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Original Article

Exposure to cold airflow alters skin pH and epidermal filaggrin degradation products in children with atopic dermatitis

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AD, Atopic dermatitis; FDP, Filaggrin degradation protein; TEWL, Transepidermal water loss; SC, Stratum Corneum; SCORAD, Severity Scoring of Atopic Dermatitis; PCA, Pyrrolidone carboxylic acid; UCA, Urocanic acid

ABSTRACT

Background: We aimed to evaluate the influence of cold airflow from the air conditioner on skin barrier function and filaggrin degradation products (FDPs) in children with atopic deramtitis (AD).
Methods: In a case-control study, 28 children with AD and 12 normal children without AD were exposed to one of two air conditioner modes (conventional or wind-free) for 2 h. Skin temperature, transepidermal water loss (TEWL), and skin pH were measured on right cheek and forearm at pre- and post-exposure time points. We also measured filaggrin and FDPs from the volar surface of the forearm.
Results: In AD patients, skin temperature on the forearm decreased after exposure to the conventional and wind-free modes ($P < 0.001$ and $P = 0.026$), and TEWL on the cheek and the forearm decreased in the wind-free mode ($P = 0.037$ and 0.002). Skin pH on the cheek increased only after exposure to the conventional mode in AD group ($P = 0.002$). However, no changes in TEWL and skin pH were found after exposure to either the conventional or the wind-free mode in the control group. In AD children, the levels of pyrrolidone carboxylic acid (PCA) and *cis*-urocanic acid (UCA) were reduced only after exposure to the conventional mode (all $P = 0.033$). The percent changes of PCA and *cis*-UCA were higher in the AD group than those in the control group after exposure to conventional mode ($P = 0.029$ and 0.046).
Conclusions: Skin barrier function in children with AD may be altered by the exposure to cold airflow from a conventional air conditioner.

Introduction

Atopic dermatitis (AD) is a chronically relapsing inflammatory skin disease that affects about 10–20% of children.[1] Although the exact pathophysiology of AD is not completely understood, environmental and genetic factors contribute to the development and exacerbation of AD.[2] For the proper management of AD, it is important to avoid indoor environmental triggers such as temperature, humidity, allergens or pollutants, because children spend most of the day in indoor environments.[3,4]

Air conditioners are widely used in patients with AD in the summer. However, even healthy adults could feel dry and uncomfortable under a conventional type of air conditioner.[5] In a study with healthy female adults, a decrease in skin hydration and an increase in dryness score were found after the exposure to cold and dry wind.[6] A previous study showed that the stratum corneum (SC) hydration of human skin decreased together with temperature with stronger relationship in low relative humidity.[7] Another study reported a positive correlation between transepidermal water loss (TEWL) and skin temperature and between skin hydration and relative humidity.[8] When human skin is exposed to dry environment, it could be more susceptible to mechanical stress.[9,10] Factory workers and aircrew personnel who work in ultra-dry working

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conditions showed more frequent skin symptoms and higher prevalence of AD than those of controls.[11,12] Although it is believed that home appliances that produce cold airflow have negative effects on allergic diseases, there is no study regarding the effects of air conditioners on skin barrier function in children with AD.

Recently, a new air conditioner that disperses cold air through numerous micro air holes using wind-free cooling technology has been invented. Therefore, we aimed to evaluate whether the exposure to cold wind affects skin barrier functions in patients with AD and normal control subjects by comparing conventional and wind-free modes of air conditioners.

Methods

Study population

We enrolled 28 Korean children with AD and 12 Korean normal control subjects matched for age and sex. AD was diagnosed by a pediatric allergist, based on diagnostic criteria of Hanifin & Rajka.[13] Patients were excluded if they used systemic corticosteroid or immunosuppressant within 4 weeks, or had acute exacerbation of allergic diseases or skin diseases other than AD. The control group was determined when subjects had no personal history of AD and skin diseases. Written informed consents were obtained from the patients and their parents, and the study was approved by the institutional review board of Samsung Medical Center (SMC IRB file No. 2016-06-088 and 2019-08-141). The study protocol was registered in the WHO International Clinical Trials Registry Platform (ICTRP) with the registration number KCT0003982.

Study design

A total of 28 AD cases and 12 normal controls were randomly allocated and exposed to one of two air conditioner modes (conventional or wind-free) for 2 h (Fig. 1A). For the exposure to each air conditioner mode, all subjects stayed in an experimental room which was maintained at a constant temperature of 24 °C and humidity of 40%. An air conditioner A3050 (Samsung Electronics, Suwon, Korea) that can be switched to conventional or wind-free mode was installed in the experimental room. The study participants were not allowed to wear long sleeves and were sitting on chairs one meter away from the air conditioner. The local air velocity around the participant's head was measured using by Velocicalc 9545/9545-A (TSI, Minnesota, USA).

