A Long-Term Study to Evaluate Acidic Skin Care Treatment in Nursing Home Residents: Impact on Epidermal Barrier Function and Microflora in Aged Skin

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Key Words
Skin aging · Stratum corneum pH · Epidermal barrier function · Skin flora · Skin care

Abstract
Background: The pH of the stratum corneum (SC) in the elderly is elevated and linked to impaired SC function. Therefore, this paper addresses the question of whether acidic skin care generates positive clinical, biophysical, and microbiological effects in aged skin. Methods: This study was performed to assess skin care effects in nursing home residents (aged 80–97 years). Visual, biophysical, and microbiological methods were used. Subjects were randomly assigned to 1 of 2 groups and treated over 7 weeks with skin care products adjusted to a pH of 4.0 (group A) or a pH of 6.0 (group B). Results: Compared to baseline, SC integrity improved significantly in group A (p = 0.007), whereas there was no change in group B (p = 0.672). SC recovery 24 h after perturbation increased significantly in group A (p = 0.004) compared to baseline. The SC recovery in group B was not significant compared to baseline (p = 0.327). Conclusion: Long-term treatment with pH 4.0 skin care results in a significant improvement in epidermal barrier function compared to identical products with a pH of 6.0. In addition, effects on skin dryness and resident flora were demonstrated, but without significant differences, between the 2 groups. Based on these results, we recommend adjustment of skin care products for the elderly to a pH of 4.0 to maintain the health of aged skin.

Introduction
Severe xerosis is a common problem in the elderly and a serious dermatological challenge; however, its prevalence depends on the clinical setting [1] (e.g., it is present in up to 77% of nursing home residents) [2]. Due to the combination of low humidity and very high room temperatures, the prevalence of skin dryness can increase to up to 95% during winter [3]. Moreover, dry, scaly, rough skin is frequently accompanied by itching (pruritus...
senilis) [4]. Severe xerosis and pruritus in the elderly can induce the dry-skin cycle [5] and the itch-scratch cycle [6], which lead to cracked, fissured, eczematous, and inflamed skin.

Xerosis is linked to many functional and structural changes in aged skin which affect different cutaneous layers, i.e. the dermis, the epidermis and its outermost layer, and the stratum corneum (SC), where the epidermal permeability barrier (EPB) is located [7]. Furthermore, sebum and sweat production [8], together with the lipid content [9] and hydration [10], are decreased in aged SC.

Abnormal EPB function in aged skin has been demonstrated. Tape stripping studies on aged murine and human skin have revealed a reduced barrier integrity compared to young skin [11, 12]. In addition, irritability by an alkaline noxe is known to be significantly higher in aged skin than in young skin, with an additional significant deficit in photoaged skin [13]. Recovery of the SC after perturbation by tape stripping or acetone is significantly delayed in human and murine aged skin compared to young skin [11, 12], with a further significant delay in photoaged skin compared to chronologically aged skin [14].

One of the most important challenges in age-related EPB physiology appears to be the elevation of the skin surface pH (and SC pH). A correlation between higher pH values (5.7 ± 0.15) and age (67–95 years) has been shown in the elderly [15]. Furthermore, a higher skin surface pH was demonstrated by Zlotogorski et al. [16] in a group of subjects aged >80 years compared to younger groups. Man et al. [17] showed pH values of approximately 6.0 on the forearms and foreheads of elderly subjects (>70 years). Choi et al. [12] demonstrated a significant increase in skin surface pH on the forearms of 55 moderately aged humans (51–80 years) compared to 65 young subjects (13–21 years). The same investigation showed elevated pH at all levels throughout the SC in aged mice.

