

Transepidermal water loss in healthy and atopic dogs, treated and untreated: a comparative preliminary study

Luisa Cornegliani*, Antonella Vercelli*,
Elisabetta Salat† and Rosanna Marsella‡

*Ambulatorio Veterinario Associato, Torino, Italy

†College of Veterinary Medicine, University of Milan, Milan, Italy

‡College of Veterinary Medicine, University of Florida, Gainesville, FL, USA

Correspondence: Luisa Cornegliani, Ambulatorio Veterinario Associato, C.so Traiano 99/d, I-10135 Torino, Italy.

E-mail: gigiami@tin.it

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Conflict of Interest

No conflicts of interest have been declared.

Abstract

An impaired skin barrier function is thought to be crucial for allergic sensitization. In humans, the skin barrier is assessed by noninvasive methods, such as the measurement of transepidermal water loss (TEWL). Although limitations have been reported, measurement of TEWL has been demonstrated to be a suitable method to assess barrier function indirectly in dogs. The purposes of this prospective clinical study were twofold. The first aim was to evaluate and compare TEWL in healthy and atopic dogs. The second aim was to evaluate TEWL in a population of atopic dogs and to assess TEWL in dogs whose disease was in remission after successful therapy and compare it with dogs whose disease was not controlled or receiving treatment. One hundred and fifty dogs were selected and divided into the following three groups: 50 atopic dogs before specific treatment (group A); 50 in remission (group B); and 50 control dogs (group C). The mean values for TEWL for each group were 22.47 (g/m² h) (group A; 95% confidence interval 20.85–24.09), 12.57 (g/m² h) (group B; 95% confidence interval 11.43–13.7) and 8.81 (g/m² h) (group C; 95% confidence interval 8.09–9.52); *P*-value was 0.0001 for TEWL (groups A, B and C). This study showed a significant difference of TEWL between healthy control dogs and dogs with atopic dermatitis. Additionally, TEWL was lower in atopic dogs whose disease was in remission due to treatment. These results were consistent with reports in human medicine about TEWL.

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Introduction

Mammalian skin works as a physiological barrier between the body and the environment. Abnormalities in barrier function have been reported in atopic dermatitis in humans and dogs.^{1–3} Canine atopic dermatitis (CAD) shares clinical and immunological similarities with human atopic dermatitis.⁴ Skin barrier impairments have been demonstrated in preliminary studies in atopic dogs, using electron microscopy.^{5,6} An impaired skin barrier function is thought to be crucial for allergic sensitization because it may lead to increased allergen penetration, which in turn favours a Th2-dominated response.^{7–9}

Skin barrier function may be assessed using noninvasive methods, such as the measurement of transepidermal water loss (TEWL), which is defined as the volume of water that passes from inside to outside the body through the upper epidermal layers. In humans, it has been used to evaluate skin barrier function in various dermatological diseases.¹⁰ In CAD, TEWL is considered to be an important indicator of impaired barrier function² and is an important factor in allergic sensitization.¹¹ Although limitations have been reported with various methodologies used to assess TEWL, measurement of TEWL has been used to assess barrier functions indirectly in dogs as well as humans, with values increasing with progressive damage of the skin.³ In humans, high TEWL correlates with low epidermal barrier function. In human atopic dermatitis, TEWL may increase twofold in nonlesional and fourfold in lesional skin.^{12,13}

Several studies have been performed to assess the TEWL in dogs in controlled environmental conditions, and controversies have been reported regarding the reliability of TEWL due to variations in skin hydration at different body sites, on different days and in different dog breeds.¹⁴ Other studies suggest less variation if the TEWL is performed in specific sites, even in different breeds.¹⁵ Unfortunately, in all the reports the number of animals enrolled is limited. A further complication in the assessment of TEWL in CAD is that most clinical cases undergo a variety of systemic and/or topical treatments to control the clinical signs and such treatments may modulate the immunological response and skin hydration and reduce pruritus.^{2,16} No data are currently available on how TEWL is affected by therapy in dogs with CAD.