Clinical evaluation

At enrollment, demographic data including age, sex, height, body weight, and the presence of other allergic diseases were collected. The severity of AD was determined by scoring of atopic dermatitis (SCORAD) index.[14] A skin prick test was performed with common allergens including house dust mite (*Dermatophagoides pteronyssinus, D. farinae*), grass pollen (timothy, bermuda, meadow grass), tree pollen (alder, birch, elder, oak, Japanese cedar), weed pollen (mugwort, ragweed, short ragweed, hops Japanese), animal dander (cat, dog), mold (*Alternaria alternata*, *Aspergillus fumigatus, Cladosporium*, *Penicillium*) and food (egg white, cow's milk, peanut). Histamine and normal saline were used as a positive and negative control, respectively. All extracts were manufactured by Allergopharma (Merck KGaA, Darmstadt, Germany). A positive skin prick test response was recorded when the mean wheal diameter was ≥3 mm larger than the negative control.

Thermal imaging was obtained to estimate the skin temperature by infrared camera T650 (FLIR systems AB, Sweden). Pictures of the participants were taken one meter away from the camera. The skin temperature at right cheek was calculated in the rectangular area between the lower borders of both eyes and the lowest points of both ears. The skin temperature at right forearm was calculated in the rectangular area between the antecubital fossa and the middle point of the forearm. We measured TEWL and skin pH three times on areas of the non-lesional skin on right cheek and right forearm. From those values, we calculated the average level of TEWL and skin pH. TEWL and skin pH were measured by TM300 (Courage & Khazaka Electronic, Cologne, Germany) and Skin-pH-Meter PH 90 (Courage & Khazaka Electronic, Cologne, Germany), respectively. Samples of the SC were obtained by using the tape-stripping method with D-squame standard tape (D-Squame, 22 mm in diameter, CuDerm, Dallas, TX). We collected SC specimens from non-lesional skins of volar forearms before and after exposure to both air conditioner modes (Fig. 1B) and stored these samples at 80 °C until further analyses. After the exposure to the wind-free or the conventional mode, subjective clinical symptom regarding skin dryness was assessed by a questionnaire: "How did you feel about your skin after you were exposed to the air conditioner? (very dry, dry, normal, moist, and very moist)

Measurement of filaggrin degradation products in the SC

For protein quantification, five consecutive tape-stripped specimens were placed in glass vials containing 5 mL of 0.1% (w/v) sodium dodecyl sulphate/2% (w/v) propylene glycol in

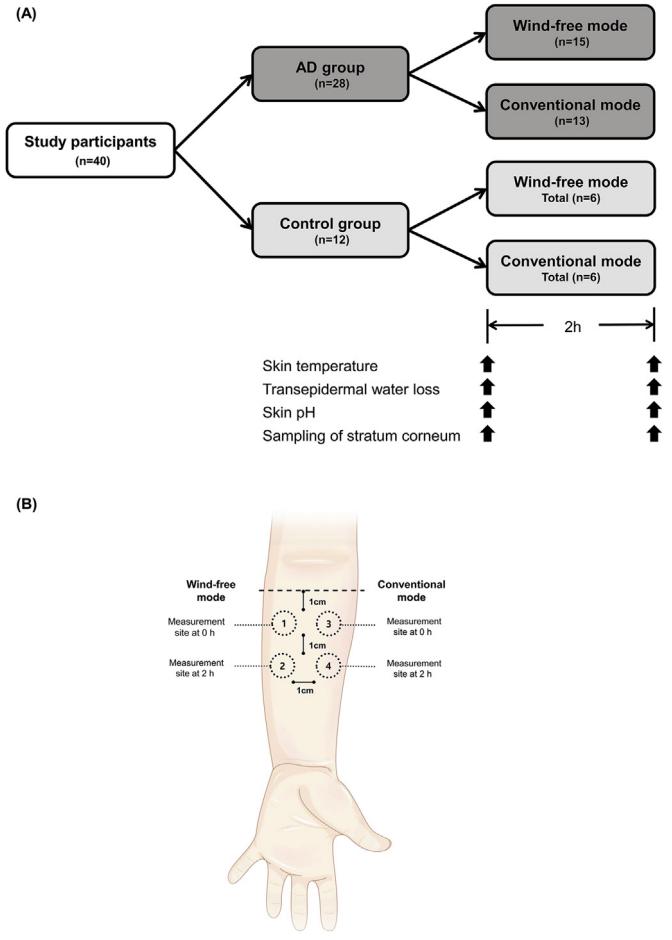

Fig. 1. Study protocol **(A)** and collection of the stratum corneum using tape-stripping method **(B)**.

phosphate buffer solutions and were sonicated for 1 h to obtain soluble proteins. The solutions were centrifuged at 12,000 rpm for 10 min at 4 °C. After the supernatant was separated, aliquots of solutions were used to quantify the amount of protein. The concentrations of soluble protein were analyzed with protein assay kit (Pierce, Rockford, IL). The sample plates were incubated for 30 min at 37 °C after which the absorbance was measured with the Microplate Reader (Spectramax 190, MDA) at 595 nm. Filaggrin degradation proteins (FDPs), including pyrrolidone carboxylic acid (PCA), *cis*-urocanic acid (UCA) and *trans*-UCA were quantitated using hydrophilic interaction liquid chromatography coupled with tandem mass spectrometry as previously.[15]