SC pH regulates at least 3 epidermal functions [18], i.e. SC integrity/cohesion, SC recovery, and antimicrobial barrier function. Desquamation is induced by a complex proteolytic cascade and depends on the activity of different kallikrein-related peptidases [19], especially kallikrein-5 (KLK5) and KLK7 [20]. As shown experimentally, both serine proteases exhibit a neutral pH optimum [21, 22]. Desquamation is linked to the degradation of desmoglein 1 (DSG1), desmocollin 1 (DSC1), and corneodesmosin (CDSN) by these KLK [20]. DSG1, DSC1, and CDSN are extracellular protein structures of corneodesmosomes which ensure SC stability. The physiological SC pH regulates the activity of KLK5 and KLK7 by reducing, but not completely inhibiting, them. Consequently, desquamation and the integrity of the SC are balanced, regulated, and maintained [18, 23].

Besides integrity, SC recovery depends on the pH gradient throughout the SC. EPB restoration is delayed if experimentally impaired skin is exposed to a neutral pH buffer [24] or to superbases [25]. These pH-induced barrier abnormalities are associated with inhibition of two lipid-processing enzymes, i.e. β-glucocerebrosidase (β-GlcCer’ase) and acid sphingomyelinase (aSMase), which exhibit a low pH optimum, and transfer of polar lipids, such as glucosylceramide and sphingomyelin, to the nonpolar barrier organization [26–29].

The relationship between skin surface pH and skin flora has been known for a long time and has been shown in many in vitro and in vivo investigations [30–35]. In vitro and in vivo studies have demonstrated that a pH of 5.0 inhibits the growth of pathogenic bacteria, such as Staphylococcus aureus; however, species of the normal resident flora are positively affected by the physiologically slightly acidic milieu [32–34, 36]. Furthermore, dissociation of endogenous bacteria from the skin surface is enhanced in alkaline conditions [30].

In summary, the age-related pH shift increases the activity of KLK5 and KLK7 and inhibits the activity of the lipid-processing enzymes β-GlcCer’ase and aSMase. This results in excessive degradation of corneodesmosomes and inadequate formation of the lamellar lipid bilayers. The functional consequences are: (i) reduced SC integrity/cohesion [25, 37, 38], (ii) delayed SC recovery [24, 25, 38], and (iii) negative effects on skin flora [30].

In a previous pilot study, we showed that topical application of an acidic emulsion (pH 4.0) normalizes the elevated skin surface pH in the elderly and improves barrier integrity as measured by tape stripping [39]. In the present work, we set out to study the effect of a long-term pH 4.0 skin care treatment in a real-life setup with regard to clinical, functional, and microbiological skin characteristics. To address this issue, we performed a randomized, controlled, double-blind, long-term study in a nursing home.

Materials and Methods

Study Population

This study was performed in cooperation with a nursing home (Haus am Berg, Osnabrück, Germany) and involved 20 residents (16 women and 4 men) aged 80–97 years (mean 87.0 ± 5.4). The inclusion criteria were: age ≥80 years, healthy skin at...
the test sites, SC hydration ≤35.0 AU, and pigment type I–III. Volunteers were excluded if they suffered from skin diseases, such as atopic dermatitis (AD) or psoriasis, or strong physical and/or psychological handicaps and/or if they had used topical and/or systemic antibiotics up to 4 weeks prior to the start of this study. The elderly from the nursing home were separated into 2 groups (A and B), without influence from the investigator, in a randomized procedure. This study started with 26 volunteers (13 in each group) and ended with 20 volunteers (group A, n = 12; group B, n = 8) because 6 volunteers dropped out due to internal diseases.

This study was approved by the Ethical Committee of the University of Osnabrück, which gave it unanimous approval (No. 4/71040/0/6). Written, informed consent was obtained from all volunteers according to the Declaration of Helsinki.

Study Design
This randomized, controlled, double-blind study was carried out in April and May. The test products [oil-in-water (O/W) cream, O/W lotion, and synthetic detergent] were applied twice a day as body care for 7 weeks by the volunteers themselves or by caregivers. Group A was treated with test products adjusted to a pH of 4.0 and group B was treated with test products adjusted to a pH of 6.0 (see suppl. material; for all online suppl. material, see www.karger.com/doi/10.1159/000437212).

