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The Influence of Two Different Foam Creams on Skin Barrier Repair of Foot Xerosis: A Prospective, Double-Blind, Randomised, Placebo-Controlled Intra-Individual Study

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Key Words

Xerosis · Skin barrier · Dry feet · Foam cream · Stratum corneum · Lipbarvis · Transmission electron microscopy · Lipid analysis

Abstract

Background/Aims: Dry skin, or xerosis, is a common condition and a key feature of skin diseases like atopic dermatitis (AD) and ichthyosis vulgaris. Foot xerosis may exist without underlying disease and could be related to very mild forms of AD or ichthyosis vulgaris. The synthesis of important skin lipids (cholesterol, free fatty acids and ceramides) is reduced in xerosis and AD, and reduced lipid synthesis is responsible for a lack of lipids and enzymes in the skin barrier. This slows down reorganisation of the lipid lamellae in the stratum corneum (SC). ***Methods:*** Skin barrier integrity was measured by morphometric analysis of the lipid lamellae in the SC after 4 weeks of treatment with a foam cream (active agent vs. placebo). ***Results:*** Significant treatment effects were shown after 2 and 4 weeks by an increasing amount of intercellular lipids in the SC. ***Conclusion:*** This study shows that a quick reorganisation of the SC lipids initiates a good restoration of the whole skin barrier after 4 weeks of treatment with a foam cream.

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Introduction

Dry skin, also called xerosis, is a common condition and a key feature of skin diseases like atopic dermatitis (AD) and ichthyosis vulgaris. In addition, various systemic diseases such as liver and kidney disease and diabetes are associated with dry skin, as are certain medications (such as retinoids, opiates, and anticancer drugs) [1] and environmental factors (such as dry and cold weather and irritants including water). However, dry skin may exist without underlying diseases. Perhaps this could be related to very mild forms of AD or ichthyosis vulgaris which remain undiagnosed. Furthermore, old age is associated with xerosis, probably due to a generally reduced metabolism.

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In addition to a reduced metabolism, the synthesis of important skin lipids (cholesterol, free fatty acids and ceramides) is also reduced in AD and xerosis [2]. Reduced lipid synthesis in the stratum spinosum is responsible for a lack of lipids and enzymes at the interface between the stratum granulosum (SG) and stratum corneum (SC). This slows down the reorganisation of the lipid lamellae in the intercellular space in the SC [3].

Barrier repair therapy involves the use of medicated or non-medicated formulations, depending of the severity of the barrier disorder [4]. In order to measure the effect of medicated or non-medicated formulations, various clinical and biophysical procedures are proposed. The clinical procedures include disease severity (SCORAD) and quality of life instruments (Dermatology Life Quality Index) [5]. The biophysical measurements include transepidermal water loss (TEWL) and skin hydration [6]. The results of these measurements describe the condition of the epidermal barrier indirectly, either by improved clinical features, reduced TEWL or increased skin capacity. The direct evaluation of skin barrier integrity is only possible by a morphometric analysis after transmission electron microscopy (TEM) investigation. The analysis of the quantity of the intercellular lipid lamellae (ICLL) in the intercellular space completes this methodological spectrum [7, 8]. In order to combine the knowledge of the amount of lipid lamellae in the intercellular space in the SC with a chemical analysis of the SC lipids, high-performance thin-layer chromatography (HPTLC) was applied. This allows discrimination between topically applied lipids, such as ingredients in the foams, and endogenous lipids synthesised during the epidermal barrier repair process.

In this study, TEM analysis of the lipid lamellae was combined for the first time with lipid analysis via HPTLC. These analyses were completed by measurements of TEWL and skin capacitance to investigate the influence of skin care foams (placebo vs. active agent) to repair the epidermal barrier in subjects with dry skin on their feet without other chronic diseases.

Materials and Methods

This open clinical trial was conducted following European Community Good Clinical Practice standards. It is a prospective, double-blind, randomised, placebo-controlled intra-individual single-centre human intervention study and was performed in accordance with the Revised Declaration of Helsinki, local laws and regulations.

Subjects

A total of 12 subjects with dry skin were included (3 male, 9 female; age: 32–58 years; skin capacitance lower than 25). The pedal skin was chosen as a test site since the pedal skin was much dryer than the arm or leg skin, and the investigated formulations were foot care products.

The investigation was carried out at Proinnovera/Münster between October and November 2014.