Immuno-dot-blot for filaggrin and caspase-14

Immuno-dot blot was performed with a Bio-Dot Microfiltration Apparatus from Bio-Rad (Hercules, USA). Protein from 14 tape strips was extracted using a buffer (1% SDS in 50 mM ammonium bicarbonate 940 uL/HALT Protease inhibitor 10 uL/200 mM DTT 50 uL). Thirty micrograms of total protein were loaded in a final volume of 50 μL TRIS-buffered saline with 0.5% Tween 20 (TBST) and allowed to bind to the nitrocellulose membrane (Bio-Rad Laboratories) by gravity filtration. The membrane was then blocked in 5% milk in TBST for 30 min at room temperature. After blocking, the membrane was incubated overnight at 4 °C with a mouse monoclonal antibody against filaggrin (Abcam, Cambridge, USA) or caspase-14 (Abcam) at 4 μg/mL in TBST. The blot was washed 3 times with TBST for 5 min and then was incubated with anti-mouse or anti-rabbit horseradish peroxidase conjugated secondary antibody (Amersham Biosciences, Buckinghamshire, UK) at a dilution ratio of 1:3000 in TBST for 1 h at room temperature. Blots were washed with TBST and then developed with ECL Western Blotting Detection Reagents (Amersham Biosciences) according to the manufacturer's protocol. The intensity of filaggrin and caspase-14 proteins was determined by digital imaged analysis using the National Institutes of Health image 1.61 software programs. Specificity of the antibodies were determined by replacing the primary antibodies with a mouse IgG1 (Southern Biotechnology, Birmingham, USA) or a rabbit IgG isotype control (Abcam). Standard curves were generated for filaggrin and caspase-14 proteins by using recombinant human filaggrin (Abcam) and caspase-14 proteins (Abcam).

Statistical analysis

Statistical analysis was performed using SPSS 24.0 software (SPSS Inc., Chicago, IL, USA). Differences in gender and allergic sensitization between the two groups were analyzed using Fisher's exact test. Age, body mass index, and SCORAD were compared between the two groups using the Mann-Whitney *U* test. The comparison of subjective skin symptoms between the wind-free mode and the conventional mode was made using Fisher's exact test. We compared the skin temperature, TEWL, skin pH, FDPs, filaggrin, and caspase-14 between pre- and post-exposure to the conventional mode and wind-free mode in AD and control groups using Wilcoxon signed rank test. The percent change was defined as the differences of the values between pre- and post-exposure divided by pre-exposure values. The differences between the percent changes of TEWL, skin pH, FDPs, filaggrin, and caspase-14 were compared between AD and control groups at wind-free mode and conventional mode using Mann-Whitney *U* test. *P* value < 0.05 was considered significant.

Results

Characteristics of subjects and experimental conditions

Median (interquartile range, IQR) ages of AD patients and control subjects were 9.2 (7.9–10.1) years and 8.5 (8.0–11.3) years, respectively. There were no significant differences in age, gender and body mass index between AD and control groups (Table 1). No significant differences in median temperature and humidity of the experimental room were observed between the wind-free mode (24.2 °C [24.1 °C–24.3 °C] and 40.8% [40.8%–40.9%]) and the conventional mode (24.2 °C [24.1 °C –24.2 °C] and 40.8% [40.7%–40.9%]) ($P = 0.378$ and 0.377, respectively). The median air velocity from air conditioner was higher in the conventional mode (0.24 m/s [0.17 m/s –0.30 m/s]) than that of the wind-free mode (0.01 m/s [0 m/s–0.02 m/s]) ($P < 0.001$).

Comparison of skin temperature and skin barrier function

In AD patients, skin temperature of the cheek and the forearm significantly decreased after exposure to the conventional mode (both $P < 0.001$), and skin temperature of the forearm was also reduced after exposure to the wind-free mode ($P = 0.026$) (Fig. 2A, B). However, no changes in skin temperature of the cheek and the forearm were found after exposure to either conventional or wind-free mode in control subjects.

In addition, in AD group, TEWL on the cheek and the forearm decreased in the wind-free mode ($P = 0.037$ and 0.002), while there was no change in the conventional mode ($P = 0.749$ and 0.340) (Fig. 3A, B). Furthermore, skin pH on the cheek increased only after exposure to the conventional mode in AD group ($P = 0.002$), while no differences were observed in skin pH of the right cheek and the forearm by the exposure to the wind-free mode ($P = 0.649$ and 0.083, respectively) (Fig. 4A, B). No changes of TEWL and skin pH on the cheek and the forearm were found when control subjects were exposed to the conventional and the wind-free modes (all $P > 0.05$). There were also no significant differences in the percent changes of TEWL on both sites for the conventional and the wind-free modes between AD and

Table 1
Demographic data of the participants (N = 40).