The existing skin care products were confiscated to avoid any possibility of confusion. Measurements were taken at baseline and after the test period in a specifically arranged skin lab within the nursing home, with comparable climatic conditions (baseline: temperature 22.4 °C ± 0.6, relative humidity 45.1% ± 1.9; after the test period: temperature 22.9 °C ± 0.4, relative humidity 47.9% ± 1.9). Volunteers refrained from using any skin care or cleansing products or other cosmetics for at least 24 h before measurements. Furthermore, contact between the test areas and water was avoided for at least 12 h. The measurements started after a 30-min acclimatization period.

Clinical Assessment
Clinical assessment in this context meant determination of skin dryness via visual scoring according to Serup [40]. Visual examination of the complete integument was performed by a trained assessor (N.S.) and expressed as a dry-skin area and severity index (DASI). Based on this guidance, the severity of scaling, roughness, fissures, and redness was assessed as absent (0), slight (1), moderate (2), severe (3), or very severe with eczema (4). This score was calculated in the following 4 regions: the lower extremities, the trunk, the upper extremities, and the head and neck. After adding the scores, DASI values ranged between 0 and 1,600.

Functional Assessment
The following biophysical measurements were taken on the volar forearm of the volunteers based on relevant guidelines [41–43]: SC hydration (Courmometer® CM825; Courage and Khazaka, Cologne, Germany), skin surface pH (Skin-pH-Meter® P905; Courage and Khazaka), and transepidermal water loss (TEWL; DermaLab® Transepidermal Water Loss Module; Cortex Technology ApS, Hadsund, Denmark).

Assessment of EPB function included: SC integrity, cohesion, and recovery as defined previously [44]. The baseline TEWL was measured, followed by sequential tape stripping (D-Squame Blue Standard; CuDerm Corporation, Dallas, Tex., USA) until the TEWL increased by 3-fold, reflecting barrier perturbation. The integrity of the SC was expressed as the number of tape strips required to increase the TEWL by 3-fold. The TEWL measurement, taken 24 h after SC perturbation, reflects EPB restoration and is calculated as the percentage recovery rate. To study SC cohesion, every second strip of the first 15 that were taken (i.e. D-Squame No. 1, 3, 5, 7, 9, 11, 13, and 15) was analyzed by: (i) infrared densitometry (SquamScan™ 850A; Heiland Electronic, Wetzlar, Germany) and (ii) Bradford protein quantification. Eight D-Squares were selected and stored at 5 °C. Absorption (%) of the D-Squares was measured with an infrared densitometer at a wavelength of 850 nm. Absorption was correlated in a linear manner with the protein content [45]. Furthermore, the protein amount was extracted from the D-Squares and assessed using a Bio-Rad Protein Assay Kit 1 (Bio-Rad Laboratories GmbH, Munich, Germany). The procedure was a modification of a previously described approach [38, 46]. Before stripping the SC, the skin surface was cleaned with an ethanol wipe. The D-Squares were treated and shaken with 1 ml of 1 M NaOH for 1 h at 37 °C to remove the corneocytes from the adhesive tape site. After neutralization with 1 ml of 1 M HCl, 0.2 ml of the solution was transferred to tubes and shaken with 0.2 ml of Bio-Rad protein dye and 0.6 ml of ddH2O for 5 min. To measure the absorption at 595 nm by spectrophotometry (U-1900; Hitachi Ltd., Tokyo, Japan), the reagent was transferred into semi-micro cuvettes (PMMA; Brand GmbH and Co. KG, Wertheim, Germany). The amount of protein was calculated as micrograms removed per tape, and it represents the mean value of the 8 analyzed D-Squares. Empty D-Squares were used as a negative control and their absorption was involved in the calculation.

