Effects of Serum Vitamin E Levels on Skin Vitamin E Levels in Dogs and Cats*

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ABSTRACT

Skin problems are common in small animal practice. Oxidative stress, or the imbalance between prooxidants and the body's antioxidant defense system, likely plays a role in the development of skin disease. According to this study, increasing amounts of vitamin E in foods for dogs and cats increases serum and cutaneous concentrations of vitamin E. Based on available scientific data, these increases in vitamin E concentration are likely to be beneficial. However, the relationship between increases in serum and skin vitamin E concentrations and the prevention, development, and treatment of skin disease remains to be elucidated by intervention studies.

INTRODUCTION

According to surveys, between 20% and 75% of small animals seen in a typical veterinary practice have skin problems as a chief or concurrent owner complaint.[1] Fleabite hypersensitivity, skin cancer, bacterial pyoderma, and seborrhea were among the most common canine skin disorders documented in case loads of 17 North American veterinary teaching hospitals.[2] According to other data, the most common feline skin disorders include abscesses, parasitic dermatoses, allergy (i.e., fleabite hypersensitivity, atopy), and miliary dermatitis.[3,4] Despite regional differences, skin and hair disorders are clearly an important part of small animal practice.

The skin is the largest organ of the body: Skin, hair, and dermis make up 24% of the body weight of a newborn puppy and 12% of an adult dog's body weight.[5] Because of its role as a barrier, the skin is uniquely challenged by oxidants (i.e., free radicals).[6] It is exposed to high oxygen tension, which increases its vulnerability to oxidative damage. As the outermost layer of the skin, the stratum corneum is continuously exposed to an oxidative environment, including air pollutants, ultraviolet (UV) radiation, oxidants released as a result of normal metabolism, parasites, and aerobic microorganisms. ultraviolet radiation can cause acute adverse effects (e.g., sunburn, photosensitivity, immunosuppression) as well as long-term sequelae (e.g., photoaging, malignant

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skin tumors). ultraviolet radiation can also cause tissues to produce reactive oxygen species, eicosanoids, and cytokines.

A study of 991 beagles living in outdoor pens with doghouses in a high-altitude, high-sunshine environment revealed that 36.6% had solar dermatosis and more than 15% developed skin neoplasms during their lifetime. Tumor types and solar dermatosis were highly correlated.[7] In another study, dogs with short coats and lightly pigmented skin had more hemangiomas and hemangiosarcomas of the dermis than dogs with variable length coats and pigmentation. The authors of this report suggested an association between solar radiation and the biologic properties of glabrous skin in the genesis of these tumors.[8] As another example, white cats have a 13 times greater risk of developing squamous cell carcinoma than pigmented cats.[9]

Oxidative stress, or an imbalance between the production of free radicals and other reactive species (i.e., reactive oxygen species, reactive nitrogen species) and the natural antioxidant protective systems, can lead to oxidation of lipids, nucleic acids, and proteins. Animals have developed complex, integrated extracellular and intracellular defense systems to counter oxidative stressors and ameliorate their injurious effects. The primary defensive compounds are antioxidants that can interact with and quench reactive radical species and enzymes that can inactivate these species and their byproducts. The protective enzymes include, among others, superoxide dismutase, catalase, and the selenium-containing glutathione peroxidases. Other antioxidant mechanisms include stimulation of the expression of antioxidant or repair enzymes as well as chelation of transition metals.

Nutritional antioxidants such as vitamins E and C, carotenoids such as β-carotene, and the mineral selenium are vitally important in vivo antioxidants. Vitamin E is a chain-breaking antioxidant that prevents the propagation of free-radical reactions. The vitamin is a peroxyl radical scavenger and especially protects polyunsaturated fatty acids within membrane phospholipids and in plasma lipoproteins.[10] Tissues depend on plasma vitamin E levels.[11] Vitamin E is a significant constituent of sebum and is continuously secreted for delivery to upper layers of the skin.

Because of the high lipid content of the stratum corneum, lipophilic antioxidants such as α-tocopherol (i.e., vitamin E) are expected to play a major role in scavenging reactive oxygen species during oxidative stress. Vitamin E protects against UV-induced skin photodamage through a combination of antioxidant and UV-absorptive properties. However, topically applied vitamin E is rapidly depleted by UVB radiation (the specific portion of the sun's energy that reaches the earth) in a dose-dependent manner.[12] Nevertheless, numerous human studies have convincingly demonstrated pronounced photoprotective effects of natural and synthetic antioxidants when applied before UV exposure.

