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Supplemental Vitamin C Appears to Slow Racing Greyhounds[1,2]

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ABSTRACT During strenuous exercise, markers of oxidation increase and antioxidant capacity decreases. Antioxidants such as vitamin C may combat this oxidation stress. The benefits of vitamin C to greyhounds undertaking intense sprint exercise has not been investigated. The objective of this experiment was to determine whether a large dose (1 g or 57 mmol) of ascorbic acid influences performance and oxidative stress in greyhounds. Five adult female, trained racing greyhounds were assigned to receive each of three treatments for 4 wk per treatment: *1*) no supplemental ascorbate; *2*) 1 g oral ascorbate daily, administered after racing; *3*) 1 g oral ascorbate daily, administered 1 h before racing. Dogs raced 500 m twice weekly. At the end of each treatment period, blood was collected before and 5 min, 60 min and 24 h after racing. Plasma ascorbate, α-tocopherol, thiobarbituric acid-reducing substances (TBARS) and Trolox equivalent antioxidant capacity (TEAC) concentrations were measured and adjusted to compensate for hemoconcentration after racing. TBARS, TEAC and α-tocopherol concentrations were unaffected by supplemental vitamin C. Plasma ascorbic acid concentrations 60 min after racing were higher in dogs that received vitamin C before racing than in dogs that either received no vitamin C or received vitamin C after racing. The dogs ran, on average, 0.2 s slower when supplemented with 1 g of vitamin C, equivalent to a lead of 3 m at the finish of a 500-m race. Supplementation with vitamin C, therefore, appeared to slow racing greyhounds. J. Nutr. 132: 1616S–1621S, 2002.

KEY WORDS: • *antioxidants* • *vitamin C* • *exercise* • *dogs*

Free radicals and other reactive oxygen species (ROS[4]) are produced during normal metabolism. These molecules are highly reactive with phospholipid membranes, triglycerides, nucleic acids, proteins and polysaccharides (1). Nutritive-reducing substances and scavenging enzymes inhibit ROS production and are normally present in sufficient quantities to prevent cell damage. During exercise, however, the production of ROS and markers of oxidation, such as malondialdehyde and TBARS, appears to increase in proportion to the severity of exercise, whereas antioxidant concentrations decrease (2,3). This oxidative stress has the potential to cause muscle damage, and several investigators have examined whether supplemental antioxidants limit exercise-induced oxidative muscle damage in human (4–9), canine (10–13) and equine (14,15) athletes.

In sled dogs, plasma concentrations of isoprostane (a marker of lipid peroxidation) and creatine kinase (a marker of muscle damage) increase and vitamin E concentrations decrease during strenuous endurance exercise (10). Supplementation of sled dogs with α-tocopheryl acetate, β-carotene and lutein daily for 1 mo decreased DNA oxidation associated with endurance exercise and increased the resistance of lipoprotein particles to oxidation in vitro (11). Daily supplementation of vitamin E (457 IU), β-carotene (5.1 mg) and vitamin C (706 mg) did not mitigate the increase in circulating creatine kinase concentrations associated with endurance exercise in sled dogs but there was a significant association between prerace vitamin E concentrations and completion of a race (12). Most studies, however, have failed to show an effect of antioxidant supplementation on performance (16).

Previous experiments in this laboratory have shown that greyhounds run a consistent race from wk to wk and that dietary manipulation can affect performance (17,18). We have also found that serum vitamin E declines and oxidative stress increases after a short sprint race but that supplementation with high (1000 IU) but not moderate (100 IU) daily doses of

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[4] Abbreviations used: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; CPK, creatine phosphokinase; ME, metabolizable energy; RBC, red blood cell; ROS, reactive oxygen species; TBARS, thiobarbituric acid-reducing substances; TEAC, Trolox equivalent antioxidant capacity; TDF, total dietary fiber; WBC, white blood cell.

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vitamin E appears to slow racing greyhounds (19). Many greyhound trainers give high doses of vitamin C to their dogs but we are unaware of any studies that have determined whether supplemental vitamin C may be of benefit to racing greyhounds.

