

Effects of age and allergen exposure on transepidermal water loss in a house dust mite-sensitized beagle model of atopic dermatitis

Katherine Hightower, Rosanna Marsella and Alison Flynn-Lurie

College of Veterinary Medicine, University of Florida, Gainesville, FL 32610-126, USA

Correspondence: Rosanna Marsella, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610-126, USA. E-mail: marsellar@vetmed.ufl.edu

Conflict of Interest

None.

Sources of Funding

This project was funded by the University of Florida, College of Veterinary Medicine, Resident Research Grant and the American College of Veterinary Dermatology Resident's Award.

Abstract

Transepidermal water loss (TEWL) was measured as assessment of barrier function in house dust mite-sensitized atopic ($n = 24$) and normal ($n = 21$) beagle dogs before and after allergen challenge. Dogs of the two groups were matched for age and housed in the same environment. Ten sites were evaluated including both areas of high and low predisposition to atopic lesion formation. On day 0 (before allergen exposure) and day 3 (3 days after allergen exposure), TEWL measurements were taken using an open-chamber evaporimeter. On day 0, significant differences (one-way ANOVA) were found between normal and atopic dogs in the chin, pinna, periorcular region, axilla, antebrachium and thorax. Within the atopic group, a significant increase (one-way ANOVA) in TEWL was found in the axilla on day 3. Such a difference was particularly marked in young dogs (chin, pinna, axilla, antebrachium and groin). Within the normal group, allergen challenge did not change TEWL except for the chin. When atopic and nonatopic sites were compared within the atopic group, a significant difference in TEWL was found on day 0. A two-way ANOVA showed a significant effect of site, a significant effect of time but no site $\times$ time (i.e. allergen exposure) interaction. It is concluded that the house dust mite-sensitized atopic beagle dogs used in this study have altered barrier function in 'atopic' areas and that such differences are more evident in young individuals and are aggravated by allergen exposure.

Accepted 31 July 2009

Introduction

Atopic dermatitis (AD) is a complex multifactorial disease in which both genetic and environmental factors play an important role.^{1,2} An impaired skin barrier function is thought to be crucial for allergic sensitization as it may lead to increased allergen penetration which in turn favours a Th2-dominated response.^{3,4} Considering all allergens, house dust mites (HDM) have the greatest ability to penetrate the skin due to their proteases and this could aggravate an already impaired cutaneous barrier.⁵ Barrier function can be assessed using noninvasive methodologies such as the measurement of transepidermal water loss (TEWL).⁶⁻⁸ Studies on human medicine have demonstrated that TEWL measured in AD patients is higher⁹⁻¹² than that of normal individuals both in lesional and nonlesional areas¹³ and that variation exists between various anatomic sites.^{14,15} In humans, TEWL is also affected by age, being higher in infants¹⁶ and decreasing with age.^{17,18} Thus, penetration of allergens may be especially increased at a young age.

Canine AD shares many immunological and clinical similarities with human AD including the same distribution of lesions.¹⁹ In dogs, the epicutaneous route of allergen exposure is accepted as being the most important for sensitization and development of lesions.^{20,21} Pilot studies have also documented ultrastructural differences between atopic and normal skin in dogs, although the question of whether these changes are primary or not still remains.²² Measurement of TEWL has been demonstrated to be a suitable method to assess barrier function in dogs as well as humans, with values increasing with progressive damage of the skin.²³ In dogs, TEWL is also affected by site,²⁴ but no information currently exists on the effect of age. Only one study evaluated the effect of AD on barrier function by measurement of electrical impedance,²⁵ but no study exists on the comparison of TEWL between normal and atopic dogs. As the distribution of lesions is similar between the human and canine disease and allergen epicutaneous exposure is important in both species, it is reasonable to speculate that sites predisposed to the development of AD may have higher permeability compared with other sites.

The best way to address all these questions would be to use an experimental model in which confounding factors (e.g. different diets and housing conditions) can be controlled thus requiring fewer subjects. An experimental model of canine AD has been identified in a colony of beagle dogs.²⁶ In this model, dogs are epicutaneously sensitized to HDM and pruritic lesions clinically and

immunologically compatible with naturally occurring AD²¹ can be induced by exposure to HDM.

Therefore, the purpose of the present study was to evaluate TEWL in an experimental model of canine AD and to compare it with normal beagle dogs. The hypotheses tested were that TEWL is higher in atopic dogs compared with normal age- and breed-matched controls, in young dogs compared with older dogs and at body sites with a high susceptibility for AD lesions compared with those with a low susceptibility.

Materials and methods

All procedures used in this study were approved by the Institutional Animal Care and Use Committee of the University of Florida.

Animals

Two research colonies consisting of HDM-sensitized atopic beagle dogs ($n = 24$) and normal control beagle dogs ($n = 21$) were used. Each group was further subdivided into three groups based on age (group 1 being 'young', group 2 'adults' and group 3 'older' dogs). The atopic beagles had been previously sensitized to HDM (*Dermatophagoides farinae*) by epicutaneous route and were known to reliably develop pruritus and lesions with positive serology and intradermal skin tests in response to HDM challenge.^{21,26} The normal beagles had been previously challenged with HDM and found not to develop any reaction (pilot studies).²⁶ Those dogs had also been serology tested (Heska) and intradermally skin tested against HDM and found not to have positive results. A positive result on Heska test was considered to be ≥ 150 EA (ELISA absorbance) and a positive skin test was considered a reaction $\geq 2+$ on subjective evaluation on a scale from 0 to 4+ where 0 was the score assigned to the saline injection and 4+ was the reaction of the histamine injection (1/100 000 w/v) used as positive control. Prior to enrolment in the study, each normal dog was examined and determined to be healthy based on history, physical examination and lack of history and evidence of skin lesions on dermatological examination.