Variables	AD group (n = 28)	Control group (n = 12)	*P* value
Age (yr)[†]	9.2 (7.9–10.1)	8.5 (8.0–11.3)	0.388
Male, n (%)	19 (67.9)	9 (75.0)	0.553
Body mass index (kg/m^2)[†]	18.5 (15.8–20.8)	16.0 (15.4–18.7)	0.299
SCORAD[†]	12.1 (2.0–24.9)	0	<0.001
Sensitization rate, n (%)			
House dust mites	22 (78.6)	2 (15.4)	<0.001
Animal dander (dog, cat)	20 (46.4)	2 (15.4)	0.055
Pollen (tree, grass, weed)	11 (39.3)	2 (15.4)	0.126
Mold	6 (21.4)	1 (7.7)	0.277
Egg white	10 (35.7)	0	0.013
Peanut	10 (35.7)	0	0.013
Cow's milk	7 (25.0)	0	<0.001

AD, atopic dermatitis; SCORAD, scoring of atopic dermatitis.
House dust mites included *Dermatophagoides pteronyssinus* and *D. farinae*. Pollens included grass pollen (timothy, bermuda, meadow grass), tree pollen (alder, birch, elder, oak, Japanese cedar), and weed pollen (mugwort, ragweed, short ragweed, hops Japanese, fat hen). Mold included *Alternaria alternata, Aspergillus fumigatus, Cladosporium,* and *Penicillium*.
[†] Values are presented in medians and interquartile range.

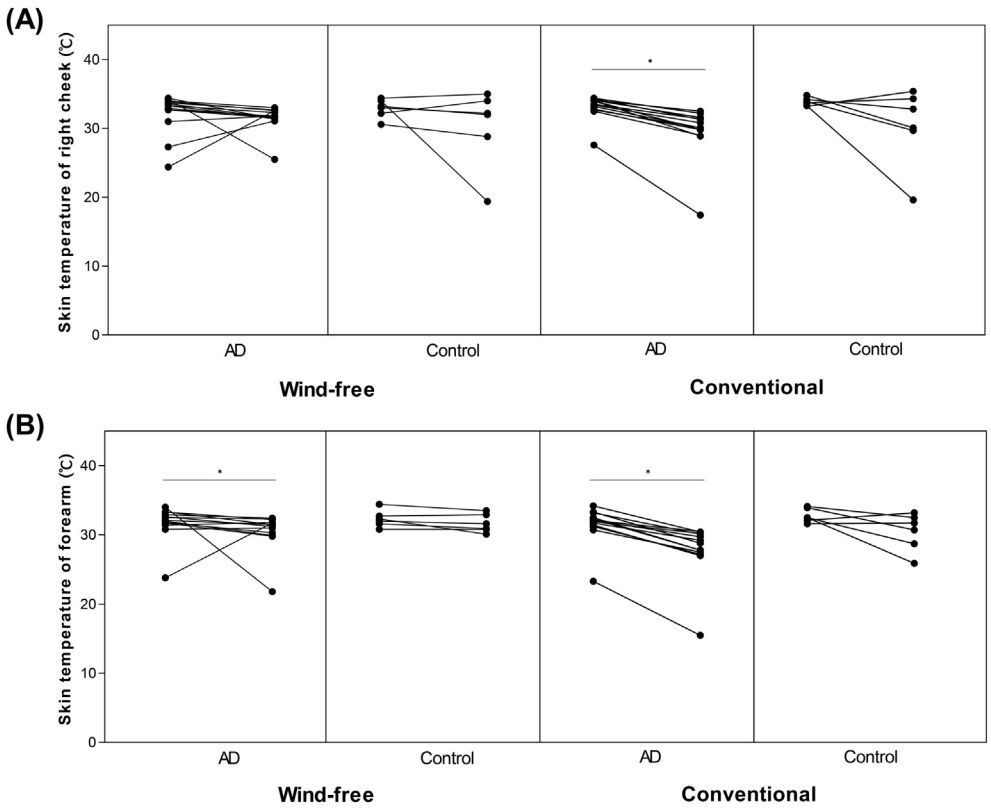

Fig. 2. Skin temperature of right cheek **(A)** and right forearm **(B)** before and after exposure to wind-free and conventional modes in AD children and control subjects. $^{*}P < 0.05$.