Vitamin C (ascorbic acid) is not an essential nutrient in sedentary dogs but the requirement for vitamin C may increase beyond the synthetic capacity of the liver during strenuous exercise. Kronfeld and Donoghue (20) have reported that plasma vitamin C concentrations decline in sled dogs undertaking strenuous endurance exercise during the racing season, and that supplementation with vitamin C benefited sled dogs fed a high-fat diet. These authors quote experiments in which sled dogs received 1 g vitamin C daily (21) and imply that dogs undertaking strenuous exercise should receive even higher doses (4 mg/kJ). The goal of this experiment, therefore, was to evaluate the effect of a daily large oral dose (1 g or 57 mmol) of ascorbic acid on plasma levels of vitamin C, vitamin E, TEAC (a measure of total antioxidant capacity) and TBARS in greyhounds before and after a short sprint race. The effect of time of supplementation relative to racing was also examined because preliminary pharmacokinetic studies in greyhounds suggested that plasma ascorbate concentrations peak within 1–2 h after oral administration, and return to baseline within 24 h.

MATERIALS AND METHODS

Animals

Five adult female greyhounds [weighing 27. 6 ± 0.6 kg (mean ± SD) and aged 2.9 ± 0.4 y old] were donated by commercial racing kennels for use in this study. All dogs had previously been trained to chase a lure on a racetrack. All dogs were healthy and had been vaccinated against rabies, distemper, leptospirosis, hepatitis and parvovirus. Dogs were cared for as previously described (18), and according to the principles outlined in the NIH Guide for the Care and Use of Laboratory Animals (22). The study was approved by the University of Florida institutional animal care and use committee. At the end of the study, all five dogs were spayed and then adopted by new owners.

Following the initial screening, the dogs began an 8-wk acclimation period, during which the dogs raced over a distance of 500 m twice weekly on a 400-m oval sand and clay track, with 10° banking at the corners. The dogs were raced in one group of three and one group of two, and were randomly assigned to each race and starting position. Race times were measured by a commercial photo-finish camera and computer program (Finish Lynx V1.2, Lynx System Developers, Woburn, MA).

Diet

Dogs were fed a commercially available kibble diet (Pedigree Performance; Kal Kan, Vernon, CA) containing approximately 280 J/kJ metabolizable energy (ME) from protein, 380 J/kJ ME from fat and 340 J/kJ ME from carbohydrate (**Table 1**). The vitamin and mineral composition of the diet conformed to the American Association of Feed Control Official (AAFCO) recommendation for adult dogs. Analysis of a representative sample of the diet during the study showed it to contain 4 mg/kg ascorbate as fed. During the 8-wk acclimation period, each dog was fed ad libitum for 30–45 min once/d. Excess food was removed after each dog had voluntarily stopped eating. Ninety percent of the average intake of each dog during this time was calculated, and this amount was fed to each dog for the remainder of the study. Food was restricted slightly in this way to maintain dogs with a lean body condition similar to that found in racing dogs and to optimize race performance.

Experimental design

Each dog received three treatments for 4 wk per treatment, in a randomized three-period crossover design. All dogs were fed after racing, but the timing and amount of vitamin C varied among treatment groups. The three treatments were: *1*) no supplemental ascorbate; *2*) 1 g (57 mmol) ascorbate daily with food; *3*) 1 g (57 mmol) ascorbate daily, administered orally 1 h before racing on race days, and with food on nonrace days. At the end of each 4 wk, blood was obtained by jugular venipuncture on a race day before racing, and at 5 and 60 min and 24 h after racing. Blood was mixed with the anticoagulant lithium-heparin for vitamin C measurements and with EDTA for measurement of blood cell variables, TBARS and TEAC. Serum from uncoagulated blood was used for other chemical analyses. Plasma and serum samples were protected from light and stored in a −70°C freezer.