Treatment, Formulation and Application

The subjects tested 2 different foam creams (active agent and placebo), one applied to the right foot and the other applied to the left foot for comparison, over a period of 4 weeks. The Allpresan foam cream with active ingredients (aqua, urea, decyl oleate, octyldodecanol, butane, panthenol, cetyl alcohol, glycerin, propane, stearic acid, glyceryl stearate, squalane, ceramide 3, allantoin, *Butyrospermum parkii* butter, potassium lauroyl wheat amino acids, palm glycerides, sorbitol, caprylic/capric triglyceride, hydrogenated lecithin, sodium lauroyl sarcosinate, capryloyl glycine, pentylene glycol) was termed the active agent, and the Allpresan foam cream without active ingredients (aqua, butane, propane, cetyl alcohol, stearic acid, caprylyl glycol, palm glycerides, sodium lauroyl sarcosinate, glyceryl stearate, potassium lauroyl wheat amino acids, capryloyl glycine) was referred to as the placebo.

Active and placebo foam creams were applied by the subjects at home twice daily (morning/evening, with a 10- to 12-hour interval between applications) under normal conditions. The instructions were given in written form as well as orally at every visit as deemed necessary by the medical personnel. The whole foot (approx. 500 cm^2) was treated with approximately 1 g foam (0.5 mg/cm^2) per application.

Testing Schedule

The test sites for all measurements were directly below the ankles of the left and right feet. Instrumental measurements (hydration, TEWL) and SC sampling were performed on day 1 (study initiation), day 15 and day 29, from sampling areas not previously used. In order to avoid disturbance of the instrumental measurements and SC sampling, subjects omitted 1 product application on days 15 and 29 before sampling.

Biophysical Measurements

After the acclimatisation phase for at least 20 min at 21 ± 1°C and 50 ± 10% relative humidity, instrumental measurements (TEWL, Corneometer) were performed laterally on the foot below the ankle.

Evaporimetry was performed with Tewameter TM 300 (Courage & Khazaka GmbH, Cologne, Germany) according to the guidelines of TEWL measurements [9] in an air-conditioned room with standardised ambient conditions (21 ± 1°C, 50 ± 10% relative humidity).

For evaluation of the moisture in the outer layer of the skin, corneometry was performed with Corneometer CM 825 (Courage & Khazaka GmbH) according to the EEMCO guidelines for the assessment of SC hydration [10] under appropriate ambient conditions of 21 ± 1°C and relative humidity of 50 ± 10%. Subjects were acclimatised to the measurement environment with the skin test areas left uncovered for at least 20 min. Measurements at each skin test area were performed in a horizontal position with a constant pressure of 1.5 N (by device).

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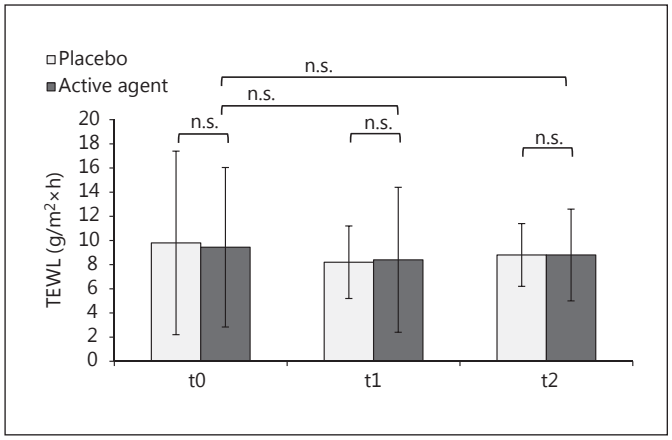

Fig. 1. Influence of treatment on the TEWL after 2 (t1) and 4 weeks (t2) of application of placebo and active agent. n.s. = Not significant; t0 = baseline.

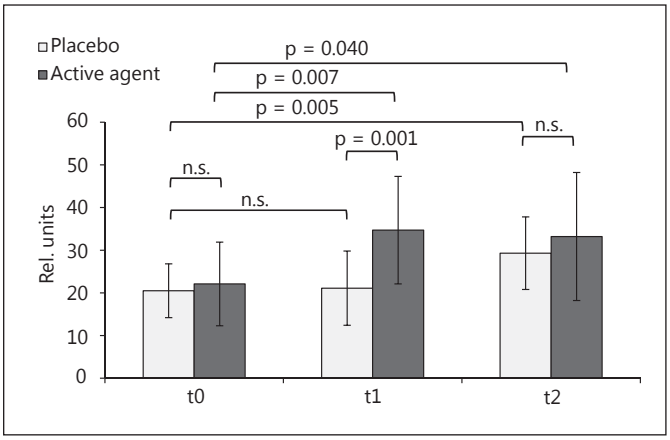

Fig. 2. Treatment effect on skin capacitance after 2 (t1) and 4 weeks (t2) of application with placebo and active foam (t0 corresponds to baseline). n.s. = Not significant; $p < 0.05$ was considered statistically significant.

