

Intrinsically defective skin barrier function in children with atopic dermatitis correlates with disease severity

Jayanta Gupta, MD,^{a,d} Emilie Grube, BA,^b Mark B. Ericksen, BS,^{b,d} Michelle D. Stevenson, MD, MS,^e Anne W. Lucky, MD,^c Anita P. Sheth, MD,^c Amal H. Assa'ad, MD,^b and Gurjit K. Khurana Hershey, MD, PhD^{b,d} Cincinnati, Ohio

Background: Recent genetic evidence supports that an underlying defect in skin barrier function contributes to the pathogenesis of atopic dermatitis (AD). The integrity of the skin barrier can be assessed objectively by measuring transepidermal water loss (TEWL). Previous investigations of TEWL as a biomarker of skin barrier function have been limited by small sample size, and studies including African American subjects are lacking.

Objective: We sought to determine whether children with AD have inherently altered skin barrier function by comparing TEWL as a measure of skin barrier function in African American and white children with AD with that in control subjects without AD.

Methods: TEWL was measured on nonlesional normal-appearing skin at 4 sites (the volar forearm, dorsal arm, lower leg, and cheek) in (1) children with AD (cases), (2) children with asthma or allergic rhinitis but without AD (allergic control subjects), and (3) nonatopic control subjects. AD severity was assessed by using the objective SCORAD index.

Results: TEWL was increased in children with AD compared with that seen in both control groups at most of the anatomic sites tested ($P < .05$). TEWL also correlated with objective SCORAD score. The presence of allergic sensitization or other allergic conditions did not affect TEWL among children with AD. TEWL was higher in white than in African American children.

Conclusion: Skin barrier function as assessed by TEWL is intrinsically compromised in children with AD but not in children with other allergic conditions. The magnitude of skin barrier dysfunction correlates with AD disease severity. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Atopic dermatitis, transepidermal water loss, objective SCORAD, African American, white

Atopic dermatitis (AD) is a chronic relapsing skin disease characterized by dry skin, pruritus, and erythematous papules and plaques that have an identifiable morphology and distribution, depending on the age of the patient.^{1,2} Its prevalence has increased recently, with up to 21% of children in industrialized nations now having the condition.³ It constitutes the first step of the "atopic march," the sequence in which atopic diseases manifest themselves preferentially at different ages in an individual's life. Generally, AD is the earliest manifestation, followed by allergic rhinitis and then asthma.⁴ It is a multifactorial disease, with multiple genes and environmental factors interacting to give rise to the typical clinical picture of AD. A complex cycle of pruritus and inflammation is orchestrated through many mediators and cell types.⁵

Recent genetic evidence provides strong support that the pathogenesis of AD is at least in part due to the presence of a defective skin barrier.⁶⁻¹¹ The skin barrier, which prevents entry of external irritants and allergens, is located in the stratum corneum of the epidermis. Transepidermal water loss (TEWL) is a biophysical method to evaluate the integrity of this barrier. Some studies have demonstrated an increase in TEWL even on clinically normal-appearing skin of patients with AD,¹²⁻¹⁸ whereas others have found no difference in TEWL between patients with AD and healthy individuals.¹⁹⁻²² Many of these studies were limited by small sample size, and the criteria used for choosing control groups for comparison varied widely between studies. Previous studies predominantly included white or Asian subjects, and data on African American children is lacking, despite the high prevalence of AD in this group.²³ Except for a single report from an Asian population,²⁴ attempts to investigate TEWL in AD subgroups based on allergic sensitization have also not been made.

In the present study we evaluated skin barrier function of children with AD, as well as the potential utility of TEWL as a biologic marker for AD in a pediatric population including white and African American subjects. By using TEWL, AD cases were compared with 2 well-characterized control groups, and the relationship between TEWL and AD severity was examined. We examined the effect of race on TEWL and compared TEWL between AD cases subdivided on the basis of sensitization to aeroallergens and the presence/absence of other allergic diseases.