Analyses

Blood cell variables and routine chemistries were measured as previously described (17). Vitamin E was measured using the proce-

TABLE 1

Proximate analysis, essential fatty acid, total dietary fiber (TDF), and estimated metabolizable energy (ME) content of the diet

Nutrient (as fed)	Amount	Nutrient	Amount
Protein, %	27.8 (28%ME)	Magnesium, *g/kg*	1.1
Fat, %	15.6 (38%ME)	Iron, *mg/kg*	261
Linoleic acid, %	2.66	Copper, *mg/kg*	14
Linolenic acid, %	0.11	Zinc, *mg/kg*	220
Arachidonic acid, %	0.05	Manganese, *mg/kg*	17
Eicosapentanoic acid, %	0.003	Selenium, *mg/kg*	0.3
Docosohexenoic acid, %	0.008	Iodine, *mg/kg*	1.8
(n-3):(n-6)	1:16	Vitamin A, *IU/g*	12.9
Nitrogen-free extract, %	33.6 (34%ME)	Vitamin E, *IU/kg*	93
Ash, %	9.1	Vitamin D3, *IU/kg*	860
Crude fiber, %	1.2	Vitamin B1, *mg/kg*	2.2
Total dietary fiber, %	6.5	Vitamin B2, *mg/kg*	5.0
Insoluble fiber, %	5.9	Vitamin B3, *mg/kg*	37
Moisture, %	8.0	Vitamin B6, *mg/kg*	1.5
ME density, *kcal/g*	3.47	Vitamin B12, *mg/kg*	0.1
Calcium, *g/kg*	19.8	Pantothenic acid, *mg/kg*	17
Phosphorus, *g/kg*	13.5	Folic acid, *mg/kg*	0.9
Sodium, *g/kg*	5.8	Choline chloride, *mg/kg*	1.3
Potassium, *g/kg*	5.9	Biotin, *g/kg*	0.1

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dure described by Browne and Armstrong (23). Samples were eluted at room temperature on a column (Supelcosil LC-18, 15 cm × 4.6 mm, 5 μm; Supelco, Bellefonte, PA) using an HPLC (Series 200, with a 235C diode array detector and ISS 200 autosampler; Perkin Elmer Cetus Instruments, Norwalk, CT). The amount injected was 50 μl; the flow rate was 1.5 mL/min; the pressure was approximately 70 kg/cm^3; absorbance was set at 292 and 325 nm; and the photodiode array detector slit width was set at 4 nm.

To stabilize vitamin C, metaphosphoric acid was added to heparinized plasma immediately after collection and then vitamin C was measured with a spectrophotometer (Shimadzu UV-1601, Kyoto, Japan) at a light wavelength of 520 nm, using the method described by Roe (24). The coefficient of variation for this assay was 5%. The concentration of TBARS was measured with a spectrofluorophotometer (Shimadzu RF-1501) at an excitation wavelength of 535 nm, emission wavelength of 552 nm, sensitivity high and slit width set at 5 nm according to the method described by Armstrong and Browne (25). The coefficient of variation for this assay was 10%. Plasma TEAC was measured after a 1:1 dilution with a spectrophotometer (Shimadzu UV-1601), on which absorbance values were recorded over 3 min, according to the method of Armstrong and Browne (25).

Calculations and statistical analysis

Observed concentrations of Vitamin C, TBARS and TEAC data at each time point were adjusted for postrace hemoconcentration, using the following formula:

$$C_{adj} = C_{obs} \times (Alb_1/Alb_2)$$

where C_{adj} is the adjusted concentration, C_{obs} is the observed concentration, Alb_1 is the prerace albumin concentration, and Alb_2 is the albumin concentration at the same time after racing as C_{obs}. Vitamin E concentrations were adjusted after racing in a similar fashion but using changes in cholesterol concentration after racing instead of changes in albumin concentration. Both observed data and data adjusted for fluid shifts were analyzed separately.

