Effect of Increasing Dietary Antioxidants on Concentrations of Vitamin E and Total Alkenals in Serum of Dogs and Cats*

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ABSTRACT

Oxidative damage to DNA, proteins, and lipids has been implicated as a contributor to aging and various chronic diseases. The presence of total alkenals (malondialdehyde and 4-hydroxyalkenals) in blood or tissues is an indicator of lipid peroxidation, which may be a result of in vivo oxidative reactions. Vitamin E functions as a chain-breaking antioxidant that prevents propagation of free radical damage in biologic membranes. This 6-week dose–titration study was conducted to assess the effect of selected dietary vitamin E levels on byproducts of in vivo oxidative reactions in dogs and cats. Forty healthy adult dogs and 40 healthy adult cats were assigned to four equal groups per species in a complete random block design. A control group for both dogs and cats was fed dry food containing 153 and 98 IU vitamin E/kg of food (as fed), respectively. Canine and feline treatment groups were fed the same basal dry food with vitamin E added at three different concentrations. The total analyzed dietary vitamin E levels for the canine treatment groups were 293, 445, and 598 IU vitamin E/kg of food, as fed. The total analyzed dietary vitamin E levels for the feline treatment groups were 248, 384, and 540 IU vitamin E/kg of food, as fed. Increasing levels of dietary vitamin E in dog and cat foods caused significant increases in serum vitamin E levels compared with baseline values. Although all treatments increased concentrations of vitamin E in serum, all were not effective at decreasing serum alkenal levels. The thresholds for significant reduction of serum alkenal concentrations in dogs and cats were 445 and 540 IU vitamin E/kg of food, respectively, on an as-fed basis. The results of this study show that normal dogs and cats experience oxidative damage and that increased dietary levels of antioxidants may decrease in vivo measures of oxidative damage.

INTRODUCTION

Oxygen functions as the final electron acceptor in the process of biologic fuel conversion within cells. This results in transformation of biologic molecules such as protein, carbohydrate, and fat to water and carbon dioxide plus...
energy. The resultant energy produced is captured efficiently via oxidative phosphorylation in the phosphodiester bonds of adenosine triphosphate (ATP). However, in the conversion process that results in water and carbon dioxide, highly reactive intermediates, known as reactive oxygen species (ROS), are produced within mitochondria. ROS may be produced in other organelles as well, such as peroxisomes, or by the interaction of environmental toxins (radiation, tobacco smoke, pollutants) with biologic molecules. The majority of ROS production is believed to occur within the mitochondrial matrix.

ROS are highly reactive in nature and, when present in a complex biologic system, may result in nonenzymatic reactions that damage DNA, lipids (especially polyunsaturated fatty acids [PUFA]), and proteins. When defense mechanisms for oxidative damage are reduced, and/or oxidative stress is increased, ROS concentrations within cells are increased, resulting in increased damage to biomolecules with subsequent production of lipid peroxidation byproducts, which are the measured alkenals (malondialdehyde [MDA], 4-hydroxynonenal [4HNE]), protein carbonyls, DNA adducts, and carbohydrate adducts. Lipid peroxidation destroys PUFA, which may impart specific cellular dysfunction, produces secondary radicals that may propagate the chain reaction of oxidative damage, and produces aldehydes (MDA, 4HNE) that may act as secondary toxins by forming Schiff’s bases with proteins, thus imparting dysfunctional characteristics. These noncontrolled oxidative reactions may result in damaged or nonfunctional biomolecules, which in turn may result in abnormal cellular function. There is a large and growing body of laboratory, clinical, epidemiologic, and intervention studies that suggests ROS may eventually result in mutation, neoplastic transformation, loss of cellular function, cellular aging, and, ultimately, cellular death.

In light of this body of knowledge, much interest has been focused on the potential to modulate or enhance the capability of cells to defend against the production of oxidative damage: collectively termed antioxidant defense. The term antioxidant defense refers to the ability of a compound to scavenge or protect/sequester cells from oxidants and reduce the overall number of oxidants in a system, thereby inhibiting oxidation. The antioxidant defense system is complex and includes enzymes (superoxide dismutase, catalase, reductase, and glutathione peroxidase), free radical scavengers (vitamins A [carotenoids], C [ascorbic acid], and E [tocopherols]), and metal chelators. In addition, antioxidants may act synergistically and have sparing effects in which one antioxidant protects another against oxidative destruction. For example, vitamin C regenerates vitamin E after it has reacted with a free radical. Antioxidants protect biomolecules by scavenging oxygen-derived species, minimizing formation of oxygen-derived species, binding metal ions that may be used to make poorly reactive species more dangerous, repairing damage to target tissues, and destroying badly damaged target molecules and replacing them with new ones.