Microbiological Assessment
The combined wash-scrub method of Williamson and Kligman [47] was used to sample the skin flora. A glass chamber (Ø 2.4 cm) was pressed to the skin surface, 1 ml of phosphate-buffered solution [0.0075 M NaH2PO4 (8.5 ml), 0.0075 M Na2HPO4 (91.5 ml), and Triton-X-100 to 0.1% (v/v); pH 7.9] was applied, and a scrub procedure with a Teflon spatula was carried out for 1 min. Thereafter, the solution was aspirated and transferred to sterile tubes using a single-channel pipette. This procedure was repeated once. The tubes were directly stored at 5 °C. Within 4 h, a serial dilution of the each sample was produced. 0.1 ml was plated onto different agar media, i.e. COS, PVX, MCK, and SGC2 (bioMérieux SA, Marcy-l’Étoile, France), and plates were incubated at 37 °C for 48 h. The skin flora was quantitatively and qualitatively analyzed via established culture-based microbiological methods. Based on this procedure, the number of colony-forming units (CFU) per square centimeter of skin was calculated and the isolated bacteria were identified.

Statistical Analyses
Statistical analysis was performed using SPSS statistic version 19.0 (IBM SPSS, Chicago, Ill., USA). To compare the nonparametric-paired values of each test site, a Wilcoxon signed-rank test was used. Differences between test sites were analyzed using a Mann-Whitney U test for nonnormally distributed, nonpaired data. p ≤ 0.05 was considered statistically significant. The data are represented in box plots and in table form as medians, 25th and 75th percentiles, and ranges.
Results

Clinical Assessment

No significant differences were noted in skin dryness, calculated based on the DASI, between groups A and B before treatment (i.e. at baseline; \( p = 0.616 \)). Significantly reduced skin dryness was observed after 7 weeks of treatment with the test products in groups A (\( p = 0.002 \)) and B (\( p = 0.036 \)) (fig. 1). The differences between both groups were not significant (\( p = 0.297 \)) after treatment. In summary, a strong decrease in skin dryness was observed after long-term treatment in both groups, with only small differences between the groups.

Functional Assessment

Before treatment, no significant differences in skin surface pH (\( p = 0.461 \)), SC hydration (\( p = 0.334 \)), or TEWL (\( p = 0.643 \)) between the groups were observed, illustrating the homogeneity of the volunteers. After treatment, the skin surface pH decreased specifically in group A (i.e. from 5.57 to 5.17, \( p = 0.003 \)). The skin surface pH in group B remained at the baseline level (i.e. above 5.5). Furthermore, statistical analyses revealed significant (\( p < 0.001 \)) differences between the groups after treatment (fig. 2a). In both groups, the SC hydration after treatment was higher than at baseline, but the increase was significant (\( p = 0.005 \)) only in group A.
Skin hydration (AU) increased from 30.5 to 36.3 in group A and from 28.3 to 30.7 in group B, respectively (fig. 2b). Evaluation of the TEWL showed no significant differences between groups or time points. The TEWL showed an increase from 4.25 to 4.60 g/m²/h in group A and from 4.65 to 4.50 g/m²/h in group B (fig. 2c).

We then performed dynamic studies on epidermal barrier integrity/cohesion and recovery of the aged volunteers. Statistical analyses of baseline SC integrity (p = 0.699), recovery (p = 0.700), and cohesion (p = 0.396; p = 0.643) revealed no significant differences between groups A and B. Before the long-term treatment, 16.0 (group A) and 14.0 (group B) tape strippings were required to perturb the epidermal barrier of the elderly. Compared to baseline, the SC integrity in group A significantly (p = 0.007) improved after 7 weeks as indicated by the higher number of tape strippings required to disturb the EPB. Moreover, after treatment, significant (p = 0.025) differences in SC integrity were shown between groups A and B (fig. 3a). The amount of protein removed per strip (125.8 vs. 143.6 μg; p = 0.396) and the percentage absorbance (12.4 vs. 11.8%; p = 0.643) displayed no significant differences between groups A and B. After treatment, the amount of protein per strip (173.2 μg; p = 0.025) and the percentage absorbance (14.3%; p = 0.025) were significantly enhanced in group A. The percentage recovery and barrier recovery showed no significant differences between groups A and B before and after the treatment (p = 0.021 and p = 0.025, respectively).