Therefore, the purposes of this clinical study were twofold. The first aim was to evaluate and compare TEWL, using routine clinic conditions, for healthy and atopic dogs of various breeds and ages. The second aim was to evaluate TEWL in a population of atopic dogs and to assess TEWL in cases whose disease was in remission after successful therapy and compare it with cases that were not controlled and had not received treatment. The two

treatments used in this study were allergen-specific immunotherapy (ASIT) and ciclosporin (CsA).

Materials and methods

This study was designed as a prospective controlled study. All TEWL measures were performed with the owners' consent and under good clinical practice [ethical guidance published in national Gazzetta Ufficiale (G.U.) no. 289; 10 December 1996, 47–53].

Animals and inclusion criteria

Atopic dogs

Nonseasonally atopic dogs were selected according to the following inclusion criteria: flea and tick control with monthly fipronil plus S-methoprene spot on (Frontline Combo® spot on; Merial, Assago (MI), Italy), clinical and cytological absence of concurrent microbial infection (yeast and bacteria), elimination diet trial with commercial food diet (Z/D® Allergen free Hill's prescription diet or Hypoallergenic Royal Canin®) for at least 2 months, absence of otitis or/and concurrent topical treatments for at least 1 month. Diagnosis of CAD was made by exclusion of other pruritic skin diseases, according to Willemse's criteria, and a positive intradermal test. The intradermal test was carried out with intradermal injections of 0.05 mL standard solution of Artu® allergens (Artuvetrin® Skin Test Set; Artu Biologicals, Almere, The Netherlands); positive reactions were >2+ using subjective evaluation on a scale from 0 to 4+, where 0 was the score assigned to the negative control and 4+ was the reaction of histamine injection (1/100,000 w/v) used as positive control. Only dogs positive to house dust and/or storage mites were selected. Animals with intradermal positive reactions for seasonal allergens were excluded.

Normal dogs

Control dogs were dogs attending the clinic for annual vaccination without any history or presence of skin disease. Dogs <1 year of age were excluded because it was deemed possible for additional sensitization to aeroallergens and changes in skin conditions. Dogs older than 10 years were excluded because of possible concurrent diseases related to age (e.g. cardiac or renal failure).

Group allocation

Atopic dogs were assigned to group A, composed of atopic dogs before starting ASIT or ciclosporin administration, or to group B, comprising dogs in remission, with specific ASIT (Artuvetrin® therapy; Artu Biologicals) or ciclosporin [Atoplus caps; Novartis Animal Health SpA, Origgio (VA), Italy] for at least 1 year, to group C, composed of normal dogs (control group). Remission of clinical signs was assessed using the Canine Atopic Dermatitis Extent and Severity

Index,¹⁷ and the patients enrolled in the study were considered under control with a CADESI value <50.

Measurement of TEWL

The study was performed in winter, from December to February, in northern Italy. This decision was made arbitrarily to avoid possible changes in skin hydration related to seasonality, although there are no data published in veterinary medicine concerning this point.

All dogs were acclimated to the measurement conditions for at least 60 min before measurements were performed at the base of the inner, concave face of the left pinna in all the animals. No clipping was performed, to avoid any changes in skin hydration and to determine the values in clinical conditions. Room conditions were stable at 22–23°C ambient temperature and 44–50% ambient relative humidity. Transepidermal water loss was measured with a VapoMeter® SWL-3 (Delfin Technologies Ltd, Kuopio, Finland), a battery-operated, portable, lightweight (150 g) instrument with a standard adaptor and 11 mm aperture, according to the manufacturer's instructions. The VapoMeter® SWL-3 has a sensitive humidity sensor inside a cylindrical measurement chamber (close chamber). This is closed during the measurement period and is unaffected by ambient airflows, decreasing variability in measurements. The evaporation rate value is calculated in grams of water per square metre per hour.

The VapoMeter® was activated by pressing a single button, after which it was placed directly onto the skin, perpendicular to the surface. The device automatically determined the measurement duration (10 s) and signalled completion with an audible tone. It was arbitrarily decided to make only two measurements on the same side and to record the median value manually. Variance greater than ±5% between the two measurements was not accepted, and they were automatically repeated. All the measurements were carried out by a single operator in a climate-controlled room.

