Effect of α-tocopheryl acetate supplementation on vitamin E concentrations in Greyhounds before and after a race

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Objectives—To determine effect of α-tocopherol supplementation on serum vitamin E concentrations in Greyhounds before and after a race.

Animals—8 adult racing Greyhounds.

Procedure—Dogs were given 2 capsules of α-tocopheryl acetate (total, 680 units [0.5 g]) with food that contained ≤ 15 mg of vitamin E/kg each morning for 7 days. Dogs were exercised in a 30 × 30-m grass paddock for 15 minutes twice a day and raced for 500 m twice a week. Blood samples were collected before and 5 minutes after a race, before supplementation was begun, and after 7 days of supplementation. Blood and diet samples were analyzed for tocopherols and α-tocopheryl acetate.

Results—Before supplementation, serum α-tocopherol concentration after racing (mean ± SD, 6.7 ± 2.4 mg/L) was significantly lower than before racing (12.2 ± 3.1 mg/L). After supplementation, α-tocopherol concentrations were significantly higher overall, although values obtained before (26.6 ± 5.2 mg/L) and after (29.8 ± 3.6 mg/L) racing were not significantly different.

Conclusions and Clinical Relevance—Supplementation with α-tocopheryl acetate increased serum α-tocopherol concentrations and eliminated the decrease in α-tocopherol concentration that was detected after a race, which may decrease oxidation during exercise and improve performance or recovery. (*Am J Vet Res* 2001;62:1118–1120)

Vitamin E was first found to be an essential nutrient for dogs in 1939.[1] The 8 known forms of vitamin E vary in activity. The 2 main categories, the tocopherols and the tocotrienols, each include 4 subgroups (α, β, γ, and Δ). Tocotrienols are generally present in low concentrations and are less active than are tocopherols. α-Tocopherol is the most active form and is found in the body as the D stereoisomer; it is available commercially as a racemic mixture of D and L isomers. This commercial mixture has been given a rating of 1 U/mg, and all other forms are ranked on the basis of relative vitamin E activity in comparison with this commercial form. Delta-tocopherol, for example, has approximately 1% of the activity of α-tocopherol.[2]

Thirty units of α-tocopherol/kg of diet prevents signs of vitamin E deficiency in Beagle pups.[3] The **National Research Council (NRC)** recommends that at least 20 U of α-tocopherol/kg of dry matter be included in any diet and that at least 0.5 mg of α-tocopherol/g of polyunsaturated fat be included in any diet that contains high polyunsaturated fat.[4] Exercise may increase the requirement for vitamin E in some dogs, such as racing Greyhounds.[5] Aerobic metabolism produces reactive oxygen radicals that have the potential to damage lipids, proteins, and nucleic acids.[6,7] Vitamin E protects cell membranes from free-radical damage[2,8] and is thought to protect against oxidative stress in muscles during exercise.[6,7]

Serum creatine kinase concentrations, possibly reflecting muscle damage, correlate with superoxide release from neutrophils, and vitamin E supplementation (800 U/d) suppresses the increase in concentration of interleukin-1β observed in young men after exercise.[6,7] Vitamin E supplementation also eliminates the greater neutrophilia and higher plasma creatine kinase concentrations observed in older men, compared with younger men, after exercise.[6] Serum vitamin E concentrations decrease in sled dogs undertaking endurance exercise, and serum creatine kinase concentrations in these dogs correlate with increased urinary excretion of the oxidation markers, isoprostanes[9]; however, to the authors' knowledge, results of studies that have measured changes in serum vitamin E concentrations in Greyhounds after a sprint race have not been published.

In Greyhounds[5] and Beagles,[10-12] plasma tocopherol concentrations correlate with dietary vitamin E concentrations when dietary vitamin E concentrations are at or below the NRC requirement; however, there is not a direct correlation between dietary and plasma tocopherol concentrations when vitamin E is supplemented above the requirement. Many Greyhound trainers give vitamin E supplements to their dogs,[5] but to our knowledge, results of studies that compare the effect of vitamin E supplementation on vitamin E concentrations in racing Greyhounds before and after a race have not been published.

The purpose of the study reported here was to determine the effect of vitamin E supplementation on serum α-tocopherol concentrations in Greyhounds before and after a race.