Fig. 3. Epidermal barrier function in both groups before (baseline) and after treatment with the test products. SC integrity (a), SC recovery (b), and SC cohesion assessed by protein quantitation (c) and percentage absorbance (d). SC integrity was assessed as the number of D-Squame tape strippings required to increase the TEWL by 3-fold. The TEWL was measured immediately and 24 h after tape stripping and percentage recovery was calculated as described previously. SC cohesion is defined as the amount of protein per D-Squame and was measured as previously described. * Extreme value; ° outlier.
B compared to baseline values. We determined no significant changes in barrier cohesion compared to baseline in group A regardless of the assessment technique (fig. 3c, d).

EPB restoration was assessed 24 h after tape stripping and was expressed as percentage recovery. In both groups, SC recovery was enhanced after a 7-week treatment with the test products. Group A showed a significant increase (–0.31 vs. 55.69%; p = 0.004) in contrast to the changes in group B (19.99 vs. 22.22%; p = 0.327). Furthermore, statistical analyses showed significant (p = 0.021) differences between both groups after treatment (fig. 3b). The clinical scored and biophysical data are summarized in table 1.

Table 1. Clinical scored and physiological data

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th></th>
<th>definition</th>
<th>p</th>
<th>Group B</th>
<th></th>
<th>definition</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>after treatment</td>
<td></td>
<td></td>
<td>baseline</td>
<td>after treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DASI</td>
<td>230.0</td>
<td>30.0</td>
<td>improved</td>
<td>0.002</td>
<td>285.0</td>
<td>70.0</td>
<td>improved</td>
<td>0.036</td>
</tr>
<tr>
<td>SC hydration, AU</td>
<td>32.1</td>
<td>36.1</td>
<td>improved</td>
<td>0.005</td>
<td>27.6</td>
<td>31.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEWL g/m²/h</td>
<td>4.25</td>
<td>4.60</td>
<td></td>
<td>NS</td>
<td>4.65</td>
<td>4.50</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>Skin surface pH</td>
<td>5.55</td>
<td>5.20</td>
<td>improved</td>
<td>0.003</td>
<td>5.75</td>
<td>5.50</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>SC integrity</td>
<td>16</td>
<td>21</td>
<td>improved</td>
<td>0.007</td>
<td>14</td>
<td>16</td>
<td></td>
<td>NS</td>
</tr>
<tr>
<td>SC cohesion</td>
<td>125.82</td>
<td>120.39</td>
<td></td>
<td>NS</td>
<td>143.60</td>
<td>173.17</td>
<td>impaired</td>
<td>0.025</td>
</tr>
<tr>
<td>SC cohesion</td>
<td>12.39</td>
<td>11.94</td>
<td></td>
<td>NS</td>
<td>11.78</td>
<td>14.27</td>
<td>impaired</td>
<td>0.025</td>
</tr>
<tr>
<td>SC recovery</td>
<td>–0.4</td>
<td>55.7</td>
<td>improved</td>
<td>0.004</td>
<td>19.0</td>
<td>22.3</td>
<td></td>
<td>NS</td>
</tr>
</tbody>
</table>

NS = Not significant. a Number of tape stripings. b Protein amount per strip (μg). c Absorbance (%). d Recovery rate (%). e Classification is based on the corresponding literature (see references).