METHODS

Subjects

Children were sequentially recruited from the outpatient clinics of Cincinnati Children's Hospital Medical Center between July 2004 and

From ^athe Division of Epidemiology and Biostatistics, Department of Environmental Health, University of Cincinnati College of Medicine; and ^bthe Division of Allergy and Immunology, ^cthe Division of Pediatric Dermatology, ^dthe Institute of Personalized and Predictive Medicine, and ^ethe Division of Emergency Medicine, Cincinnati Children's Hospital Medical Center, and the Department of Pediatrics, University of Cincinnati College of Medicine.

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Reprint requests: Gurjit K. Khurana Hershey, MD, PhD, Institute of Personalized and Predictive Medicine and Division of Allergy and Immunology, Department of Pediatrics, Cincinnati Children's Hospital Medical Center, 3333 Burnet Ave, MLC 7028, Cincinnati, OH 45229. E-mail: Gurjit.Hershey@cchmc.org.

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Abbreviations used

AD: Atopic dermatitis
TEWL: Transepidermal water loss

September 2006. Children with AD, recruited from allergy and dermatology clinics, fulfilled the diagnostic criteria of Hanifin and Rajka.¹ AD severity was assessed by using the SCORAD index²⁵ by the same trained investigator in all cases. Two groups of control subjects were used for this study. First, children with asthma, allergic rhinitis, or both but without present or past AD were recruited from the allergy clinics and served as allergic control subjects. Asthma was diagnosed according to the guidelines of the American Thoracic Society.²⁶ Children with allergic rhinitis had symptoms of the condition and were sensitized to at least 1 aeroallergen based on allergy testing. Second, children without any personal or family history of AD or other allergic disorders were recruited from outpatient dental, dermatology, and orthopedic clinics and served as healthy control subjects. Their nonatopic status was confirmed by negative allergy skin prick test responses to a panel of aeroallergens.

Informed consent was obtained from all participants, their parents, or both. The study was approved by the Institutional Review Boards of the Cincinnati Children's Hospital Medical Center and the University of Cincinnati.

Allergy testing

Allergic sensitization was determined in all subjects by means of skin prick tests or RASTs (Quest Diagnostics, San Juan Capistrano, Calif). The allergens tested included mold mixes, ragweed pollen, grass pollen mix, tree pollen mixes, mite mix, cockroach, cat, and dog, with histamine as positive and saline as negative controls. A skin prick test response was considered positive when the wheal diameter exceeded that of the negative saline control by 3 mm or more after 15 minutes. The RAST result was positive when the allergen-specific antibody level was greater than 0.35 kU/L. For 3 children who had allergy tests done outside the facility, the test results were standardized according to the above-mentioned criteria. Four children with AD had testing only to a subset of the above allergens based on their clinical history.

Questionnaires

Detailed questionnaires to collect medical and demographic information were completed by the parent/guardian accompanying the child during the clinic visit.^{3,27} In addition, patients' medical records were checked for additional and missing information (eg, previous skin prick test/RAST results or missing data in questionnaire) and to confirm questionnaire responses.

Measurement of TEWL

TEWL was measured by using the Dermalab instrument (cyberDERM; Cortex Technology, Media, Pa). Measurements were made according to the published guidelines of Pinnagoda et al.²⁸ The temperature and relative humidity of the rooms in which measurements were made varied between approximately 20°C and 27°C and 10% and 60%, respectively. Median values of temperature and relative humidity did not differ significantly either between cases and control subjects or between races (data not shown). Subjects had not used any topical ointment, cream, lotion, or moisturizer for at least 12 hours before the measurement by their report. TEWL measurements were taken after an acclimatization period of at least 15 minutes. Measurements were recorded as grams per meter squared per hour after the rate of TEWL had stabilized, usually after 60 seconds, and when the SD became 0.2 or less. Four body sites were assessed for TEWL: the cheek, the volar surface of the forearm, the dorsal surface of the arm, and the medial surface of the lower leg. In patients with AD, TEWL was measured only on normal-appearing skin at least 1 inch away from any lesional skin. Normal-appearing skin was defined as skin with no erythema, edema/papulation, oozing/crusting, excoriation, lichenification, or palpable dry patches. The same investigator measured TEWL on all study subjects.