Housing and diet

As diet may affect TEWL,^{27,28} both colonies were placed on the same maintenance diet (Hill's Science Diet Adult Maintenance kibble formulation; Hill's Pet Nutrition Inc., Topeka, KS, USA) 8 weeks prior to and for the duration of the study. All dogs were housed indoors in the same temperature- and humidity-controlled research facility (72–75 °F; relative humidity of 68–72%) for the duration of the study. Dogs were housed in individual cement runs which were cleaned daily using high-temperature and high-pressure wash. Stuffed toys, carpets, soft bedding or anything that could trap dust were not allowed. Walls of the runs and filters were checked monthly to ensure the absence of HDM in the environment between challenges. Results were always below the detection level of the test used (MITE-T-FastTM Allergen Detection System; Aveho Biosciences, <http://www.avehobiosciences.com>). The atopic beagles were regularly housed indoors, while the normal beagles were moved to the indoor facility purposely for the study.

Allergen challenge

Allergen challenge was done using 50 mg of HDM antigen (*D. farinae*, Greer) daily for three consecutive days, as previously described.²⁶ The HDM solution was sprayed onto the bottom of air kennels and the dogs remained in their kennels for 3 h each day. Allergen challenge was done in all atopic beagles ($n = 24$) and in only six normal dogs due to logistic problems with animal care services.

Evaluation of clinical signs and transepidermal water loss measurements

Evaluation of clinical signs and TEWL measurements were done on day 0 (before allergen exposure) and day 3 (6 h after beginning the

last allergen exposure). TEWL was measured using an open-chamber evaporimeter (DermaLab[®] TEWL probe; Cortex Technology, Hadsund, Denmark). As the normal dogs had changed environment, TEWL was measured twice in these dogs on day 0 (12 h apart from each other). All TEWL readings were done in triplicate and the mean of the readings was used for analysis. Data were recorded electronically with a laptop computer and specified software (DASYLab[®]; cyberDERM Inc., Media, PA, USA).

Ten skin sites, consisting of eight canine AD predilection sites and two sites not typically affected by AD, were measured. For sites that were bilateral, only the left site was measured. Atopic sites included the chin, concave surface of the pinna, superior periocular region, axilla, antebrachial fossa, lateral groin and palmar and plantar interdigital spaces between the second and third phalanx. The nonatopic sites included the dorso-caudal lumbar spine and lateral thorax.

Readings were performed by parting the hair coat to ensure good contact between the probe and the skin. The probe consisted of a cylindrical open chamber measuring system with a diameter of 10 mm covering an area of 0.79 cm². Two sensors mounted at different points in a diffusion chamber measured relative humidity and temperature. The difference in water vapour pressure measured at each point in the chamber was used to calculate the water loss rate over a constant skin area (Figure 1).

Statistical analysis

Transepidermal water loss values were analysed using mixed model analysis of variance (ANOVA) with within-subject factor of time (i.e. allergen exposure) and between-subject factor of group (normal versus atopic; various age groups; atopic sites versus nonatopic sites) using STATVIEW statistical software (SAS, Cary, NC, USA). Follow-up contrasts employed paired *t*-tests. A value of $P < 0.05$ was considered statistically significant. Bonferroni correction was applied to control for multiple comparisons. To control for family-wise error rates, correction was made for three comparisons (i.e. across groups, within each sampling site and time), yielding a Bonferroni-corrected *p*-value of $0.05/3 = 0.0167$.

Results

Animals

The atopic ($n = 24$; 10 males and 14 females; mean age 2.5 years) and normal ($n = 21$; 10 males and 11 females; mean age 3.4 years) beagle colonies were similar for the



Figure 1. The DermaLab[®] Open-Chamber Evaporimeter consists of a cylindrical hand-held probe with paired sensors in a diffusion chamber which measures relative humidity and temperature when placed perpendicular to the skin surface. The vapour pressure gradient in the chamber is used to calculate evaporated water loss over a constant skin area.

Table 1. Demographic data for house dust mite-sensitized atopic and normal beagles for the various age groups (group 1 = young; group 2 = adult; group 3 = older dogs)

	Atopic (<i>n</i> = 24)			Normal (<i>n</i> = 21)		
Group	1	2	3	1	2	3
Dogs	11	7	6	10	7	4
Age (years)	1	2	6	1–2	3–4	6–10
Male:female	6:5	2:5	1:5	6:4	2:5	2:2

number of dogs, age range, sex and age group assignment (Table 1). No dogs were excluded and all dogs finished the study.

TEWL measurements

Normal dogs

On day 0, no significant difference was found in two TEWL measurements taken 12 h apart from each other, except for the groin (paired *t*-test, *P* = 0.0336) where TEWL decreased in the second measurement. No difference in TEWL between days 0 and 3 was found for any of the body sites of normal dogs except the chin (two-way ANOVA, *P* = 0.0258) where TEWL was decreased on day 3.

Normal vs. atopic dogs.

On day 0, significant differences were found in TEWL between the two groups at six sites (chin, pinna, periocular region, axilla, antebrachium and thorax) (Figure 2). There was no significant effect of time, no significant effect of group and no group × time interaction (two-way ANOVA, *P* > 0.05 for all analyses).