The primary purpose of this study was to determine the effect of feeding foods with varying amounts of vitamin E on serum and skin concentrations of vitamin E in dogs and cats.

MATERIALS AND METHODS

All protocols were approved by the Hill's Animal Use and Care Committee and complied with US Department of Agriculture (USDA) guidelines for use of laboratory animals.

Dogs

Twenty adult male and female beagles ranging in weight from 8.1 to 16.6 kg (mean 11.09 ± 0.53) were enrolled in the study. All dogs were fed a dry dog food (Pedigree® Mealtime® Small Crunchy Bites, Waltham) for 3

weeks before onset of the study. At the end of the prefeeding period (Day 0), dogs were stratified within sex into two groups based on vitamin E concentrations in the skin and body weight, so that averages of these parameters and standard deviations were similar between the two groups.

One group of 10 dogs was fed the dry food to which they were acclimated. When analyzed, this food was found to contain 217 IU of total vitamin E/kg of food (low vitamin E food; Table 1). The other group of 10 dogs was fed a different dry food (Science Diet® Sensitive Skin Canine Adult, Hill's Pet Nutrition). This food was found to contain 654 IU of total vitamin E/kg of food (high vitamin E food; Table 1). Both foods were fed for the remaining 8 weeks of the study.

All dogs were fed to maintain current body weight, based on weekly body weight recordings and historical food intake. Food was available ad libitum for 23 hours daily, and food intake was monitored daily. Animals were housed in individual cages conforming to USDA standards for laboratory animals. Water was available ad libitum. The room temperature was controlled between 20°C and 22°C.

Cats

Twenty adult male and female domestic shorthaired cats ranging in weight from 3.8 to 5.8 kg (mean 4.9 ± 0.13) were enrolled in the study. All cats were fed a dry cat food (Purina® Cat Chow®, Nestlé Purina Petcare) for 3 weeks before onset of the study. At the end of the prefeeding period (Day 0), cats were stratified into two groups in the same manner as for the dogs.

One group of 10 cats was fed the dry food to which they were acclimated. When analyzed, this food was found to contain 86 IU of total vitamin E/kg of food (low vitamin E food; Table 1). The other group of 10 cats was fed a different dry food (Science Diet® Sensitive Skin Feline Adult, Hill's Pet Nutrition). This food was found to contain 709 IU of total vitamin E/kg of food (high vitamin E food; Table 1). Both foods were fed for the remaining 8 weeks of the study.

TABLE 1. Measured Vitamin E Amounts in Foods Fed to Dogs and Cats (IU of Total Vitamin E/kg of Food)

Foods	*Dogs*	*Cats*
Low vitamin E food	217[a]	86[b]
High vitamin E food	654[c]	709[d]

[a]Pedigree® Mealtime® Small Crunchy Bites, Waltham.
[b]Purina® Cat Chow®, Nestlé Purina Petcare.
[c]Science Diet® Sensitive Skin Canine Adult, Hill's Pet Nutrition, Inc.
[d]Science Diet® Sensitive Skin Feline Adult.

All cats were fed to maintain current body weight, based on weekly body weight recordings and historical food intake. Food was available ad libitum for 23 hours daily, and food intake was monitored daily. Animals were housed in individual cages conforming to USDA standards for laboratory animals. Water was available ad libitum. The room temperature was controlled between 20°C and 22°C.

Anesthesia

Dogs and cats were anesthetized before blood samples and ethanol extracts from skin were collected for vitamin E determinations. Anesthesia was induced in dogs with a 2.5% solution of thiopental sodium administered intravenously (IV) at 15 mg/kg of body weight. Dogs were then intubated, and anesthesia was maintained with isoflurane delivered in oxygen. Cats were anesthetized with a combination of 0.01 mg atropine sulfate, 7 mg ketamine hydrochloride, and 0.1 mg acepromazine/kg of body weight administered

IV. Following extraction of vitamin E from the skin, dogs and cats were returned to their cages and monitored until sternal recumbency was achieved.

Samples

All dogs and cats were deprived of food overnight before serum samples and ethanol extracts from skin were collected. Blood samples were drawn aseptically from each animal on Days -3, 14, 28, and 56 of the study. Blood samples were placed on ice in serum separation tubes for at least 20 minutes but no longer than 2 hours.

