

# Improvement of Mild Inflammatory Changes of the Facial Skin Induced by Winter Environment with Daily Applications of a Moisturizing Cream

## A Half-Side Test of Biophysical Skin Parameters, Cytokine Expression Pattern and the Formation of Cornified Envelope

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### Key Words

Stratum corneum · Interleukin 1 · Interleukin 1 receptor antagonist · Barrier function · Moisturizing cream

### Abstract

**Objective:** Based on our previous findings that, reflecting mild inflammation, the exposed facial skin shows much poorer functional properties of the stratum corneum (SC) in the dry and cold winter than those evaluated in the same individuals in the warm and humid summer time, we conducted a half-side test on the face to determine how the facial skin changes induced by a winter environment are improved by daily applications of a moisturizing cream as assessed by non-invasive biophysical and cytological methods. **Methods:** One side of the face of 16 young females was treated with a moisturizing cream twice daily for 6 weeks, with the other side serving as the non-treated control. Before treatment, 3 and 6 weeks after the start of the treatment, high-frequency conductance as a parameter of the skin surface hydration, trans-epidermal water loss (TEWL), a parameter of the water barrier function of the skin, and the skin surface lipid level were measured on the cheeks. Obtaining the SC from

the skin surface by adhesive tape, interleukin (IL) 1 $\alpha$  and IL-1 receptor antagonist (IL-1ra) in the SC and cornified envelope (CE) maturation were determined. **Results:** At first, baseline measurements conducted before treatment showed rather high TEWL values suggestive of an impaired skin barrier. During the treatment with the moisturizing cream, significantly higher conductance values and lower TEWL values were found on the moisturizer-treated side, accompanied by a decreasing IL-1ra/IL-1 $\alpha$  ratio and immature CEs. **Conclusion:** These results suggested that the daily application of a moisturizing cream is effective in improving mild subclinical inflammation that is induced on the facial skin by the winter environment.

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### Introduction

Recently, we have reported that the daily application of moisturizing creams or emollients for 5 days increased skin surface hydration depending on their efficacy that persisted at least for several days after the cessation of the treatment [1]. These findings indicated that even a simple

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1018–8665/03/2073–0269\$19.50/0

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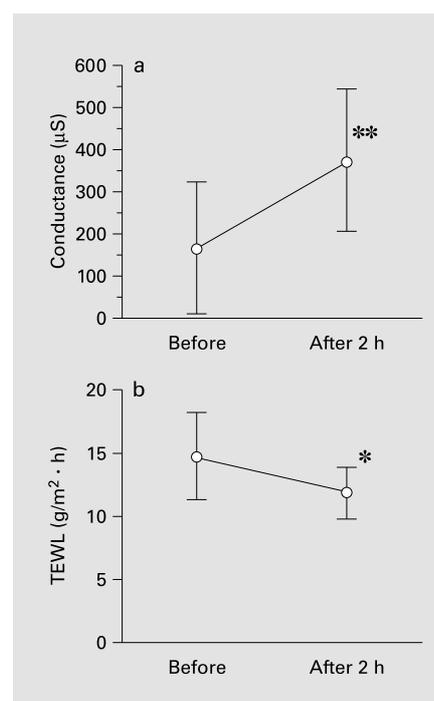
application of topically effective moisturizers not only affects the surface portion of the stratum corneum (SC) but also exerts a much more far-reaching effect than expected, suggesting that such a therapeutic modality can be designated as corneotherapy. In fact, the application of a moisturizing cream is effective not only for the treatment of dry pruritic skin but also for the prevention of exacerbations of inflammatory dermatoses such as atopic dermatitis [2]. It is also useful for immature neonatal skin [3], whose barrier function is not intact. Moreover, a moisturizing cream has been reported to prevent the development of occupational hand dermatitis due to gloves [4] and experimental irritant dermatitis induced by detergents [5].

As compared with the skin of the other body regions, the skin of the face is unique because it is always exposed to the environment, although it is covered with a thinner SC accompanied by a poorer barrier function than the skin of other locations [6]. Evaluations of the properties of the SC of inflammatory dermatoses have greatly improved with recent advances in non-invasive biophysical methods as well as cytological analyses of cytokines in the SC [7, 8] and the differentiation end-products such as the cornified envelope (CE) [9], enabling us to detect changes that are unrecognizable clinically. By using biophysical measurements, we have recently found that the poorly protected facial skin showed unexpected deterioration in the functional properties of the SC in the dry and cold winter time when compared with those evaluated in the warm and humid summer time in the same individuals, probably reflecting subclinical underlying inflammation [10]. Thus, we conducted a half-side test on the face to determine how facial skin changes induced by a winter environment are improved by daily applications of a moisturizing cream as assessed by non-invasive biophysical and cytological methods. Employing those biophysical and cytological analyses of the SC, we performed a half-side comparative study in which we treated one side of the face of young Japanese females in the winter with a moisturizing cream for 6 weeks, with the other side serving as the non-treated control.