Atopic dogs

When all age groups of the atopic group were combined, no significant difference in TEWL between days 0 and 3 was found, except for the axilla (*t*-test; axilla *P* = 0.0042) where TEWL increased on day 3. Table 2 shows TEWL measurements.

A three-group-by-two-time ANOVA was then used to evaluate how TEWL changed among and within the different age groups. When TEWL in atopic and nonatopic predilection sites was compared among the age groups (group 1 = young; group 2 = adult; group 3 = older) on day 0, significant increase was found in more body sites of older dogs (group 3) (Table 3).

When TEWL in atopic and nonatopic predilection sites was compared among the age groups on day 3, significant

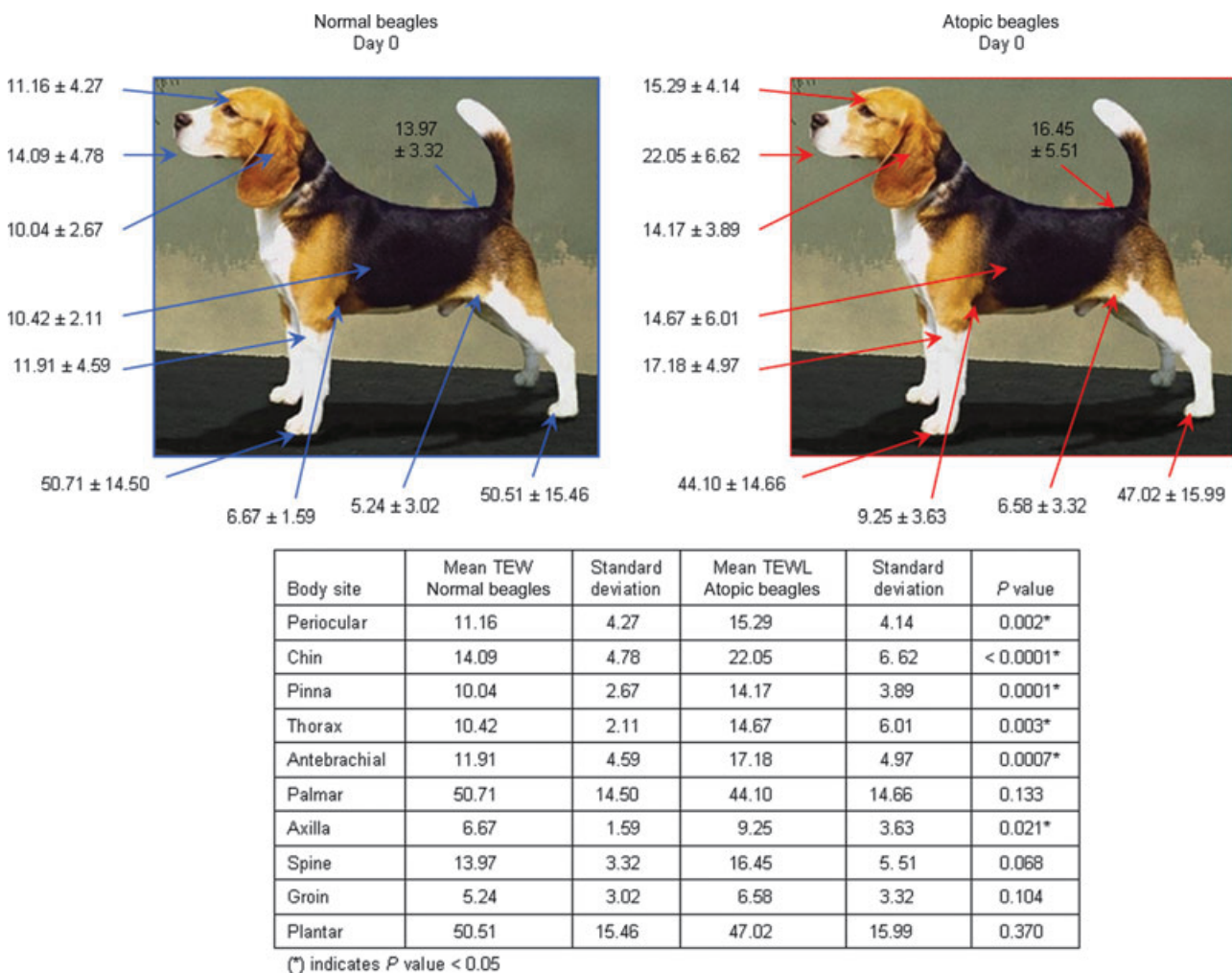


Figure 2. Mean (±standard deviation) transepidermal water loss (TEWL) (g/m²/h) and *P*-value in normal and house dust mite-sensitized atopic beagles according to body site before (day 0) allergen exposure. Significant differences in TEWL between the two groups were noted at the chin, pinna, periocular, axilla, antebrachial and thoracic sites.

Table 2. Mean ($\pm$ standard deviation) transepidermal water loss (TEWL) ($\text{g}/\text{m}^2/\text{h}$) for all house dust mite-sensitized atopic beagles according to body site before (day 0) and after allergen exposure (day 3)

	Mean TEWL day 0	Standard deviation	Mean TEWL day 3	Standard deviation
Atopic sites				
Chin	22.051	6.625	27.070	10.503
Pinna	14.178	3.897	16.115	4.898
Periocular	15.298	4.141	16.487	4.277
Axilla*	9.254	3.630	12.883	6.365
Antebrachium	17.180	4.973	17.775	5.699
Groin	6.582	3.322	8.657	4.986
Palmar	44.106	14.661	43.351	11.317
Plantar	47.024	15.991	48.784	12.486
Nonatopic sites				
Spine	16.456	5.510	19.112	6.796
Thorax	14.673	6.013	14.973	5.067

*Site for which comparison yielded a value of $P < 0.005$.

increase was found in more body sites of young dogs (group 1) (Table 3).