Statistical analysis

Because normality of data could not be assumed for all study groups and subgroups, the nonparametric Wilcoxon rank sum test for comparing 2 variables and the Kruskal-Wallis test for comparing more than 2 variables were used to analyze difference between groups. The Bonferroni correction for multiple comparisons was used wherever appropriate. χ^2 Tests were used to compare categorical variables. The Spearman rank correlation test was used to examine the correlation between variables. The statistical analysis system (SAS Version 9.1; SAS, Institute Inc, Cary, NC) was used for all analyses. A *P* value of less than .05 was defined as statistically significant.

RESULTS**Subjects**

Although subjects from all races were approached for the study, only the white and African American races had sufficient numbers with valid TEWL and allergy test data for statistical analysis. Fifteen children with race identity that was not white or African American were thus excluded. Because all children in the control groups were 3 years of age or older, 19 children with AD who were younger than 3 years were also not included in the final analysis. Thus statistical analyses were performed on a final sample size of 250 children. Age differed significantly among cases and control subjects in the white group, whereas the African American cases and control subjects were significantly different with respect to sex (Table I). However, subject age was not correlated to TEWL in any of the study groups. Similarly, no significant difference in TEWL between sexes was detected (data not shown). White children had higher TEWL than African American children at a majority of the body sites tested, and the difference reached statistical significance at the volar forearm consistently (Fig 1).

TEWL in normal-appearing skin is altered in children with AD

TEWL in AD cases was significantly higher compared with that seen in both control groups in a majority of the sites, but there was no significant difference in TEWL between the 2 control groups (Table II). White children with AD had increased TEWL at each site tested compared with one or both control groups (Fig 2, A). Similarly, TEWL was increased in African American children with AD when compared with the African American control groups at 3 of the 4 sites (Fig 2, B).

Effect of presence of allergic sensitization and other allergic conditions on TEWL in children with AD

Children with AD were subclassified according to allergy testing on the basis of skin prick test or RAST results. On comparison, no significant difference was observed in TEWL between the 2 subclasses (Table III and see Fig E1 in the Online Repository at www.jacionline.org). There was also no significant difference in TEWL between AD cases without asthma/allergic rhinitis and AD cases with asthma/allergic rhinitis (Table IV and see Fig E2 in the Online Repository at www.jacionline.org).

TEWL correlates with AD disease severity

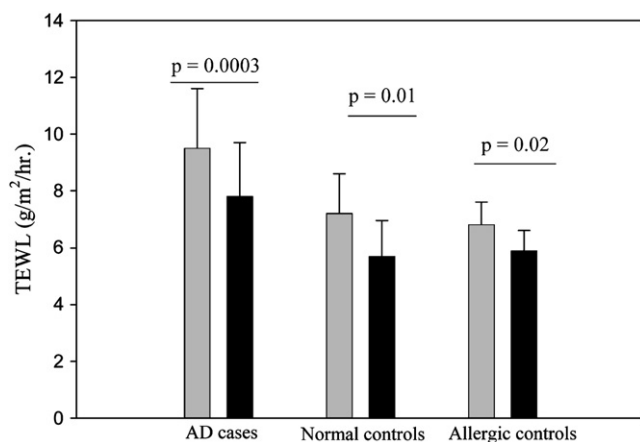
TEWL measured on the volar forearm positively correlated with AD severity in both white and African American children (Fig 3; Spearman coefficient of 0.41 and *P* value of .001 in white subjects; Spearman coefficient of 0.38 and *P* value of .003 in African American subjects).

