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ORIGINAL RESEARCH

Maintenance of an Acidic Skin Surface with a Novel Zinc Lactobionate Emollient Preparation Improves Skin Barrier Function in Patients with Atopic Dermatitis

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ABSTRACT

Introduction: The skin of patients with atopic dermatitis (AD) is characterised by elevated pH. As a central homeostatic regulator, an increased pH accelerates desquamation and suppresses lipid processing, resulting in diminished skin barrier function. The aim of this study was to determine whether a novel zinc lactobionate emollient cream can strengthen the skin barrier by lowering skin surface pH.

Methods: A double-blind, forearm-controlled cohort study was undertaken in patients with AD. Participants applied the test cream to one forearm and a vehicle cream to the other (randomised allocation) twice daily for 56 days. Skin surface pH and barrier function (primary outcomes) were assessed at baseline and after 28 days and 56 days of treatment, amongst other tests.

Results: A total of 23 adults with AD completed the study. During and after treatment, a sustained difference in skin surface pH was observed between areas treated with the test cream and vehicle (4.50 ± 0.38 versus 5.25 ± 0.54, respectively, $p < 0.0001$). This was associated with significantly reduced transepidermal water loss (TEWL) on the test cream treated areas compared with control (9.71 ± 2.47 versus 11.20 ± 3.62 g/m^2/h, $p = 0.0005$). Improvements in skin barrier integrity, skin sensitivity to sodium lauryl sulphate, skin hydration, and chymotrypsin-like protease activity were all observed at sites treated with the test cream compared with the control.

Conclusion: Maintenance of an acidic skin surface pH and delivery of physiologic lipids are beneficial for skin health and may help improve AD control by reducing sensitivity to irritants and allergens.

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Keywords: Acid mantle; Atopic dermatitis; Eczema; Emollient; pH; Skin barrier; Protease

Key Summary Points
The barrier function of the skin, which prevents the entry of irritants and allergens, is impaired in atopic dermatitis.
Skin pH, which regulates the biochemical and functional properties of the skin, is elevated in atopic dermatitis.
This study aimed to assess whether pH changes in the skin induced by emollient use affect skin barrier function.
Following treatment with emollients at differing pH, skin barrier function is improved and skin sensitivity reduced at sites with a lower pH.
The pH of formulations used for eczema treatment could have an important role in determining the efficacy of treatment.

INTRODUCTION

Skin surface pH (pH_{SS}) varies between individuals and is influenced by skin pathology, age, gender and exposure to environmental factors such as washing regimes and treatment usage [1]. In healthy individuals, pH_{SS} is acidic, ranging from 4.1 to 5.8 [1]. Maintenance of an acidic stratum corneum (SC) pH (pH_{SC}) is crucial to skin homeostasis. Experimental modification of pH_{SC} has revealed significant impacts on lipid metabolism [2–5] and the activity of extracellular proteases involved in desquamation (the normal shedding of the uppermost mature corneocytes from the skin surface) [3–6]. Lipids released into the extracellular space from lamellar bodies produced by developing keratinocytes are incorporated into lamellar bilayer structures. Embedded within this lipid matrix are the terminally differentiated corneocytes; attached to one another through intercellular junctions called corneodesmosomes. This highly interconnected arrangement of flattened and cornified cells surrounded by structural lipids forms a barrier to water loss and to the entry of exogenous irritants and pathogens. pH_{SC} regulates the formation of this barrier, its protective function and its interaction with the microbiome [7–9].

Changes to pH_{SS} have been implicated in a number of inflammatory skin conditions, including atopic dermatitis (AD) [10–12]. AD is a multifactorial skin disease with both inherited and environmental components. It is likely that an underlying dysfunction of the skin barrier combined with heterogeneous environmental triggers, microbial dysbiosis and a dysregulated immune response all contribute to development of the clinical phenotype [13]. AD has a significant prevalence in many populations worldwide [14]. The chronic nature of the disorder and the prominent characteristics of skin lesion, persistent itch and sleep disturbance have a significant impact on psycho-social wellbeing and patient reported quality of life [15–18]. Increased pH_{SS} is observed in areas affected by dermatitis compared with unaffected skin in the same individuals [19]. Lowering pH_{SS} is therefore expected to alleviate barrier impairment and improve symptoms. Acidification of murine pH_{SC} improves skin barrier integrity, cohesion and barrier recovery following disruption [5]. Further evidence in mice also suggests that skin acidification could prevent primary AD development [20].

