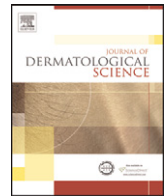




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Letter to the Editor

Altered expression of fatty acid desaturases in the skin of dogs with atopic dermatitis

Canine atopic dermatitis (AD) is a chronic inflammatory (skin) disease which shares several characteristics with its human counterpart e.g. the genetic predisposition to develop the disease, the early age of onset, the predilection sites of the affected skin and

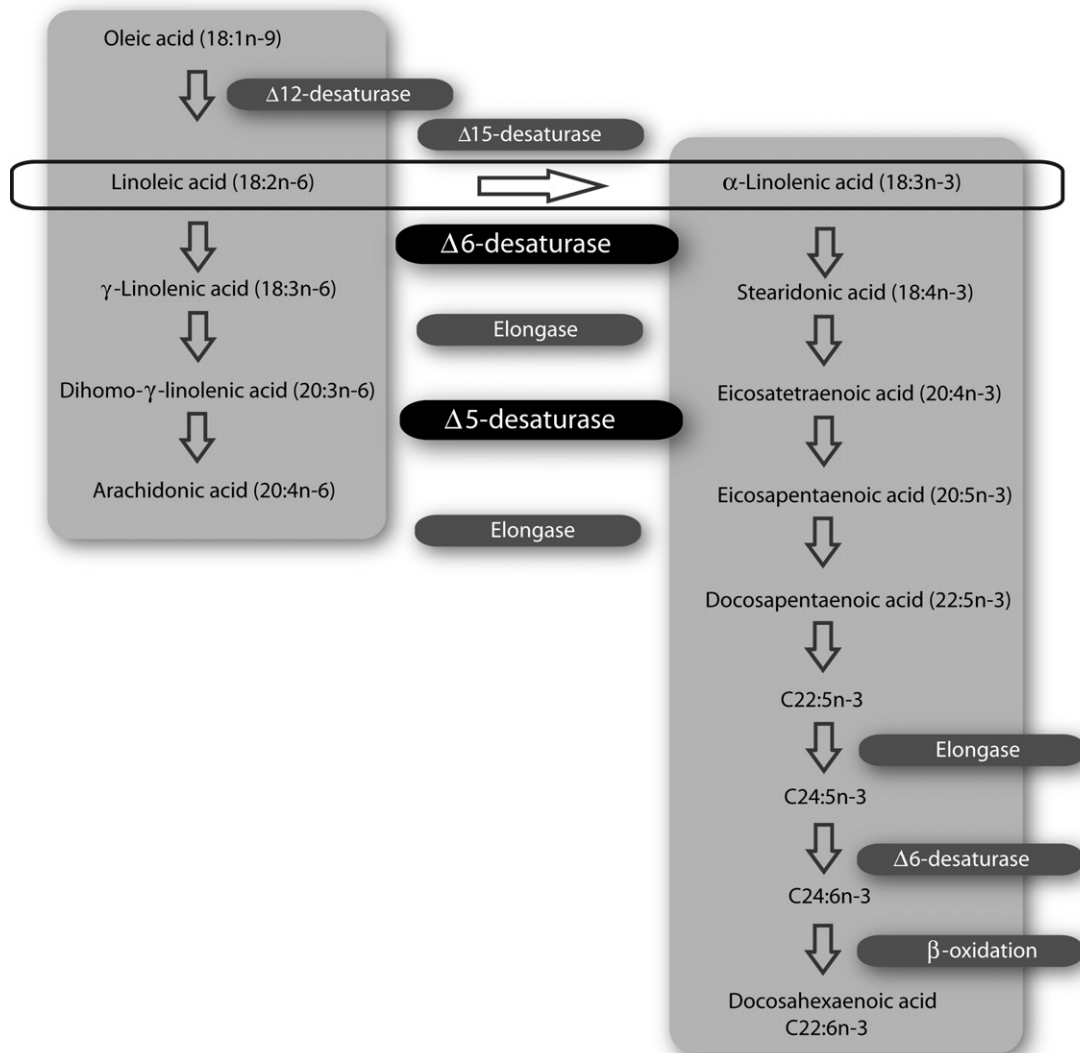


Fig. 1. Pathway of biosynthesis and metabolism of polyunsaturated fatty acids. All mammals can synthesize FA *de novo* from acetyl coenzyme A. The end product of this synthesis is palmitic acid (16: 0) which can be elongated to stearic acid (18:0). Stearic acid can easily be converted to oleic acid (18:1 *n*-9) by Δ -9 desaturase, universally present in cells of both plants and animals. Mammalian cells lack Δ -12 desaturase and thus are not able to convert oleic acid to LA (LA; 18:2 *n*-6). They also lack Δ -15 desaturase, the enzyme responsible for the synthesis of α -linolenic acid (ALA; 18:3 *n*-3) out of LA. To obtain these two essential FA (EFA) mammals are completely dependent on their diet. Once consumed, these EFA can be metabolised to other FA. Thus, LA, the parent precursor for the *n*-6 pathway, can be converted via γ -linolenic acid (GLA; 18:3 *n*-6) and dihomogamma-linolenic acid (DGLA; 20:3 *n*-6) to arachidonic acid (AA; 20:4 *n*-6). For the *n*-3 pathway the same enzymes are used and the parent precursor of this pathway, α -linolenic acid (ALA; 18:3 *n*-3) can be metabolised via stearidonic acid (SDA; 18:4 *n*-3) and eicosatetraenoic acid (ETA; 20:4 *n*-3) to eicosapentaenoic acid (EPA; 20:5 *n*-3).