Table 2. Cell count before (baseline) and after treatment by study group

<table>
<thead>
<tr>
<th></th>
<th>Time point</th>
<th>Deviation, %</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>baseline</td>
<td>after treatment</td>
<td></td>
</tr>
<tr>
<td>Group A (n = 12)</td>
<td>Median</td>
<td>92</td>
<td>207</td>
</tr>
<tr>
<td></td>
<td>25th percentile</td>
<td>48.0</td>
<td>74.25</td>
</tr>
<tr>
<td></td>
<td>75th percentile</td>
<td>191.0</td>
<td>356.75</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>8–1,796</td>
<td>20–3,846</td>
</tr>
<tr>
<td>Group B (n = 8)</td>
<td>Median</td>
<td>480</td>
<td>1,336</td>
</tr>
<tr>
<td></td>
<td>25th percentile</td>
<td>175.0</td>
<td>566.0</td>
</tr>
<tr>
<td></td>
<td>75th percentile</td>
<td>2,501.5</td>
<td>3,879.0</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>80–3,625</td>
<td>293–5,040</td>
</tr>
</tbody>
</table>

p value<sup>a</sup> = 0.015 0.003

Values are presented as CFU/cm² of skin unless otherwise stated. The volar aspect of the forearm was the test site. a Based on Mann-Whitney U test. b Based on Wilcoxon signed-rank test.

Microbiological Assessment

Microbiological analysis revealed that group A and group B both showed quantitative differences (p = 0.015) prior to treatment with the test products. A median of 92 CFU/cm² of skin was detected via cell counting in group A, compared to 480 CFU/cm² of skin in group B (table 2). This difference was also significant after treatment (p = 0.003), but it was significantly higher, i.e. up to 207 (group A) and 1,336 (group B) CFU/cm² of skin. In summary, the 7-week-long application of the test products led to higher cell counts in both groups.

Table 3 displays the results of the qualitative microbiological evaluation. Overall, 8 different genera of bacteria and 1 fungus were detected. Altogether, coagulase-nega-
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Discussion

Xerosis is a serious dermatological problem for the elderly, especially for nursing home residents [1]. Therefore, it is necessary to improve the epidermal barrier function in the elderly to maintain the health of aged skin and to prevent serious skin problems and/or disorders such as severe xerosis, pruritus, eczema craquelé, and skin infection and inflammation. The correlation between xerosis and barrier dysfunction is commonly accepted [7] and prompted the presented research. The following question was addressed: is it possible to improve the epidermal barrier function in nursing home residents over 80 years of age?

This question is based on the described age-related enhancement of SC pH [15–17], leading to impaired SC integrity/cohesion and restoration [12]. No increase in skin surface pH was noted in the oldest group in a recent study; however, volunteers above the age of 80 were not included [48]. Therefore, it appears that the increase in SC pH starts at about the age of 70 years and is measurable, particularly in subjects over 80 years old.

A single application of an acidic O/W emulsion led to normalization of the increased pH in aged skin over a 7-hour time period [39]. Based on this finding, we addressed the question here of whether long-term acidic skin care treatment results in positive clinical, biophysical, and microbiological effects on aged skin. In a randomized, controlled, double-blind study, 2 groups of aged residents were nursed over 7 weeks – taking the age-related decelerated epidermal turnover time into account – with skin care products adjusted to a pH of 4.0 (group A) or a pH of 6.0 (group B). In contrast to Kim et al. [49], we discovered that skin hydration was significantly improved by application of the acidic products (p = 0.005) but not by treatment with cosmetics such as emollients.
adjusted to a pH of 6.0 (p = 0.161). The different designs of the 2 studies do not allow a direct comparison. To our knowledge, no equivalent investigation with elderly volunteers has been performed previously.

The increase in SC hydration is related to the observed improvement in EPB, which in turn correlates with the normalized and stabilized SC pH. In addition, the loss of water-retaining osmolytes (natural moisturizing factor) seems to be enhanced in aged skin due to the reduced EPB function [11, 50, 51]. Thus, the described improvement in EPB likely reduces the loss of soluble natural moisturizing factor in aged skin and thereby enhances corneal water binding. Another key factor in the epidermal water balance is the water- and glycerol-transporting channel aquaporin AQP3 [52, 53]. To our knowledge, until now, age-related changes in epidermal AQP3 expression have not been studied. Nevertheless, an age-related decline in the expression of aquaporins in other mammalian tissues has been demonstrated [54, 55]. Based on the reported pH-sensitivity of AQP3 [56], it could be postulated that age-related changes in epidermal AQP3 expression were compensated for by the slight SC pH modification investigated in the present study. SC pH improvement likely leads to a stabilized AQP3 activity and thereby optimizes the SC water distribution. Further analysis is required to address questions concerning AQP3 expression in aged skin and the impact of acidic microdomains in SC on these important glycerol and water channels.