Statistics

Calculations were executed with SPSS (version 17; SPSS Inc., Chicago, IL, USA). As the distribution of TEWL was not normal, statistical analysis was carried out with the Kruskal–Wallis test, and a *P*-value <0.05 was considered to be significant (one-way ANOVA). The test was applied to TEWL measurements and the sex and breed of the dogs.

Results

One hundred and fifty dogs were selected and divided into three groups: 50 atopic dogs before ASIT (group A), 50 in remission (group B) and 50 control dogs (group C). Breeds were represented by mixed breeds, West Highland white terriers, German shepherds and Labrador

Table 1. Dogs enrolled in the study; for each group the number and breeds are reported

No.	Group A (dogs with atopic dermatitis before treatment)	No.	Group B (dogs with atopic dermatitis under remission)	No.	Group C (control dogs)
9	Mixed breed	9	Labrador retriever, West Highland white terrier,	12	Mixed breed
7	German shepherd, Labrador retriever, West Highland white terrier	6	Mixed breed	9	Dachshund
3	Dachshund, pit bull terrier	3	Boxer, German shepherd, pit bull terrier	4	Miniature pinscher, Jack Russell terrier
2	English bulldog, Siberian husky	2	American bull terrier, miniature pinscher, Yorkshire terrier	3	Labrador retriever
1	American Staffordshire bull terrier, poodle, beagle, boxer, American bull terrier, French bulldog, fox terrier, Jack Russell terrier, shi tzu, shar-pei	1	American Staffordshire bull terrier, poodle, dachshund, beagle, French bulldog, Chihuahua, Jack Russell terrier, schnauzer, Italian hound, shar-pei, shi-tzu	2	Cavalier King Charles spaniel, schnauzer, English setter, West Highland white terrier, Yorkshire terrier
				1	Akita inu, bulldog, Chihuahua, cocker spaniel, Dogue, Deutsche drahthaar, Italian hound, German shepherd

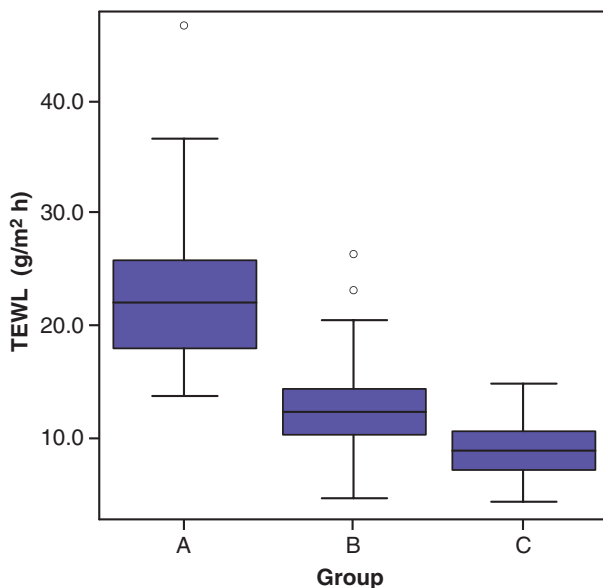


Figure 1. Transepithelial water loss (TEWL) values for group A (atopic dermatitis before specific treatment), group B (atopic dermatitis in remission) and group C (control group). The bottom and top of the purple boxes are the 25th and 75th percentile (the lower and upper quartiles, 1st and 3rd respectively); the bands in the middle of the boxes are the median (the 50th percentile). Circles are the outlier measures. Black whisker cross hatches represent the lowest datum within the lower quartile and the highest datum within the upper quartile.

retrievers, as reported in Table 1. The median age was 5.7 ± 3.7 years in group A, 6.5 ± 3.0 years in group B and 5.6 ± 2.8 years for group C; for sex and breed there was no statistical correlation, and the P -value was >0.05 . The mean TEWL values of the three groups were 22.47 ($\text{g}/\text{m}^2 \text{ h}$) (group A; 95% confidence interval 20.85 – 24.09), 12.57 ($\text{g}/\text{m}^2 \text{ h}$) (group B; 95% confidence interval 11.43 – 13.7) and 8.81 ($\text{g}/\text{m}^2 \text{ h}$) (group C; 95% confidence interval 8.09 – 9.52). The one-way ANOVA P -value was 0.0001 for TEWL (groups A, B and C; see Figure 1).