## Materials and Methods

### Subjects

Sixteen Japanese females aged 19–37 years with an average age of 21 years who did not routinely use a moisturizing cream participated in the study after submitting informed consent. Those who had atopic dermatitis, acne or other skin dermatoses were excluded from the study.



**Fig. 1.** A significant increase in the values of high-frequency conductance (**a**), a parameter for hydration of the SC, and a decrease in TEWL values (**b**) were found 2 h after a single application of the moisturizing cream ( $n = 6$ ). Error bars indicate standard deviations; \*  $p < 0.05$ , \*\*  $p < 0.01$ .

### Moisturizing Cream

A moisturizing cream containing glycerol and erythritol as the main moisturizing agents (d program<sup>®</sup> cream AD, Shiseido, Tokyo, Japan) and formulated for sensitive skin was used. This cream was confirmed to exert no sunscreen effect. In our preliminary study conducted on the cheek skin of 6 females, it produced a significant increase in high-frequency conductance and a decrease in transepidermal water loss (TEWL), 2 h after a single application of the cream (fig. 1).

### Study Design

The study was conducted from January 9 to February 26, 2001. The subjects were instructed to apply the moisturizing cream to the same side of their facial skin twice daily for 6 weeks. Continued usage of make-up cosmetics was allowed to those who intended to use them on their whole face, and these subjects were supplied with a make-up cleansing and washing cream of the same brand (d program) during the test period. The use of any other moisturizer on the face was prohibited. Before the start of the treatment as well as 3 and 6 weeks after the start of the treatment, clinical examinations and non-invasive measurements were performed to evaluate the skin conditions of the right and left sides of the face. During the treatment period, the subjects were asked not to apply the test moisturizing cream on the morning before the measurements.

### Clinical Assessment

Scaling/dryness, erythema and papules were scored on a 0–4 grading basis (0 = none; 1 = slight; 2 = mild; 3 = moderate; 4 = severe) by a trained dermatologist.

### Biophysical Skin Parameters Obtained by Non-Invasive Measurements

High-frequency conductance, which is a parameter for the hydration state of the skin surface [11], was measured with a skin surface hygrometer (Skicon® 200, IBS Ltd., Hamamatsu, Japan) with a probe of 2-mm inner diameter and 4-mm outer diameter electrodes. TEWL, a parameter for the water barrier function of the SC, was measured with DermaLab® (Cortex Technology, Hudsund, Denmark). The skin surface lipid level was measured with a Sebumeter® SM 810 (Courage & Khazaka, Köln, Germany). All measurements were performed on the mid portion of the cheek after washing followed by a 30-min acclimatization time in a climate chamber the room temperature and relative humidity of which were adjusted to  $21 \pm 1^\circ\text{C}$  and  $50 \pm 3\%$ , respectively.

### Analysis of Inflammatory Cytokines and CE Maturation from the Surface SC Samples

After the biophysical measurements, the surface portion of the SC was stripped from the cheek with adhesive tape twice consecutively. The first strip was used for the analysis of interleukin (IL) 1 $\alpha$  and IL-1 receptor antagonist (IL-1ra), and the second strip was used for analysis of the CE maturation to avoid contamination of the CE samples by other insoluble materials such as dusts from the skin surface.

For the analysis of IL-1 $\alpha$  and IL-1ra, the SC samples with adhesive tape were cut into small pieces and immersed into Dulbecco's phosphate-buffered saline supplemented with penicillin, streptomycin and Fungizone. Extraction was performed with an ultrasound sonifier, equipped with a probe, on ice for 2 min, and then the contents of IL-1 $\alpha$  and IL-1ra were measured by using specific enzyme-linked immunosorbent assay kits, Quantikine human IL-1 $\alpha$  and IL-1ra (R&D Systems Inc., Minneapolis, Minn., USA), respectively. Each obtained value was divided by the amounts of soluble proteins in the SC samples to calculate the IL-1ra/IL-1 $\alpha$  ratio as reported before [7].

