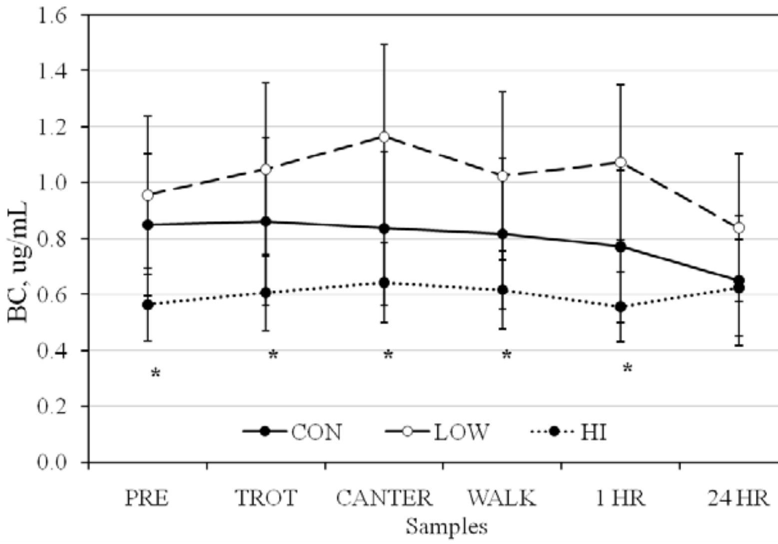

Figure 2 - Plasma concentrations of β-carotene (BC) in horses supplemented with a HI (10,000 IU/d), MOD (5,000 IU/d), or CON (no supplemental vitamin E) dose of vitamin E.
* Indicates significant (P<0.05) difference between HI and MOD. Reprinted from Williams & Carlucci (2006).

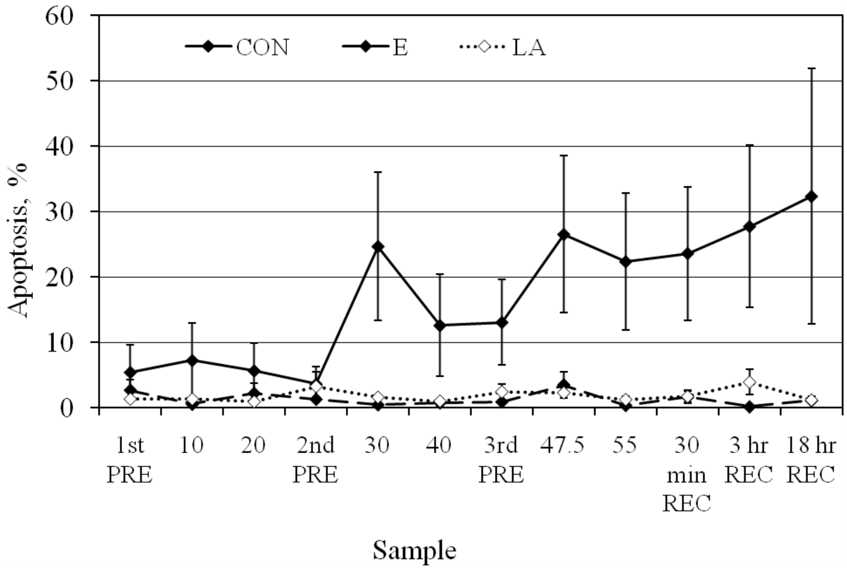

Figure 3 - White blood cell apoptosis for the control (CON; n = 3), vitamin E (E; n = 3), and lipoic acid (LA; n = 3) groups. Reprinted from Williams et al. (2004a).

aided the white blood cells in scavenging the reactive oxygen species triggering the apoptosis in these cells.

Antioxidants are linked together in various ways; this explains the increase in antioxidant status with supplementation of vitamin E and lipoic acid. In the present study lipoic acid increased the total glutathione concentrations in whole blood compared to control (Williams et al., 2004a). The lipoic acid group also had increased levels of ascorbic acid and α-tocopherol in the plasma throughout the study. Both the vitamin E and lipoic acid groups had about 40% more total glutathione, 30% more α-tocopherol, and 15% more ascorbic acid than the control group. This illustrates recycling and scavenging of antioxidant radicals using the exogenous sources of the vitamin E and lipoic acid.

Other exercises studies

Older horses are another group that might require antioxidant supplementation, especially if in combination with exercise. Evidence of a disequilibrium oxidant balance during exercise and aging was not made clear in a previous study (Williams et al., 2008). In old horses (22 ± 2 years), the amount of lipid peroxidation and blood antioxidant concentrations are similar to those found in mature but younger (12 ± 2 years) horses. Neither group had lipid peroxidation changes with either acute exercise or 8-weeks of training, but there was a higher concentration of total glutathione in the pre- vs. post-training tests in both age groups (Figure 4). The observation that more total glutathione was needed during the pre-training graded exercise test for both old and younger groups of horses suggests that training helped the horses prime their systems for the intense post-training exercise tests. The study also found that white blood cell apoptosis was significantly lower in the younger than in the older horses, signifying that age might have more of an impact on the immune system than on the oxidant/antioxidant system.

