

Transient and reversible reduction of stratum corneum filaggrin degradation products after allergen challenge in experimentally mite-sensitised atopic dogs

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Background – A defective skin barrier occurs in dogs with atopic dermatitis, and there is controversy over whether this defect pre-exists, or is secondary to allergic inflammation.

Objectives – To study if an allergen challenge decreases the natural moisturising factor (NMF), which contains the main filaggrin degradation products.

Animals – Four house dust mite (HDM)-sensitised adult atopic dogs from a research colony.

Methods and materials – Dogs were challenged epicutaneously with HDMs on the right lateral abdomen while the left thorax served as control. We swabbed the skin surface before, and at days (D)1, D2, D3, D7 and D28 after challenge, on both selected sites; swabs were soaked in detergent and frozen until assayed. The NMF components were measured by liquid chromatography-tandem mass spectrometry (LC/MS-MS).

Results – The allergen challenge induced moderate skin lesions at the application sites, and also mild erythema at the control areas. The allergen provocation led to significant decreases in the total NMF and its components trans-urocanic acid (t-UCA), pyrrolidone carboxylic acid (PCA) and serine on both sites. Lesion scores abated by D7 and the NMF concentrations had re-increased by D28. Skin lesion scores correlated negatively with the total NMF, t-UCA and PCA concentrations.

Conclusions and clinical importance – In this experimental model, a single epicutaneous allergen challenge led to a transient and reversible decrease in skin surface NMF and its components, and concentrations were negatively correlated with skin lesion scores. These observations suggest that some of the skin barrier anomalies seen in atopic dogs likely develop secondarily to the underlying cutaneous allergic inflammation.

Introduction

In humans, atopic dermatitis (AD) is a common, chronically relapsing pruritic allergic skin disease that is heterogeneous both genetically, phenotypically and immunologically.¹ This syndrome is stratified into various endotypes based on the severity of clinical signs, the age of onset, genetic factors and various immunological biomarkers.^{1–3}

In spite of this heterogeneity, endotypes of AD share a similar pathogenesis consisting of the complex interplay of a triad of overlapping parameters: a T-helper (Th)-2-predominant allergic inflammation, an epidermal barrier dysfunction and itch.^{4,5}

Dry skin has been known for decades to be an essential component of human AD, yet it is with the discovery of

loss-of-function mutations in the filaggrin-encoding gene (*FLG*) in AD-affected individuals,⁶ that the skin barrier took centre stage in modern investigations on the pathogenesis of this disease.

Of profound consequences for the pathogenesis of AD is that allergic inflammation itself, with its epidermal keratinocyte- and Th2-derived cytokines, also worsens the hereditary filaggrin deficiency.

One of the modern dogmas of AD pathogenesis thus posits that genetic mutations in *FLG* (and likely those of genes encoding other superficial epidermal structural proteins including *FLG2*) result in a defective epidermal barrier with an abnormal water loss and an increased permeability to allergens. In turn, the epidermal-penetrating allergens trigger a Th2-centred inflammation that leads to a secondary reduction in filaggrin/filaggrin-2 expression to further exacerbate the stratum corneum (SC) barrier defects. This self-amplifying loop was proposed as the “outside-inside-outside” theory.⁷

That a skin barrier dysfunction in canine natural (i.e. spontaneous) AD also exists is now beyond controversy; the various anomalies have been summarised in several reviews on this topic.^{8–11} At this time, however, we could not find any evidence for mutations in *FLG* — or for that

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matter, in any gene encoding proteins involved in skin barrier function — to be convincingly associated with AD in any canine breed.

Using a colony of experimentally HDM-sensitised atopic dogs, we first showed that a single epicutaneous HDM challenge led to a transient and reversible decrease in the SC ceramides. This reduction in skin lipids occurred not only at the site of allergen application, but also at a site away from it; such an observation suggests that a local allergen-triggered inflammation also has distant repercussions.¹²

In the present experiment, we aimed at expanding the observations above with the study of the effect of a single allergen challenge on the expression of the SC natural moisturising factors (NMF), the small compounds that represent the ultimate degradation products of filaggrins. The value of looking at NMF is not only to explore the consequences of an allergen provocation on the expression of filaggrin and filaggrin-2 (the NMF precursors), but also to assess the enzymatic processing into their final metabolites. Our study's results converge with the previous demonstration of a transient ceramide deficiency¹² to suggest that the induction of acute, focal, allergic skin lesions triggers several, rapid, widespread — yet temporary and reversible — defects in the epidermal barrier of atopic dogs.

Methods and materials

All *in vivo* aspects of this experiment were approved beforehand by our institution's Animal Care and Use Committee (NCSU IACUC protocol 17-005-O).

Study subjects

We determined that four dogs would enable this study to have a 90% power to detect a 50% difference in the expression of NMF components between before and after allergen challenge, each having a standard deviation of 30%; we used one-tailed calculations due to the unidirectional hypothesis of a significant reduction in NMF after allergen provocation ($P < 0.05$) (www.powerandsamplesize.com; page last accessed, September 7, 2020).

We thus selected four 3–8-year-old Maltese-beagle atopic dogs that had been sensitised to the *Dermatophagoides farinae* (Df) HDM epicutaneously, as in other studies.¹³ There were two females and two male dogs (all intact); these four dogs shared the same environment and ate the same diet, and these had not been changed in the month preceding this experiment. Furthermore, to avoid any interference resulting from previous interventions, these dogs had not been subjected to any allergen challenge in the preceding three months. They had not received any antiallergic drug or topical product, or been bathed in the month before this study.

Allergen challenges

We performed an HDM challenge, as described previously.¹⁴ In brief, the right lateral abdomen was clipped one day before we applied 25 mg Df-HDM in 1 mL mineral oil to this area. We also clipped the hair on a small patch on the left lateral thorax, where we applied 1 mL mineral oil to serve as the negative control.

Before [Day 0 (D0), Time 1 (T1)], and one (D1, T2), two (D2, T3), three (D3, T4), seven (D7, T5) and 28 (D28, T6) days post-provocation with allergen, we graded the local skin lesions on the right abdomen (challenge) and left thorax (control), as done previously and as follows: we scored erythematous macules, oedema, papules/pustules, and excoriations as 0 (absent), 1 (faint, mild), 2 (moderate) or 3 (strong, severe). The grades for each lesion were added to yield a skin lesion score (SLS) that had a possible maximum of 12; we photographed all areas at all time points.

Sample collection and quantification of NMFs

Before allergen provocation and after lesion scoring, both challenge and control areas were rubbed with pressure, twice in a row for 45 s each, using cotton-tipped swabs that had been soaked in a solution containing a nonionic detergent. The two tips were cut and put in Eppendorf tubes containing the same solution kept on crushed ice. All tubes then were stored at -80°C until shipment to the laboratory on dry ice.

Quantification of NMFs

In order to measure the total NMF and some of its components pyrrolidone-5-carboxylic acid (PCA), trans-urocanic acid (t-UCA) and the amino acid serine, we employed a liquid chromatography with tandem mass spectrometry (LC/MS-MS) analysis.