When TEWL was compared among sites within each age group, it was significantly higher on day 3 compared with day 0 in the chin, pinna, axilla, antebrachium and groin of young dogs (group 1). Table 4 shows TEWL measurements.

Table 3. Comparisons between transepidermal water loss (TEWL) measurements at the various body sites among young (group 1), adult (group 2) and older (group 3) house dust mite-sensitized atopic beagles before (day 0) and after (day 3) allergen exposure.

	Day 0			Day 3		
	Group 1 vs. group 2	Group 1 vs. group 3	Group 2 vs. group 3	Group 1 vs. group 2	Group 1 vs. group 3	Group 2 vs. group 3
Atopic sites						
Chin	2 > 1	3 > 1	3 > 2	1 > 2	1 > 3	3 > 2
Pinna	1 > 2	3 > 1	3 > 2*	1 > 2*	1 > 3	3 > 2
Periocular	1 > 2	3 > 1	3 > 2	1 > 2	1 > 3	3 > 2
Axilla	1 > 2*	1 > 3	3 > 2*	1 > 2*	1 > 3*	3 > 2
Antebrachium	1 > 2	1 = 3	3 > 2	1 > 2*	1 > 3	3 > 2
Groin	1 > 2	1 = 3	3 > 2	1 > 2*	1 > 3	3 > 2
Palmar	1 > 2	1 > 3	2 > 3	1 > 2	3 > 1*	3 > 2
Plantar	1 > 2	1 > 3	2 = 3	1 > 2	1 > 3	3 > 2
Nonatopic sites						
Spine	1 > 2	1 > 3	3 > 2	1 > 2	1 > 3	3 > 2
Thorax	1 > 2	1 > 3	3 > 2	1 > 2*	1 > 3	3 > 2

* $P < 0.0167$.

Table 4. Mean transepidermal water loss (TEWL) ($\text{g}/\text{m}^2/\text{h}$) for young (group 1), adult (group 2) and older (group 3) house dust mite-sensitized atopic beagles

	Group 1		Group 2		Group 3	
	Day 0	Day 3	Day 0	Day 3	Day 0	Day 3
Atopic sites						
Chin	21.171	30.296*	21.453	20.601	24.362	28.278
Pinna	13.842	18.213*	11.782	11.619	17.591	17.515
Periocular	15.922	18.075	12.342	14.529	17.602	15.863
Axilla	11.295	17.146*	5.601	8.162	9.775	10.576
Antebrachium	18.480	21.855*	13.874	13.543	18.652	14.722
Groin	7.001	11.691*	5.584	5.041	6.981	7.311
Palmar	47.917	49.483	43.078	40.015	38.320	35.999
Plantar	50.376	48.260	45.010	45.102	43.954	54.041
Nonatopic sites						
Spine	17.941	21.348	13.065	15.511	17.196	19.216
Thorax	17.761	18.250	10.553	11.435	13.819	13.093

Day 0 = before allergen exposure; day 3 = after allergen exposure. * $P < 0.05$ from day 0 to day 3.

Atopic vs. nonatopic predilection sites in AD group

A two-way ANOVA evaluating how atopic and nonatopic predilection sites differed over time as function of HDM exposure within the atopic beagles found that there was a significant effect of site ($P = 0.0001$, nonatopic site mean 16.23, SE 0.083; atopic site mean 22.8, SE 0.083), significant effect of time ($P = 0.001$) and no site $\times$ time interaction. Thus, atopic predilection sites had a higher TEWL and those values changed as a function of HDM exposure.

Discussion

The results of this study showed that in normal beagle dogs TEWL measurements were repeatable at two separate times (12 h apart) after a change in environment had occurred. This study also found that HDM exposure did not alter TEWL in normal beagles in all sites but one (chin). HDM-sensitized atopic beagles had significantly different TEWL values compared with normal dogs on day 0 and significant increases were noticeable on day 3 in young individuals.

In this study, an open-chamber evaporimeter was used. This methodology was chosen as it had been previously validated in dogs,²⁴ while, at the time of the study, no validation of the closed-chamber instruments could be

found in the veterinary literature despite the fact that these evaporimeters are generally thought to be more reliable and less affected by environmental factors. Studies on human subjects have validated a variety of open and closed types of devices in both comparative and reproducibility tests.^{29,30} One human study compared a newer portable closed-chamber device with the Derma-Lab[®] evaporimeter (Derma Lab Inc., Highland Park, IL, USA) used in this study and found that both devices correlated well and had similar sensitivity at multiple identical skin sites in AD and control patients.³¹

In this study, precautions were taken to prevent and minimize environmental factors that could alter measurements. All readings were taken in a closed room with a consistently controlled temperature and relative humidity, away from drafts and air currents. The instrument was calibrated between different dogs and the staff took measures not to heat or breathe on the probe. As in prior canine studies, TEWL measurements with open-chamber devices were optimized by minimizing dog movement with light restraint²⁷ or by training the dogs to stand reasonably still during readings,²⁴ minimal restraint was purposely used in this study. In addition, as the hair coat could interfere with the contact between the probe and skin surface, the hair coat was manually parted just prior to placement of the probe. Altering the hair coat by removal was avoided as clipping has been shown to decrease TEWL by 44%.²⁴