Elite three-day event horses competing internationally at a CCI** or CCI*** were found to have no differences between divisions for cortisol, α-tocopherol, retinol, β-carotene, aspartate aminotransferase, and erythrocyte glutathione peroxidase (Williams & Burk, 2007). Total glutathione, however, was higher in the horses competing in the CCI** than horses in the CCI***. Total glutathione also peaked immediately after the cross-country phase returning to baseline after 18 to 24 hr of recovery. Other measures including creatine kinase, aspartate aminotransferase, erythrocyte glutathione peroxidase, β-carotene, retinol, cortisol and lactate also peaked immediately after the cross-country phase and were typically lower before the competition started compared to 24 h after the cross-country. Overall these results provided the first report of antioxidant status of horses competing in either a CCI** and CCI*** 3-day event (Williams & Burk, 2007). Pre-event nutritional surveys were also undertaken to determine the intake level of antioxidants and other nutrients that would affect the level of stress during competition (Williams & Burk, 2010). Actual intake of vitamin E was higher than recommended intakes (1587 ± 230 vs. 1065 ± 16 IU/d). Intake of vitamin E negatively correlated with inflammatory markers, serum nitric oxide and tumor necrosis factor-α. Plasma BC assessed at competition and amount of pasture consumed prior to competition were positively correlated. Complete dietary management results from the CCI** and CCI*** 3-day event horses during competition was previously published (Burk & Williams, 2008).

The effects of superoxide dismutase on oxidative stress and inflammation in exercising horses systemically and locally (synovial fluid) was recently completed (Lamprecht, 2009). Previous studies in rats (Radak et al., 1995) and humans (Arent et al., 2009, 2010) have shown beneficial results; however, these horse studies failed to show similar results. Studies have tested a superoxide dismutase derivative on exhaustively exercising rats and found that it provided effective protection against oxidative stress in the liver and kidney along with skeletal muscle in exercising rats (Radak et al., 1995). Recently, in humans, the effects of an oral superoxide dismutase supplement on preseason collegiate soccer players (Arent et al., 2010) and football players (Arent et al., 2009) determined that performance

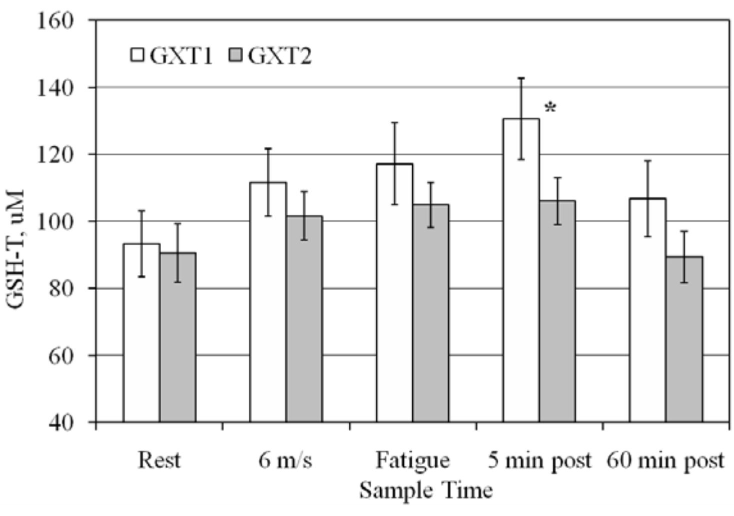

Figure 4 - Total red blood cell glutathione (GSH-T) concentrations for pre- and post-training (GXT1 and GXT2, respectively). The data from the mature and older horses have been combined due to lack of age group differences.
* Indicates a difference between pre- and post-training tests (*P*<0.05). Reprinted from Williams et al. (2008).

improved by a greater magnitude in the soccer players supplemented with superoxide dismutase, where the football players had greater improvements in peak power and lowered muscle breakdown, measured with creatine kinase, along with lower levels of 8-isoprostane.

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.

Implications

Overall these exercise studies have shown that oxidative stress was observed during endurance, intense, and treadmill exercise. Supplementing antioxidants like vitamin E, vitamin C, and lipoic acid is beneficial to 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 before they are asked to perform under intense conditions. However, caution needs to be taken if supplementing above and beyond the recommended levels due to the possible interference with the absorption of other nutrients and before assuming efficacy from other species horse specific studies should be performed.

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