similarities in immunopathogenic mechanisms [1]. Sinke et al. [1] reviewed the immune dysregulation of canine AD and stated that, similar to human AD, it is probably the resultant of a systemic component, the atopic constitution, and a tissue-specific component, i.e. altered reactivity of the skin [2]. With respect to altered skin reactivity both allergen-specific cellular mechanisms and an impaired epidermal barrier in atopic subjects likely contribute to the onset and perpetuation of AD in man [3]. In the beginning of the last century it was proposed that AD is related to abnormal FA metabolism since linoleic acid (LA) deficiency in human and rodents leads to marked abnormalities of the skin of AD patients [4]. More recent studies confirm changes in the lipid organization of the stratum corneum of AD patients [5]. It has been well established that in AD patients LA concentrations tend to be elevated in blood and adipose tissue, however, several studies reported that the levels of downstream metabolites of LA and also

of α -linolenic acid (ALA) were found to be reduced [6,7]. Both delta-5-desaturase (FADS1) and delta-6-desaturase (FADS2) are responsible for the synthesis of highly unsaturated n -3 and n -6 FA from LA and ALA (Fig. 1.). Thus deficit amounts of LA and ALA metabolites in AD have been attributed to reduced Δ -6- and Δ -5 desaturase activity [6]. Human, rat and guinea pig epidermis have been shown to lack enzymatic activity of both desaturases which implies that several important members of epidermal fatty acids, e.g. arachidonic acid (AA), are derived from extra-epidermal sites [8]. To date very few studies focused on the characteristics and metabolism of skin lipids in dogs with respect to a possible epidermal lipid barrier defect in canine AD. We hypothesize that an abnormal lipid metabolism contributes to the pathogenesis of canine AD, potentially as a result of a defect in the epidermal lipid barrier. The aim of the present study was to find evidence for this association in dogs by the analysis of the mRNA expression of these