The SC samples were also extracted with dissociation buffer for the analysis of CE as described by Hirao et al. [9]. In short, the CE suspension was dropped onto a slide glass and then air-dried followed by fixation with acetone at  $-20^\circ\text{C}$  for 10 min. An antihuman involucrin (1:100, clone SY5, Novocastra, Newcastle upon Tyne, UK) antibody was allowed to react at  $4^\circ\text{C}$  overnight, then FITC-labelled anti-mouse Ig (1:100, Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) was applied. After washing, CEs were stained with Nile red. By a fluorescence microscope (Olympus, Tokyo), the number of involucrin-positive CEs was evaluated using grading scores from 0 to 4. Since morphologically fragile CEs were stained with anti-involucrin but not with Nile red, the maturation of the CEs was evaluated utilizing this double staining with Nile red and anti-involucrin.

### Statistics

Scores of the clinical assessment and CEs were compared with the Wilcoxon signed-ranks test between the treated side and non-treated control side. The values of the skin surface lipid levels, conductance, TEWL and the IL-1ra/IL-1 $\alpha$  ratio were compared between the treated side and non-treated control side with the paired t test.

## Results

### Clinical Assessment

One of the 16 subjects showed pruritic erythematous and papular changes on the moisturizer-treated side at the 3-week examination. We dismissed this subject from the study group and excluded her data from further analyses suspecting a case of contact dermatitis to the test cream, although no further confirmatory measures such as patch testing were performed. Except for this case there was no significant difference in the scores of erythema and papules obtained at 0, 3 and 6 weeks. In contrast, the scaling/dryness scores were significantly lower on the treated side as compared with the control side at both 3 and 6 weeks although there was no statistically significant difference at 0 weeks before the start of the treatment.