Biomarkers of oxidative DNA damage and lipid peroxidation can be used to establish the role of antioxidants in health and disease. In this study alkenals were chosen as biomarkers because they are significant products of peroxidation and cause further cellular damage. This dose–titration study was conducted to assess the effect of various dietary vitamin E levels on in vivo markers of oxidative stress in normal dogs and cats.

MATERIALS AND METHODS

All protocols were approved by the Hill’s animal use and care committee and complied
with United States Department of Agriculture (USDA) guidelines for use of laboratory animals.

**Dogs**

Forty adult beagles were used in this phase of the study. All dogs were fed a base (control) dry food for 2 weeks before the study began. Dogs were then assigned in a complete random block design to one of four dietary groups of ten dogs each, using age and sex as stratification factors (Table 1). Animals were ranked by age within gender and applied to treatments to balance both age and gender. All foods fed to control and treatment groups had the following average nutrient levels: moisture, 6.8%; protein, 24.4%; fiber, 2.0%; ash, 4.4%; and fat, 14.9%. Analyzed nutrient levels in all products were within published analytical variances of the means, showing that all foods, including the control, had the same composition.

Treatment foods varied only in levels of dietary antioxidants. Foods fed to dogs in groups 1 through 3 contained 70 parts per million (ppm) vitamin C and 1.5 ppm β-carotene. Vitamin E was added as vitamin E acetate.

**Cats**

Forty adult cats were used in this phase of the study. All cats were fed a base (control) dry food for 2 weeks before the treatment period. Cats were assigned in a complete random block design to one of four dietary groups of ten cats each, using age and sex as stratification factors (Table 2). Animals were ranked by age within gender and applied to treatments to balance both age and gender. All foods fed to control and treatment groups had the following average nutrient levels: moisture, 5.4%; protein, 33.8%; fiber, 1.3%; ash, 4.6%; and fat, 19.3%. Analyzed nutrient levels in all products were within published analytical variances of the means, showing that all foods, including the control, had the same composi-

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**TABLE 1. Canine Treatment Groups**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Total Dietary Vitamin E (IU/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>153</td>
</tr>
<tr>
<td>1†</td>
<td>293</td>
</tr>
<tr>
<td>2†</td>
<td>445</td>
</tr>
<tr>
<td>3†</td>
<td>598</td>
</tr>
</tbody>
</table>

*Hill’s® Science Diet® Canine Maintenance®, dry. Nutritional adequacy of the control food was demonstrated by successful completion of an AAFCO animal feeding trial.

†Hill’s® Science Diet® Canine Maintenance®, dry with 70 parts per million (ppm) vitamin C and 1.5 ppm β-carotene. Vitamin E was added as vitamin E acetate.

**TABLE 2. Feline Treatment Groups**

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Total Dietary Vitamin E (IU/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control*</td>
<td>98</td>
</tr>
<tr>
<td>1†</td>
<td>248</td>
</tr>
<tr>
<td>2†</td>
<td>384</td>
</tr>
<tr>
<td>3†</td>
<td>540</td>
</tr>
</tbody>
</table>

*Hill’s® Science Diet® Feline Maintenance®, dry. Nutritional adequacy of the control food was demonstrated by successful completion of an AAFCO animal feeding trial.

†Hill’s® Science Diet® Feline Maintenance®, dry with 70 parts per million (ppm) vitamin C and 1.5 ppm β-carotene. Vitamin E was added as vitamin E acetate.
Treatment foods varied only in levels of dietary antioxidants. Foods fed to cats in groups 1 through 3 contained 70 ppm vitamin C and 1.5 ppm β-carotene added above basal levels; vitamin E as α-tocopherol acetate also was added as shown in Table 2. All cats were fed to maintain current body weight; body weights were recorded weekly. Cats had free access to controlled amounts of food for 23 hours daily. Food was fed ad libitum with a controlled amount based on recorded weight and historical food intake. Animals were individually housed in identical cages conforming to the USDA standards for laboratory animals. Water was available ad libitum. The temperature was controlled between 20˚C and 22˚C.

Samples
All dogs and cats were deprived of food overnight before blood collection. Blood samples were drawn aseptically from each animal at the beginning and end of the 6-week study period. Blood for complete blood counts and serum for biochemistry analyses were analyzed immediately. Blood was centrifuged at 5000 g, and serum was harvested and maintained at −70˚C until analyses for vitamin E and total alkenal concentrations could be completed.