Statistical analyses were performed using a standard statistical software package (SAS, version 8.0; SAS Institute, Cary, NC). Variables that were not normally distributed at all time points or with unequal variances were logarithmically transformed before analysis. Hematological and blood chemistry data were analyzed by repeated-measures analysis of variance. Vitamin E, vitamin C, TBARS and TEAC concentrations, the ratio of TEAC/TBARS, body weight and race times were analyzed as a three-period crossover design with a within-period repeated-measures factor, using generalized least-squares estimation (26). This was an unbalanced design with five dogs, three periods and three treatments. Only race times for wk 2–4 of each treatment period were included in the analysis. Week 1 was excluded to allow an adjustment period after changing treatments. The last race was also excluded because blood drawn before racing could have affected performance. Measurements over time within each period (body weights and race times) and measurements before and after racing (blood analyses) were treated as within-subject repeated measures. A standard goodness-of-fit criterion (i.e., Schwarz's Bayesian criterion) indicated that a spatial power-law structure was appropriate for the unequally spaced repeated measures, whereas an autoregressive order 1 structure was chosen for equally spaced repeated measures (27). A random effect was included for each dog. Period, treatment, time, treatment*period and treatment*time were used as fixed effects in the model. A Bonferroni adjustment was used for post hoc comparisons. Values were considered significant at a $P < 0.05$.

RESULTS

There was an interaction between time and treatment for both observed and adjusted mean ascorbate concentrations ($P = 0.002$; **Table 2**). In dogs that received vitamin C after racing, there was no evidence of a change in adjusted plasma ascorbate concentrations over time. In dogs that received no supplemental vitamin C, adjusted vitamin C concentrations were higher 5 min after racing compared to those before or 24 h after racing ($P < 0.02$). In dogs that received vitamin C before racing, however, adjusted ascorbate concentrations were higher at 5 min and 1 h after racing compared to those before or 24 h after racing ($P < 0.02$). Adjusted mean ascorbic acid concentrations were higher 1 h after racing in these dogs receiving vitamin C before racing than in those dogs that received no vitamin C or received vitamin C after racing ($P < 0.001$). Observed concentrations showed a similar pattern but were affected by fluid shifts.

There was an effect of treatment on race times ($P = 0.004$). Race times were shorter and dogs were faster over 500 m (**Table 3**) when dogs were not supplemented with vitamin C than when they received vitamin C before racing ($P = 0.03$) or after racing ($P = 0.005$). There was no evidence that the time of vitamin C supplementation relative to racing (i.e., prerace vs. postrace) affected race times. There was a significant interaction between period and treatment with respect to

TABLE 2

The effect of supplemental vitamin C on observed and adjusted[1] plasma ascorbate concentration[2] (μmol L^{-1}) in greyhounds before and after racing

	Time			
Treatment	Before racing	5 min postrace	1 h postrace[3]	24 h postrace
Observed concentration				
No C	39 ± 19[a]	74 ± 17[b]	48 ± 11[ab]	28 ± 10[a]
C before racing	47 ± 19[a]	109 ± 38[b]	124 ± 61[b]	29 ± 12[a]
C after racing	54 ± 22[ab]	73 ± 24[a]	62 ± 22[ab]	37 ± 14[b]
Adjusted concentration				
No C	39 ± 19[a]	62 ± 15[b]	52 ± 12[ab]	29 ± 11[a]
C before racing	47 ± 19[a]	90 ± 31[b]	134 ± 66[b]	30 ± 12[a]
C after racing	54 ± 22	61 ± 21	66 ± 24	39 ± 14

[1] Values are adjusted for hemoconcentration using changes in albumin concentration from before to after racing.

[2] Mean concentration ± 1 SD; $n = 5$. There was a significant time by treatment for both observed and adjusted mean ascorbate concentrations ($P < 0.002$).

[3] Mean concentrations at 60 min after a race (both observed and adjusted) are significantly different in dogs receiving vitamin C before racing compared to dogs not supplemented with vitamin C and dogs receiving vitamin C after racing ($P < 0.05$).

[a,b] Means within a row with different superscripts are significantly different ($P < 0.05$).