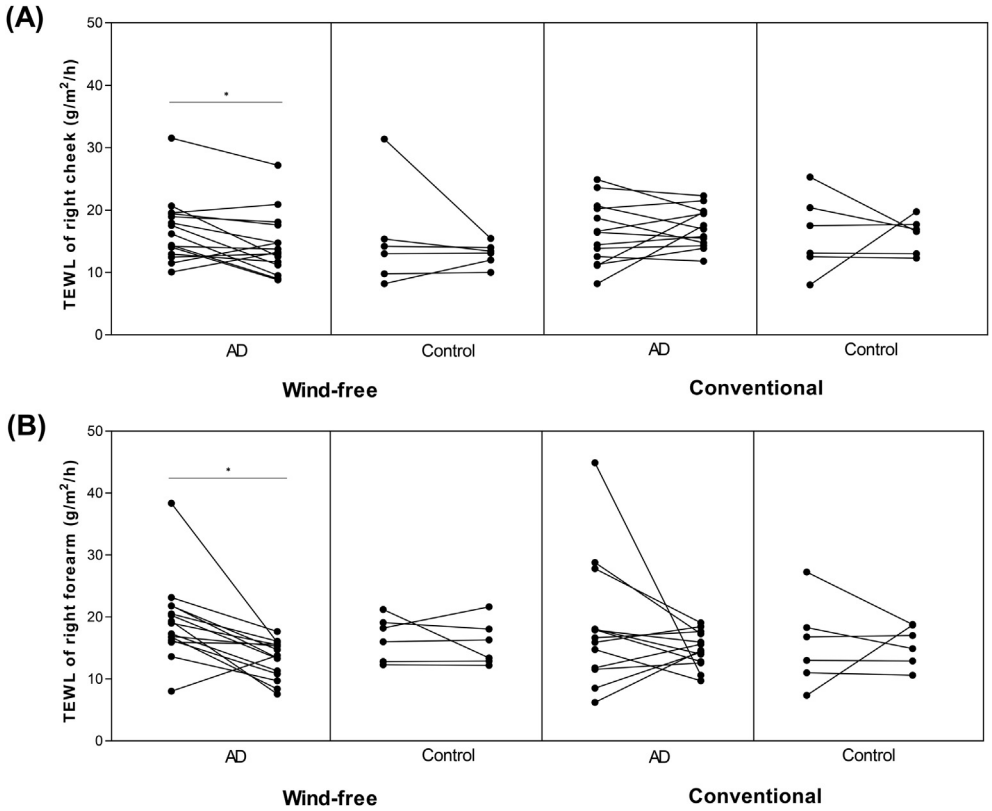

Fig. 3. Transepidermal water loss of right cheek **(A)** and right forearm **(B)** before and after exposure to the wind-free and the conventional modes in AD children and control subjects. TEWL, transepidermal water loss. $^{*}P < 0.05$.

control group (all $P > 0.05$) (Fig. 5A). However, the percent changes of skin pH on the cheek of AD group were higher than those of control group in the conventional mode ($P = 0.022$) (Fig. 5B). Noticeable erythema or pruritus was not induced after the exposure to either the wind-free or the conventional air conditioning mode in both groups. There was no difference in the distribution of subjective symptoms regarding skin dryness between two air conditioner modes in AD group and control group ($P = 0.103$ and 0.065) (Table 2).

Comparison of filaggrin, caspase-14, and filaggrin degradation products

Using immuno-dot-blot technique, the levels of filaggrin and caspase-14 were evaluated in samples from skin tape stripping method. In both AD and control groups, there were no differences in epidermal filaggrin and caspase-14 levels between pre- and post-exposure in either conventional or wind-free air conditioner mode (all $P > 0.05$, data not shown). In AD children, however, the levels of PCA and *cis*-UCA were significantly reduced after the exposure to the conventional mode (all $P = 0.033$) (Fig. 6A, B), but no changes of FDPs were noted in the wind-free mode ($P = 0.847$). In the control group, there were no changes in levels of PCA, *cis*-UCA, *trans*-UCA after exposure to neither the conventional ($P = 0.063$, 0.219, and 0.222, respectively) nor the wind-free air conditioner mode ($P = 0.563$, 0.313, and 0.688, respectively) (Fig. 6A, B. C). The percent changes of PCA and *cis*-UCA were higher in AD patients than those in control subjects only after exposure to conventional mode ($P = 0.029$ and 0.046) (Fig. 6D). However, the percent changes of *trans*-UCA showed no significant difference between AD and control groups after exposure to both air conditioner modes (all $P > 0.05$).

Discussion

To the best of our knowledge, this is the first study to investigate the effect of cold airflow from the air conditioner on functional and biophysical parameters of skin barrier in children with AD. We found that skin temperature decreased more significantly in the conventional mode than that in the wind-free mode of the air conditioner in patients with AD. Interestingly, exposure to cold airflow from the conventional mode reduced epidermal FDPs and increased skin pH when compared to the wind-free mode only in AD patients, although obvious clinical reactions like erythema or pruritus were not observed. It suggests that skin barrier function of AD patients may be altered by the exposure to cold airflow from the air conditioner, which is commonly used in homes and various indoor spaces.

Filaggrin in the skin undergoes subsequent processing by variable proteases, including caspase-14 and histidase, into FDPs such as PCA, *cis*-UCA, and *trans*-UCA.[16] They are the major components of natural moisturizing factors which contribute to skin hydration, UV protection, and pH modulation.[16,17] It is well known that FDPs are major factors in maintaining SC pH as an acidic condition.[18] Therefore, our observation of FDPs reduction in SC could be a possible mechanism of an elevated skin pH after exposure to the conventional mode. These findings are meaningful because the increase in skin pH is associated with a reduced antimicrobial activity, impaired SC integrity, and possible epidermal lipid abnormality.[18–20] However, it still remains unclear how the cold airflow lowers FDPs in SC. In a previous study, there was a positive correlation between the quantities of caspase-14 and PCA in AD patients, suggesting that decreased activity of caspase-14 cause deficiency of FDPs.[21] However, we failed to find a significant change in caspase-14 and filaggrin expression in the present study. This