One strength of this study lies in the availability of research colonies that could be housed in the same controlled environment, fed the same diet and the accessibility to a temperature- and humidity-controlled environment for all the measurements allowing to control, as much as possible, for confounding factors that could have made the interpretation of the results challenging in a clinical setting. All dogs in the study were fed the same commercial diet for eight consecutive weeks prior to and for the duration of the study as prior data had shown a metabolic skin steady state after 7 weeks.²⁷ Dogs were matched for age and breed in the attempt to minimize variability due to these factors. Additionally, the availability of an experimental model for canine AD using dogs that are predisposed to allergic sensitization but that have minimal clinical signs when maintained in a HDM-free environment allowed for discrimination between barrier function differences that may pre-exist obvious clinical lesions and the effect of allergen exposure and development of clinically obvious allergic inflammation.

Some findings in this study were unexpected, such as the decrease in TEWL in normal dogs after they were housed indoors for 24 h (groin) and after allergen challenge (chin). One plausible explanation (chin) may be due to the fact that these dogs were accustomed to drinking water from bowls and had to learn how to use water spigots in the new environment. Many of the differences at baseline (day 0) between the normal and atopic dogs were expected (e.g. chin, pinna, periocular region, axilla and antebrachium) and suggests that impaired barrier function exists in atopic dogs. The findings parallel the results of human studies, where TEWL of uninvolved skin of patients with AD was significantly increased when

compared with normal controls.^{9,11} Whether the increased TEWL was the result of a low-grade inflammation (not clinically obvious) present in atopic skin or due to structural defects need further investigation. Preliminary ultrastructure evaluation of the stratum corneum of non-lesional canine AD skin has shown abnormalities in the continuity and thickness of the intercellular lipid lamellae when compared with normal dogs.²² A second ultrastructure study characterized the nonlesional skin of AD dogs as having a disorganized and reduced number of lamellar lipids present in the stratum corneum when compared with normal controls.³² Such findings support the suggestion of a constitutive skin barrier defect. Alternatively, the existence of a defect in cutaneous permeability is viewed by others as a downstream consequence of immunologic and inflammatory changes ('inside-out' hypothesis).^{33,34} As the atopic dogs had already been sensitized at the time of the study, future research should focus on newborn puppies prior to any contact with allergens to further assess this issue.

This study also demonstrated that allergen exposure in young atopic beagle dogs resulted in a TEWL increase in atopic sites. These findings are consistent with human studies where TEWL in AD patients is not only elevated in lesional skin but also correlates with the degree of clinical activity.^{35–37} As HDM possess proteases that can potentially alter the barrier function of the skin it was important to challenge normal dogs. This study found that HDM exposure *per se*, in the absence of an allergic reaction, did not impair barrier function in any body site. Thus, an allergic sensitization and inflammatory response is needed in order to have changes in TEWL. This was evident in the axilla in the atopic dogs. When atopic were compared with nonatopic predilection sites, this difference was quite significant. Thus, it is conceivable that the distribution of lesions in AD may be due to the fact that these sites appear to be more prone to the epicutaneous absorption of allergens. As epicutaneous route of allergen exposure has been demonstrated to be the single most important of the routes,²⁰ these findings may help shed some light to explain the characteristic distribution of lesions in AD. A possible explanation for this is that in general, AD predilection sites are anatomically less haired regions of the body or may have differences in the thickness or structure of the stratum corneum;³⁸ therefore, they are more permeable and allow more allergen exposure.

Aging has also been demonstrated to affect TEWL. In this study, one interesting finding was that the youngest atopic beagle dogs (group 1) had the highest TEWL measurements for most of the body sites both before and after allergen challenge. An explanation for TEWL differences between age groups may be that the pathophysiology of the disease varies with the age of the animal and that younger dogs are more susceptible to development of lesional alterations from AD. These findings are in line with human studies that showed the highest TEWL in infants^{12,16} supporting the hypothesis that sensitization of predisposed individuals occurs at a very young age.³⁹ In humans, TEWL generally also declines with aging⁴⁰ most likely due to an overall decrease in the lipid composition,^{41,42} thus a decrease in skin hydration and lower

TEWL. Whether similar changes in lipid composition occur in older dogs is currently unknown.

In this study, an increase in TEWL at AD predilection sites was observed with allergen challenge in a colony of HDM-sensitized AD beagle dogs. It is important to remember that statistical significance does not necessarily imply clinical relevance. As large variation was seen for some measurements, the findings of this study will need to be confirmed and expanded with larger controlled studies. This study emphasizes the need for further investigations of skin barrier in canine AD. Although it is tempting to speculate that a primary defect may be present in atopic individuals, particularly in atopic sites, more studies are warranted before final conclusions can be made. From the clinical standpoint, it is interesting to hypothesize that normalization of the barrier function could result in clinical benefit and, potentially, alter the course of the disease.

In summary, in this study, TEWL was increased in HDM-sensitized atopic dogs prior to the development of skin lesions and this was particularly evident in the younger dogs. Allergen exposure had no effect on TEWL in normal dogs while worsened TEWL in young atopic dogs. Collectively, these findings suggest that at least in this experimental model of canine AD-increased skin permeability is detectable in atopic nonlesional sites of young individuals possibly explaining how easily these dogs are sensitized by epicutaneous exposure.