TABLE I. Characteristics of study subjects (n = 250)

	White subjects			P value	African American subjects			P value
	AD cases	Healthy control subjects	Allergic control subjects		AD cases	Healthy control subjects	Allergic control subjects	
Age (y)								
n	60	18	60	<.0001*	63	14	35	NS*
Median	5.2	9.3	10.5		7.2	11.5	10.0	
Interquartile range	2.7	7.7	5.7		6.5	9.1	6.3	
Sex				NS†				.03†
Male (%)	40 (66.7)	11 (61.1)	32 (53.3)		29 (46.0)	3 (21.4)	22 (62.9)	
Female (%)	20 (33.3)	7 (38.9)	28 (46.7)		34 (54.0)	11 (78.6)	13 (37.1)	

NS, Nonsignificant.

*P value obtained by using the Kruskal-Wallis procedure.

†P value obtained by using the χ^2 test/Fisher exact test.**FIG 1.** Comparison of TEWL measured at the volar forearm between white and African American children. Gray bars, Median TEWL in white children; black bars, median TEWL in African American children. Error bars represent semi-interquartile range. All P values were obtained by using the Wilcoxon rank sum test.

DISCUSSION

Impaired function of the epidermal barrier is characteristic of AD. This was commonly thought to be a consequence of the inflammatory phenotype, the “inside-out” hypothesis, whereby inflammation in the epidermis and dermis was postulated to result in defective skin barrier function.^{29,30} IL-4, a key cytokine involved in the allergic response, has actually been found to suppress barrier homeostasis, leading to a decrease in the ceramide component (essential for maintaining barrier integrity) of the stratum corneum in the lesional skin of patients with AD.²⁹ Recently, this hypothesis has been challenged by considerable evidence supporting a primary defect in the skin barrier. Genetic associations have been found between AD and genes encoding proteins critical in skin barrier function, including serine protease inhibitor Kazal-type 5,⁶ stratum corneum chymotryptic enzyme,⁷ and filaggrin.⁸⁻¹¹ This has lent support to the alternative “outside-in” hypothesis that an intrinsic defect in skin barrier function is responsible for the pathogenesis of AD.³¹ This study of white and African American children found that children with AD have abnormal skin barrier function in normal-appearing nonlesional skin, as assessed by TEWL. Moreover, TEWL correlates with AD disease severity. Thus it supports this alternative outside-in hypothesis that an intrinsic defect in barrier function is responsible for the pathogenesis of AD.

TABLE II. Comparison of TEWL values (g/m²/h) between case and control groups

Body site	White subjects			African American subjects		
	AD cases	Healthy control subjects	Allergic control subjects	AD cases	Healthy control subjects	Allergic control subjects
Volar forearm						
n	59	18	60	60	14	35
Median	9.5	7.2†	6.8†	7.8	5.7†	5.9†
Interquartile range	4.2	2.8	1.6	3.8	2.5	1.4
Dorsal arm						
n	58	18	60	57	14	35
Median	8.5	7.3	5.6†	7.4	5.6*	4.9†
Interquartile range	5.1	3.7	2.7	3.3	2.8	3.3
Lower leg						
n	60	18	60	62	14	34
Median	8.5	5.1†	4.8†	6.4	5.4	4.8
Interquartile range	3.8	2.4	3.3	3.8	3.3	2.7
Cheek						
n	53	17	60	59	14	35
Median	12.7	9.1	8.3†	10.5	8.0	8.0*
Interquartile range	6.2	6.4	5.8	6.1	6.9	2.8

All P values were obtained by using the Wilcoxon rank sum test with the Bonferroni correction for multiple comparisons.

*P < .05 when compared with AD cases.

†P < .0004 when compared with AD cases.