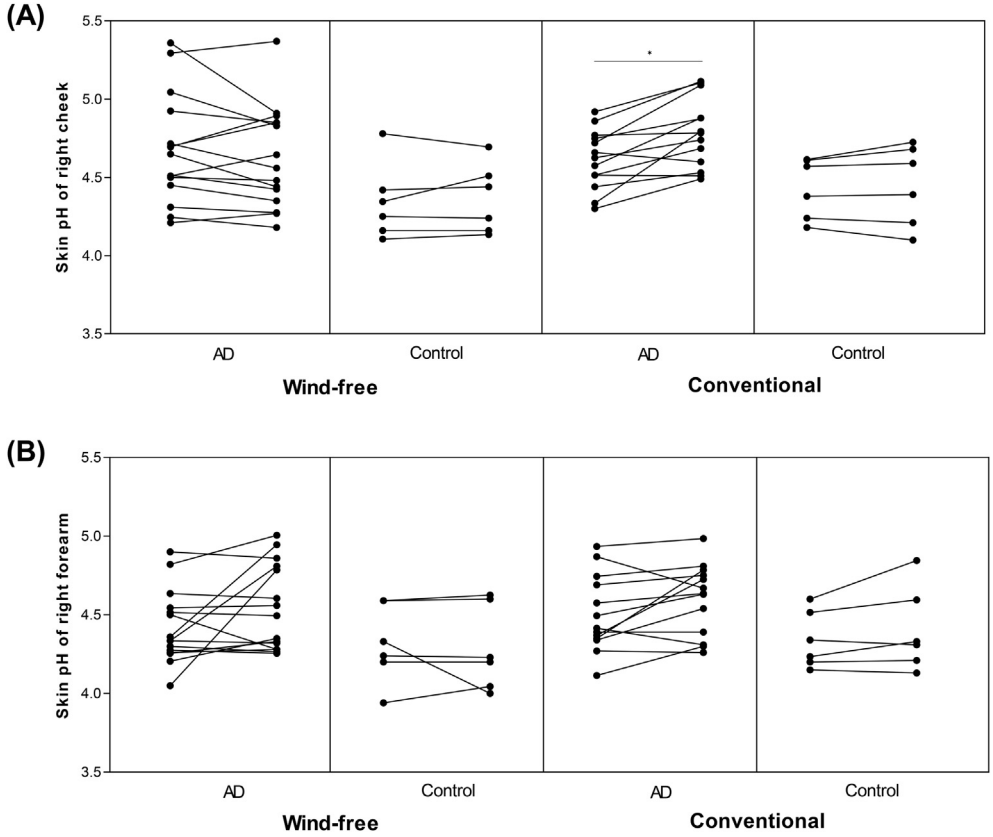

Fig. 4. Skin pH of right cheek **(A)** and right forearm **(B)** before and after exposure to the wind-free and the conventional modes in AD children and control subjects. *$P < 0.05$.