Analyses
Reproducibility was determined by performing assays for 10 days under identical experimental conditions. With standard concentrations from 0 to 20 µm, the SEM values obtained were less than 5%. Total alkenal concentrations were assayed using a commercial colorimetric kit (Calbiochem®, Calbiochem-Novabiochem Corp, San Diego, CA) and an automated robotic chemistry analyzer. Total alkenals were defined as the combined concentrations of MDA and 4HNE in serum. Vitamin E in serum was analyzed by high-performance liquid chromatography with UV detection after methanol/hexane extraction. Complete biochemistry profiles were performed by an automated analyzer (Hitachi 912-Roche, Indianapolis, IN). Complete blood counts were performed by an automated analyzer calibrated for dogs and cats (Serona System 9000, ABX Diagnostics Inc, Irvine, CA).

Statistics
Data were analyzed using the general linear model procedure of the Statistical Analysis System. Effects of treatment on changes in vitamin E and alkenal concentrations were evaluated by using treatment as a discrete variable. This allowed each treatment to be analyzed for the effect of that antioxidant bundle on these variables. Treatments were considered to have a significant effect on the analyzed variable if there was a significant (P < .05) probability associated with the change of that variable between the beginning and the end of the study.

RESULTS
Dogs
Results are expressed as means. Differences between initial and final serum concentrations of vitamin E and total alkenals were not significant in the control group (Table 3). Vitamin E concentrations increased significantly in all groups receiving increased dietary supplementation of vitamin E (Table 3). Only the treatment groups receiving 445 and 598 IU vitamin E/kg food had significantly reduced serum alkenal concentrations (Table 3). Complete blood counts and serum biochemistry values remained within normal physiologic ranges.

Cats
Results are expressed as means. Differences between initial and final serum concentrations of vitamin E and total alkenals were not significant in the control group (Table 4). Vitamin E concentrations increased significantly in all
groups receiving increased dietary supplementation of vitamin E (Table 4). Only the treatment group receiving 540 IU vitamin E/kg food had significantly reduced serum alkenal concentrations (Table 4). Complete blood counts and serum biochemistry values remained within normal physiologic ranges.

**DISCUSSION**

The results of this study reveal that increasing the inclusion level of antioxidants in foods for cats and dogs reduced serum concentrations of total alkenals, an indicator of oxidative damage to PUFA. The control foods were formulated to contain enough vitamin E to meet the nutrient requirements of dogs and cats, both of which require a dietary source of vitamin E. Vitamin E dosages were determined based on previous work that showed that these were effective levels. Physiologically, vitamin E functions primarily as a biologic antioxidant and is critical for preventing oxidation of PUFA in membranes. A maximum level of 1000 IU vitamin E/kg of food is listed for dogs; no maximum levels are given for cats. Thus, the levels chosen for inclusion fall within the approved safe and effective range. The treatment groups were fed increasing levels of vitamin E to evaluate the effect of this dietary antioxidant on indicators of biologic antioxidant activity.

Increased levels of dietary vitamin E in dog
and cat foods significantly increased concentrations of vitamin E in serum. Initial mean concentrations of vitamin E in serum ranged from 27.30 to 36.74 µg/mL in dogs and 14.55 to 16.52 µg/mL in cats (Tables 3 and 4). These values are similar but not identical to concentrations of vitamin E in plasma in middle-aged and older American men and women (i.e., mean α-tocopherol levels of 27 and 26 µg/mL, respectively). The difference in baseline concentrations of vitamin E in dogs compared with those of cats may be related to the content of vitamin E in the base foods; the canine base food was approximately 56% higher in vitamin E content than the feline base food. However, differences in metabolism and excretion of vitamin E between dogs and cats may exist and could account for differences in baseline concentrations.

Vitamin E is reported to have relatively low toxicity in both animals and humans. Several reports conclude that the use of vitamin E supplements at dosages from 200 to 2400 IU/day (about 13 to 160 times the current recommended dietary allowance), administered to humans for up to 4.5 years, are safe. Patients with Parkinson’s or Alzheimer’s disease received 2000 IU vitamin E/day for 2 years without significant side effects. The reported acute oral median lethal dose (LD₅₀) for all-rac-α-tocopherol acetate in rats, mice, and rabbits is >2 g/kg body weight. There is minimal evidence that levels above 1000 IU vitamin E/kg of food are toxic to dogs. However, when very high levels of vitamin E (≥5000 IU/day) are administered, signs of antagonism to other fat-soluble vitamin actions have been documented and include impaired bone mineralization, reduced hepatic storage of vitamin A, and coagulopathies. The study reported here revealed no adverse physiologic changes in serum biochemistry or complete blood count values at the levels of antioxidants tested.