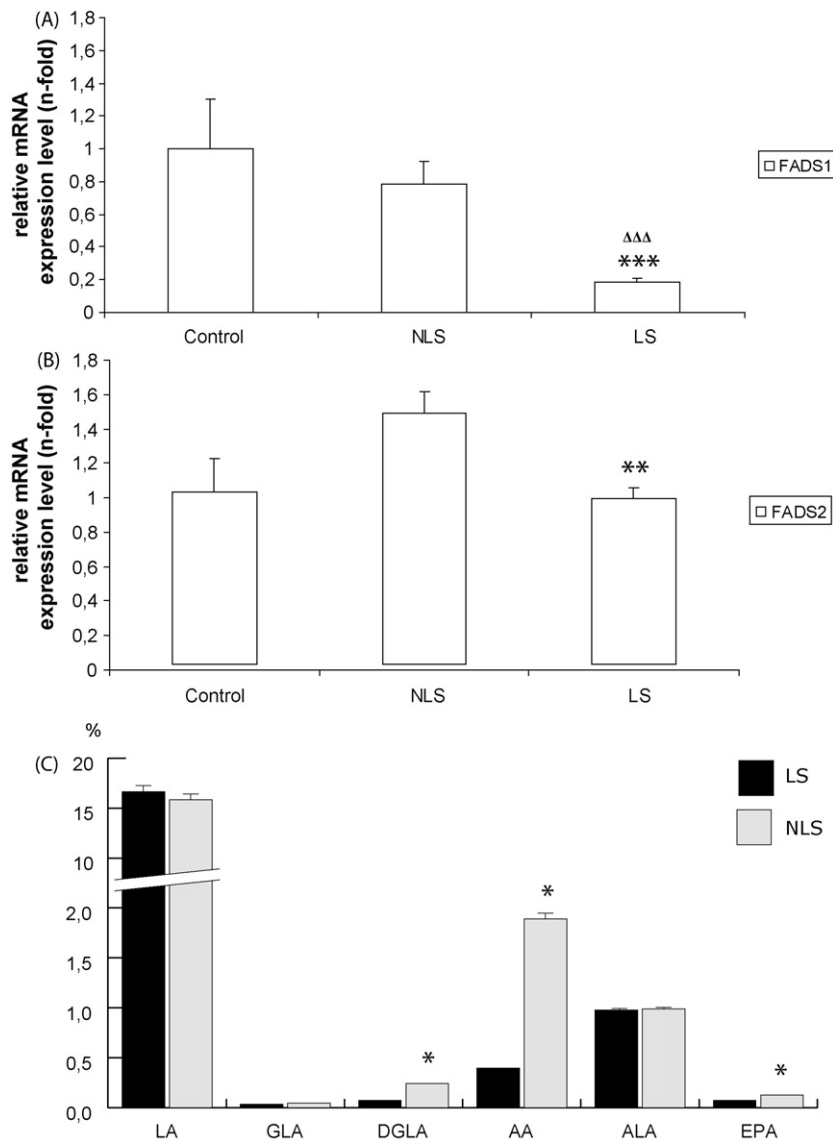


Fig. 2. (A) Relative mRNA levels of Δ -5 desaturase (FADS1) in healthy skin, non-lesional atopic skin (NLS) and lesional atopic skin (LS). The control biopsies were taken from healthy dogs ($n = 7$) and the NLS and LS biopsies were taken from atopic dogs ($n = 28$). RNA was isolated and mRNA analysis was performed using Real-time qPCR. Data represent mean \pm SEM. A statistically significant difference in Δ -5 desaturase was noted between the LS AD group versus the control group ($\Delta\Delta\Delta$ p-value < 0.0001 , Multivariate ANOVA) and between the LS AD group versus the NLS AD group (***) p-value < 0.0001 ; repeated measures analysis of the GLM). (B) Relative mRNA levels of Δ -6 desaturase (FADS2) in healthy skin, non-lesional atopic skin (NLS) and lesional atopic skin (LS). A statistically significant difference in Δ -6 desaturase was noted between the LS AD group versus NLS AD group (** p-value < 0.001 , repeated measures analysis of the GLM). (C) Fatty acid composition (mean of percentage \pm SEM) of skin lipids in NLS and LS (* p-value ≤ 0.05 when NLS and LS are compared) as measured by gas chromatography of fatty acid methyl esters. Fatty acid concentration is expressed as a percentage of total fatty acids. Significant differences between NLS and LS were found for the amount of DGLA (dihomo-gamma-linolenic acid), AA (arachidonic acid) and EPA (eicosapentaenoic acid). No significant differences were found for GLA (gamma linolenic acid) and ALA (α -linolenic acid).