Acknowledgements

The authors thank Gary Grove and his group at cyberDERM Inc. for their very generous support in providing an open-chamber evaporimeter with computerized support, William Perlestein for time and assistance with all the statistical analyses, Dana Liska, Abby Foust, Elizabeth Creary, Paul Dutcher and Jason Alpert for their patience in the handling and restraining of animals during TEWL measurements and without whom this study would not have been possible.

References

- Hill PB, DeBoer DJ. The ACVD task force on canine atopic dermatitis (IV): environmental allergens. *Veterinary Immunology and Immunopathology* 2001; 81: 169–86.
- Sousa CA, Marsella R. The ACVD task force on canine atopic dermatitis (III): genetic factors. *Veterinary Immunology and Immunopathology* 2001; 81: 153–7.
- Leung DYM, Boguniewicz M, Howell MD *et al.* New insights into atopic dermatitis. *The Journal of Clinical Investigation* 2004; 113: 651–7.
- Palmer CNA, Irvine AD, Terron-Kwiatkowski A *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nature Genetics* 2006; 38: 441–6.
- Wan H, Winton HL, Soeller C *et al.* Der p 1 facilitates transepithelial allergen delivery by disruption of tight junctions. *Journal of Clinical Investigation* 1999; 104: 123–33.
- Fluhr JW, Feingold R, Elias PM. Transepidermal water loss reflects permeability barrier status; validation in human and rodent *in vivo* and *ex vivo* models. *Experimental Dermatology* 2006; 15: 483–92.
- Wilson DR, Maibach H. *Transepidermal Water Loss. A Review. Cutaneous Investigation in Health and Disease. Non-Invasive Methods and Instrumentation*, 1st edn. New York: Dekker, 1989.
- Pinnagoda J, Tupker RA, Agner T *et al.* Guidelines for transepidermal water loss (TEWL) measurement. A report from the Standardization Group of the European Society of Contact Dermatitis. *Contact Dermatitis* 1990; 22: 164–78.
- Loden M, Olsson H, Axell T *et al.* Friction, capacitance and transepidermal water loss (TEWL) in dry atopic and normal skin. *British Journal of Dermatology* 1992; 126: 137–41.
- Lee C-H, Chuang H-Y, Shih C-C *et al.* Transepidermal water loss, serum IgE and β -endorphin as important and independent biological markers for development of itch intensity in atopic dermatitis. *British Journal of Dermatology* 2006; 154: 1100–7.
- Seidnari S, Giusti G. Objective assessment of the skin of children affected by atopic dermatitis: a study of pH, capacitance and TEWL in eczematous and clinically normal skin. *Acta Dermatologica Venereologica* 1995; 75: 429–33.
- Boralevi F, Hubiche T, Leaute-Labreze C *et al.* Epicutaneous aeroallergen sensitization in atopic dermatitis infants – determining the role of epidermal barrier impairments. *Allergy* 2008; 63: 205–10.
- Werner Y, Lindberg M. Transepidermal water loss in dry and clinically normal skin in patients with atopic dermatitis. *Acta Dermatologica Venereologica* 1985; 65: 102–5.
- Kim DW, Park JY, Na GY *et al.* Correlation of clinical features and skin barrier function in adolescent and adult patients with atopic dermatitis. *International Journal of Dermatology* 2006; 45: 698–701.
- Nicander I, Nyren M, Erntestam L *et al.* Baseline electrical impedance measurements at various skin sites – related to age and sex. *Skin Research and Technology* 1997; 3: 252–8.
- Nikolovski J, Stamatas GN, Kollias N *et al.* Barrier function and water-holding and transport properties of infant stratum corneum are different from adult and continue to develop through the first year of life. *The Journal of Investigative Dermatology* 2008; 128: 1728–36.
- Yosipovitch G, Maayan-Metzger A, Merlob P *et al.* Skin barrier properties in different body areas in neonates. *Pediatrics* 2000; 106: 105–8.
- Wilhelm KP, Cua AB, Maibach HI. Skin aging. Effect of transepidermal water loss, stratum corneum hydration, skin surface pH and casual sebum content. *Archives of Dermatology* 1991; 127: 1806–9.
- Marsella R, Olivry T. Animal models of atopic dermatitis. *Clinics in Dermatology* 2003; 21: 122–33.
- Marsella R, Nicklin C, Lopez J. Studies on the role of routes of allergen exposure in high IgE-producing beagle dogs sensitized to house dust mites. *Veterinary Dermatology* 2006; 17: 306–12.
- Marsella R, Olivry T, Maeda S. Cellular and cytokine kinetics after epicutaneous allergen challenge (atopy patch testing) with house dust mites in high-IgE beagles. *Veterinary Dermatology* 2006; 17: 111–20.
- Inman AO, Olivry T, Dunston SM *et al.* Electron microscopic observations of stratum corneum intercellular lipids in normal and atopic dogs. *Veterinary Pathology* 2001; 38: 720–3.
- Shimada K, Yoshihara T, Yamamoto M *et al.* Transepidermal water loss correlates with skin barrier function in dogs. *Veterinary Dermatology* 2007; 18: 194.
- Watson A, Fray T, Clarke S *et al.* Reliable use of the ServoMed evaporimeter EP-2 to assess transepidermal water loss in the canine. *American Society for Nutritional Sciences* 2002; 132: 1661S–4S.
- Chesney CJ. Measurement of skin hydration in normal dogs and in dogs with atopy or a scaling dermatosis. *Journal of Small Animal Practice* 1995; 36: 305–9.

