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To cite this article: Alja Plevnik Kapun , Janez Salobir , Alenka Levart , Gabrijela Tavčar Kalcher ,
Alenka Nemec Svete & Tina Kotnik (2013) Plasma and skin vitamin E concentrations in canine
atopic dermatitis, Veterinary Quarterly, 33:1, 2-6, DOI: [10.1080/01652176.2012.758395](https://doi.org/10.1080/01652176.2012.758395)

To link to this article: <http://dx.doi.org/10.1080/01652176.2012.758395>



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Accepted author version posted online: 14
Dec 2012.
Published online: 17 Jan 2013.



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ORIGINAL ARTICLE

Plasma and skin vitamin E concentrations in canine atopic dermatitis

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(Received 6 June 2012; final version received 10 December 2012)

Background: Altered homeostasis of vitamin E has been demonstrated in human atopic dermatitis. Data on plasma and skin vitamin E concentrations in canine atopic dermatitis (CAD) are not available.

Objective: To determine vitamin E concentrations in plasma and skin of atopic dogs.

Animals and Methods: Vitamin E concentrations in plasma and full-thickness skin biopsies of 15 atopic dogs were related to CAD extent and severity index (CADESI-03) scores and compared to the equivalent concentrations in 17 healthy dogs. Statistically significant differences of measured parameters between the two groups were determined by the nonparametric Mann Whitney U test and correlations between CADESI-03 scores and vitamin E concentrations were evaluated by the Spearman rank test. A value of $P < 0.05$ was considered significant.

Results: Plasma concentrations of vitamin E were significantly lower in atopic dogs than in healthy dogs, with median values of 29.8 and 52.9 $\mu\text{mol/L}$, respectively. Skin vitamin E values did not differ significantly between patients and healthy controls. The median concentration of skin vitamin E in atopic dogs was higher than that in healthy dogs. No significant correlations were found between CADESI-03 score and plasma vitamin E or skin vitamin E concentrations.

Conclusions: Significantly lower plasma vitamin E concentrations in atopic dogs than in healthy controls indicate altered homeostasis of vitamin E in CAD.

Clinical importance: Further investigation into vitamin E supplementation in CAD is warranted.

Keywords: dog; canine; atopic dermatitis; antioxidants; vitamin E; CADESI

1. Introduction

Canine atopic dermatitis (CAD) is the most frequent canine dermatosis defined as a genetically predisposed inflammatory and pruritic skin disease with characteristic clinical features associated with IgE antibodies most commonly directed against environmental allergens (Halliwell 2006). Genetic factors, impairment of the skin barrier followed by antigen trespassing, immune system deviation and microbial superinfections are proven to be involved in the pathogenesis of CAD (Olivry et al. 2010; Marsella et al. 2011). In the last decade, oxidative stress (OS) has been well documented to play an important role in the pathogenesis of human AD (Tsuboi et al. 1998; Omata et al. 2001; Antille et al. 2002; Niwa et al. 2003; Nakai et al. 2009). Hydrogen peroxide, a potent reactive oxygen species (ROS), is indispensable for T-cell activation upon triggering of the T-cell receptor by antigen presenting cells (Kamiński et al. 2010). During their first encounter with antigen, memory T-lymphocytes, driven by IL-4, differentiate into Th2-lymphocytes producing IL-4, IL-5, IL-6, IL-10, and IL-13 (Bickers & Athar 2006). IL-4 promotes B cell activation and isotype switching to IgG1 and IgE. Elevated levels of IL-4 in human AD coincide with increased IgE levels mediating hypersensitivity reactions (Avgerinou et al. 2008; Bieber 2008). Recently published data showed involvement of oxidative stress in the pathogenesis of CAD (Plevnik Kapun et al. 2012). Significantly higher plasma malondialdehyde (MDA) concentrations

were found in atopic dogs compared to healthy ones. MDA is the most commonly used marker of the overall lipid peroxidation level and the presence of oxidative stress (Grotto et al. 2009). It has been suggested that oxidative stress with increased lipid peroxidation could be involved in the pathogenesis of atopic dermatitis in dogs (Plevnik Kapun et al. 2012), therefore there is an increasing interest in the use of antioxidants in its treatment.