enzymes and the PUFA composition in non-lesional skin (NLS) and lesional skin (LS) of atopic dogs in comparison to healthy controls. Gene expression levels of Δ -5 desaturase (FADS1) and Δ -6 desaturase (FADS2) were measured by quantitative PCR in biopsies from non-lesional and lesional skin of canine AD patients ($n = 28$) and from control skin of healthy dogs ($n = 7$). The mRNA expression level of FADS1 was significantly lower in lesional skin compared to healthy control skin (5.5-fold) and non-lesional atopic skin (4-fold) (Fig. 2 (A)). With respect to FADS2 mRNA expression a significant decrease (1.5-fold) was found in lesional AD skin when compared to non-lesional AD skin (Fig. 2B). Both FADS1 and FADS2 show high correlation in expression in non-lesional ($n = 0.57$; $p < 0.01$) as well as lesional skin ($n = 0.47$; $p < 0.05$). We assume that the low mRNA levels found for FADS1 and FADS2 in lesional skin coincide with a lower enzymatic activity as is shown in literature [9,10]. Our study implies that suppression already takes place at the mRNA level. It is plausible that the decreased mRNA expression of Δ -5 desaturase in atopic skin results from changed regulation by SREBP-1c and PPAR- α . These transcription factors play important roles in the regulation of both Δ -5 and Δ -6 desaturases [4]. Based on the pathway of PUFA biosynthesis it was expected that a decrease in Δ -5-desaturase activity would lead to reduced production of AA from DGLA and of EPA from eicosatetraenoic acid (ETA) (Fig. 1). In contrast our FA analysis showed significant increased levels not only of AA, but also of EPA in lesional skin when compared to non-lesional skin (Fig. 2C). Besides the transcription factors mentioned, (dietary) PUFA are major regulators of the expression levels of both Δ -5 and Δ -6 desaturase indicating that these enzymes are involved in feedback regulation with respect to the production of e.g. AA, EPA and DHA [4]. This PUFA inhibition of desaturases is mediated by SREBP-1c whereby PUFA suppress the target gene transcription (e.g. of desaturases) by reducing the active form of SREBP-1c [4]. The decreased expression of Δ -5 desaturase mRNA observed in our study might be explained by the high amounts of AA and EPA found in lesional skin. In this respect also the decreased mRNA expression level for Δ -6 desaturase in lesional skin if compared to non-lesional skin (NLS; $p \leq 0.001$) could have been expected. However, this expression is not comparable to that of for Δ -5 desaturase mRNA ($p \leq 0.0001$). An explanation might be that the final enzymatic step in the synthesis of AA and EPA is the direct result of Δ -5 desaturase activity and thus these products might be more important in negative feedback regulation of Δ -5 than of Δ -6 desaturase. GLA and stearidonic acid (SDA) are the direct metabolites of Δ -6 desaturase metabolism and as FA analysis did not reveal a significantly higher amount of GLA in lesional versus non-lesional skin this might explain why Δ -6 desaturase was not suppressed by GLA to the same extent as Δ -5 desaturase by AA and EPA.

In conclusion: we assume the presence and activities of both these enzymes in the skin which results in the synthesis of PUFA in the skin itself based on the local differences in skin PUFA composition together with different expression of both desaturases in non-lesional versus lesional skin. It is likely that both transcription factors SREBP-1c and PPAR- α play important roles in mediating the (feedback) regulation of Δ -5 and Δ -6 desaturase by

PUFA in the skin. Since the desaturases play a role in production of an effective epidermal lipid barrier their dysfunction in the skin may result in a defective epidermal lipid barrier in atopic dermatitis and emphasizes the need for further research into the lipid metabolism in the skin of atopic humans and dogs.

Acknowledgement

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Yvette M. Schlotter^{a,*}
Ton Willemsse^{a,b}
Frank Riemers^a
Victor P.M.G. Rutten^{b,c}
Edward F. Knol^d
Gary Davenport^e

^aFaculty of Veterinary Medicine, Department of Clinical Sciences of Companion Animals, Utrecht University, Netherlands

^bFaculty of Veterinary Medicine, Department of Infectious diseases and Immunology, Utrecht University, Netherlands

^cDepartment of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, South Africa

^dUniversity Medical Center Utrecht, Department of Dermatology/Allergology, Utrecht, Netherlands

^eP&G Pet Care, Lewisburg Technical Center, Lewisburg, OH, USA

*Corresponding author. Tel.: +31 30 2531011; fax: +31 30 2518126
E-mail address: Y.M.Schlotter@uu.nl
(Y. M. Schlotter)

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