26. Marsella R, Olivry T, Nicklin C *et al.* Pilot investigation of a model for canine atopic dermatitis: environmental house dust mite challenge of high-IgE-producing beagles, mite hypersensitive dogs with atopic dermatitis and normal dogs. *Veterinary Dermatology* 2006; 1: 24–35.
27. Hester SL, Rees CA, Kennis RA *et al.* Evaluation of corneometry (skin hydration) and transepidermal water-loss measurements in two canine breeds. *The Journal of Nutrition* 2004; 134: 2110S–3S.
28. Watson AL, Fray TR, Bailey J *et al.* Dietary constituents are able to play a beneficial role in canine epidermal barrier function. *Experimental Dermatology* 2006; 15: 74–81.
29. Shah JH, Hongbo Z, Maibach HI. Comparative evaporimetry in man. *Skin Research and Technology* 2005; 11: 205–8.
30. Barel AO, Clarys P. Study of the stratum corneum barrier function by transepidermal water loss measurements: comparison between two commercial instruments: evaporimeter and tewameter. *Skin Pharmacology* 1995; 8: 186–95.
31. Tagami H, Kobayashi H, Kikuchi K. A portable device using a closed chamber system for measuring transepidermal water loss: comparison with the conventional method. *Skin Research and Technology* 2002; 8: 7–12.
32. Piekutowska A, Pin D, Rème CA *et al.* Effects of a topically applied preparation of epidermal lipids on the stratum corneum barrier of atopic dogs. *Journal of Comparative Pathology* 2008; 138: 197–203.
33. Leung DYM. Atopic dermatitis: new insights and opportunities for therapeutic intervention. *Journal of Allergy and Clinical Immunology* 2000; 105: 860–76.
34. Elias PM, Wood LC, Feingold KR. Epidermal pathogenesis of inflammatory dermatoses. *American Journal of Contact Dermatitis* 1999; 10: 119–26.
35. Shahidullag M, Raffle EJ, Frain-Bell W. Transepidermal water loss in patients with dermatitis. *British Journal of Dermatology* 1969; 81: 722–30.
36. Allto-Korte K. Improvement of skin barrier function during treatment of atopic dermatitis. *Journal of the American Academy of Dermatology* 1995; 33: 969–72.
37. Kolbe L, Kligman AM, Schreiner V *et al.* Corticosteroid-induced atrophy and barrier impairment measured by non-invasive methods in human skin. *Skin Research and Technology* 2001; 7: 73–7.
38. Ya-Xian Z, Suetake T, Tagami H. Number of cell layers of the stratum corneum in normal skin: relationship to the anatomical location on the body, age, sex and physical parameters. *Archives of Dermatological Research* 1999; 291: 555–9.
39. Taieb A. Hypothesis: from epidermal barrier dysfunction to atopic disorders. *Contact Dermatitis* 1999; 41: 177–80.
40. Cua AB, Wilhelm KP, Maibach HI. Frictional properties of human skin: relation to age, sex and anatomical region, stratum corneum hydration and transepidermal water loss. *British Journal of Dermatology* 1990; 123: 473–9.
41. Rogers J, Harding C, Mayo A *et al.* Stratum corneum lipids: the effect of ageing and the seasons. *Archives of Dermatological Research* 1996; 288: 765–70.
42. Ghadially R, Brown BE, Sequeira-Martin SM *et al.* The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in human and a senescent murine model. *The Journal of Clinical Investigation* 1995; 95: 2281–90.

Résumé La perte d'eau trans-épidermique (TEWL) a été mesurée afin d'évaluer la fonction barrière cutanée de chiens atopiques sensibilisés aux acariens de la poussière de maison ($n=24$) et de chiens normaux ($n=21$) avant et après exposition à l'allergène. Les chiens des deux groupes ont été répartis en fonction de leur âge et placés dans le même environnement. Dix zones ont été évaluées incluant des régions hautement et faiblement prédisposées à la formation de lésions d'atopie. Au jour 0 (avant exposition à l'allergène) et au jour 3 (3 jours après exposition), la TEWL a été mesurée par un évaporimètre à chambre ouverte. Au jour 0, des différences significatives (ANOVA à un facteur) ont été trouvées entre les chiens normaux et les atopiques au niveau du menton, du pavillon auriculaire, du pli axillaire, de l'avant-bras, du thorax et en région péri-oculaire. A J3, une augmentation significative (ANOVA à un facteur) de la TEWL a été observée au niveau du pli axillaire des chiens atopiques. Cette différence était surtout marquée chez les jeunes chiens (menton, pavillon auriculaire, plis axillaire, avant-bras et pli inguinal). Au sein du groupe normal, l'exposition allergénique n'a pas modifié la TEWL à l'exception du menton. Au sein du groupe atopique, les zones cutanées atopiques et non-atopiques ont été comparées, révélant une différence significative de la TEWL à J0. Un test ANOVA à deux facteurs a montré une influence significative de la zone et du temps mais pas des facteurs combinés zone et temps (i.e., exposition allergénique). Nous pouvons en conclure que les beagles atopiques sensibilisés aux acariens de la poussière de maison utilisés dans cette étude ont une fonction barrière cutanée altérée dans les « zones atopiques », que ces différences sont plus évidentes chez les jeunes et sont aggravées par l'exposition à l'allergène.