SC Sampling, Preparation and TEM

The removal of the SC samples using Lipbarvis and the processing of the samples for the TEM analysis is described by Dähnhardt-Pfeiffer et al. [7]. The TEM analysis shows the intercellular lipids. In the subsequent morphometric analysis, the lengths of the ICLL in the intercellular space were set into a relationship of a reference value of 1,000 nm^2 (nICLL); this quotient allowed for a semi-quantitative analysis of the quality of the skin barrier. Values between 40 and 70 were measured for very dry skin, and values between 70 and 100 were measured for dry skin. Healthy skin ranged from about 180 and higher.

Lipid Analysis

At the beginning of the lipid extraction, a photo was taken of the carrier with the covered corneocytes. Using the Image J software (www.nih.gov), the corneocyte layer was measured and identified on the sample surface. The number of cell layers on the carrier was assessed from the TEM data so that the extracted amount of lipids was referenced to a well-defined circular carrier surface (13 mm diameter) and corneocyte layers. The extraction of lipids from the corneocytes adhered on the carrier (here also referred to as slide) as well as their separation using HPTLC according to Imokawa et al. [11]. For the chromatographic analysis, Nano-Sil 20 plates (10 × 10 cm, Macherrey & Nagel) were used. The standard used contained the lipids cholesterol, ceramides EOS, NP and NH and free fatty acids. The free fatty acids mixture contained: (i) palmitic acid 25%, (ii) stearic acid 25%, (iii) linolenic acid 16.7%, (iv) linoleic acid 16.7% and (v) oleic acid 16.7%.

The completed HPTLC plates were densitometrically measured and quantitatively analysed.

Statistical Analysis

The examined parameters SCORAD, TEWL, skin capacitance, nICLL and lipids were analysed using the Shapiro-Wilk tests for normal distribution at the 3 measurement time points: before treatment (t0), after 2 weeks (t1) and after 4 weeks (t2) of treatment. In cases of significant deviation from normal distribution, non-parametric methods were used for further statistical analysis, otherwise parametric procedures were used.

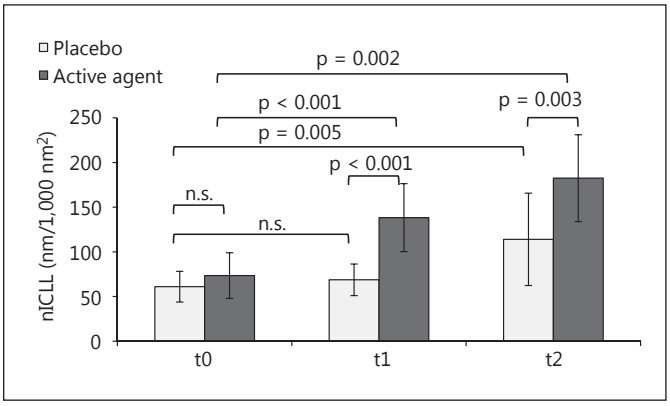

Fig. 3. Influence of treatment on nICLL after 2 (t1) and 4 weeks (t2) of application of placebo and active foam (t0 corresponds to baseline). n.s. = Not significant; $p < 0.05$ was considered statistically significant.

Accordingly, measurements at t0, t1 and t2, as well as the t0 differences, were compared to each other or between products (active agent vs. placebo) using the Wilcoxon matched-pairs test for cases of significant deviation from normal distribution. For all other samples, the matched-samples t test was carried out.

Two-sided analyses were performed and a level of significance of 5% was presumed. An alpha adjustment for multiple tests was not performed; the results correspondingly have an explorative and descriptive character. Statistical analyses were performed with SPSS Statistics 22 (SPSS Inc., IBM, Chicago, Ill., USA).