It has been proposed that nutrient recommendations, in humans and other animals, would benefit from a health promotion paradigm rather than a disease treatment one. Multiple studies indicate that intakes greater than the recommended dietary allowances of certain vitamins and minerals such as calcium, folic acid, vitamin E, selenium, and chromium reduce the risk of certain diseases for some people. Limitation of intakes to the recommended dietary allowances would preclude reductions in disease risk from these nutrients. Vitamin C and β-carotene are two nutrients in which health benefits are reported in response to increased supplementation. These were added to supply a base antioxidant value for the antioxidant comparison. It is commonly reported that antioxidants should be considered as a group. Vitamin C and β-carotene were added to increase the antioxidants of the foods studied and were therefore not added to the control group. Vitamin E levels were based on previous work showing this to be an efficacious concentration.

Epidemiologic data and limited clinical intervention studies indicate vitamin E levels in excess of 100 IU are needed for prevention of disease in humans. The Cambridge Heart Antioxidant Study reported strong protection by vitamin E doses against the risk of fatal and nonfatal myocardial infarction. Researchers from the Harvard School of Public Health are conducting long-term studies of groups of more than 80,000 female nurses and more than 40,000 male dentists and other health care professionals. In both of these large study populations, persons with high intakes of vitamin E were found to have reduced risks of coronary heart disease. Intakes equivalent to 100 to 400 IU vitamin E have been shown to reduce oxidation of low-density lipoprotein cholesterol and lower the risk of heart disease in humans; however, the recommended dietary

allowance for adult males and females is 15 mg/day of naturally occurring α-tocopherol.  

Results of a recent study suggest that dogs with idiopathic dilated cardiomyopathy have high oxidative stress with alterations in their endogenous antioxidant system. Compared with healthy dogs, dogs with idiopathic dilated cardiomyopathy had increased mean MDA levels. Furthermore, vitamin E concentrations were negatively correlated with the severity of the disease.

Oxygen free radicals are highly reactive and unstable, especially in a biologic matrix. Consequently, analytical methods have been developed that allow indirect measures of oxidative damage. These tests measure serum and tissue breakdown products of DNA, protein, or lipid as indices of in vivo oxidative stress. Increased levels of these biomarkers have been proposed to correlate with increased oxidative stress. Concentrations of total alkenals in serum are one indicator of lipid peroxidation. Total serum alkenal concentrations were selected as the biomarkers in these studies because serum alkenals are measurable in blood and indicative of generalized free radical damage. In addition, aldehydes from lipid peroxidation such as the alkenals MDA and 4HNE have been shown to be cytotoxic and genotoxic. Specifically, 4HNE has been shown to be cytotoxic to cholinergic neurons and increases neuronal susceptibility to oxidative stress.

Although all treatments with increased dietary vitamin E resulted in increased circulating concentrations of vitamin E in the study reported here, they were not all effective at decreasing serum alkenal levels. According to our findings, the thresholds for significant reduction of serum alkenal concentrations in dogs and cats were 445 and 540 IU vitamin E/kg food, respectively, on an as-fed basis. A possible explanation for this finding may be the relative uptake of vitamin E into tissue. Concentrations of vitamin E in serum are known to increase with intake; however, vitamin E accumulation in tissues other than adipose tissue follows more of a logarithmic function. In dogs, differential uptake of vitamin E into tissues has been demonstrated with varying intakes of vitamin E. The central nervous system is most resistant to changes due to vitamin E deficiency; however, it has much less absolute vitamin E content than muscle or adipose tissue. In contrast, supplementation of dogs with vitamin E resulted in the greatest increase of vitamin E in peripheral nerves and muscle, with a relatively smaller increase in liver and renal tissue, compared with tissue levels in dogs receiving foods with adequate levels of vitamin E. Thus, it may be necessary to increase concentrations of vitamin E in serum to an extent that allows for adequate accumulation into various tissues to see effective protection against oxidative stress that occurs within tissues.

The findings of this study show that normal dogs and cats experience oxidative stress and that increased dietary levels of vitamin E can improve antioxidant status and modulate oxidative stress in vivo. Similar decreases in plasma lipid peroxide levels have been reported to occur in diabetic humans and rats after vitamin E supplementation.

**CONCLUSION**

Biomarkers can be used to investigate which foods and dietary constituents decrease levels of oxidative DNA and lipid damage in vivo. They also can be used to investigate the optimum intake of any dietary component found to be protective. Thus, biomarkers can help establish the roles and levels of antioxidants and provide a rational basis upon which to conduct long-term interventional studies.

Incremental increases in dietary vitamin E resulted in incremental increases in serum vita-
A dietary level of 445 IU vitamin E/kg food resulted in reduced in vivo lipid peroxidation in dogs as shown by a reduction in serum alkenal concentrations. Likewise, 540 IU vitamin E/kg food resulted in reduced in vivo lipid peroxidation in cats based on reductions in the same biomarker.

REFERENCES