Before LC/MS-MS analysis, samples were centrifuged at 20 000g for 10 min to wring out the swabs. The supernatant was placed in a vial, and the internal standard (imidazole-4-acetic acid sodium) was added. The samples were shaken (vortex), and 20 μL were injected for LC/MS-MS analysis.

A Symmetry RP18-LC column (Waters; Milford, MA, USA) was used with the mobile phase consisting of Buffer A (0.1% formic acid in methanol) and Buffer B (0.1% formic acid in deionised water). A portion was injected at 0.40 mL/min into the LC system. The column was re-equilibrated at initial conditions for 10 min. The MS-MS analysis was carried out on a Quattro Micro (Waters) with electrospray ionisation in the positive ion mode.

We calculated the concentrations of NMF and its components by plotting the nominal concentrations versus the NMF response factor (peak area of NMF/peak area of internal standard). The LC/MS-MS program was carried out with MASSLYNX software v4.0 (Waters). The protein concentration was determined in each sample with a Bradford protein assay kit (Abcam; Cambridge, UK). Finally, we standardised and expressed the content of NMF as its concentration (μg) per mg total proteins.

Statistical analyses

In order to compare the concentrations of total NMF and its components over time, at either HDM application or control sites, we used a nonparametric repeated-measures ANOVA with uncorrected Dunn's post-tests. At each time point, we also compared values between HDM application and control sites using one-tailed paired *t*-tests, as the hypothesis was that the concentrations of NMF would be lower at the allergen application site compared to those of the control. Finally, the correlations between the SLS and the total and individual NMF components were calculated using nonparametric Spearman rank-order correlation. We used PRISM 7 (GraphPad Software; San Diego, CA, USA) and a significance level of 5%.

Results

Skin lesion scores

The single painting of HDM in oil on D0 (T1) led to a rapid increase in erythematous skin lesions at the allergen application site Figure 1, left half (HDM). Because of the rise in inflammation beyond the level agreed with the animal care and use committee, we treated the dogs with oclacitinib (Apoquel, Zoetis; Parsippany, NJ, USA) at the dose of 0.5 mg/kg twice daily on D1, D2 and D3; all lesions disappeared by D7. Skin lesion scores were significantly different over time (Friedman repeated-measures ANOVA, $P = 0.002$), being significantly higher on D1 (uncorrected Dunn's test, $P = 0.038$) and D2 ($P = 0.003$) compared to those before challenge.

Of interest is that in two (on D1), and then three dogs (on D2 and D3), the SLS also increased at the control sites, albeit to values lower than those where HDM was applied, suggesting that a mild allergic reaction of

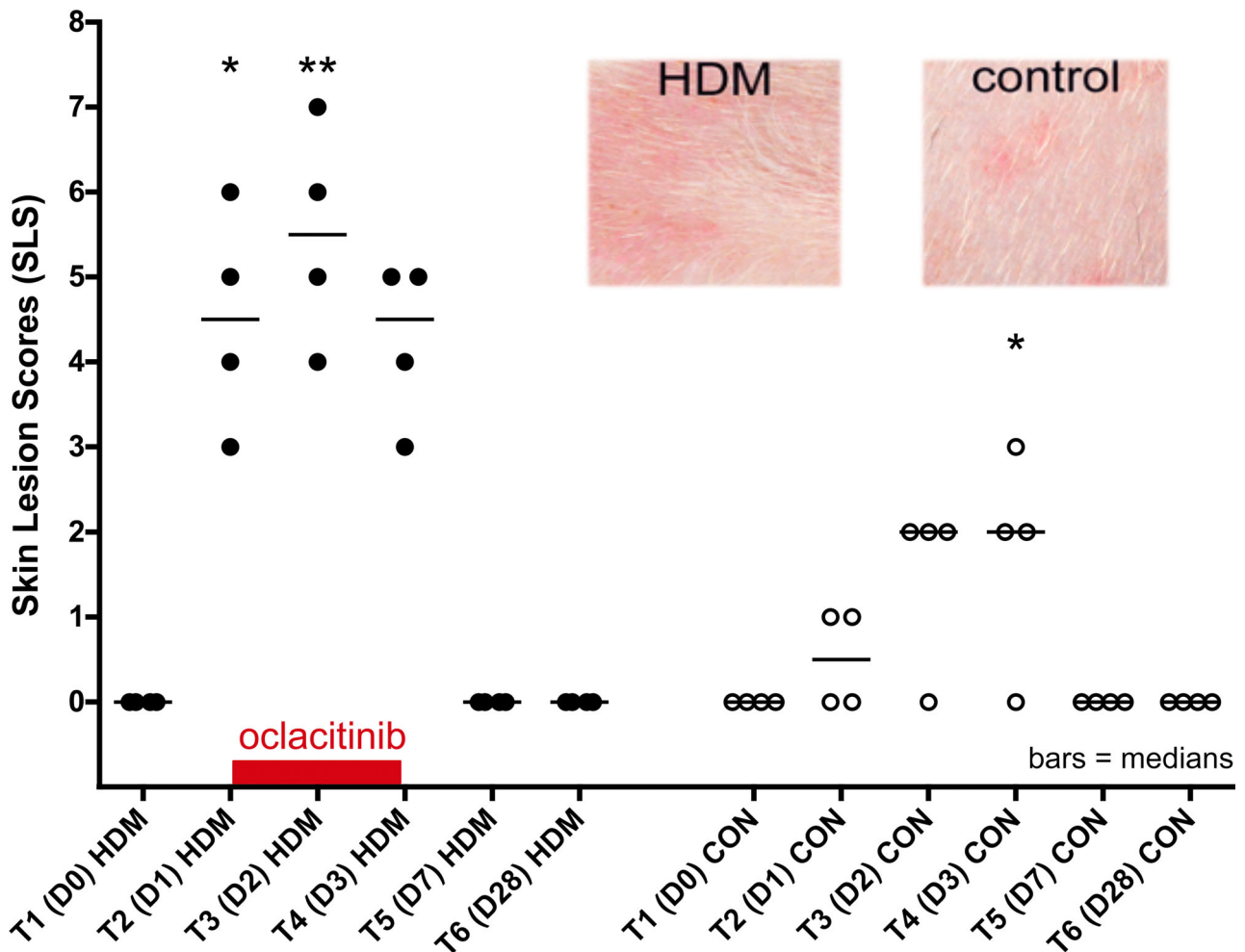


Figure 1. Evolution of skin lesion scores after allergen challenge.

The skin lesion scores (SLS) increased markedly after a single house dust mite (HDM) challenge not only at the application site (closed circles, left), but also — and to a minor degree — at the control area (open circles, right). We show an example of erythematous lesions in the two regions at the same time and on the same dog. Asterisks depict the significance level of the difference in values compared to those at baseline [Time (T)1, Day (D)0]: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

systemic origin also had occurred there [Figure 1, right half (CON)]. The SLS also were significantly different over time at the control sites (Friedman repeated-measures ANOVA, $P = 0.015$), only being significantly higher on D3 ($P < 0.047$) compared to before the challenge.

Compared to those at the control sites, the scores at the HDM application sites were higher, not significantly so, on D1, D2 and D3 after challenge (Wilcoxon matched-pairs signed-rank test, $P = 0.065$ for each of these three days).