Resumen La pérdida de agua transepidermal se midió como una valoración de la función de barrera en perros Beagle atópicos sensibilizados para el ácaro del polvo ($n = 24$) y normales ($n = 21$), antes y después de la exposición al alérgeno. Se evaluaron 10 sitios incluidas áreas de alta y baja predisposición a la formación de lesiones atópicas. En el día cero (antes de la exposición al alérgeno) y en el día tres (tres días después de la exposición al alérgeno), la pérdida de agua transepidermal se midió utilizando un evaporizador de cámara abierta. En el día cero, se observaron diferencias significativas (ANOVA de una vía) entre perros normales y perros atópicos en la barbilla, oreja, región periorcular, axila, antebrazo y tórax. En el grupo de perros atópicos, hubo un incremento significativo (ANOVA de una vía) en la pérdida de agua transepidermal en la axila en el día tres. Esta diferencia fue particularmente marcada en perros jóvenes (barbilla, oreja, axila, antebrazo e ingle). Dentro del grupo normal, la exposición al alérgeno no produjo un cambio en la pérdida de agua transepidermal a excepción de la barbilla. Cuando los sitios atópicos y no atópicos se compararon en los perros del grupo atópico, hubo una diferencia significativa en la pérdida de agua transepidermal en el día cero. Una prueba ANOVA de dos vías mostró un efecto significativo del lugar, un efecto significativo temporal, pero sin interacción lugar x temporal (tras exposición al alérgeno). Concluimos que los perros atópicos Beagle sensibilizados al polvo del ácaro del hogar utilizados en este estudio tienen una función de barrera alterada en las áreas "atópicas", y que tales diferencias son más evidentes en individuos jóvenes y están agravadas tras la exposición al alérgeno. La perte d'eau trans-épidermique (TEWL) a été mesurée afin d'évaluer la fonction barrière cutanée de chiens atopiques sensibilisés aux acariens de la poussière de

maison ($n = 24$) et de chiens normaux ($n = 21$) avant et après exposition à l'allergène. Les chiens des deux groupes ont été répartis en fonction de leur âge et placés dans le même environnement. Dix zones ont été évaluées incluant des régions hautement et faiblement prédisposées à la formation de lésions d'atopie. Au jour 0 (avant exposition à l'allergène) et au jour 3 (3 jours après exposition), la TEWL a été mesurée par un évaporimètre à chambre ouverte. Au jour 0, des différences significatives (ANOVA à un facteur) ont été trouvées entre les chiens normaux et les atopiques au niveau du menton, du pavillon auriculaire, du pli axillaire, de l'avant-bras, du thorax et en région péri-oculaire. A J3, une augmentation significative (ANOVA à un facteur) de la TEWL a été observée au niveau du pli axillaire des chiens atopiques. Cette différence était surtout marquée chez les jeunes chiens (menton, pavillon auriculaire, plis axillaire, avant-bras et pli inguinal). Au sein du groupe normal, l'exposition allergénique n'a pas modifié la TEWL à l'exception du menton. Au sein du groupe atopique, les zones cutanées atopiques et non-atopiques ont été comparées, révélant une différence significative de la TEWL à J0. Un test ANOVA à deux facteurs a montré une influence significative de la zone et du temps mais pas des facteurs combinés zone et temps (i.e. exposition allergénique). Nous pouvons en conclure que les beagles atopiques sensibilisés aux acariens de la poussière de maison utilisés dans cette étude ont une fonction barrière cutanée altérée dans les « zones atopiques », que ces différences sont plus évidentes chez les jeunes et sont aggravées par l'exposition à l'allergène.

Zusammenfassung Um die Barrierefunktion der Haut bei Hausstaubmilben-sensiblen atopischen ($n = 24$) und bei normalen ($n = 21$) Beagles vor und nach allergischer Provokation zu beurteilen, wurde der transepidermale Flüssigkeitsverlust (TEWL) gemessen. Die Hunde der beiden Gruppen wurden altersmäßig gleichmäßig verteilt und in derselben Umgebung gehalten. Es wurden zehn Stellen, die sowohl Zonen hoher als auch niedriger Prädisposition für die Entwicklung von atopischen Läsionen beinhalteten, evaluiert. Am Tag 0 (vor der Allergenexposition) und am Tag 3 (3 Tage nach der Allergenexposition), wurden TEWL Messungen mittels Evaporimeter mit offener Kammer gemacht. Am Tag 0 wurden signifikante Unterschiede (Einweg Varianzanalyse – ANOVA) zwischen normalen und atopischen Hunden am Kinn, an den Ohren, in der periokulären Region, der Achsel, dem Unterarm und am Thorax festgestellt. Innerhalb der atopischen Gruppe wurde am Tag 3 ein signifikanter Unterschied (Einweg Varianzanalyse – ANOVA) in der Haut der Achsel festgestellt. Diese Unterschiede waren vor allem bei jungen Hunden deutlich (Kinn, Ohren, Achsel, Unterarm und Leistengegend). Innerhalb der Gruppe der normalen Hunde veränderte die Provokation mit Allergen den TEWL außer am Kinn nicht. Beim Vergleich der atopischen und nicht-atopischen Stellen innerhalb der Gruppe der atopischen Hunde, wurde am Tag 0 ein signifikanter Unterschied des TEWL festgestellt. Ein Zweiweg ANOVA zeigte einen signifikanten Einfluss der Hautstelle und der Zeit, aber keine Wechselwirkung von Hautstelle x Zeit (das heißt Allergenexposition). Es wird daraus geschlossen, dass die Hausstaubmilben-sensiblen atopischen Beagles, die in dieser Studie verwendet wurden, eine geänderte Barrierefunktion an den „atopischen“ Hautstellen haben und dass derartige Unterschiede bei jungen Individuen deutlicher sind und bei Allergenexposition verstärkt werden.