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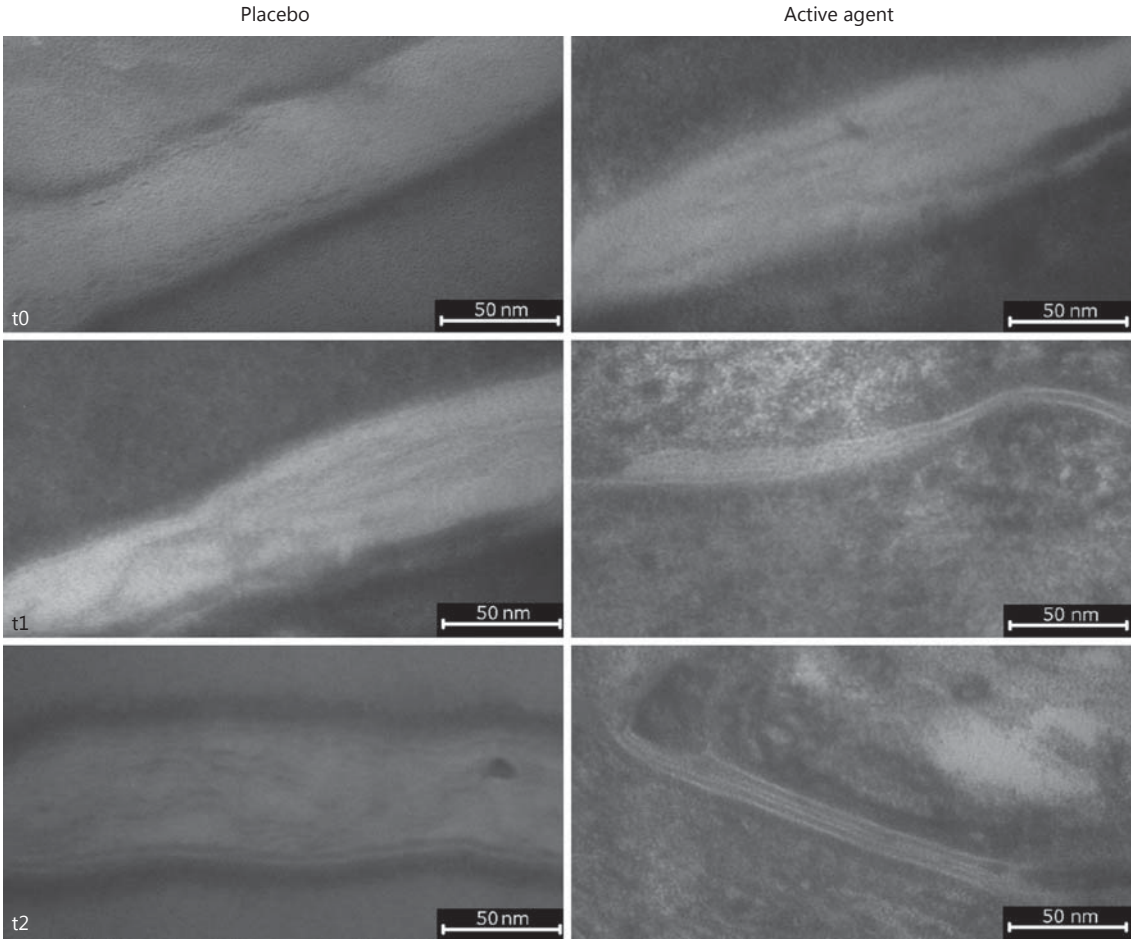

Fig. 4. Selected TEM images of the ICLL in the intercelluar space in the SC at different time points (t0 = baseline, t1 = 2 weeks of treatment and t2 = 4 weeks of treatment) under the different treatments (active agent vs. placebo).

Results

Transepidermal Water Loss

Neither treatment area showed a significant change compared to baseline for TEWL mean values in the course of the study (fig. 1).

Skin Capacitance

After 2 weeks of treatment, the skin capacitance had significantly increased in the feet treated with the active foam cream, while those feet treated with placebo foam cream did not show any significant changes in skin capacitance. After 4 weeks of treatment, the skin capacitance increased in the feet treated with the active foam cream as well as in those treated with placebo foam cream (fig. 2).