Total NMF

The total amount of NMF decreased significantly at the site of allergen application (Friedman repeated-measures ANOVA, $P = 0.007$; Figure 2, left half); the values on D2, D3 and D7 were significantly lower than those before provocation (uncorrected Dunn's test, $P = 0.008$, 0.001 and 0.008, respectively). By D28, the NMF concentrations had increased again, and they then were only slightly lower than those before allergen application.

A similar NMF reduction pattern also was seen at the control sites (left thorax), which were not contiguous with those challenged with the HDM on the right abdomen

(Friedman repeated-measures ANOVA, $P = 0.022$; Figure 2, right half). Of note is that the reduction in NMF concentrations in the control areas appeared slightly delayed compared with that at the provocation sites, as the values were significantly lower only on D3 and D7 compared to those at the study's onset ($P = 0.03$ and 0.08, respectively). By D28, the concentrations were still less than half those at baseline.

On D1, D3, and D7, the total NMF concentrations were lower at the allergen application sites than at the same dog's control areas. Still, the values were not significantly different ($P = 0.188$, 0.125, 0.1888 and 0.188, respectively).

Combining the values obtained at both allergen and control application sites, we found the SLS and total NMF concentrations to be inversely correlated (Spearman $r = -0.38$, $P = 0.007$; Figure 3), which suggested that the local skin inflammation increases had led to a proportional reduction in the SC total NMF amounts at these sites.

Individual NMF components

In addition to the skin surface concentrations of NMF, we also determined those of three of its components, the

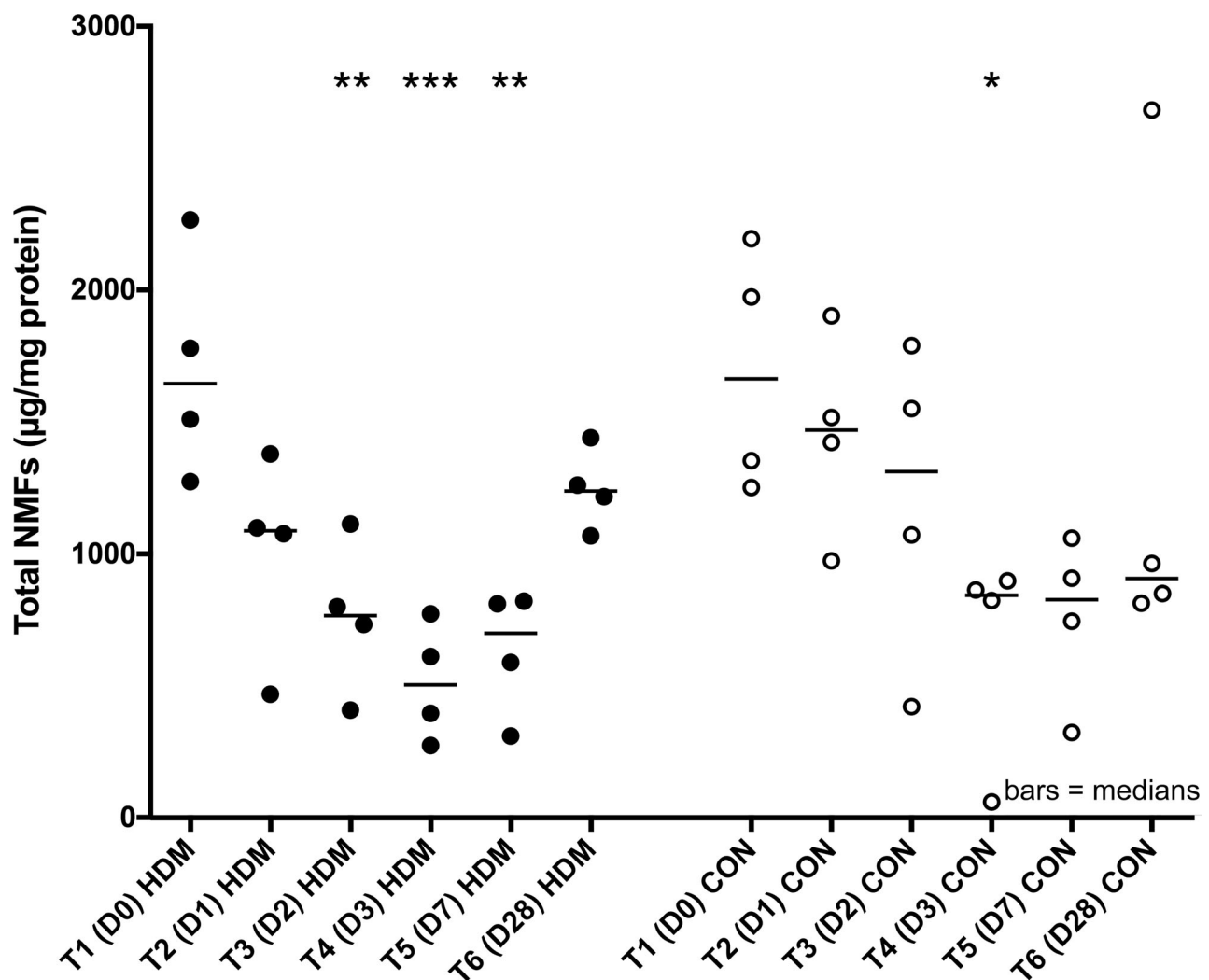


Figure 2. Evolution of total natural moisturising factor (NMF) skin surface concentrations after allergen challenge.

After a house dust mite (HDM) challenge, the total NMF concentrations decreased at the allergen application site and away from it. By four weeks postapplication [Day (D) 28], the values had increased from their trough. Asterisks depict the significance level of the difference in values compared to those at baseline [Time (T)1, Day (D)0]: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

t-UCA, PCA and serine. We saw the same NMF component reduction pattern after HDM provocation at both allergen and control sites (see Supporting information Figure S1). Likewise, the surface concentrations of the t-UCA, PCA and serine also were inversely correlated with the SLS (Figure S2).

As for the total NMF, the median surface skin concentrations of t-UCA, PCA and serine were nearly always lower on D1, D2, D3 and D7 at the HDM application sites compared to those of controls, and the differences also never reached the threshold for significance.