The inside-out hypothesis would predict abnormal barrier function in only affected AD skin. We found that normal-appearing skin of children with AD exhibited defective barrier function, and this observation was specific to children with AD. This intrinsic defect in barrier function might be a primary factor in the inflammation observed in AD rather than the inflammation leading to the barrier defect (outside-in mechanism). In fact, it has been shown that barrier disruption in the skin results in the production of T_H2 cytokines, including IL-4 and IL-5,^{32,33} and the initiation of an inflammatory cascade resulting in a milieu conducive to the pathogenesis of AD.³² In the context of underlying barrier dysfunction in AD, minimal exogenous skin trauma might be sufficient to activate epidermal cytokines and activate disease in clinically normal skin. However, the presence of a barrier defect does not necessarily lead to a T_H2-dependent cytokine cascade,

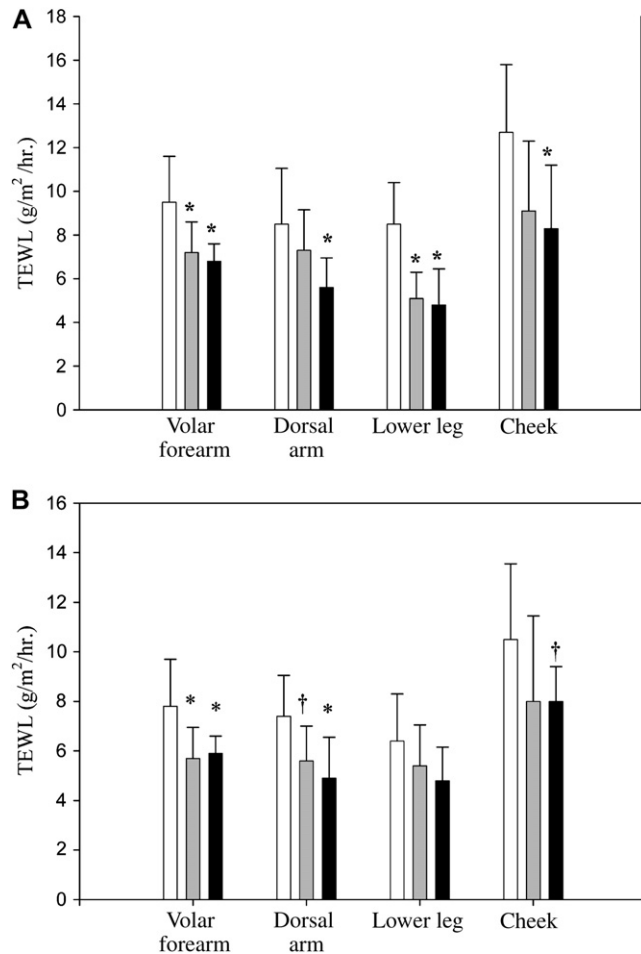


FIG 2. Comparison of TEWL between white (A) or African American (B) children with AD and racially matched control groups. *White bars*, Median TEWL in AD cases; *gray bars*, median TEWL in healthy control subjects; *black bars*, median TEWL in allergic control subjects. * $P < .0004$ compared with AD cases. † $P < .05$ compared with AD cases. Error bars represent semi-interquartile range. All P values were obtained by using the Wilcoxon rank-sum test with the Bonferroni correction for multiple comparisons.

such as in psoriasis.³⁴ Thus other modifying factors, both environmental and genetic, are important in determining the AD phenotype.

In this study TEWL in normal-appearing skin correlated with AD disease severity defined by objective SCORAD score in both white and African American subjects, corroborating similar findings by Chamlin et al.³⁵ This suggests that the intrinsic skin barrier dysfunction not only contributes to disease pathogenesis but also directly affects disease severity. It is interesting to speculate that the severity of the skin barrier dysfunction might correlate with a reduction in the threshold for the induction of cytokines and inflammation in response to minimal skin trauma. Regardless of the underlying mechanism for this association, it raises the possibility of the potential clinical utility of TEWL in the management of AD. Interventions aimed at enhancing the skin barrier function of clinically normal unaffected skin in children with increased TEWL might prevent or attenuate AD disease progression. The defective skin barrier observed in AD might contribute to allergic sensitization caused by allergen exposure through the skin and potentially explain the atopic march. It is