Materials and Methods

Eight Greyhounds (4 sexually intact females and 4 sexually intact males, 2 to 4 years old) that weighed 31.7 ± 2.7

Received Jun 19, 2000.
Accepted Aug 18, 2000.
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Partially funded by the Waltham Centre for Pet Nutrition and the University of Florida Center for Veterinary Sports Medicine.
Published as University of Florida, College of Veterinary Medicine Journal Series No. 590.

kg (mean ± SD) were used in this study. All dogs were determined to be clinically normal and were cared for as described,[13,14] according to principles outlined in the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. This study was approved by the University of Florida Institutional Animal Care and Use Committee. Dogs were exercised for 15 minutes twice daily in a 30 × 30-m grass paddock and raced in randomized pairs twice a week. Race distance was 500 m (five sixteenths of a mile) on a 400-m (quarter mile) oval soft sand-clay track with 10° banking on the corners. Dogs chased a mechanical lure.

Dogs were fed once daily after their morning exercise, and water was available at all times. Each dog was offered food in excess of its estimated requirement and was allowed to eat for 30 to 40 minutes. Excess food was removed after each dog had voluntarily stopped eating. Dogs were fed either a moderate-protein (24% of energy as protein) or high-protein (37% of energy as protein) experimental diet. Both diets contained 33% of energy as fat, of which 14% was polyunsaturated fat. These diets contained 0.4 and 0.5 mg of selenium/kg, as fed, respectively. Dogs had been fed these 2 diets for 26 weeks prior to the onset of this study as part of a previous cross-over dietary protein study. Design of the previous study and the composition of the diets are published elsewhere.[14] During the 8 days of the vitamin E study reported here, dogs continued to receive the diet to which they had been randomly assigned for the previous 11 weeks. Each diet group contained 2 males and 2 females. Each diet was manufactured as a single batch and did not contain added α-tocopheryl acetate. A representative 1-kg sample of each diet was stored at –20 C for subsequent analysis.

Dogs received vitamin E for 7 days; 2 gelatin capsules that each contained 0.25 g of D-α-tocopheryl acetate[a] were given orally to each dog once daily in the morning with food for a total daily dose of approximately 680 U/dog. Blood samples were obtained from each dog by jugular venipuncture in the kennel before racing and at the racetrack 5 minutes after racing on days 1 (before supplementation) through 8. Blood was drawn directly into a 6-ml serum separation tube[b] that contained gel for clot separation and silica coating for clot activation, using a 20-gauge 1.5-inch needle and holder. Samples were immediately placed in crushed ice, and serum was separated by centrifugation at 500 × g for 15 minutes at 4 C within 1 hour of collection.

Sample preparation—One milliliter of ethanol was added to 500 µl of serum in a 10-ml glass centrifuge tube and vortexed to precipitate protein. Two milliliters of petroleum ether was added, and the tube was vortexed again and centrifuged at 500 × g for 5 minutes. The petroleum ether layer was placed into another 10-ml glass tube and kept in ice. An additional 2 ml of petroleum ether was added to the original tube, vortexed, and centrifuged again. The petroleum ether layer was removed and added to the tube in ice. This extract was evaporated to dryness under a stream of nitrogen at 25 C. The residue was dissolved in 1 ml of isooctane and stored at –20 C until analysis.

Diet samples were prepared for vitamin E analysis in a similar manner. Each diet sample was ground to the consistency of powder. A representative 1.5-g test sample of this ground diet was suspended in 2 ml of deionized water. The remaining preparation steps were identical to those performed on the serum samples, beginning with the addition of ethanol.

Sample analysis—Samples were analyzed by use of **high pressure liquid chromatography (HPLC)**, using an automatic injector,[c] pump,[d] 4.6-mm × 25-cm normal phase silica column,[e] and a fluorescence detector.[f] Sample size for injection was 20 µl. The mobile phase was HPLC-grade 90% isooctane, 9.5% tetrahydrofuran, and 0.5% glacial acetic acid[g] with a flow rate of 1.0 ml/min. α-Tocopherol and α-tocopheryl acetate were separated, and peaks were identified at an excitation wavelength of 290 nm and an emission wavelength of 330 nm with 2.5-mm slit by coelution with external standards (D,L-alpha-tocopherol, D-alpha-tocopheryl acetate).[a]

Statistical analyses—α-Tocopherol and α-tocopheryl acetate concentrations were compared by use of the general linear models procedure of a computerized statistics program[h] with before versus after racing and supplementation as repeated measures variables and sex and diet as nonrepeated measures variables. Because there was no evidence of an effect of diet or sex, a 2-way ANOVA was performed with before versus after racing and supplementation as repeated measures variables. Post-hoc comparisons of before versus after racing were performed before and after supplementation, using paired *t*-tests. Type I error was set at 0.05. Values are expressed as mean ± SD.