Discussion

This paper reports the skin surface concentrations of NMF after a single HDM challenge in experimentally sensitised atopic dogs. Despite the small number of dogs — a limitation of this study — we demonstrated that the allergen provocation led to a rapid reduction of NMF, not only at the site of a challenge, but also on the other side of the body in an area distant from it. After the skin lesions abated following oclacitinib therapy, the NMF

concentrations increased again, nearing normal values 28 days postchallenge. Altogether, the total and individual NMF values were inversely correlated with the SLS. These dynamic changes in NMF after allergen challenge mirror those in the SC ceramide concentrations, which we observed in a study with a nearly identical design.¹²

As we reported before, the HDM application onto sensitised atopic dogs' skin leads to inflammation at the site of allergen challenge.^{13,14} The detection of lesions of lower severity on an area distant from the provocation site suggests that the focal allergen painting had led to systemic inflammation. This observation is compatible with what has been shown recently in humans with AD, in whom the severity of skin lesions correlates with plasma concentrations of the C-reactive protein, a biomarker of systemic inflammation.¹⁵ Indeed, AD is now considered a systemic disease, as human adults with atopic skin lesions have a high risk for several internal illnesses, including cardiovascular complications.^{16,17} That inflammation was seen in our dogs not only at the site of provocation, but also away from it, parallels what occurs in dogs with spontaneous AD in which both the visibly

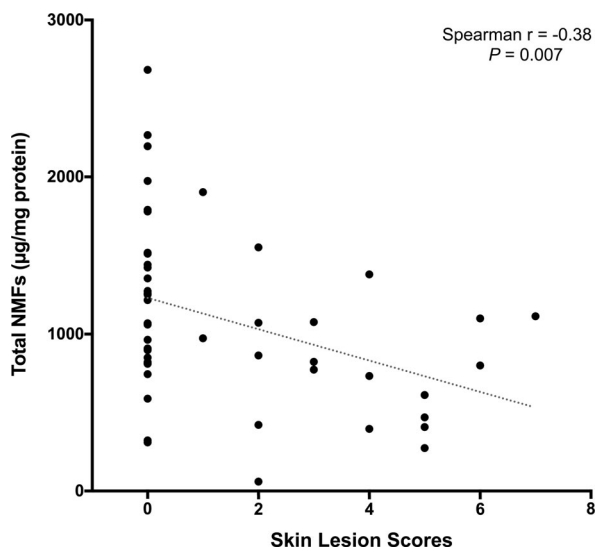


Figure 3. Correlation between skin lesion scores and total natural moisturising factor (NMF) skin surface concentrations. After combining the values obtained at both house dust mite (HDM) application and control sites, we found that the total NMF concentrations and skin lesion scores negatively correlated.

lesional skin and that in distant areas is inflamed. Indeed, the so-called "nonlesional" has a higher level of dermal inflammatory cells,¹⁸ and it is also rich in pro-allergic and pro-inflammatory cytokines.^{19,20}

The observation of a decrease of skin NMF concentrations after an allergen challenge in sensitised atopic dogs suggests either a downregulation of the production of filaggrins, and/or a reduction in the proteolysis of filaggrins by their degrading enzymes (caspase-14, calpain-1, bleomycin hydrolase and histidase for the generation of t-UCA).²¹ While the first hypothesis is unlikely as a consequence of the very rapid reduction in NMF and its component after allergen challenge, there is support for the second possibility. For example, the classic Th2 cytokines interleukin (IL)-4 and -13 can inhibit the expression of *CASP14* mRNA in human keratinocyte cell lines,²² and there is a reduction in the immunoreactivity of bleomycin hydrolase in the lesional epidermis of humans with AD.^{23,24}

In two different experimental canine AD models, the expression of mRNA encoding the filaggrin-degrading enzymes was not significantly different after an allergen challenge.^{25,26} The data on caspase-14 protein expression are contradictory, however, as the epidermal immunoreactivity for this enzyme was unchanged over time after an allergen provocation in one experiment,²⁷ while it was reported to increase in another study using dogs from the same colony.²⁶ The reasons for these discrepancies between mRNA and protein expressions remain unexplained. A possible hypothesis for our results is that the observed low concentrations of NMF could be to the result of a decreased function — and not the expression — of these enzymes.

As discussed in the introduction to this paper, the dysfunction of the epidermal barrier in atopic dogs is firmly established.^{8–11} However, the controversy arises whether this barrier dysfunction is a primary (genetic)

defect — one that might need a therapeutic correction — or one simply resulting from the underlying allergic inflammation (i.e. a secondary deficiency).

At this time, there is increasing support for the latter theory. For example, in dogs with spontaneous AD, the transepidermal water loss (TEWL), an imperfect yet widely used measure of barrier dysfunction, is markedly increased on visibly inflamed atopic skin lesions compared to normal canine skin, yet after treatment with oral ciclosporin or topical hydrocortisone aceponate, the TEWL returns to near normal values.^{28,29}

Results from allergen challenge experiments in HDM-sensitised dogs also support the concept of a reversible, inflammation-induced secondary barrier dysfunction. We previously showed that the SC ceramides transiently decreased after an allergen challenge before spontaneously returning to normal, days after the visible inflammation abated.¹² In this paper, we demonstrate a temporary and reversible epidermal NMF concentration reduction after allergen challenge. Likewise, the skin surface bacterial dysbiosis, which sometimes is included in descriptions of "atopic barrier dysfunction," also was reversible in our dogs, as a transient increase in the population of staphylococci (including *Staphylococcus pseudintermedius*) occurred after allergen challenge, and numbers returned to normal weeks after the lesions were no longer visible.³⁰

Finally, in another canine model of HDM-induced AD, the immunoreactivity for filaggrin/filaggrin-2 was shown to decrease rapidly after an allergen challenge, before rebounding later on,³¹ and the successful treatment with ciclosporin led to an increase in filaggrin expression compared to that before treatment.²⁶

In summary and at this time, the existing evidence altogether converges to support the dogma that the skin barrier dysfunction present in dogs with AD is most likely secondary to the underlying allergic cutaneous inflammation. All studied parameters, including SC lipids, filaggrins and staphylococci, eventually return to or near typical values after the inflammation subsides, either spontaneously or after treatment with antiallergic drugs. Thus, one could argue that the prompt resolution of atopic skin lesions with potent anti-inflammatory drugs is likely to have a more robust and faster impact on the correction of any existing skin barrier dysfunction than the use of topical or systemic barrier-restoring interventions. Nevertheless, one cannot rule out that barrier restorative therapeutic strategies might have a synergic benefit in addition to with that provided by antiallergic drugs; the possible added value of such adjunctive therapy would need to be unequivocally proven, however.

Author contributions

Thierry Olivry: Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Supervision, Validation, Visualization, Writing-original draft, Writing-review & editing. **Judy Paps:** Investigation, Methodology, Project administration. **Nicolas Amalric:** Methodology, Resources, Writing-review & editing.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Evolution of the skin surface concentrations of three NMF components after allergen challenge. The concentrations of t-UCA (a), PCA (b) and serine (c) evolved similarly to those of the total skin surface NMF after an allergen challenge (Figure 2). CON, control; D, Day; T, Time.

Figure S2. Correlation between skin lesion scores and skin surface concentrations of NMF components. The skin surface concentrations of t-UCA (a) and PCA (b) — and not those of serine (c) — were negatively correlated with SLS, as seen with total NMF concentrations (Figure 3).

Résumé

Contexte – Un défaut de barrière cutanée existe chez le chien atopique et il y a controverse si ce défaut préexiste ou s'il est secondaire à une inflammation allergique.

Objectifs – Etudier si une provocation allergénique diminue le NMF (natural moisturising factor) qui contient les principaux produits de dégradation de la filaggrine.

Sujets – Quatre chiens atopiques sensibilisés aux acariens de poussière de maison (HDM) d'une colonie de recherche.