Transmission Electron Microscopy and Morphometric Analysis of the Intercellular Lipids

The most distinct differences between treatment with verum foam cream and placebo foam cream were seen under TEM and with the semi-quantitative analysis of the ICLL of the SC. At the baseline analysis, the organisation of the ICLL was clearly disturbed in both treatment groups (fig. 3, 4). Few lipid lamellae were found in the intercellular space between the corneocytes in the SC (fig. 4). After 2 and 4 weeks of treatment with the active foam cream, a distinct significant increase in the number of lipid lamellae in the intercellular space was seen. After 4 weeks of use, the number of intercellular lipids, compared to the baseline value, had tripled so that the feet treated with the active foam cream showed a barrier similar to healthy skin. ICLL showed no significant changes

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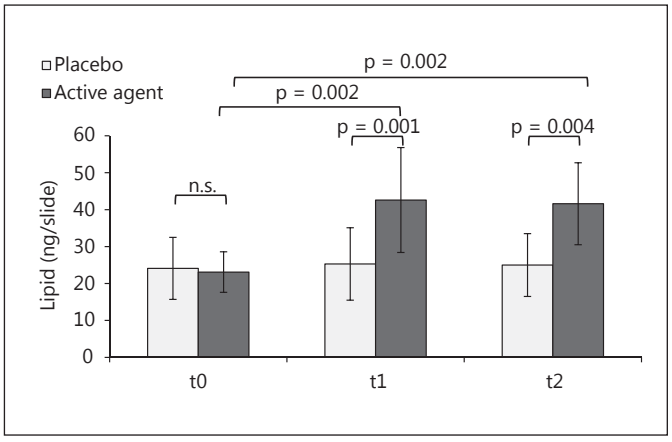

Fig. 5. Treatment effect on sum of lipid level after 2 (t1) and 4 (t2) weeks of application of placebo and active foam. n.s. = Not significant; $p < 0.05$ was considered statistically significant.

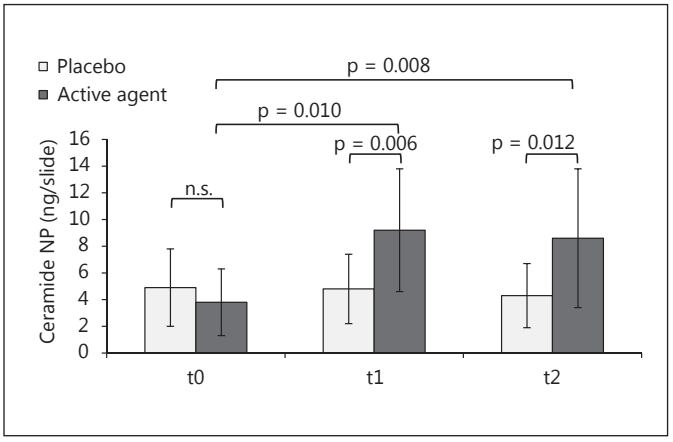

Fig. 6. Influence of treatment on the ceramide NP level after 2 (t1) and 4 (t2) weeks of application of placebo and active foam (t0 corresponds to baseline). n.s. = Not significant; $p < 0.05$ was considered statistically significant.

in subjects treated 2 weeks with the placebo foam cream. After 4 weeks of treatment with the placebo foam cream, the amount of lipid lamellae had doubled compared to baseline values. After 2 and 4 weeks of treatment, the differences between treatment with the active agent and placebo foam creams were highly significant (fig. 3).

Lipid Analysis

After 2 and 4 weeks of treatment with the active foam cream, the content of total lipids increased significantly compared to baseline values, while no changes could be seen in feet treated with placebo foam cream (fig. 5). The higher content of total lipids in the feet treated with the active foam cream was mainly due to a significantly higher content of ceramide NP (fig. 6), as well as higher free fatty acids and a higher content of total ceramides (data not shown) compared to those feet treated with placebo foam cream.

Discussion

Xerosis of the feet is a common symptom that is present not only in specific skin diseases, such as AD or the diabetic foot, but can also be induced or worsened by environmental factors, including frequent showering or bathing [12, 13]. The proliferation of dry skin increases with advancing age, which is due to changes in the physiological and immunological properties of the skin [14]. Although many people suffering from dry skin of the feet do not have a chronic illness such as diabetes, there are only a few studies regarding pedicure preparations for the foot [15].

In the present study, dry pedal skin was treated using special foot care foam cream (active agent) and placebo foam cream in order to study the reorganisation of the skin barrier by determining the TEWL, the skin capacitance, the lipid lamella length within the intercellular space, and the lipid content in the SC of the pedal skin.