Results

Total tocopherol concentrations in the high- and low-protein diets were similar (15.3 and 13 mg/kg, respectively). Tocopherol in both diets was > 95% δ-tocopherol and < 5% α-tocopherol. The high-protein diet contained 0.5 mg of alpha-tocopherol/kg and 14.7 mg of delta-tocopherol/kg. The low-protein diet contained 0.7 mg of α-tocopherol/kg and 12.5 mg of δ-tocopherol/kg. α-tocopheryl acetate was not detected in either diet; thus, there was little vitamin E activity in either diet.

There was no evidence of an effect of diet (high-protein vs low-protein) or sex on serum α-tocopherol concentration. α-Tocopheryl acetate supplementation, however, increased serum α-tocopherol ($P < 0.001$) before and after racing. Before supplementation, serum α-tocopherol concentration before racing was 12.2 ± 3.1 mg/L and after racing was 6.7 ± 2.4 mg/L ($P < 0.001$); after supplementation, there was no significant difference between concentration before (26.6 ± 5.2 mg/L) and after (29.8 ± 3.6 mg/L) racing.

Discussion

Results of this study indicated that serum alpha-tocopherol concentrations decreased substantially after a short sprint race of 500 m in Greyhounds that did not receive supplemental α-tocopheryl acetate. This decrease developed despite hemoconcentration and a substantial decrease in plasma volume reflected in an increase in mean PCV and serum albumin concentration from 56% and 3.0 g/dl, respectively, before racing to 66% and 3.8 g/dl, respectively, after racing.[14] The decrease in serum α-tocopherol concentration after racing suggests that there may be increased oxidative stress in Greyhounds during a sprint race. Measures of oxidative stress were not measured, but α-tocopherol may have been oxidized to protect cell membranes. In addition, because samples were obtained 5 minutes after racing, there may not have been sufficient time for the oxidized moieties to be reduced by other blood compounds such as glutathione and ascorbic acid.

Supplementation with 680 units of alpha-tocopheryl acetate daily for a week substantially increased serum α-tocopherol concentrations. Supplement-

ation also mitigated the decrease in α-tocopherol concentration after a race observed in dogs that did not receive supplemented food; however, PCV increased by 20% with racing, so plasma volume and the total mass of tocopherol in the plasma probably decreased with racing, even after supplementation.

The prerace unsupplemented serum α-tocopherol concentrations reported here are similar to those reported in Greyhounds and other dogs. Snow and Frigg[5] observed higher plasma α-tocopherol concentrations in Greyhounds from kennels that supplemented diets with vitamin E than in Greyhounds from kennels that gave little additional vitamin E to their dogs. Nevertheless, the vitamin E content of the basic diets fed in these kennels was not measured. Pillai et al[11] reported 2-fold higher plasma α-tocopherol concentrations (25 ± 6 g/ml) in Beagles fed a standard diet that contained 114 mg of vitamin E/kg than in Beagles fed a standard diet without vitamin E added. The diets used to feed Greyhounds in our study contained only 15 mg of vitamin E/kg, mostly as delta-tocopherol, which has low activity.

α-Tocopheryl acetate was used for supplementation, because it is quite stable, whereas unesterified forms of vitamin E are relatively unstable in stored foods such as kibble dog food. α-Tocopheryl acetate has no antioxidant activity until hydrolyzed to α-tocopherol but is a useful source of vitamin E, because it is hydrolyzed within the body. Results of the study reported here suggest that alpha-tocopheryl acetate appears to be absorbed and increases circulating α-tocopherol concentrations in Greyhounds.

The daily dose of α-tocopheryl acetate (680 units) used to supplement dogs was high. A typical Greyhound consuming 300 g (dry matter)/d of a food containing the minimum amount of α-tocopherol (20 U/kg [dry matter]) recommended by the NRC would consume only approximately 6 U/d. It remains to be determined, therefore, whether lower doses of vitamin E are adequate to prevent the decline in vitamin E concentrations observed in after racing in dogs that do not receive a supplemented diet.[4] A further limitation of our study was that it did not employ a cross-over design. Vitamin E is stored in the body, which made a cross-over design impractical for this short study.

[a]Sigma Chemical Co, St Louis, Mo.
[b]Vacutainer tubes, Becton-Dickinson, Rutherford, NJ.
[c]ISS-100, Perkin-Elmer, Norwalk, Conn.
[d]Spectroflow 400, ABI Analytical Kratos Division, Ramsey, NY.
[e]EQC 10 m S1 60A, Whatman Ltd, Maidstone, Kent, England.
[f]LS-4 fluorescence spectrometer, Perkin-Elmer, Norwalk, Conn.
[g]Fisher Scientific, Pittsburgh, Pa.
[h]SAS/STAT, version 6.04, SAS Institute Inc, Cary, NC.

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