Méthodes – Les chiens ont été provoqués par test épicutané avec HDMs sur l'abdomen latéral droit

pendant que le thorax gauche servait de contrôle. Nous avons prélevés la surface de la peau avant et après provocation à D1, D2, D3, D7 et D28 sur les deux faces sélectionnées; les écouillons ont été trempés dans un détergent et congelés jusqu'aux tests. Les composés NMF ont été mesurés par LC/MS-MS (liquid chromatography-tandem mass spectrometry).

Résultats – La provocation allergénique a induit des lésions modérées aux sites d'application, et aussi un érythème modéré aux zones contrôles. Le test de provocation a mené à des diminutions significatives du NMF total et ses composés t-UCA (trans-urocanic acid), PCA (pyrrolidone carboxylic acid) et sérine sur les deux sites. Les scores lésionnels se calmaient à D7 et les concentrations de NMF ré-augmentaient à D28. Les scores de lésions cutanées corrélaient négativement avec les concentrations de NMF total, t-UCA et PCA.

Conclusions et importance clinique – Dans ce modèle expérimental, un test allergénique epicutané unique mène à une diminution réversible et transitoire de NMF de la surface cutanée et de ses composés et les concentrations sont négativement corrélées avec les scores de lésion cutanée. Ces observations suggèrent que certaines des anomalies de barrière cutanée observées chez les chiens atopiques pourraient se développer secondairement à l'inflammation cutanée allergique sous jacente.

Resumen

Introducción – en perros con dermatitis atópica se observa una barrera cutánea defectuosa, y existe controversia sobre si este defecto existe antes o es secundario a la inflamación alérgica.

Objetivos – Estudiar si una exposición con alérgenos disminuye el factor de hidratación natural (NMF), que contiene los principales productos de degradación de la filagrina.

Animales – cuatro perros adultos atópicos sensibilizados contra los ácaros del polvo doméstico (HDM) de una colonia de investigación.

Métodos – los perros fueron expuestos epicutáneamente con HDM en el abdomen lateral derecho mientras que el tórax izquierdo sirvió como control. Limpiamos la superficie de la piel antes y en los días (D) 1, D2, D3, D7 y D28 después de la exposición, en ambos sitios seleccionados; los hisopos se empaparon en detergente y se congelaron hasta su análisis. Los componentes de NMF se midieron mediante cromatografía líquida-espectrometría de masas en tándem (LC/MS-MS).

Resultados – la exposición al alérgeno indujo lesiones cutáneas moderadas en los lugares de aplicación y también eritema leve en las áreas de control. La provocación del alérgeno condujo a disminuciones significativas en el NMF total y en sus componentes ácido transurocánico (t-UCA), ácido pirrolidona carboxílico (PCA) y serina en ambos sitios. Las puntuaciones de las lesiones disminuyeron al D7 y las concentraciones de NMF habían vuelto a aumentar en el D28. Las puntuaciones de las lesiones cutáneas se correlacionaron negativamente con las concentraciones totales de NMF, t-UCA y PCA.

Conclusiones e importancia clínica – en este modelo experimental, una sola exposición a alérgeno epicutáneo condujo a una disminución transitoria y reversible en el NMF de la superficie de la piel y sus componentes, y las concentraciones se correlacionaron negativamente con las puntuaciones de las lesiones cutáneas. Estas observaciones sugieren que algunas de las anomalías de la barrera cutánea observadas en perros atópicos probablemente se desarrollen de forma secundaria a la inflamación alérgica cutánea subyacente.

Zusammenfassung

Hintergrund – Bei Hunden mit atopischer Dermatitis besteht eine defekte Hautbarriere und es besteht eine Kontroverse darüber, ob dieser Defekt wirklich vor-existiert oder ob er sekundär durch die allergische Entzündung verursacht wird.

Ziele – Es sollte untersucht werden, ob eine Provokation mit dem Allergen den natürlichen Feuchthalte-Faktor (NMF), der die hauptsächlichen Abbauprodukte von Filaggrin enthält, reduziert.

Tiere – Vier erwachsene atopische Hunde aus einer Forschungskolonie, die mit Hausstaubmilben (HDM) sensibilisiert worden waren.

Methoden – Die Hunde wurden epikutan mit den HDMs rechts lateral am Abdomen provoziert, während der linke Thorax als Kontrolle diente. Wir entnahmen vorher und an den Tagen (D) 1, D2, D3, D7 und D28 nach der Provokation von beiden ausgewählten Seiten Tupferproben; die Tupfer wurden in Detergens getränkt und bis zur Untersuchung eingefroren. Die NMF Komponenten wurden mittels Hochleistungsflüssigkeitschromatografie Tandem Massenspektrometrie (LC/MS-MS) gemessen.

Ergebnisse – Die Allergen Provokation induzierte moderate Hautveränderungen an den Applikationsstellen, sowie ein mildes Erythem an den Kontrollstellen. Die Allergen Provokation führte auf beiden Seiten zu signifikanten Abnahmen der totalen NMF und seiner Komponenten Trans-Urocaninsäure (t-UCA), Pyrrolidoncarbonsäure (PCA) und Serin. Die Läsionswerte nahmen mit D7 ab und die NMF Konzentrationen hatten mit D28 wieder zugenommen. Die Werte der Hautläsionen korrelierten negativ mit den Gesamt NMF, t-UCA und PVA Konzentrationen.

Schlussfolgerungen und klinische Bedeutung – In diesem experimentellen Modell führt eine einzige epikutane Allergenprovokation zu einer vorübergehenden und reversiblen Zunahme der NMF der

Hautoberfläche und seiner Komponenten, die Konzentrationen waren mit den Hautwerten negativ korreliert. Diese Beobachtungen weisen darauf hin, dass einige der Anomalien der Hautbarriere, die bei atopischen Hunden gesehen werden, sich sekundär aufgrund der zugrundeliegenden allergischen Hautentzündung entwickeln.

要約

背景 – アトピー性皮膚炎の犬では皮膚バリアの欠損が見られるが、この欠損が以前から存在していたのか、あるいはアレルギー性炎症に二次的に生じたものなのかについては議論が分かれている。

目的 – 本研究の目的は、アレルゲン負荷が、主なフィラグリン分解産物を含む天然保湿因子 (NMF) を減少させるかどうかを研究することであった。

被験動物 – 研究用コロニーのハウスダストマイト (HDM) 感作アトピー成犬4頭。

方法 – 犬の右側腹部にHDMを付着させ、左胸部はコントロールとした。負荷前、負荷後1日目、2日目、3日目、7日目、28日目に、選択した両部位の皮膚表面を綿棒で採取し、綿棒は洗剤に浸して測定するまで凍結させた。

NMFの成分は、液体クロマトグラフィー・タンデム質量解析法 (LC/MS-MS) で測定した。

結果 – アレルゲン負荷は、塗布部位に中程度の皮膚病を誘発し、コントロール部位にも軽度の紅斑が見られた。アレルゲンの誘発により、両部位のNMFおよびその成分であるtrans-urocanic acid (t-UCA)、pyrrolidone carboxylic acid (PCA)、serineが有意に減少した。皮膚病変のスコアはD7までに減少し、NMFの濃度はD28までに再び上昇した。皮膚病変のスコアは、NMF、t-UCAおよびPCAの合計濃度と負の相関があった。