TABLE III. TEWL values ($\text{g}/\text{m}^2/\text{h}$) in AD cases with positive and negative allergy test results

Body site	White AD cases		African American AD cases	
	Positive allergy test result	Negative allergy test result	Positive allergy test result	Negative allergy test result
Volar forearm				
n	51	8	53	7
Median	9.5	9.8	7.5	8.2
Interquartile range	4.5	2.8	3.7	6.0
Dorsal arm				
n	50	8	50	7
Median	8.5	7.3	7.4	7.0
Interquartile range	5.0	4.5	3.3	7.2
Lower leg				
n	51	9	55	7
Median	8.5	8.5	6.3	7.7
Interquartile range	4.1	2.8	3.4	7.1
Cheek				
n	46	7	52	7
Median	13.6	10.5	10.5	11.0
Interquartile range	7.0	4.5	5.5	7.5

All comparisons of TEWL between AD cases with positive and those with negative allergy test results were nonsignificant.

TABLE IV. TEWL values ($\text{g}/\text{m}^2/\text{h}$) in AD cases without asthma/allergic rhinitis and AD cases with asthma/allergic rhinitis

Body site	White AD cases		African American AD cases	
	Without asthma/allergic rhinitis	With asthma/allergic rhinitis	Without asthma/allergic rhinitis	With asthma/allergic rhinitis
Volar forearm				
n	18	41	16	44
Median	9.0	9.8	8.2	7.6
Interquartile range	3.7	4.2	5.0	3.5
Dorsal arm				
n	18	40	17	40
Median	7.7	9.1	7.2	7.5
Interquartile range	5.0	5.2	3.6	3.6
Lower leg				
n	18	42	17	45
Median	8.2	8.6	6.9	5.9
Interquartile range	4.8	3.7	4.4	3.3
Cheek				
n	16	37	17	42
Median	12.9	12.7	12.6	9.5
Interquartile range	7.5	6.3	4.5	5.6

All comparisons of TEWL between AD cases without asthma/allergic rhinitis and AD cases with asthma/allergic rhinitis were nonsignificant.

intriguing to speculate that interventions aimed at enhancing the skin barrier function of clinically normal unaffected skin in children with increased TEWL might prevent the atopic march.

We did not observe any difference in TEWL among white or African American allergen-sensitized children with AD when compared with nonsensitized children with AD. Thus a primary