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TABLE 3

The effect of supplemental vitamin C on racing time and speed in racing greyhounds[1]

	Treatment			
	No C	C prerace	C postrace	SE
Race time, *s*[2]	32.52[a]	32.69[b]	32.73[b]	0.18
Speed, *m/s*[2]	15.38[a]	15.30[b]	15.28[b]	0.08
Body weight, *kg*	27.8	28.1	28.0	0.4

[1] Least-squared means; $n = 5$ greyhounds during wk 2–4 of each diet period.

[2] Means with different superscripts are significantly different with respect to treatment ($P < 0.05$).

body weight ($P = 0.04$): dogs receiving vitamin C before racing were slightly heavier than dogs receiving vitamin C after racing during period 1 only ($P = 0.03$). The mean body weight of all dogs increased slightly (~0.3 kg) from period 1 to period 2 ($P = 0.007$), and remained elevated during period 3. The mean body weight of dogs not receiving vitamin C was slightly (~0.3 kg) less than that of dogs receiving vitamin C, although this difference was not significant ($P = 0.06$).

Most hematologic or serum biochemical variables increased or decreased after racing, but there was no evidence of an effect of treatment or a significant interaction between time and treatment (**Tables 4** and **5**). There was no evidence of an effect of treatment or a time-treatment interaction on either observed or adjusted α-tocopherol, TEAC or TBARS concentrations or the ratio of TEAC to TBARS (**Table 6**). There was, however, an effect of time relative to racing on some of these variables. Observed but not adjusted α-tocopherol and TBARS concentrations increased after racing ($P = 0.03$), which suggests that the changes in observed concentrations were an effect of fluid shifts. There was no evidence of an effect of racing on observed TEAC concentrations or the ratio of TEAC to TBARS but adjusted TEAC concentrations (Table 6) declined slightly after racing and were lower at 5 min than at 60 min after racing ($P \leq 0.04$).

DISCUSSION

Performance

Greyhound trainers commonly give high doses of antioxidants such as vitamin C and E to their dogs in the belief that these supplements will improve performance or will reduce the risk of injury. In the present study, however, the dogs ran, on average, 0.2 s slower over 500 m when supplemented daily with a high dose (1 g) of vitamin C. Although this time difference was small, it equates to a lead of 3 m at the finish of a 500-m race, and may represent the difference between winning and losing. In a previous experiment, greyhounds also ran slower when supplemented with a high daily dose (1000 IU) of vitamin E but not when supplemented with a more moderate daily dose (100 IU) of vitamin E (19). Together, these experiments suggest that high doses of antioxidants may be detrimental and should not be given to racing greyhounds. More moderate doses may still have a beneficial effect, however, without slowing dogs down. It is also possible that combinations of antioxidants may work together to have a beneficial action without detrimental side effects where individual antioxidant supplements do not.

Others have found that antioxidant vitamins may have a deleterious effect at high doses. Podmore and colleagues (28) report that vitamin C may exhibit prooxidant properties at high doses. Childs and colleagues (29) found that supplemen-

TABLE 4

The effect of supplemental vitamin C on serum biochemical variables in greyhounds before and after racing[1]

	Treatment						
	No C		C before racing		C after racing		
	Before racing	5 min after racing	Before racing	5 min after racing	Before racing	5 min after racing	SE
ALP, *nkat* L^{-1}*	0.73	0.80	0.59	0.81	0.70	0.88	0.11
AST, *nkat* L^{-1}*	0.66	1.02	0.73	1.06	0.71	1.00	0.08
ALT, *nkat* L^{-1}*	0.81	0.93	0.71	1.15	0.77	1.08	0.15
CPK, *nkat* L^{-1}	2.8	5.0	6.0	4.2	3.0	3.6	0.8
Bilirubin, μ*mol* L^{-1}	6	8	6	7	6	8	1
Total protein, *g* L^{-1}*	59	72	61	74	59	72	1
Globulin, *g* L^{-1}	26	32	26	33	26	32	1
Creatinine, μ*mol* L^{-1}	121	144	137	137	131	145	9
BUN, *mmol* L^{-1}*	6.9	7.6	7.1	7.4	6.5	7.0	0.3
Glucose, *mmol* L^{-1}*	5.0	7.8	4.7	7.0	4.7	7.5	0.2
Calcium, *mmol* L^{-1}*	2.42	2.62	2.43	2.62	2.45	2.59	0.02
Sodium, *mmol* L^{-1}*	152	158	152	158	151	158	1
Potassium, *mmol* L^{-1}*	4.1	4.5	4.2	4.5	4.2	4.3	0.1
Chloride, *mmol* L^{-1}*	111	105	110	104	110	105	1
Phosphorus, *mmol* L^{-1}	0.82	0.86	0.84	0.88	0.85	0.91	0.07
Carbon dioxide, *mmol* L^{-1}*	24	8	24	8	23	7	1
Anion gap, *mmol* L^{-1}*	22	50	22	51	21	50	2