結論と臨床上的重要性 – この実験モデルでは、アレルゲンの単回外皮投与により、皮膚表面のNMFとその成分が一過性かつ可逆的に減少し、その濃度は皮膚病変のスコアと負の相関を示した。これらの観察結果は、アトピー犬に見られる皮膚バリアの異常のいくつかは、根底にある皮膚のアレルギー性炎症に続いて発症する可能性が高いことを示唆している。

摘要

背景 – 特应性皮炎犬の皮膚屏障存在缺陷, 对于该缺陷是预先存在还是继发于过敏性炎症存在争议。

目的 – 研究过敏原激发是否会降低含有主要丝聚蛋白降解产物的天然保湿因子(NMF)。

动物 – 来自一个研究种群的4只屋尘螨(HDM)致敏的成年特应性犬。

方法 – 在犬右侧腹部用HDM进行皮外激发, 同时左胸作为对照。我们在激发前和激发后第(D)1、D2、D3、D7和D28天, 在两个选定部位擦拭皮肤表面; 将拭子浸泡在洗涤剂中并冷冻直至测定。通过液相色谱-串联质谱法(LC/MS-MS)测定NMF组分。

结果 – 过敏原激发在用药部位引起中度皮肤病变, 在对照区域也引起轻度红斑。过敏原激发导致两个位点的总NMF及其组分反式尿烷酸(t-UCA)、吡咯烷酮羧酸(PCA)和丝氨酸显著降低。病变评分在D7时减轻, NMF浓度在D28时重新增加。皮肤病变评分与总NMF、t-UCA和PCA浓度呈负相关。

结论和临床重要性 – 在该实验模型中, 单次表皮过敏原激发导致皮肤表面NMF及其组分一过性和可逆性降低, 且浓度与皮肤病变评分呈负相关。这些观察结果表明, 在特应性犬中观察到的一些皮肤屏障异常可能继发于潜在的皮肤过敏炎症。

Resumo

Contexto – Defeitos de barreira cutânea ocorrem em cães com dermatite atópica, e há controvérsias se esse defeito é pré-existente, ou secundário à inflamação alérgica.

Objetivos – Estudar se um desafio alérgico é capaz reduzir o fator hidratante natural (NMF), que contém os principais produtos de degradação de filagrina.

Animais – Quatro cães atópicos adultos sensibilizados por ácaros da poeira doméstica (HDM) de uma colônia de pesquisa.

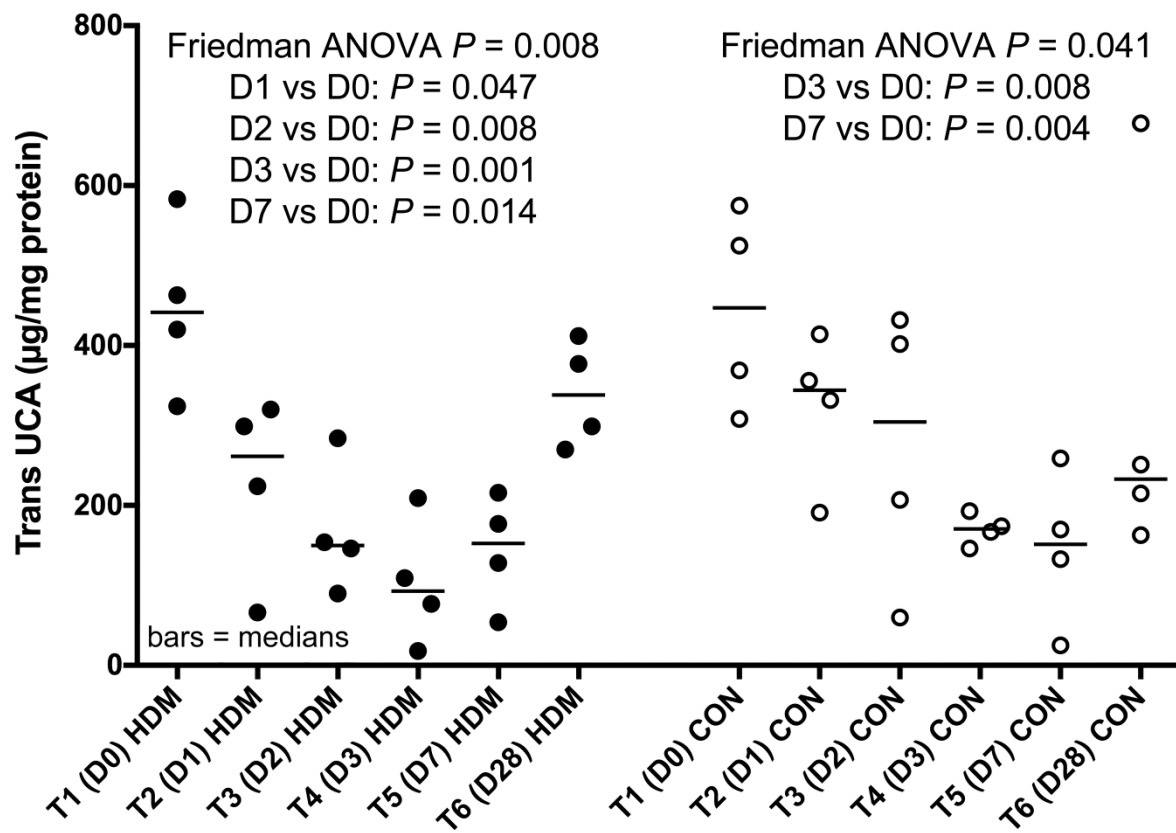
Métodos – Os cães foram desafiados por via epicutânea com HDMs no abdômen lateral direito, enquanto o tórax esquerdo serviu como controle. Friccionamos a superfície da pele com *swabs* antes, e nos dias (D) 1, D2, D3, D7 e D28 após o desafio, em ambos os locais selecionados; os *swabs* permaneceram embebidos em detergente e congelados até serem processados. Os componentes NMF foram medidos por espectrometria de massa de cromatografia líquida-tandem (LC/MS-MS).

Resultados – O desafio alérgico induziu lesões de pele moderada nos locais de aplicação, e também eritema discreto nas áreas de controle. O desafio alérgico levou a diminuições significativas no NMF total e em seus componentes ácido trans-urocânico (t-UCA), ácido carboxílico pirrolidona (PCA) e serina em ambos os locais. Os escores de lesão diminuíram no D7 e as concentrações de NMF aumentaram novamente no D28. Os escores de lesão de pele correlacionaram negativamente com as concentrações de NMF total, t-UCA e PCA.