Discussion

This study showed a significant difference of TEWL between healthy control dogs and dogs with CAD. Additionally, this study showed a lower TEWL in atopic dogs whose disease was in remission due to treatment. These results are consistent with reports in human medicine about TEWL, where this value may be a useful tool for monitoring human patients with allergic skin diseases.¹³ Previous studies have reported TEWL values in dogs, including determination of the best sites to measure TEWL.^{3,9,15} It has been demonstrated that TEWL is greater in dogs with CAD compared with normal dogs.¹¹ No data are available to assess how TEWL might be affected by specific treatment for CAD. Furthermore, no studies have been done to prove the suitability of this measure as an indirect assessment of disease control.

In children, TEWL values are markedly different depending on the season and the location on the body; they are higher in winter than in summer.¹⁸ Recently, it has been reported that measurements are variable during the day and at different body sides in dogs, but there

were no data about seasonal variation.¹⁴ To avoid this possible problem, it was decided to perform the study during one season for 3 months; it will be interesting to perform measures throughout the year to evaluate seasonal variation. Winter was chosen because it was considered unlikely that sensitization to new seasonal allergens would occur and lead to changes in clinical parameters of selected animals.

In dogs, Lau-Gillard *et al.*¹⁴ reported variability of TEWL during the day, and the animals were allowed to acclimate for 15 min. Arbitrarily, in our study it was decided to increase the period of acclimation to 1 h in an attempt to decrease variation. No data are available about the changes of TEWL during a 1 h period, and it was not possible to check every 15 min owing to owner compliance. In future studies, it would be interesting to monitor TEWL every 15 min in standard conditions to increase the data about its possible variability. Many conditions can change TEWL in humans and dogs, such as ambient humidity or temperature. All the measures of this study were performed after leaving the animals for 1 h in a separate waiting room with a controlled climate (air-conditioning system). No other animals or people were allowed to stay in the same room, and no food intake was permitted. These controls were used to minimize changes in ambient conditions and in skin hydration.

In previous canine studies, investigators repeated measurements of TEWL three to 10 times in a row in selected skin regions (e.g. axillae, ear).^{8,11,14} This is the standard method used in human medicine for patients with skin diseases. In veterinary dermatology, there are no comparative studies to assess whether there are statistically significant different results when comparing one, two, five or 10 measurements in a row at the same site. In the present study, TEWL measurements were arbitrarily made twice for each dog at the inner surface of the left ear. The decision to adopt this method was related to the difficulty of repeating many measurements in privately owned dogs owing to owner compliance, the personality of the dogs and the challenge in keeping them sufficiently still during the assessment. The inner surface of the ears is considered the most appropriate site, because the TEWL values from this anatomical region seem to represent the body site with the lowest variability and are less affected by movement.¹⁵ Another reason for choosing the ear was that this is one of the affected areas in dogs with CAD.¹¹

The dogs included in this study were of various ages, sexes and breeds. In humans, there is a difference in skin surface physiology between children of different age groups, reflecting growth stage, but no significant differences between sexes was observed in the age range examined.¹⁸ In dogs, the age can influence skin hydration, and TEWL seems to increase in older dogs;¹¹ it would be interesting to evaluate TEWL in young and old dogs with CAD. In our clinical study, there were no statistically significant differences related to age or sex (data not shown). The control group C enrolled in this study was composed of dogs aged between 1 and 10 years, as for groups A and B; also the median age was similar among groups A, B and C.

In human atopic dermatitis, the TEWL is increased two-fold in nonlesional skin and fourfold in lesional skin, com-

pared with the skin of healthy control subjects.^{13,19} This difference is mostly related to inflammation of the skin, disturbed lipid composition of the stratum corneum, scratching and secondary bacterial infection (increased destruction of epidermal lipids).¹³ In the present study, we obtained similar results in dogs, albeit limited to a single body area. Similar mechanisms seem to influence the skin barrier in animals, but there are few data to assess the influence of these factors in terms of increased or decreased values for TEWL.