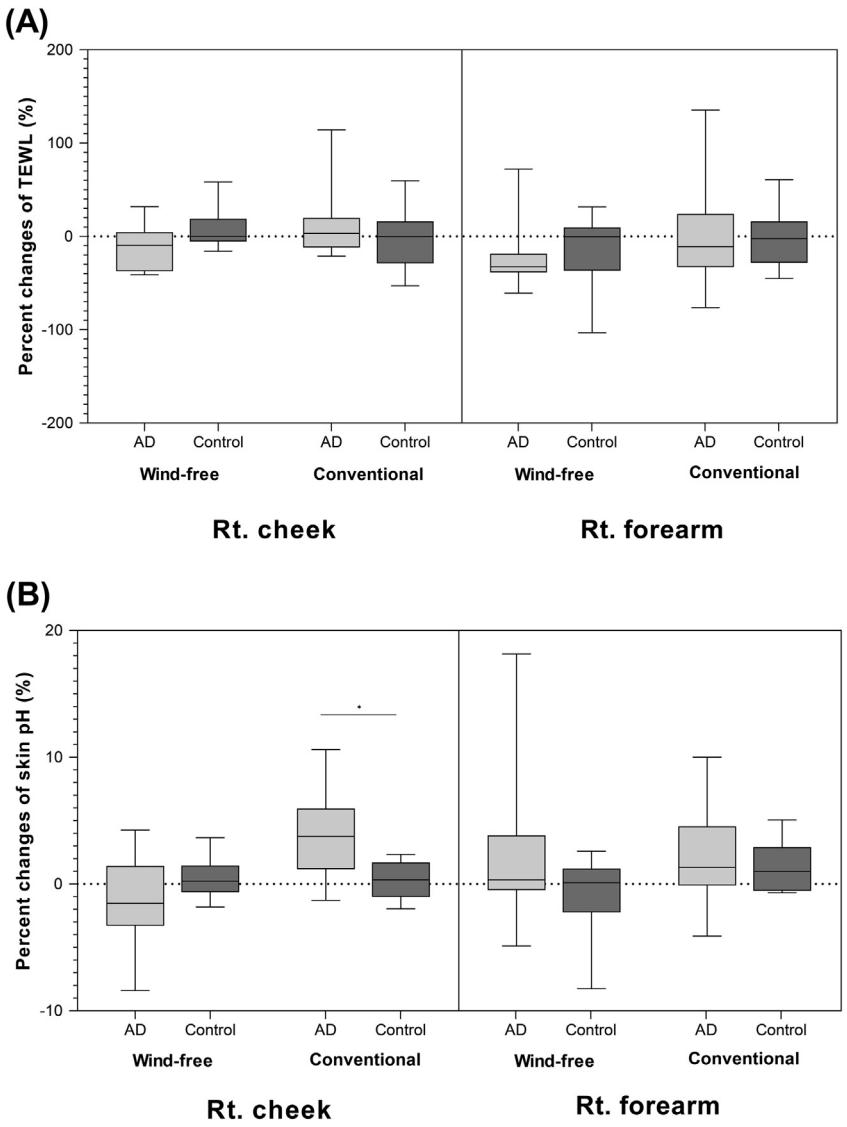

Fig. 5. Percent changes of transepidermal water loss **(A)** and skin pH **(B)** after exposure to the wind-free and the conventional modes in AD children and control subjects. The bars represent box plots with median value (horizontal line), interquartile range (box) and values (whiskers). TEWL, transepidermal water loss. $^{*}P < 0.05$.

finding may be due to short exposure time of cold airflow. Further studies are needed to elucidate the mechanism by which cold airflow affect the skin barrier in AD.

Filaggrin expression and free amino acid content in SC decreased 24 h after moving mice from a normal to a dry condition.[22] However, the free amino acid level recovered to the baseline within 3 days, suggesting adaptation of skin barrier function to an unfavorable environment.[22] Similarly, windy condition is likely to induce dryness around the skin surface, resulting in a reduction of FDPs and altered skin barrier function in our present study. However, our results are different from those of Yamaguchi *et al.*, who compared caspase-14 levels between healthy young male adults exposed to an exhaust type air conditioner (high local air velocity) and those exposed to a whole ceiling type air conditioner (low local air velocity) for 5 consecutive days.[23] Epidermal caspase-14 concentrations increased with the exhaust air conditioner and were higher when exposed to the high local air velocity than when exposed to the low local air velocity on the day 3 and day 5.[23]

Table 2
Subjective clinical symptom after the exposure to the wind-free or the conventional mode.

	AD group		*P* value	Control group		*P* value
	Wind free (n = 15)	Conventional (n = 13)		Wind free (n = 6)	Conventional (n = 6)	
Very dry	0	0	0.103	0	0	0.065
Dry	4 (26.7)	3 (23.1)		0	2 (28.6)	
Normal	7 (46.6)	10 (76.9)		3 (50.0)	5 (71.4)	
Comfortable	4 (26.7)	0		3 (50.0)	0	
Very comfortable	0	0		0	0	

AD, atopic dermatitis.