Vitamin E is the predominant antioxidant in human stratum corneum (Thiele et al. 2001) and functions almost exclusively as a peroxyl radical scavenger, therefore protecting against lipid peroxidation (Traber & Atkinson 2007). The initial oxidation product of tocopherol is the metastable tocopheroxyl radical which can be reduced back to tocopherol by co-antioxidants (ascorbate, glutathione, coenzyme Q₁₀) or may react with another lipid peroxyl radical, yielding tocopherol quinone. Since lipid peroxyl radicals react with the tocopherols several orders of magnitude faster than their reactions with acyl lipids, one molecule of vitamin E can protect about 10^3 – 10^8 polyunsaturated fatty acid molecules at low peroxide levels (Kamal-Eldin & Appelqvist 1996). Altered homeostasis of vitamin E has been demonstrated in human AD (Antille et al. 2002; Hozyasz et al. 2004). Significantly higher concentrations of vitamin E and lower concentrations of lipid peroxides in the skin of atopic human patients were measured compared to healthy controls (Antille et al. 2002). This was attributed to chronic OS in

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This article was originally published with errors. This version has been corrected. Please see Erratum (<http://dx.doi.org/10.1080/01652176.2013.800699>).

AD that may provoke distribution of vitamin E into the target tissue. In human patients with AD, receiving vitamin E, significant improvement of clinical parameters with decrease of serum IgE levels have been shown in placebo-controlled studies (Tsourelis-Nikita et al. 2002; Javanbakht et al. 2011).

No data are available on homeostasis of vitamin E in CAD. The aim of the present study was, therefore, to determine concentrations of vitamin E in blood and skin of atopic dogs and compare them with those in healthy dogs.

2. Materials and methods

2.1. Animals

Fifteen atopic dogs (7 females and 8 males), aged from 1 to 5 years and of different breeds, and diagnosed to have atopic dermatitis according to current diagnostic procedures (Olivry 2010), were recruited into the study. A control group comprised of 9 female and 8 male age-matched healthy dogs of different breeds (Table 1). In order to exclude symptoms of food allergy, atopic dogs were, for 3 months prior to the study, fed a home-produced, individually adjusted elimination diet. Control dogs were fed a home-produced food.

Written consent of the owners was obtained and procedures complied with the relevant Slovenian governmental regulations (Animal Protection Act, Official Gazette of the Republic of Slovenia, No. 43/2007).

2.2. Inclusion and exclusion criteria

Other causes of pruritic skin disease, such as parasitic (scabies, demodicosis), infectious (bacterial folliculitis, *Malassezia* dermatitis) and allergic diseases (food hypersensitivity, flea bite hypersensitivity), were excluded or controlled prior to the study. Dogs were not allowed to be treated with prostaglandins (1 week before inclusion), antihistamines (2 weeks before inclusion), oral or topical

corticosteroids and cyclosporine (4 weeks before inclusion), injectable corticosteroids (8 weeks before inclusion), and essential fatty acids and vitamin E (12 weeks before inclusion).

2.3. Clinical examination

Clinical lesions were scored using Canine Atopic Dermatitis Extent and Severity Index (CADESI-03) (Olivry et al. 2007) and a value of 25 was set as the threshold. Concomitant diseases were excluded by history, physical examination as well as haematological and biochemical analyses. Based on the same approach, dogs of the control group were considered healthy (data not shown).

2.4. Blood sampling and processing

Venous blood samples for determination of plasma vitamin E concentration were collected into a 2 ml tube containing lithium heparin as anticoagulant (Vacuette, Greiner Bio-One, Kremsmünster, Austria) and immediately centrifuged at $1500 \times g$ for 15 minutes at 4°C . Plasma was separated and immediately frozen at -80°C and stored until analysis. Blood samples for biochemical analysis and complete blood count (CBC) were collected into serum separator tubes (Vacuette, Greiner Bio-One, Kremsmünster, Austria) and EDTA-containing tubes (Vacuette, Greiner Bio-One, Kremsmünster, Austria). Samples in serum separator tubes were stored for 30 min at 4°C to clot and then centrifuged ($1300 \times g$ for 10 min) to separate the serum. Serum samples were assayed on the day of collection to determine concentrations of creatinine and urea as well as activities of alanine aminotransferase (ALT) and alkaline phosphatase (AP). EDTA blood samples for CBC and white cell differential count (WDC) determinations were stored at room temperature and analysed between 1 and 3 hours following sampling.