After 4 weeks of maintenance treatment, neither the active nor the placebo foam cream resulted in any change in the TEWL. At the beginning of the study, the 2 treatment groups showed very large differences in the TEWL values (active group: minimum value 2.5 $g/m^2 \times h$, maximum value 26.6 ($g/m^2 \times h$; placebo group: minimum value 4 $g/m^2 \times h$, maximum value 26.4 $g/m^2 \times h$), even though visual assessment by the study nurse showed a comparable dryness of the pedal skin. This effect is probably due to the fact that the thickness of the pedal SC differs in a pronounced way and varied among the subjects included in this study. Even though the high standard deviation in the TEWL values measured at baseline indeed became smaller over the course of the treatment, no significant differences were found between the treatment groups. As shown in this study, the determination of pedal TEWL (below the ankle) is not an appropriate parameter for describing the skin barrier. Similar results were also found by Lodén et al. [15] in a study of dry pedal skin. While the tested treatment preparation changed the skin thickness, moisture content and erythema of the feet, no

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differences were found in this study in terms of the TEWL. Because these difficulties are well known in the literature, pedicure preparations have often been tested on other parts of the body, such as the forearms [16].

In contrast to the measured TEWL values, however, skin hydration clearly showed a significant effect. After a 2-week treatment using the active foam cream, the treated feet showed significantly improved skin hydration. This was significantly better than at baseline and significantly higher than in those feet treated with the placebo foam cream. This effect is probably due to the additional ingredients compared to placebo foam cream, enabling the active foam cream to normalise the skin moisture much faster. A crucial role in skin hydration is played by the natural moisturising factors [17–19]. Both of the tested foam creams include natural moisturising factors in the form of wheat amino acids.

The improved skin hydration in both treatment groups was accompanied by an increase in the length of the lipid lamellae in the intercellular space. With the active foam cream treatment, however, the barrier repair happened faster and more efficiently than in those treated with the placebo foam cream. After just 2 weeks of treatment, the length of the lipid lamellae had doubled (baseline: 73.4 nm, t1: 138.3 nm). After 4 weeks of treatment using the active foam cream, the length of the lipid lamellae continued to increase in the intercellular space and equalled that of healthy skin (t2: 182.4 nm). With the placebo foam cream treatment, there was also a significant increase in the length of the lipid lamellae after 4 weeks (baseline: 61 nm, t2: 114 nm); however, the reorganisation of the skin barrier was considerably slower and significantly less than the barrier repair in those feet treated with the active foam cream.

Reorganisation of the lipid lamellae in the intercellular space presumably occurs in several steps. Penetration of the lipophilic components from the active foam cream into the intercellular space presumably allows a first reorganisation phase which leads to homeostasis in the epidermis. This causes the water transport to be normalised in the SC and SG, and causes the environmental conditions of many important biomolecules (proteins, enzymes) to be restored, resulting in a barrier repair which favours the production and transport of lamellar bodies from the SG to SC, and an increased funnelling and incorporation of lipids into the intercellular space [20]. The importance of the quality of the first step of the reorganisation is evidenced by the differences in the topically applied drugs. For feet treated with the active foam cream, the combination of ceramide NP and other lipids probably allowed a faster first reorganisation phase in the SC (leading to increased total lipid content after only 2 weeks of treatment) compared to those feet subjected to placebo treatment. The patient feet treated with the placebo foam cream likely reached this first phase of reorganisation after 28 days of treatment.

In summary, this study showed for the first time that a quick reorganisation of SC lipids favours homeostasis in the SC of feet and leads to the restoration of an intact barrier after 4 weeks of treatment with active foam cream.

Statement of Ethics

This open clinical trial was conducted following European Community Good Clinical Practice standards. It is a prospective, double-blind, randomised, placebo-controlled intra-individual single-centre human intervention study and was performed in accordance with the Revised Declaration of Helsinki, local laws and regulations.

Disclosure Statement

D.D. and S.D.-P. have no conflicts of interest to declare. They are employees of the Microscopy Services Dähnhardt GmbH in Flintbeck. J.S.-W. and E.H. are employed as scientists at neubourg skin care GmbH & Co. KG (nsc), but they do not hold shares in the company. T.N. is the owner of nsc and therefore has an equity interest in this company. nsc may potentially benefit from the research results in this article. He is also the inventor of the technology described in the article for which patents have been granted in many countries worldwide. C.S. has no conflicts of interest to disclose. M.B. declares no disclosable financial arrangement or interests related to the tested product or to nsc (sponsor). R.F.-H. is employed as a physician at the University Hospital of Schleswig-Holstein, Campus Kiel, Department of Dermatology, Venerology and Allergology. She does not have any shares in pharmaceutical companies and neither in nsc. This year, however, she has received lecture fees from pharmaceutical companies such as Infectopharm, Novartis or Johnson & Johnson.

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