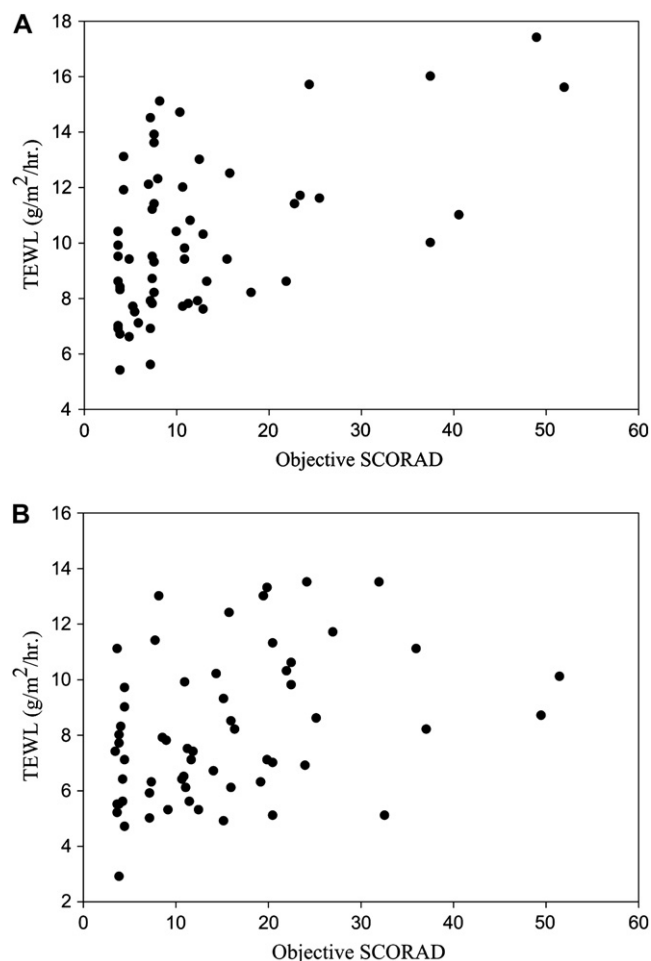


FIG 3. Scatter plot demonstrating correlation between TEWL and objective SCORAD score in white (A) or African American (B) children with AD (Spearman coefficient of 0.41 and *P* value of .001 in white subjects; Spearman coefficient of 0.38 and *P* value of .003 in African American subjects).

defect in skin barrier function, presumably genetically driven, might be sufficient to give rise to the clinical manifestations of AD, even in the absence of external allergen sensitization. Indeed, up to two thirds of children with AD might not be sensitized to allergens.³⁶ However, the number of children without allergen sensitization in our study was small, and the study was underpowered to address the role of the skin barrier in allergic sensitization. Prospective studies in infants are needed to relate the role of the skin barrier, assessed by TEWL, in the atopic march. We did not observe any difference in TEWL between children with allergic rhinitis or asthma (without AD) and nonatopic control subjects. These data corroborate the findings of Conti et al¹⁶ and Löffler and Effendy,¹⁷ who also did not find evidence of skin barrier disruption in respiratory atopic subjects in 2 separate European populations. Our results are based on findings from 2 different racial groups and support that the skin barrier defect was unique to AD in both white and African American subjects and not to atopic individuals in general.

Most studies investigating the racial difference in skin barrier function have found baseline TEWL to be higher in dark skin, whereas a few have reported similar or higher baseline TEWL in white skin.^{37,38} However, previous investigators focused only on

healthy individuals and were limited by small sample sizes. In our population TEWL was higher in white children when compared with values in African American children. The observed difference in TEWL between races was evident not only for the nonatopic healthy population but also for the AD cases and allergic control subjects. Interestingly, white children had higher TEWL values despite the fact that African American children have more severe AD. Thus absolute TEWL measurements in a given individual need to be compared with the normal values for individuals of the same race to determine whether the TEWL is increased. This might be determined based on pigmentation rather than race. Black skin has higher melanin content than white skin, which might offer some protection against evaporative water loss,³⁹ and black skin also possesses a higher number of corneocyte cell layers and is richer in lipid content,⁴⁰ all of which might have contributed to the lower TEWL observed in this study. Studies across racial and pigmentation groups will be needed to better understand this and set normal standards.

In summary, our study provides evidence that the skin barrier function in children with AD is intrinsically defective and that the level of dysfunction, quantified by TEWL, correlates with AD disease severity. TEWL might be a clinically useful biomarker of AD.

Clinical implications: TEWL might be a useful biomarker for AD.