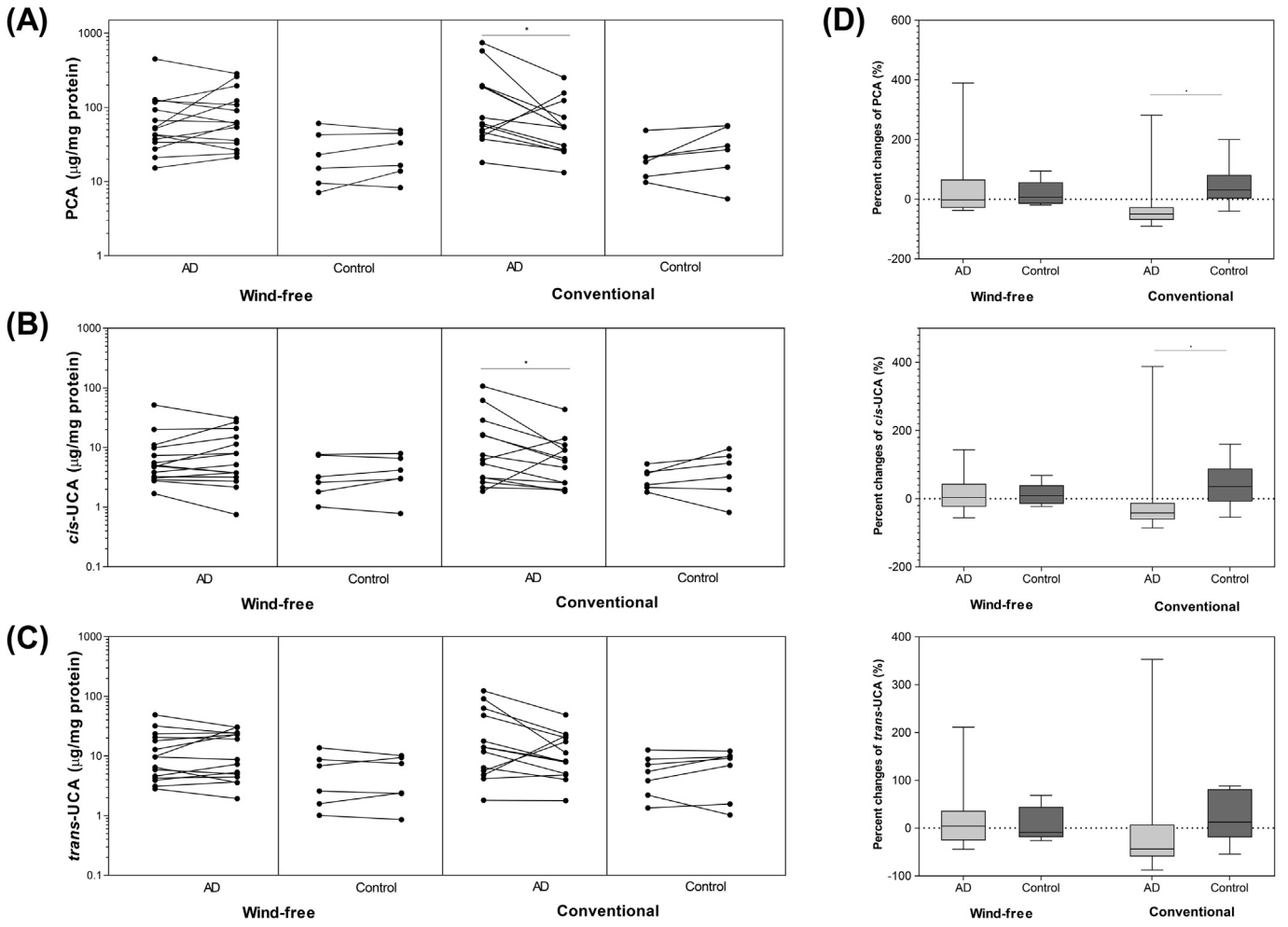

Fig. 6. Levels of pyrrolidone carboxylic acid **(A)**, *cis*-urocanic acid **(B)**, and *trans*-urocanic acid **(C)** in the stratum corneum of right forearm before and after exposure to the wind-free and the conventional modes and percent changes of these molecules after exposure to each mode **(D)** in AD children and control subjects. The bars represent box plots with median value (horizontal line), interquartile range (box) and values (whiskers). PCA, Pyrrolidone carboxylic acid; UCA, urocanic acid. $^{*}P < 0.05$.

However, the results by Yamaguchi *et al.* are difficult to compare directly with those of our present study since their study targeted normal adults and was performed in settings similar to chronic exposure rather than acute exposure. AD patients would lack of adaptation for dry environment, and skin inflammation might inhibit the action of proteolytic enzymes.[21,24,25]

Our present study showed that TEWL was decreased after exposure to the wind-free mode in AD patients, while there was no change in the conventional mode. These results are in agreement with those of a previous study which aimed to evaluate the effects of mist on skin hydration and showed reduction of TEWL on the face during the 2 h in control subjects.[26] In that study, the participants of the control group were placed in mist-free, air-conditioned rooms where temperature (24 °C) and humidity (35%) were similar to those of our study. A similar change in TEWL on the cheek was also reported by another study in which subjects were exposed to dry environment with a relative humidity of 10%,[27] supporting that TEWL is altered depending on the skin condition and environmental humidity.[26,28] Additionally, an experimental observation study showed a positive correlation between TEWL and skin temperature in healthy adult subjects.[8,29] Therefore, it is postulated that reasons for the TEWL reduction in our present study are due to decrease in skin temperature and equilibrium between evaporation and supply of water in the skin while subjects were in the experimental room.

The primary limitations of our study were the short duration of exposure to air flow from air conditioners as well as close distance between subjects and the air conditioner. Our findings should be interpreted carefully because people are usually a few meters away from the air conditioner. However, it was realistically difficult for children to sit still in the same seats for a long time. Another limitation was that we used non-lesional skin samples. However, altered lipid composition, low expression of filaggrin, and excessive infiltration of $CD3^{+}$T-cells are found in non-lesional area similar to lesional area in patients with AD.[30] Consequently, the results of the present study are clinically meaningful in that the environmental factors over a short period of time led to changes even in non-lesional skin of AD patients.

In conclusion, our results suggest that the exposure to cold airflow from a conventional air conditioner may act as an aggravating factor of AD by reducing FDPs and increasing skin pH. It could be beneficial to minimize a direct exposure to cold airflow with high air velocity from air conditioning appliances for children with AD.

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Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

MJ, JK, and KA contributed to the conception and design of the study. MJ, IK, JYL, HMK, MKw and JK contributed to acquisition of data. MKi, KML and PSK participated to analysis of data. MJ and IK carried out the interpretation of data and draft the manuscript. IK, MJ and JK coordinated and supervised data collection. KA, PSK, and JK critically reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agreed to be accountable for all aspects of the work.

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