To extract vitamin E from the skin, dogs and cats were placed on a bed in right lateral recumbency. On Day -3, hair was shaved from an area of the left flank measuring 5 × 10 cm using clippers that had been rinsed in ethanol. A glass ring was pressed firmly to the skin. Two ml of ethanol was added to the glass ring. A glass rod was used to gently stir the ethanol in the glass ring for 2 minutes. Afterward, the ethanol in the glass ring was transferred to a 50-ml polypropylene tube using a plastic transferring pipette. The same skin spot was again extracted with 2 ml of ethanol. On Days 14, 28, and 56, a different skin spot in the shaved area was extracted twice for vitamin E using the same technique described above. The skin extraction spots were at least 2 cm apart at each of the four measurements.

The ethanol from the two skin extraction spots was combined in the same tube, covered with a screw cap, and analyzed for vitamin E content within 6 hours of extraction. All personnel wore latex surgical gloves, and all glassware was rinsed thoroughly with ethanol to minimize contamination. The analytical method of determining vitamin E concentrations in ethanol extracts from skin was validated by measuring samples for precision, specificity, and accuracy.[13]

Analyses

Serum was separated by centrifugation and stored at −70°C until analysis. Vitamin E in serum was analyzed by high-performance liquid chromatography with UV detection following methanol/hexane extraction using a modified method.[14]

Ethanol was used to extract vitamin E from skin as described above. After evaporation and reconstitution with ethanol, samples were analyzed by high-performance liquid chromatography with UV detection.

Statistics

Data were analyzed using the general linear model procedure of the Statistical Analysis System (SAS).[15] Effects of treatment on changes in serum vitamin E concentrations were evaluated using treatment as a discrete variable. A significant effect occurred on the analyzed variable if there was significant ($P < .05$) probability associated with the change of that variable between the beginning and the end of the study.

The influence of serum vitamin E level on skin vitamin E concentration was evaluated using the same general linear model procedure of the SAS. The model tested the effects of initial skin vitamin E concentration, body surface area, and final serum vitamin E concentration. Effects of the analyzed variables were determined to be significant if $P < .05$. Body surface area and species were confounding variables that made data interpretation difficult when both were included in the statistical model. Body surface area accounted for more variation than species. Therefore, it was used as a continuous variable in the model. No species effects on skin vitamin E concentrations resulted when body surface area was included in the model. Thus species was not used as a variable.

TABLE 2. Serum Concentrations (± SEM) of Vitamin E in Dogs and Cats (µg/ml)

Foods	*Day –3*	*Day 14*	*Day 28*	*Day 56*
Dogs				
Low vitamin E	26.7 ± 2.2	31.6 ± 4.3	23.7 ± 1.7	24.8 ± 2.4
High vitamin E	26.1 ± 2.4	44.3 ± 3.1[b]	39.0 ± 2.1[b]	42.0 ± 3.1[a,b]
Cats				
Low vitamin E	15.1 ± 1.3	13.1 ± 1.2	11.9 ± 1.2	12.1 ± 1.0
High vitamin E	15.5 ± 1.5	27.6 ± 2.6[b]	27.3 ± 3.0[b]	29.7 ± 2.9[a,b]

[a]Significantly different from initial concentration ($P < .05$).
[b]Significantly different from the low vitamin E food ($P < .05$).

RESULTS

Serum Vitamin E Concentrations

Results are expressed as means (± standard error of the mean). There were no significant differences in serum vitamin E concentrations between the groups for either species at the beginning of the study (Table 2). Any dietary effects on serum vitamin E levels occurred within the first 2 weeks of the study in both species, and the significant ($P < .05$) changes that occurred during that period persisted until the study was ended.

At each subsequent time point, serum concentrations of vitamin E in dogs and cats fed the high vitamin E foods were significantly ($P < .05$) different from serum concentrations of dogs and cats fed the low vitamin E foods (Table 2). In addition, final (Day 56) serum concentrations of vitamin E in both species were significantly ($P < .05$) higher than the initial (Day –3) values in the groups receiving the high vitamin E food (Table 2). Values increased 61% in dogs and 92% in cats in these groups over the course of the study. Conversely, final serum concentrations of vitamin E in the groups fed the low vitamin E food were statistically similar to their initial values. Numerically, mean serum vitamin E values decreased slightly from the beginning to the end of the study in dogs and cats fed the low vitamin E food.

One cat died of a uterine infection during the study; data from this animal were not included in the analyses. All other animals remained healthy and maintained body weight for the duration of the study.

Pooled Skin Vitamin E Concentrations

There were no significant differences in skin vitamin E concentrations between the groups for either species at the beginning of the study. Because species was not a significant factor for skin vitamin E concentrations, data for both species were pooled. At the end of the study, vitamin E concentrations in skin were significantly ($P < .05$) influenced by initial skin vitamin E concentration, body surface area, and final serum vitamin E concentration (Figure 1).