### Biophysical Skin Parameters Obtained by Non-Invasive Measurements

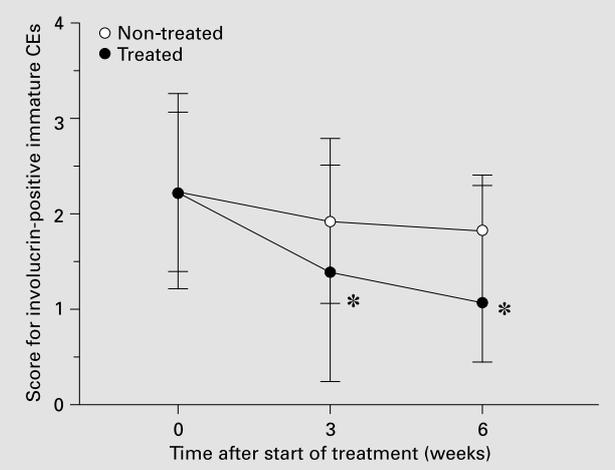
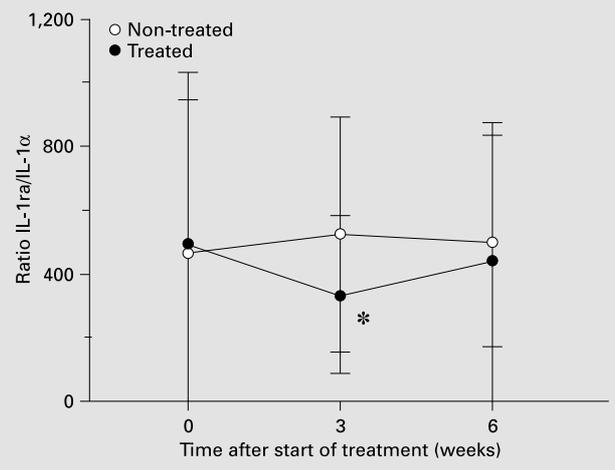
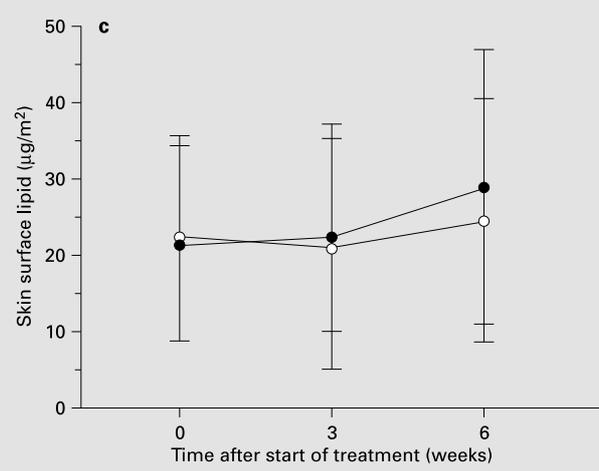
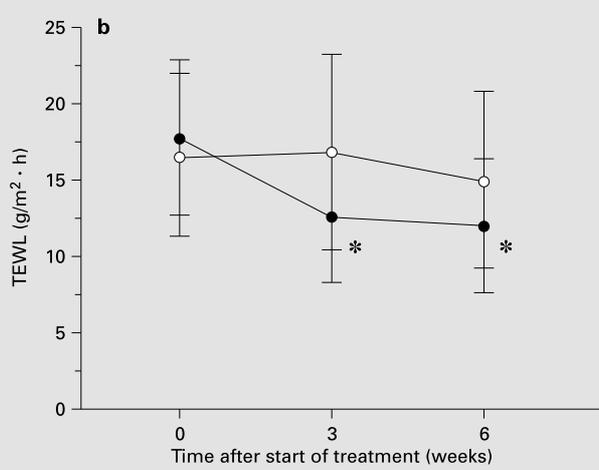
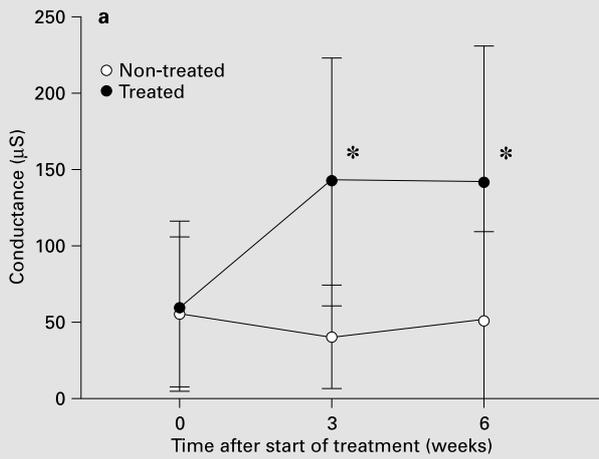
The obtained high-frequency conductance values were significantly higher on the treated side than on the control side (fig. 2a) and the TEWL values were significantly lower on the treated side than on the control side (fig. 2b) at both 3 and 6 weeks after the start of the treatment when there was no difference in these parameters between the right and left cheeks. In contrast, there was no significant difference in the values of the skin surface lipid levels at 0, 3 and 6 weeks (fig. 2c).

### Inflammatory Cytokines

Although there was no statistical difference in the IL-1ra/IL-1 $\alpha$  ratio before the treatments, it decreased on the treated side as compared to that on the control side at the 3-week measurement. However, later it rose on the treated side to values that were not significantly different from those of the control side (fig. 3).

### CE Maturation

There was no significant difference in the scores of involucrin-positive immature CEs between the treated and control sides before the start of the treatment (fig. 4, 5). With the repeated applications of the moisturizing cream, the score of immature CEs became significantly lower on the treated side as compared to that on the control side at 3 and 6 weeks (fig. 4, 5).



**Fig. 2.** Biophysical parameters measured on the cheek. Closed circles indicate values on the side treated with a moisturizing cream; open circles indicate values on the non-treated side. The values of conductance were remarkably higher on the treated side as compared to those on the non-treated side at 3 and 6 weeks (a). TEWL, a parameter for the water barrier function of the SC, was significantly lower on the treated side as compared to the non-treated side at 3 and 6 weeks (b). No significant difference was noted in the values of the skin surface lipid levels between the treated and non-treated sides (c). Error bars indicate standard deviations; \*  $p < 0.001$ .