Table 1. Breed, gender and age distribution of atopic and healthy dogs.

Atopic dogs			Healthy dogs		
Age (months)	Gender	Breed	Age (months)	Gender	Breed
47	M	mixed	51	F	labrador
25	F	labrador	47	M	mixed breed
54	F	golden retriever	46	M	mixed breed
60	F	golden retriever	60	M	mixed breed
42	F	american cocker spaniel	37	F	mixed breed
41	M	dalmatian	33	F	dalmatian
49	M	labrador	60	F	german shepherd
57	F	dobermann	35	F	mixed breed
30	F	Staffordshire terrier	13	M	shetland sheepdog
56	M	golden retriever	21	M	mixed breed
14	M	great dane	13	F	golden retriever
34	M	cocker spaniel	26	M	illirian shepherd
50	F	flat coated retriever	12	F	airedale
42	M	german shepherd	59	F	mixed breed
53	M	dogo argentino	12	F	mixed breed
			15	M	australian kelpie
			12	M	bull terrier

2.5. Skin sampling

Atopic dogs were sedated with medetomidine hydrochloride (Domitor®; Pfizer animal health) in manufacturer recommended dosage. For local anaesthesia, 2% lidocaine (Xylocaine®; Astra Zeneca) was used in a dosage of 4 mg/kg body weight. Five to 10 minutes after the injection, three to five 8-mm punch biopsies (Biopsy Punch; Kruuse, Marslev, Denmark) from lesional and nonlesional skin were taken in the area of the sternum and abdomen. Fat tissue was eliminated from biopsied skin samples by repeated scraping of the ventral part of the dermis immediately after the specimen was taken as described previously (Niwa et al. 2003). Skin samples were snap-frozen in liquid nitrogen and kept in a freezer at -80°C until further processing.

Skin from healthy dogs was taken during the routine ovarioectomy/castration procedure and processed the same way as in atopic dogs.

2.6. Determination of vitamin E in plasma samples

Vitamin E was determined according to the previously described method (Salobir et al. 2010) with a minor modification. Vitamin E isomers were separated using a Luna PFP (Phenomenex, 5 mm, 4.6 mm i.d., 250 mm length) instead of a Prodigy ODS2 (Phenomenex) column. The mobile phase consisted of methanol and water in a ratio 95:5 and was run at 1.2 ml/min.

2.7. Determination of vitamin E in skin biopsy samples

The procedure was based on analytical methods described elsewhere (Antille et al. 2002; Rosa et al. 2007). Weighed skin samples (0.03–0.09 mg) were placed in glass tubes. To each sample, 0.25 ml of butylated hydroxytoluene (BHT) solution (2.5g BHT; Merck) dissolved in 50 ml of methanol, 1.25 ml of 2 M KOH solution (5.6 g KOH dissolved in 50 ml of deionised water) and 1.25 ml of ethanol was added. The mixtures were saponified in a water bath at 80°C for 30 minutes. After cooling, they were extracted twice with 2 ml portions of hexane. The hexane was evaporated to dryness under vacuum (Syncore Reactor R-48; Büchi) at around 60°C and the residues re-dissolved in 0.5 ml of methanol. The concentration of vitamin E was determined by HPLC with fluorescence detection using an external standard of α -tocopherol (Sigma, Saint Louis, MO, USA). The HPLC system (Waters Alliance 2695, Waters, Milford, MA, USA) was equipped with a μ Bondapak C18 column, 10 μm , 3.9×300 mm (Waters, Milford, MA, USA), a fluorescence detector Waters 474, and a computer with the program Millennium for system control and data processing. Methanol, at a flow rate of 2 ml/min, was used as the mobile phase. The injection volume was 20 μl . The column was kept at the ambient temperature, and detection was carried out at an excitation wavelength of 295 nm and emission wavelength of 330 nm. The concentration of vitamin E in skin biopsy samples was expressed as $\mu\text{g/g}$ skin.