Group C was composed of dogs receiving treatment with ASIT or CsA for at least 1 year. The decision to enrol dogs with different treatments was related to the difficulty of selecting dogs with CAD in remission in a short time period. There are no data to evaluate whether the use of ASIT instead of CsA, and vice versa, is a limiting factor. It will be interesting to enrol a greater number of dogs to evaluate such differences.

In dogs, variation in TEWL values is the major obstacle to introduction of TEWL measurement as a method to monitor the improvement or worsening of CAD and its treatments. In human atopic dermatitis, each patient has his/her personal file, where TEWL values are routinely entered during rechecks.¹³ This enables assessment of changes in the patient's skin disease and response to treatment. In atopic dogs, it will be useful to carry out longitudinal studies to determine whether there is an increase in skin hydration with therapy, using each dog as its own control. The main limitation of this preliminary study was that the dogs in groups A and B were not their own control because this was only a 3 month study. Despite this limitation, the differences between groups A and B was statistically significant. In a future longitudinal study, it will be useful to perform TEWL measurements over at least 2 years to monitor the effectiveness of specific CAD treatments. To the best of the authors' knowledge, this is the first report comparing dogs with or without CAD, with and without treatment. The results of this study show a significant difference in TEWL values in normal and atopic dogs. Furthermore, the decrease of TEWL in patients with CAD under remission might suggest the possibility of using this value as a tool to monitor disease severity and evolution.

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Résumé Une barrière cutanée endommagée est supposée cruciale pour la sensibilisation allergique. Chez l'homme, la barrière cutanée est évaluée par des méthodes non-invasives telles que la mesure de la perte hydrique trans-épidermique (TEWL). Bien que des limites aient été rapportées, il a été montré que les mesures de la TEWL sont valables pour évaluer indirectement la fonction de barrière cutanée chez le chien. Cette étude clinique prospective avait un double objectif. Le premier était d'évaluer et de comparer la TEWL chez les chiens sains et atopiques. Le second était d'évaluer la TEWL dans une population de

chiens atopiques et pour les chiens en rémission après un traitement efficace et de comparer avec les résultats des chiens non contrôlés ou en cours de traitement. Cent cinquante chiens ont été inclus et répartis en trois groupes : 50 chiens atopiques avant traitement spécifique (groupe A), 50 chiens en rémission (groupe B), 50 chiens contrôles (groupe C). Les valeurs moyennes de TEWL pour chaque groupe étaient (groupe A, 95% intervalle de confiance 20.85-24.09), 12.57 (groupe B, 95% intervalle de confiance 11.43-13.7), 8.81 (groupe C, 95% intervalle de confiance 8.09-9.52); P-value : 0.0001 pour TEWL (groupes A, B, C). Cette étude a montré une différence significative de TEWL entre les chiens sains et les chiens atopiques. En outre, la TEWL était plus faible chez les chiens atopiques dont la maladie était en rémission grâce au traitement. Ces résultats sont en accord avec les données de médecine humaine sur la TEWL.

Resumen Una función deteriorada de la barrera de la piel puede ser crucial para la sensibilización alérgica. En seres humanos, la función de barrera de la piel es determinada por métodos no invasivos tales como la medida de la pérdida de agua transepidermal (TEWL). Aunque se han indicado limitaciones del método, las medidas de TEWL han demostrado ser un método conveniente para determinar indirectamente la función de la barrera en perros. El propósito de este estudio clínico prospectivo fue doble. El primer propósito fue evaluar y comparar TEWL en perros sanos y atópicos. El segundo fue evaluar TEWL en una población de perros atópicos y determinar TEWL en los perros enfermos en remisión después de un tratamiento efectivo y compararla a los perros que no fueron controlados o que no recibieron tratamiento. Ciento cincuenta perros fueron seleccionados y divididos en 3 grupos: 50 perros atópicos antes del tratamiento específico (grupo A), 50 perros en remisión (grupo B), 50 perros control (grupo C). Los valores medios para TEWL para cada grupo fueron 22,47 (grupo A, intervalo de confianza del 95% 20,85-24,09), 12,57 (grupo B, intervalo de confianza del 95% 11,43-13,7), 8,81 (grupo C, intervalo de confianza del 95% 8,09-9,52); el valor P fue de 0,0001 para TEWL (grupos A, B, C). Este estudio demostró una diferencia significativa del valor de TEWL entre los perros sanos controles y los perros con dermatitis atópica. Además, TEWL fue más bajo en los perros atópicos con enfermedad en remisión debido al tratamiento. Estos resultados fueron comparables con informes en medicina humana acerca de TEWL.