DISCUSSION

The results of this study revealed that increasing vitamin E amounts in food significantly ($P < .05$) increased vitamin E concentrations in serum and skin. There were no significant differences in serum or skin vitamin E levels between the groups of dogs or cats at the beginning of the study. Serum vitamin E concentrations increased significantly ($P < .05$)

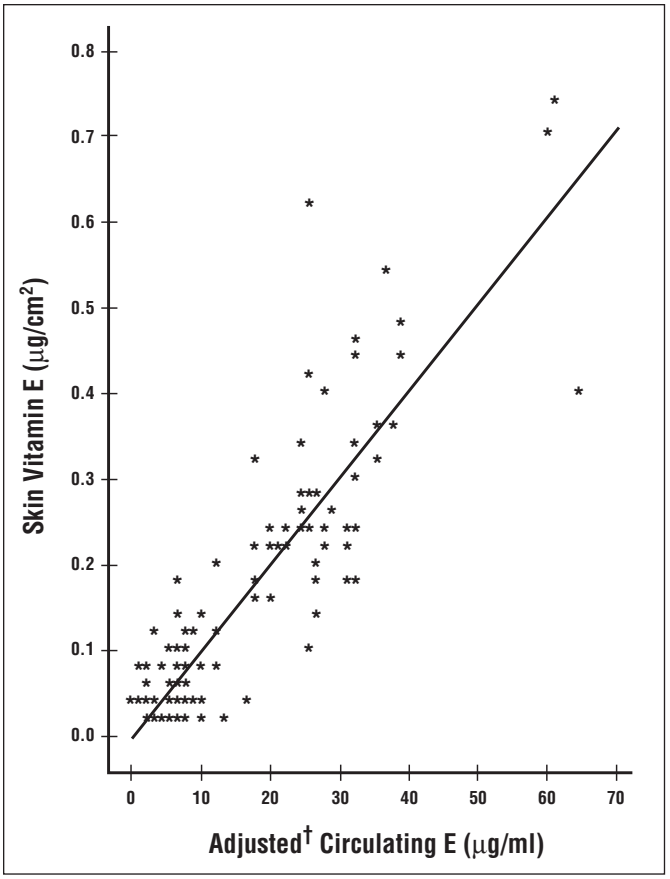

Figure 1. *Least squares analysis using initial vitamin E skin concentration* (P < *.001), body surface area* (P = *.003), and final serum E concentration* (P = *.002).*

†For every μg/ml change in serum vitamin E, there is a corresponding 1.2% change in skin vitamin E.

by Day 14 in dogs and cats fed the high vitamin E foods versus values in animals fed the low vitamin E foods. These values remained significantly ($P < .05$) different for the remainder of the study, demonstrating that increasing dietary vitamin E amounts increased serum vitamin E concentrations in dogs and cats. Furthermore, these data are similar to those obtained recently when investigators increased dietary amounts of vitamin E in foods for dogs and cats and found corresponding increases in serum vitamin E concentrations.[16] In that study, a biomarker of lipid peroxidation, malondialdehyde (MDA) generally decreased with increasing dietary amounts and serum levels of vitamin E.[16] More important, in the study reported here, serum vitamin E concentrations positively affected skin vitamin E levels when adjusted for initial skin vitamin E concentration, body surface area, and final serum vitamin E concentration.

Few, if any, terrestrial mammals have the diversity of body weight and body surface area as dogs have (viz. a Yorkshire terrier weighing 1 kg or less versus a Mastiff weighing 130 kg or more). Consequently, adjusting for body surface area is a widely accepted pharmacotherapeutic veterinary practice and is viewed to be more accurate than calculating dosages based on body weight.[17] Such adjustments can also be accurately made for cats.[17] Thus the canine and feline data for skin vitamin E concentrations were adjusted for body surface area and pooled.

All four foods used in this study 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.[18] The nutrient profiles of the Association of American Feed Control Officials (AAFCO) list minimum allowances of 50 and 30 IU of vitamin E/kg of food for dogs and cats, re-

spectively, for both growth and maintenance. A maximum amount of 1000 IU of vitamin E/kg of food is listed for dogs; no maximum amounts are listed for cats. Thus the amounts of all four foods fall within the AAFCO-approved safe and effective range.