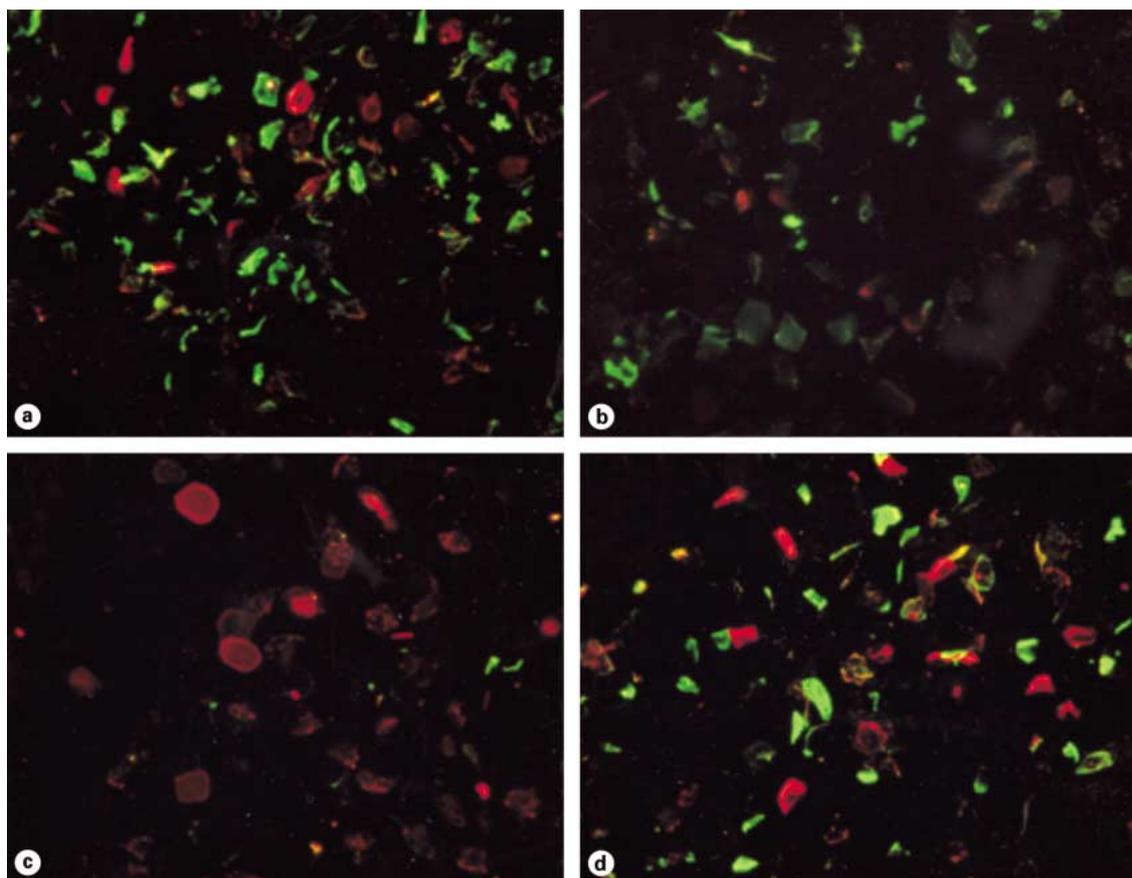
**Fig. 3.** The IL-1ra/IL-1 $\alpha$  ratio was significantly lower on the treated side (closed circles) at 3 weeks as compared with the non-treated side (open circles). Error bars indicate standard deviations; \*  $p < 0.01$ .

**Fig. 4.** Scores for involucrin-positive CEs were significantly lower on the treated side (closed circles) as compared with those on the non-treated side (open circles) at 3 and 6 weeks. Involucrin-positive CEs indicate immature CEs. Error bars indicate standard deviations; \*  $p < 0.01$ .

2

3

4



**Fig. 5.** CE maturation. The majority of CEs that were involucrin positive (green) immature ones before the treatment (**a**, the treated side at week 0; **b**, the non-treated side at week 0) decreased in number only on the treated side at week 3 (**c**) but not on the non-treated side (**d**).

## Discussion

As compared with the skin of other locations, the facial skin shows extraordinarily high TEWL values that are comparable to those noted in dermatitic skin lesions at other locations of the body [6]. In our previous study conducted in healthy individuals, we demonstrated that their skin barrier function on the face is impaired in winter, showing higher TEWL values, being accompanied by smaller sizes of the corneocytes obtained from the skin surface compared to those in summer, which reflects a quicker turnover rate of the epidermis probably due to irritation caused by the dry and cold winter environment [10]. In fact, low humidity itself was shown to stimulate the epidermal DNA synthesis, amplifying the hyperproliferative response of the epidermis to barrier disruption [12]. In the present study, we also found that the facial

skin of the normal subjects showed an impaired water barrier function as compared to that of other portions of the body. The TEWL values were  $17.7 \pm 1.3 \text{ g/m}^2 \cdot \text{h}$  on the right cheek and  $16.6 \pm 1.4 \text{ g/m}^2 \cdot \text{h}$  on the left before the start of the treatment which far exceeded those reported for other locations [6].