2.8. Statistical analysis

Data were analysed using commercial statistical software SPSS (SPSS 17.0 for Windows, Chicago, Illinois, USA). Descriptive statistics was used to describe the basic features of the data. Shapiro Wilk test was performed to test whether the data were normally distributed. Nonparametric Mann Whitney U test was used to test for statistically significant differences of all measured parameters between canine atopic dermatitis patients and healthy dogs. Spearman rank test was performed to evaluate a correlation between CADESI score and plasma and skin vitamin E concentration. In all analyses, a value of $P < 0.05$ was considered significant.

3. Results

Biochemical and haematological parameters were within the reference ranges (Kaneko et al. 1997; Bush 2005) in the group of atopic dogs as well as in the group of control dogs.

Median value of CADESI score was 104 ranging from 72 to 148. Plasma concentrations of vitamin E were significantly lower in atopic than in healthy dogs, with median values of $29.8 \mu\text{mol/L}$ and $52.9 \mu\text{mol/L}$, respectively (Figure 1). In contrast, skin vitamin E values did not differ significantly between patients and healthy controls (Figure 2). However, the median concentration of skin vitamin E in atopic dogs ($26.5 \mu\text{g/g}$) was higher than that in healthy dogs ($19.7 \mu\text{g/g}$). The results of Spearman rank test showed no significant correlation between CADESI score and either plasma vitamin E concentration or skin vitamin E concentration.

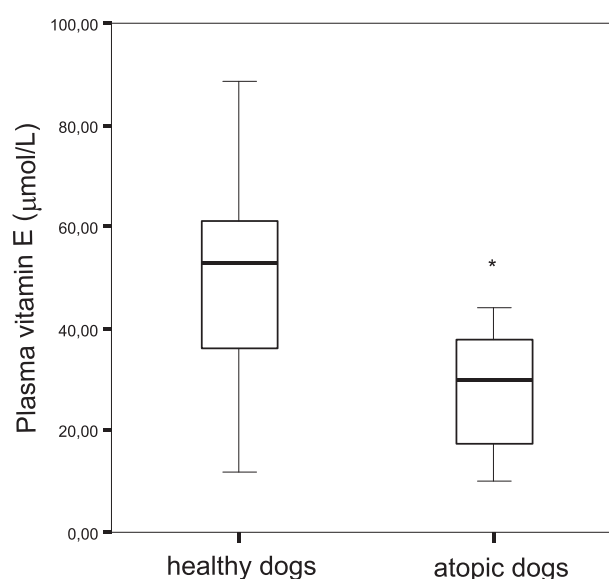


Figure 1. Box plot of plasma vitamin E concentrations ($\mu\text{mol/L}$) in atopic and healthy dogs. The boxes represent the interquartile range (i.e. 25–75% range) and the horizontal bar in the box represents the median value. For each box plot, the T-bar represents minimum and maximum of all the data. Differences between atopic and healthy dogs were significant ($P < 0.05$).

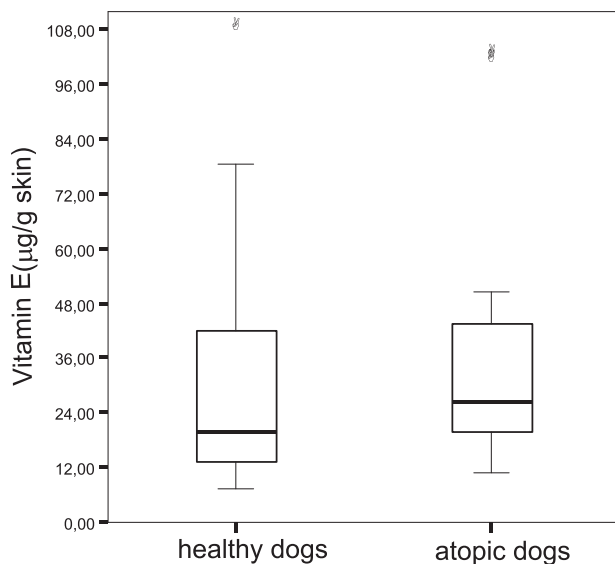


Figure 2. Box plot of concentrations of vitamin E in skin biopsy samples ($\mu\text{g/g}$) from atopic and healthy dogs. The boxes represent the interquartile range (i.e. 25–75% range) and the horizontal bar in the box represents the median value. For each box plot, the T-bar represents minimum of all the data and the largest non-outlier.