[1] ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate transaminase; CPK, creatine phosphokinase; BUN, blood urea nitrogen. Mean concentrations; $n = 5$.

*Means before racing were significantly different from those 5 min after racing ($P < 0.05$). There was no significant treatment effect or time by treatment interaction.

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TABLE 5

The effect of supplemental vitamin C on hematological variables in greyhounds before and after racing[1]

	Treatment						
	No C		C before racing		C after racing		
	Before racing	5 min after racing	Before racing	5 min after racing	Before racing	5 min after racing	SE
WBC count, $\times 10^9$ L^{-1}	5.3	5.1	5.1	5.4	5.0	5.4	0.3
RBC count, $\times 10^{12}$ L^{-1}*	8.6	10.1	8.8	10.2	8.8	10.1	0.1
Hematocrit*	0.57	0.67	0.58	0.69	0.58	0.68	0.01
Hemoglobin, *mmol* L^{-1}*	12.6	14.7	12.8	14.9	12.9	14.8	0.2
Mean corpuscular volume, *fl*	66	66	66	67	66	67	0.4

[1] WBC, white blood cell; RBC, red blood cell. Means; $n = 5$.
* Means before racing were significantly different from those 5 min after racing ($P < 0.05$). There was no treatment effect or time by treatment interaction.

tal vitamin C and *N*-acetyl cysteine during the acute phase inflammatory response to an eccentric arm injury increased lipid hydroperoxides and bioreactive iron and transiently increased tissue damage in humans. Coombes and colleagues (30) report that high levels of vitamin depress skeletal muscle force production at low stimulation frequencies. It is possible, therefore, that vitamin C was reducing performance in these greyhounds either by acting as a prooxidant and increasing tissue damage or by interfering with force production within muscle. None of the variables measured, however, showed any increase in muscle damage or oxidative stress.

The lactic acidosis observed after short-duration races may also lead to a failure in contractile machinery, and ultimately a decrease in speed (31). Vitamin C is an acid and could have exacerbated the metabolic acidosis observed in the dogs of the present study after racing. Blood pH was not measured directly but blood pH is a reflection of the relative concentrations of cations (such as sodium and potassium) and anions (such as chloride and bicarbonate). The concentration of all these ions (including bicarbonate as total carbon dioxide) and the anion gap between them were measured. The anion gap increased 125% after racing, as expected, primarily because of the increase in lactic acid concentration. Ascorbic acid could have contributed to this increase but the effect of ascorbate on the anion gap and, therefore, blood pH must have been small because there was no statistical evidence that supplemental ascorbic acid affected the anion gap or any of the anion or cation concentrations. It is unlikely, therefore, that acidosis was responsible for the difference in speed observed in the present study.