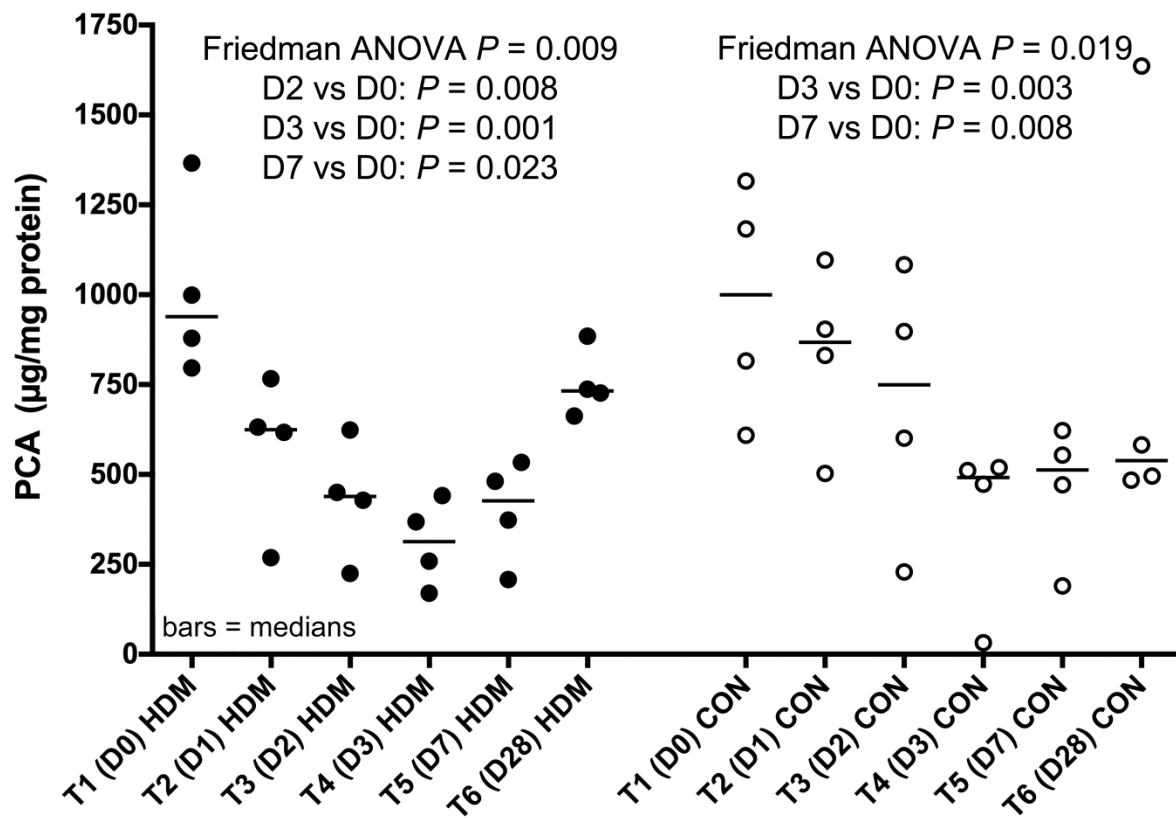
Conclusões e importância clínica – Neste modelo experimental, um único desafio de alérgico epicutâneo levou a uma redução transitória e reversível NMF superficial e seus componentes, e as concentrações foram negativamente correlacionadas com as pontuações da lesão da pele. Essas observações sugerem que algumas das anomalias de barreira da pele observadas em cães atópicos provavelmente se desenvolvem secundariamente à inflamação alérgica cutânea subjacente.

Figure S1

(a)



(b)



(c)