The known subnormal baseline TEWL in aged skin [57] was confirmed by this study, and no significant effects on water loss were measured. Xerotic skin has a lower SC water content and often shows proper barrier function, even if it is only reflected by the TEWL [58]. The conclusion can be made that static TEWL measurements are not sufficient to evaluate effects on EPB with subnormal baseline values (<5 g/m²/h) in the elderly. For this reason, it is more appropriate to measure EPB function in the elderly using dynamic methods, as previously specified [59].

Investigations on aged murine and human skin have revealed a reduced SC integrity compared to young skin [11, 12]. Besides a reduced integrity, the SC recovery rate is also significantly delayed in aged skin [11, 12]. Both SC integrity and recovery are even more reduced in photo-damaged skin compared to intrinsic aged skin [13, 14]. In the present trial, EPB integrity (p = 0.007) as well as recovery (p = 0.004) significantly improved in group A after a 7-week course of treatment with pH 4.0 products.

One possible explanation is that the measured increase in skin surface pH in group A (p = 0.003) optimized the activity of KLK5 and KLK7, leading to an improved EPB integrity compared to that in group B. Concerning the described enhancement of SC restoration following pH 4.0 skin care, the activities of the 2 key lipid-processing enzymes β-GlcCer'ase and acid aSMase are also possibly optimized. More polar lipids are transferred to nonpolar (and slightly polar) barrier lipids during the recovery period. Based on the normalized SC pH, the enhanced lamellar body lipid secretion after barrier disruption is followed by a sufficient enzyme-controlled repair process within the SC lipid matrix. The reduction in skin surface pH to a physiological level strengthens the SC structure and accelerates EPB repair mechanisms in the elderly, as described in mice by Choi et al. [12]. Buraczewska and Lodén [60] demonstrated that treatment with an acidic emulsion (pH 4.0) compared to treatment with a pH-neutral product (pH 7.5) did not result in positive effects on SC recovery in younger volunteers. In that age group (21–54 years), physiological skin surface pH levels and a sufficient skin buffering system putatively override pH modulation by acidic products. Therefore, pH modulation by products adjusted to a pH of 4.0 might not be possible as in the elderly [39] and are instead not necessary in healthy young skin.

Serine proteases, like KLK5 and KLK7, are not only regulated by the SC pH via their pH optimum but are also inhibited, for instance, by metal ions or LEKTI (lympho-epithelial kazal-type related inhibitor) [61]. The SC pH also affects LEKTI-KLK5 and LEKTI-KLK7 binding properties directly. SC acidic microdomains lead to dissociation of the LEKTI-KLK complex and thereby indirectly to the degradation of corneodesmosomes in superficial SC layers [62]. To our knowledge, no studies have been performed to investigate LEKTI and pH interactions in aged skin. It is still unclear whether age-related SC pH perturbation impacts LEKTI inhibition on KLK5 and KLK7. In addition, it is unknown whether the described SC acidification in the present work leads to stimulation or inhibition of KLK. Nevertheless, in terms of functional assessment data, i.e. on SC integrity and cohesion (table 1), we propose that KLK5 and KLK7 were slightly inhibited by SC pH normalization, which in turn is associated with homeostatic desquamation and enhanced SC integrity/cohesion [23]. This mechanism seems to overlap with the KLK activation breakdown of the LEKTI-KLK complex. However, the exact interactions between the mentioned key factors were not addressed and are still unclear.