Zusammenfassung Es wird vermutet, dass eine beeinträchtigte Funktion der Hautbarriere ausschlaggebend für eine allergische Sensibilisierung ist. Beim Menschen wird die Hautbarriere durch nicht-invasive Methoden, wie die Messung des transepidermalen Wasserverlustes (TEWL) untersucht. Obwohl von Limitierungen berichtet wird, haben die Messungen des TEWL sich als passende Methode erwiesen, um indirekt die Barrierefunktion bei Hunden zu bestimmen. Die Ziele dieser prospektiven Studie waren zweierlei: Das erste Ziel war es die TEWL in einer Population atopischer Hunde zu erfassen und die TEWL bei Hunden zu untersuchen, deren Erkrankung nach einer erfolgreichen Therapie in Remission war und diese Ergebnisse mit jenen von Hunden zu vergleichen, deren Krankheit nicht kontrolliert war oder die gerade in Behandlung waren. Einhundertfünfzig Hunde wurden ausgewählt und in 3 Gruppen aufgeteilt: 50 atopische Hunde vor einer speziellen Behandlung (Gruppe A), 50 Hunde in Remission (Gruppe B), 50 Kontrollhunde (Gruppe C). Die durchschnittlichen Werte für den TEWL für jede Gruppe lagen bei 22,47 (Gruppe A, 95% Konfidenzintervall 20,85-24,09), 12,57 (Gruppe B, 95% Konfidenzintervall 11,43-13,7), 8,81 (Gruppe C, 95% Konfidenzintervall 8,09-9,52); der P-Wert betrug 0,0001 für den TEWL (Gruppen A, B, C). Diese Studie zeigte einen signifikanten Unterschied beim TEWL zwischen gesunden Kontrolltieren und Hunden mit atopischer Dermatitis. Zusätzlich war der TEWL bei atopischen Hunden, deren Krankheit aufgrund erfolgreicher Behandlung in Remission war, niedriger. Diese Ergebnisse waren im Einklang mit Berichten über TEWL aus der Humanmedizin.

要約 障害された皮膚バリア機能はアレルギー感作にとって極めて重要と考えられている。ヒトでは、皮膚バリア機能は経表皮水分蒸散量 (TEWL) という非侵襲方法で評価されている。TEWL の測定はある程度の制約つきではあるが、犬で間接的にバリア機能の評価する方法として適していることが証明されている。今回の前向き臨床研究の目的は2つある。最初の目的は健康犬とアトピー犬で TEWL を測定して比較することである。2 つ目の目的は、アトピー性皮膚炎の治療に成功し症状が安定している犬と、管理ができていないあるいは治療を受けていない犬の TEWL を比較することである。150 頭の犬を選定し、3つのグループに分類した: 特定の治療をする前のアトピー犬 50 頭 (グループ A)、50 頭の治療によって症状が安定している犬 (グループ B)、50 頭の対照犬 (グループ C) である。それぞれのグループでの TEWL の平均値は 22.47 (グループ A、95%信頼区間 20.85-24.09)、12.57 (グループ B、95% 信頼区間 11.43-13.7)、8.81 (グループ C、95%信頼区間 8.09-9.52) ; TEWL (グループ A、B、C 間の) の P-value は 0.001 であった。この研究では健康な対照犬とアトピー性皮膚炎の犬の間の TEWL に有意な差を示した。さらに TEWL は治療の結果、症状が安定している状態のアトピー犬でより低かった。これらの結果はヒトの医学の TEWL の報告と同様である。