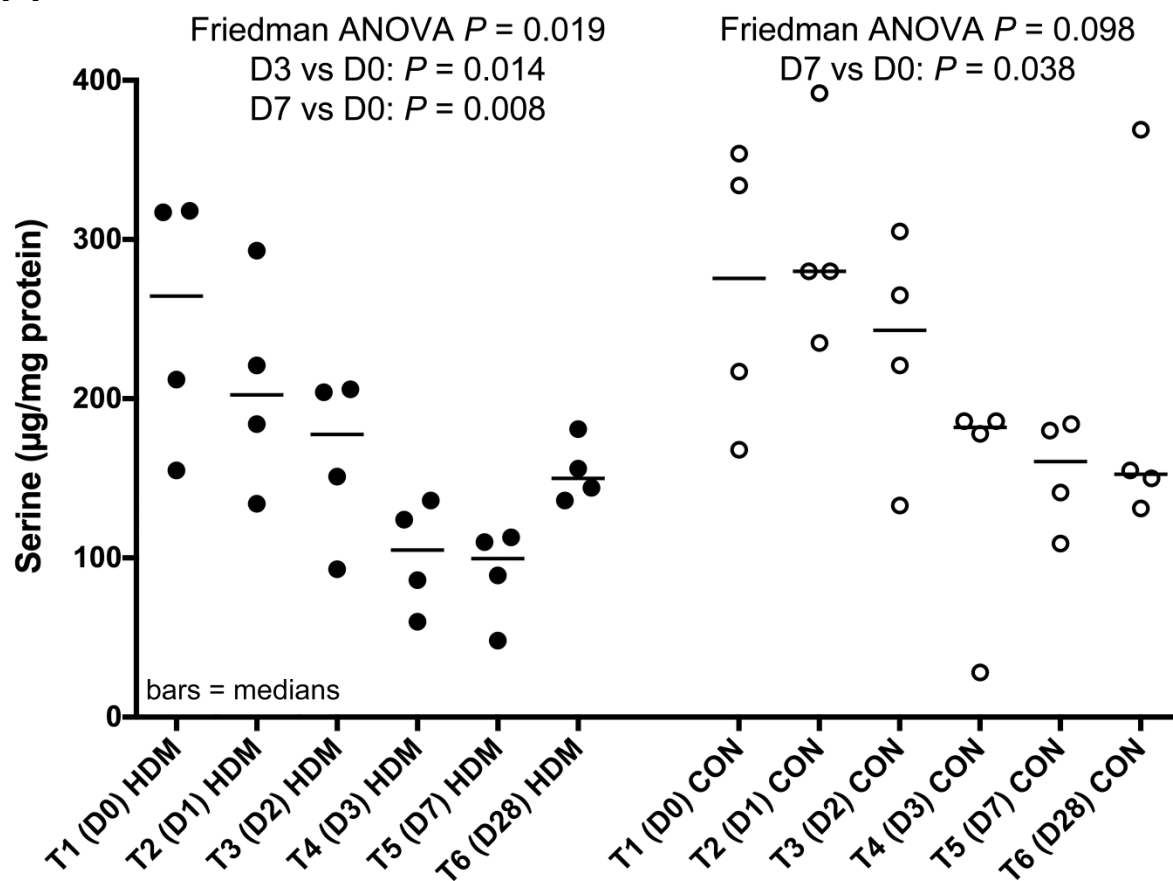
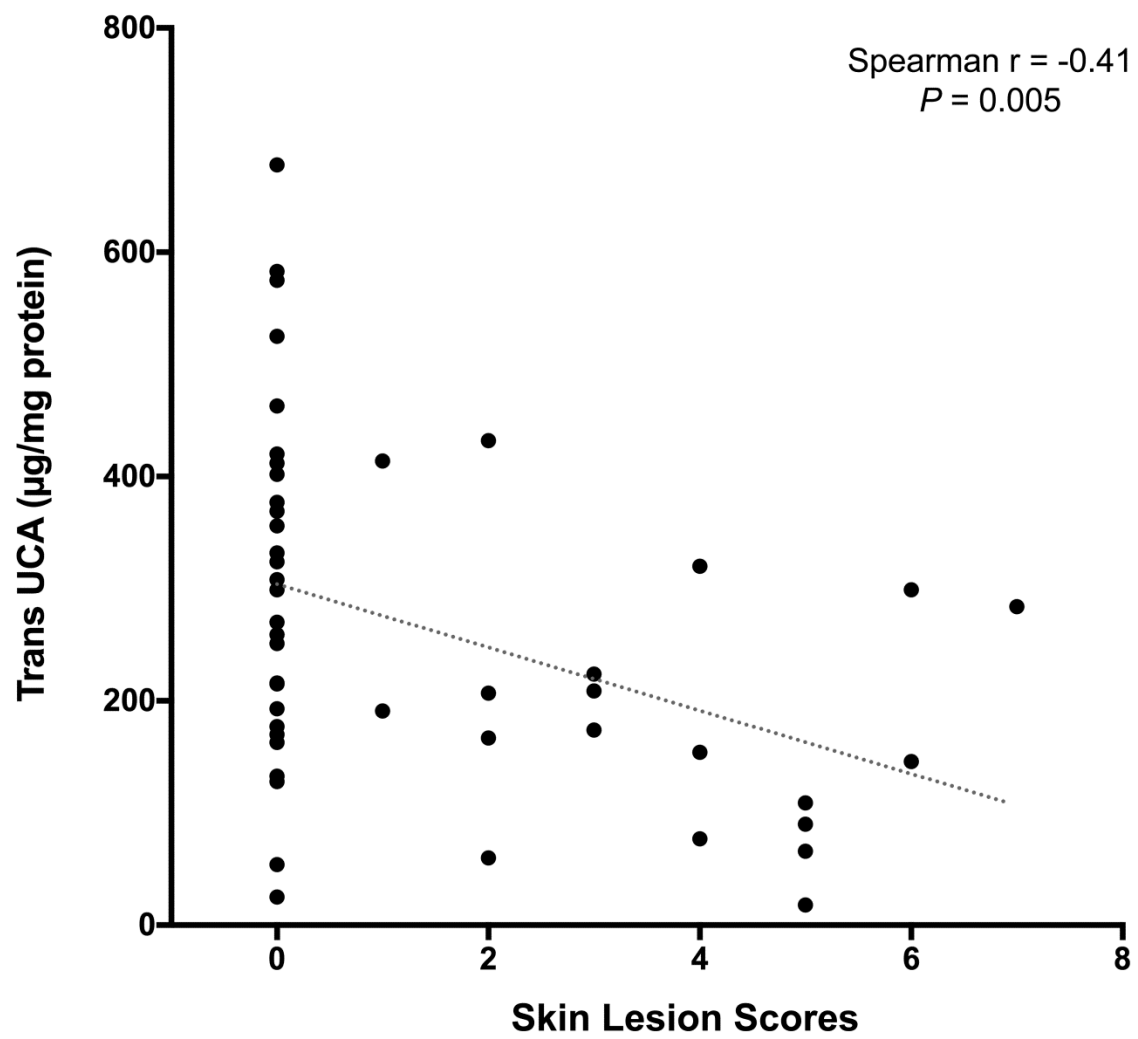
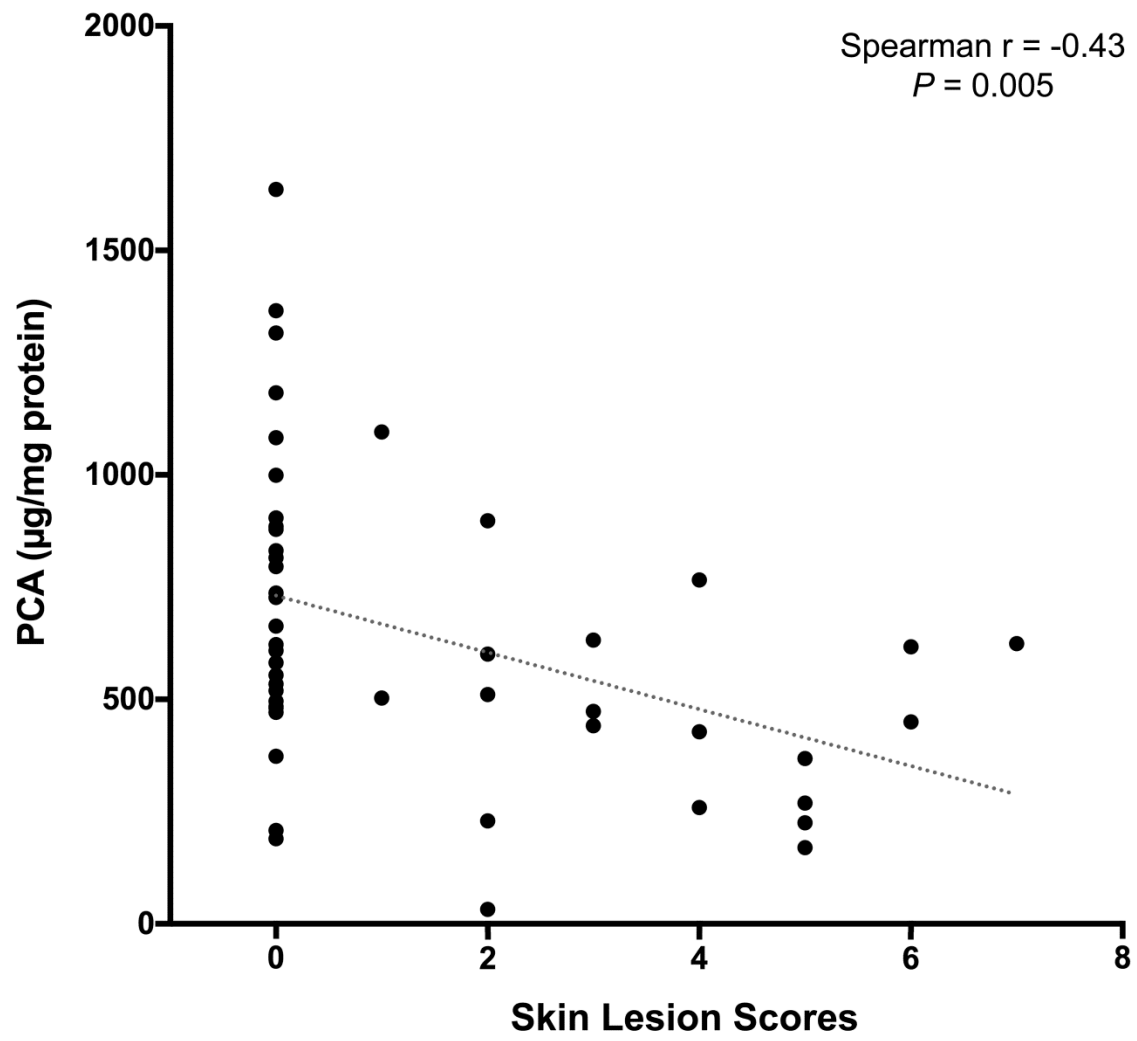


Figure S1

(a)



(b)



(c)