Concentrations of serum vitamin E increase with intake; however, vitamin E accumulation in tissues other than adipose tissue follows more of a logarithmic function.[19,20] In dogs, differential uptake of vitamin E into tissues has been demonstrated with varying intakes of vitamin E.[21] The central nervous system is most resistant to changes resulting from vitamin E deficiency; however, it has much less absolute vitamin E content than muscle or adipose tissue.[21] 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 amounts in dogs receiving foods with adequate amounts of vitamin E.[21] Therefore, it may be necessary to increase concentrations of vitamin E in serum to an extent that allows adequate accumulation into various tissues to realize effective protection against oxidative stress that occurs within tissues.

In humans, 300 mg vitamin E supplementation increased plasma α-tocopherol concentrations threefold and at least doubled most tissue concentrations, whereas supplementation with 30 mg had little effect on either plasma or tissue concentrations.[22] These data suggest that tissue α-tocopherol concentrations largely reflect changes in plasma concentrations of α-tocopherol and that larger doses increase tissue α-tocopherol concentrations, including those in nervous tissues.[19] Of importance, even though it was given for more than 1½ years, the lower dose did not increase tissue α-tocopherol concentrations.[22]

As the outermost barrier of the body, the stratum corneum is frequently and directly exposed to a prooxidant environment, including UV solar radiation.[23] The stratum corneum is remarkably susceptible to UV radiation-induced depletion of vitamin E. Investigators often use two parameters to determine oxidative stress in skin: vitamin E depletion and MDA production.

Ozone exposure damages cutaneous lipids, an effect that can be attenuated by vitamin E application.[24] In the upper epidermis, ozone substantially depletes α-tocopherol and ascorbic acid. These antioxidants are unchanged by ozone in the lower skin layers. More remarkable, MDA increases 10-fold in the upper epidermis and twofold in the lower epidermis and remains unchanged in the dermis. Consequently, exposure to ozone depletes ascorbic acid and α-tocopherol and strongly induces lipid peroxidation in the skin.[25] Human stratum corneum reveals characteristic antioxidant and protein gradients with increasing antioxidant depletion and protein oxidation toward the outer layers.[26]

Ultraviolet radiation of skin destroys its antioxidants; however, previous application of vitamin E as the tocotrienol-rich fraction of palm oil preserves vitamin E.[27] Topical α-tocopherol treatment increased dermal superoxide dismutase (i.e., a major antioxidant enzyme) activity by 30% and protected epidermal glutathione peroxidase and superoxide dismutase (both antioxidant enzymes) from depletion after UV radiation. Total and reduced glutathione levels in the epidermis increased by 50% after topical treatment, whereas dermal ascorbate levels increased by 40%. The topical treatment increased α-tocopherol levels in the epidermis by 62-fold and the dermis by 22-fold. α-Tocopherol also reduced the formation of epidermal lipid peroxides after UV radiation. These results demonstrate that topical administration of α-tocopherol protects cuta-

neous tissues against oxidative damage induced by UV radiation in vivo and suggest that the underlying mechanism of this effect involves the up-regulation of a network of enzymatic and nonenzymatic antioxidants.[28]

α-Tocopherol, the major antioxidant in stratum corneum, is an early and sensitive biomarker of environmentally induced oxidation.[29] Furthermore, levels of protein oxidation increase toward the outer stratum corneum layers.[29] Topical application of α-tocopherol inhibits UV-induced photocarcinogenesis and DNA photodamage in mice.[30]

Although most research deals with topically applied vitamin E in mice or humans, oral vitamin E also has photoprotective actions. Oral β-carotene and α-tocopherol were orally administered to healthy volunteers for 12 weeks. Erythema was induced by illumination with a blue-light solar simulator. Serum β-carotene and α-tocopherol concentrations increased with supplementation, and erythema was significantly diminished after Week 8. Erythema suppression was greater when a combination of β-carotene and vitamin E administered than with β-carotene alone.[31] In another study, oral vitamins E (1000 IU/day) and C (2 g/day) reduced the sunburn reaction in humans.[32]

Antioxidant defenses highly depend on adequate nutrition. Inadequate ingestion of dietary antioxidants, such as vitamins C and E, mimics the effects of radiation exposure.[33–35] The balance between oxidants and antioxidants is crucial for health and is an important determinant of immune function, particularly for maintaining the integrity and functionality of membrane lipids, cellular proteins, and nucleic acids.

According to this study and others, increasing amounts of dietary vitamin E in foods for dogs and cats increases serum and skin concentrations of vitamin E and decreases serum levels of some of the biomarkers associated with oxidative stress. Based on available scientific data, these increases in vitamin E concentration are likely to be beneficial. However, the relationship between increases in serum and skin vitamin E concentrations and the prevention, development, and treatment of skin diseases remains to be elucidated by intervention studies.

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