Epidermal keratinocytes produce IL-1 $\alpha$  constitutively; however, in inflamed skin they produce IL-1ra to inhibit the pro-inflammatory effect of the former. Thus, the IL-1ra/IL-1 $\alpha$  ratio increases in the SC of various inflammatory dermatoses such as atopic dermatitis and psoriasis [13]. The IL-1ra/IL-1 $\alpha$  ratio in the SC was also found to be higher on the exposed facial skin than on the unexposed upper arm skin [7]. In the present study, we found a decreasing tendency in the IL-1ra/IL-1 $\alpha$  ratio on the treated facial skin that became statistically significant after 3 weeks of treatment. These results suggest that the

increased TEWL values of the face in winter due to the mild inflammation induced by exposure to the harsh winter environment were alleviated by the application of the moisturizing cream. However, we do not have any explanation as to the question why the IL-1ra/IL-1 $\alpha$  ratio on the treated facial skin increased again to the level of the non-treated facial skin after 6 weeks of treatment, despite the findings that other parameters did not show such a recovery.

The slow and steady differentiation process taking place in the normal skin of the covered areas produces a well-matured, Nile-red-positive CE, a rigid and insoluble structure encasing the corneocytes in the SC, which is assembled by the cross-linking of several precursor proteins by transglutaminases. The CE provides the SC with a hydrophobic foundation for its barrier function. Corneocytes obtained from the skin surface by tape-stripping become Nile red negative when they have fragile and immature CEs, as noted in diseased skin where the epidermal differentiation process is rapid, whereas they begin to show positive staining to anti-involucrin or antibodies against other CE components, including loricrin, envoplakin, filaggrin and iso-peptides [9]. Such immature CEs were also reported to be found on the skin surface of the face, deeper layers of the SC of the covered area [9] and in the lesional skin of inflammatory skin diseases [Hirao T. et al., unpubl. data]. In our present study conducted in mid winter, we found immature CEs in the facial skin of young normal females at the beginning of the treatment. This finding suggested the presence of mild inflammation on the facial skin in the winter. The observed decrease in the immature CEs after the treatment suggests that an improvement of the subclinical inflammatory condition was caused by the treatment with the moisturizing cream.

In the present study, we found an increase in the hydration state of the SC when evaluated with the measurements of high-frequency conductance of the facial skin surface at 3 and 6 weeks after the start of the moisturizer treatment. Lodén et al. [2] reported that an increase in capacitance, which is also a parameter of skin surface hydration but less sensitive than high-frequency conductance [14], was observed in patients with atopic dermatitis 10 days after treatment with a moisturizing cream and that this increase was accompanied by a lowering of the TEWL values. Moisturizing creams do not only provide water to the SC but also assist in the formation of a lipid film at the skin surface which may gradually reduce the TEWL so that the water builds up beneath it [15]. However, the observed decrease in TEWL seems also to be the

result of the far-reaching effect of the repeated applications of the moisturizer that may induce changes even in the underlying epidermal tissue.

Physiological lipids have been demonstrated to penetrate into damaged skin and to influence the barrier recovery [16]. It has been reported that an increase in hydration in the SC occurred rather soon after 2 days of moisturizer application [17] and was sustained up to 7 days after the last application of a suitable moisturizing cream for 5 days [1].

Preventive and therapeutic effects of moisturizers are also reported for experimental irritant dermatitis induced by sodium lauryl sulphate [5]. In contrast, an increased susceptibility to irritants was suggested to occur by a prolonged application of a moisturizing cream [18]. There, differences seem to be dependent on the quality of moisturizers according to the report of Hachem et al. [19]; they found that long-term use of a poorly hydrating moisturizer increased skin barrier damage in allergic contact dermatitis.

In conclusion, we demonstrated that the barrier impairment as well as the decreased hydration state observed on the facial skin of normal individuals in winter might be due to the presence of mild inflammation, based on the obtained results of an inflammatory cytokine profile and the presence of immature CEs in the SC as well as the biophysical skin parameters. Moreover, application of a suitable moisturizing cream exerts therapeutic effects on this condition as shown by the non-invasive methods employed in the present study.

### Acknowledgement

The authors give special thanks to Ms. Yuko Nagahamaya for her technical support. This study was partly supported by Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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