4. Discussion

Plasma vitamin E concentrations in dogs with moderate atopic dermatitis are shown to be significantly lower than in healthy controls. However, skin vitamin E concentrations did not differ significantly between atopic and healthy dogs.

Low plasma levels of vitamin E have previously been reported in human patients with atopic dermatitis (Juhlin et al. 1982), urticaria (Briganti et al. 2001), psoriasis (Jain et al. 1988) and seborrheic dermatitis (Passi et al. 1991). The significantly lower plasma vitamin E concentrations found in atopic dogs could be ascribed to increased peripheral uptake in order to reduce ROS-mediated lipoperoxidation skin damage in CAD. It is assumed that ROS are involved in the pathogenesis of allergic inflammation, so increased ROS production may be one of the causes of tissue damage in atopic dermatitis (Tsuboi et al. 1998; Briganti and Picardo 2003; Tsukahara et al. 2003; Nakai et al. 2009). In addition, oxidative stress with increased levels of lipid peroxidation has already been confirmed in human AD (Niwa et al. 2003) and CAD (Plevnik Kapun et al. 2012). Of note, Esterbauer et al. (1992) reported that plasma vitamin E concentrations show little variation over a wide range of dietary intakes.

In contrast to our results, Hozyasz et al. (2004) observed no significant differences in plasma vitamin E concentrations between children with AD and healthy controls. However, the homeostasis of α -tocopherol in AD children was changed since its concentrations in their erythrocytes were significantly lower than in healthy controls.

The vitamin E values in our study did not differ significantly between skin biopsy samples of atopic dogs and healthy controls. However, the median concentration of skin vitamin E in atopic dogs was higher than that in

healthy dogs which could indicate an adaptive response to oxidative stress due to chronic skin inflammation (Fuchs et al. 2001; Briganti and Picardo 2003). Antille et al. (2002) have demonstrated increased vitamin E concentrations in the skin of human AD patients, although the method of sampling differed from the method used in the present study. Antille et al. (2002) collected skin samples via skin scrapings that did not include the dermis, and depending on the method none or not all layers, of the epidermis, while in the present study full-thickness skin biopsies involving the removal of all skin layers and possibly also subcutaneous fat were used. As a consequence, it cannot be excluded that different sampling methods may have influenced the results.

Olivry et al. (2008) proposed intervals of CADESI scores (remission: 0–15; mild AD: 16–59; moderate AD: 60–119; and severe AD: ≥ 120). According to this reference, a moderate level of CAD intensity was found in the majority of investigated atopic dogs (13 out of 15). In human AD patients, Javanbakht et al. (2011) found a significant negative association between plasma α -tocopherol and SCORAD (SCORing Atopic Dermatitis) (Stalder et al. 1993). Contrary to our expectations no significant correlation between CADESI score (Olivry et al. 2007) and plasma concentration of vitamin E were found in our study. Additionally, no significant correlation between CADESI and skin vitamin E was found. This could be due to small sample size and moderate CADESI score predominance.

The present study has demonstrated significantly lower plasma vitamin E concentrations in canine atopic dermatitis patients than in healthy controls thereby indicating altered homeostasis of vitamin E in CAD. These low plasma concentrations warrant further investigation in view of the use of vitamin E supplementation in canine atopic dermatitis patients.

Funding

Slovenian Research Agency (research programme P4-0053) provided financial support.

Welfare and ethical considerations

Written consent of the owners was obtained and procedures complied with the relevant Slovenian governmental regulations (Animal Protection Act, Official Gazette of the Republic of Slovenia, No. 43/2007).

Conflicts of interest

No conflicts of interest.

Acknowledgements

The authors thank Professor Roger Pain for review of English.

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