It is also possible that body weight was partly responsible for the difference in race times. An unpublished study in this same laboratory showed greyhounds ran, on average, 0.4 s faster when dogs were given slightly less food and weighed, on average, 1.7 kg less. There were slight changes in body weight over time during the present study. Dogs receiving vitamin C were slightly (0.3 kg) heavier than dogs not receiving C, although this difference was small and not significant. Changes in most hematologic and serum biochemical variables observed after racing were similar to those reported previously (17,18). In previous greyhound studies in the same laboratory, however, white blood cell count increased, and phosphorus

TABLE 6

Alpha-tocopherol, TEAC, TBARS, cholesterol and albumin concentrations[1] *(both observed and adjusted for hemoconcentration*[2]*) and the ratio of TEAC/TBARS concentrations in the blood of greyhounds before and after a 500-m race*

	Time				
Variable	Before racing	5 min postrace	1 h postrace	24 h postrace	SE
Observed concentration					
α-Tocopherol, *μmol/L*	5.8[ab]	7.2[a]	5.1[b]	6.0[ab]	0.7
TEAC, *mmol/L*	1.31	1.45	1.45	1.46	0.12
TBARS, *μmol/L*	1.36[ab]	1.57[a]	1.26[b]	1.46[ab]	0.09
Cholesterol, *mmol/L*	4.2[a]	5.0[b]	3.7[c]	4.1[a]	0.3
Albumin, *g/L*	34[a]	40[b]	31[c]	32[d]	0.5
TEAC/TBARS	1028	970	1198	1056	97
Adjusted concentration					
α-Tocopherol, *μmol/L*	5.8	6.2	5.8	6.2	0.7
TEAC, *mmol/L*	1.31[ab]	1.20[a]	1.57[b]	1.52[ab]	0.11
TBARS, *μmol/L*	1.36	1.30	1.37	1.53	0.08

[1] TBARS, thiobarbituric acid-reducing substances; TEAC, Trolox equivalent antioxidant capacity. Least-squares mean concentration, $n = 5$. There was no significant treatment effect or time by treatment interaction.
[2] Concentrations of TBARS and TEAC are adjusted using changes in albumin concentration, whereas α-tocopherol concentrations were adjusted using changes in cholesterol concentrations.
[a,b,c] Means within a row with different superscripts are significantly different ($P < 0.05$).

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concentrations decreased after racing. White blood cell count and phosphorus concentration did not change in the present study. Other investigators have reported an increase in white blood cell count and an increase in phosphorus concentration after racing (31). The reason for this disparity among studies is unclear.

Oxidative stress

Supplemental oral vitamin C did not increase vitamin C in dogs given vitamin C after racing but increased plasma vitamin C concentrations 5 and 60 min after racing in dogs given vitamin C 1 h before racing. Vitamin C concentrations were higher 60 min after racing in dogs receiving vitamin C before racing compared to dogs receiving the other treatments. This confirms preliminary observations that the response to oral supplementation is transient and concentrations return to baseline within 24 h. Administration of vitamin C and changes in circulating vitamin C concentrations did not affect circulating concentrations of vitamin E, TBARS as a marker of oxidation, total antioxidant capacity as measured by TEAC or the ratio of TEAC/TBARS. Hill et al. (19) observed that adjusted serum vitamin E levels decreased, adjusted TBARS concentrations increased and the TBARS to vitamin E concentration decreased in greyhounds after racing. The present study used fewer dogs, however, and variation between dogs was high. It is possible, therefore, that subtle changes were not detected.

Over the course of the present study, two dogs ran consistently 0.5–1.0 s slower than the other dogs. It is possible, therefore, that TBARS did not increase significantly after racing because these two dogs did not run with the same intensity as the other three dogs from this study or the dogs from the previous study. The power of this study, therefore, may have been insufficient to detect small but potentially important changes in TBARS. The results from the present study agree, however, with a study in sled dogs, in which 706 mg vitamin C was given orally daily as part of an antioxidant vitamin cocktail but did not affect plasma ascorbate concentrations or total antioxidant status when these variables were measured before the evening feeding on a day when dogs were not exercised (12).

In summary, therefore, this study suggests that oral administration of high doses of vitamin C slows racing greyhounds. Oxidative stress did not increase in this study after a short sprint race, however, and vitamin C may be beneficial at lower doses, in combination with other antioxidant supplements or in situations where there is a greater oxidative stress such as might occur if untrained dogs undertake intense exercise.

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