Aside from SC integrity and cohesion, our attention was drawn to tape stripplings in 8 of the 20 elderly, which
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revealed no immediate barrier recovery. In fact, the 
TEWL continued to increase within the first 24 h after perturbation. These findings revealed an age-related initial exacerbation of barrier damage not documented in young skin [11, 63, 64]. Barrier perturbation leads to enhanced expression of different primary cytokines, such as IL-1α, IL-1β, IL-6, and TNF-α [65], which, in turn, might be disturbed due to age-related subclinical changes in immune function known as inflamm-aging [66]. Further investigations should be performed to clarify the relationship between EPB function and immunosenescence.

Apart from the present investigation, a further link between inflammation and skin surface pH has been demonstrated [67] and is commonly accepted [68]. Maintenance of an acidic normal SC pH by topically applied lactobionic acid improved the epidermal barrier function in healthy mice [25] and in an AD mouse model [69]. Further, external acidification reduced cytokine generation and normalized antimicrobial peptide expression [69]. In another AD mouse model, SC pH neutralization in turn correlated with cutaneous inflammation and restoration of the SC pH shift was delayed [70]. Recently, Lee et al. [71] described an atopic march animal model and demonstrated that topical acidification (acidic cream pH 2.8) reduced AD-like skin lesions and interestingly inhibited respiratory allergic inflammation. Based on these murine studies, SC acidification seems to be relevant not only as a specific skin care regime but also as an approach to counteract the allergic march. The present study transferred experimental data from aged mice [12] into a real-life setting in aged nursing home residents. As far as AD is concerned, it seems necessary to verify these new insights [70, 71] in human studies.

The results of the microbiological investigations are not as clear as the results concerning EPB function. Many in vitro and in vivo investigations have demonstrated a relationship between skin surface pH and skin flora and the positive effect of a slightly acidic skin surface milieu [30–35]. Due to age-related microbiological variations [72–75], the question was raised of whether it is possible to stabilize the resident flora via long-term treatment with acidic products. No significant differences between groups A and B were detected either before or after treatment. The number of detected microorganisms (CFU/cm² of skin) increased after the treatment course compared to baseline in both groups; only the number of corynebacteria appeared to increase after treatment with pH 4.0 products. This could be interpreted as a pH-related enhancement in the resident microflora. Korting et al. [36] and Lambers et al. [30] described beneficial pH-related effects on *S. epidermidis* that were not observed in the present study. Apart from the mentioned effect on corynebacteria and the increase in bacterial diversity in group A, the microbiological results might only be tendencies and should be carefully interpreted. Therefore, questions concerning the impact of long-term pH 4.0 skin care treatment on microflora in the elderly remain unanswered. One conclusion is that the documented broad distribution (in CFU/cm² skin) in both groups and the lack of a control for various influence factors on skin flora, such as nutrition and clothing, are the reasons for the unclear microbiological results.

**Conclusions**

The present investigation transferred published experimental results for mice to a real-life situation in a nursing home. Long-term treatment with a pH 4.0 skin care range significantly improved the corneophysiology in aged skin, thereby helping to keep aged skin healthy. In treatment groups A (pH 4.0) and B (pH 6.0), skin hydration and the DASI were improved, but SC integrity and SC recovery were only accelerated in group A. The results led to the hypothesis that skin moisturizing in aged skin needs to be induced by skin care products that are more acidic than those available on the market and mostly used in German nursing homes [unpubl. data].

In addition to aged skin and AD, further inflammatory skin disorders, like seborrhoeic dermatitis [76] and acne [77], are accompanied by an elevated skin surface pH. Moreover, the skin surface pH is also higher in sensitive skin [78] and after sporting activities [79].

Thus, for these skin conditions, acidic skin care treatment can also be beneficial and should be evaluated via in vivo EPB measurements in humans. Finally, to maintain health in aged skin, we recommend adjusting skin care products for the elderly to a pH of 4.0.

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**Disclosure Statement**

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