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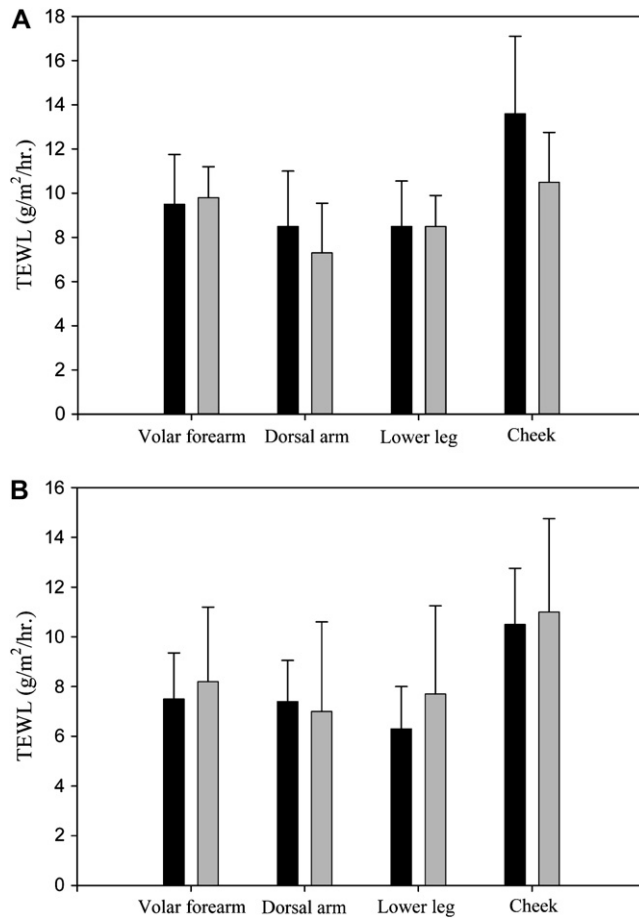


FIG E1. Comparison of TEWL between children with AD with positive and children with AD with negative allergy test results (**A**, white children; **B**, African American children). *Black bars*, Median TEWL in AD cases with positive allergy test results; *gray bars*, median TEWL in AD cases with negative allergy test results. *Error bars* represent semi-interquartile range. None of the *P* values (obtained by using the Wilcoxon rank sum test) were statistically significant.

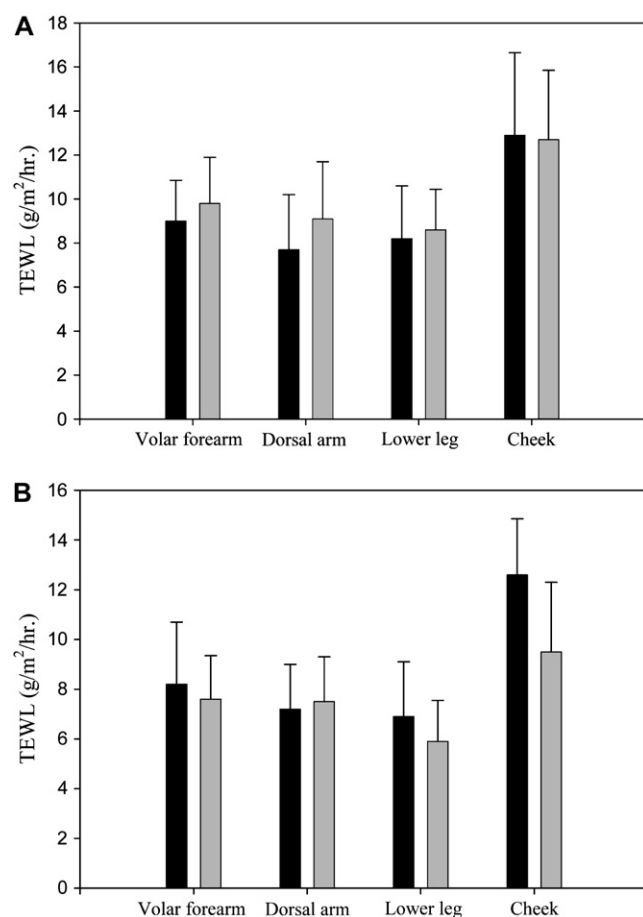


FIG E2. Comparison of TEWL between AD cases without asthma/allergic rhinitis and AD cases with asthma/allergic rhinitis (**A**, white subjects; **B**, African American subjects). *Black bars*, Median TEWL in AD cases without asthma/allergic rhinitis; *gray bars*, median TEWL in AD cases with asthma/allergic rhinitis. *Error bars* represent semi-interquartile range. None of the *P* values (obtained by using the Wilcoxon rank sum test) were statistically significant.