Emollients are widely used to treat skin dryness and to improve barrier function. However, many skincare products are formulated with a pH greater than 5.5. In a survey of commonly used emollient products, Shi et al. [21] found that more than half of the products had a pH greater than 5.5 and nearly a third had a pH greater than 6, outside the normal range for skin. Furthermore, unless treatment is formulated with sufficient buffering capacity, effects on pH_{SS} will be transient. To support optimal barrier function, alleviate dryness and maintain skin health, there is a requirement for skincare products to effectively moisturise the skin at a more favourable pH. The aim of this study was to determine whether acidification of the skin

with a novel zinc lactobionate emollient preparation (pH 4.0) results in improved barrier function.

METHODS

Study Design

The work presented here comprises a preliminary interventional study involving a single application of the test cream and a "Main study" involving repeated use of the test cream over a longer timescale.

Main Study

An interventional, within-participant (bilateral forearm) controlled, double-blind cohort study in patients with AD compared the effect of 8 weeks of skin treatment with the test cream compared with a vehicle cream. The study was conducted at the Sheffield Dermatology Research Skin Barrier Facility, The University of Sheffield Medical School, Sheffield, UK. The University of Sheffield Research Ethics Committee approved the study, under the project reference 019255. It was performed in accordance with the 1964 Declaration of Helsinki and its later amendments, and all subjects provided informed consent to participate.

The study period for each participant lasted approximately 65 days, made up of a 7-day washout period (for non-medicated, topical, leave-on products on the treatment areas) and a 58-day testing period. Participants were instructed in treatment application technique and completed the majority of applications within their own homes. Skin condition was assessed at baseline, 4 weeks and 8 weeks.

A sample size of 22 participants was targeted to give 80% power to detect a difference in transepidermal water loss (TEWL) of 2 $g/m^2/h$ assuming a within-subject standard deviation of 3.2 $g/m^2/h$. Volunteers were invited to participate in the study by open recruitment from the local community. Eligible participants had a self-reported history of eczema according to the 'UK working party diagnostic criteria' (current signs assessed by trained investigators under the supervision of a dermatologist); occurrence of an itchy skin condition within the past 12 months; pH_{SS} on the forearms above 4.75; were not receiving 'active' drug treatment for AD at the point of screening (emollient use considered inactive for the purposes of this study); treatment sites clear of lesional skin, tattoos, hyperpigmentation, obstructing the site; no use of systemic eczema treatment within 3 months, including biologics, cyclosporin, azathioprine, methotrexate, oral corticosteroids and mycophenolate; and no use of topical medication on the treatment areas within 1 month including topical corticosteroids and topical calcineurin inhibitors.

The Intervention

The products, composed of an unmedicated test cream and a vehicle cream, were supplied by Hyphens Pharma in identical plain packaging (Table 1). The test cream (LCP) was composed of an emollient base with a zinc lactobionate buffering system, glycerin and physiologic lipids (ceramide, free fatty acid and cholesterol) at pH 4.0. The vehicle was made up of the same base emollient with a pH adjusted to 7.0. Participants were trained to accurately measure a finger-tip unit (FTU) of treatment and instructed to apply 2 FTU twice daily to the whole volar surface of the forearm from the elbow crease to the wrist (the treatment area). The test cream was applied to one forearm and the vehicle to the other. Participants recorded treatment use in a diary. During visits participants were observed in the use of treatments and provided with additional training and guidance where necessary. Participants were instructed to apply treatments 12 h before the skin assessment visits and to avoid applying treatments before washing.

As commonly used wash products can affect the pH of the skin, we asked participants to use a standardised wash product (Simple bar soap, Unilever, London, UK) on their arms for 7 days before baseline assessments and throughout the study period.

Table 1 Investigational products

Name	Brand and manufacturer	Ingredients
Test cream/ LCP	Ceradan® Advanced, Hyphens Pharma	Water, hydrogenated polydecene, propylene glycol, **lactobionic acid**, behenyl alcohol, PEG-20 methyl glucose sesquistearate, **glycerin**, **hydroxypropyl bispalmitamide mea**, myristyl alcohol, polyacrylate-1 crosspolymer, methyl glucose sesquistearate, citric acid, **cholesterol**, **linoleic acid**, phenoxyethanol, ethylhexylglycerin, sodium hydroxide, **zinc oxide**. **pH 4.0** *Active ingredients in bold
CP	Hyphens Pharma	Water, hydrogenated polydecene, propylene glycol, polyacrylate-1 crosspolymer, **lactobionic acid**, PEG-20 methyl glucose sesquistearate, behenyl alcohol, methyl glucose sesquistearate, myristyl alcohol, citric acid, phenoxyethanol, **zinc oxide**, ethylhexylglycerin. **pH 3.2** *Active ingredients in bold
Vehicle cream	Hyphens Pharma	Water, hydrogenated polydecene, propylene glycol, behenyl alcohol, PEG-20 methyl glucose sesquistearate, myristyl alcohol, polyacrylate-1 crosspolymer, methyl glucose sesquistearate, citric acid, phenoxyethanol, ethylhexylglycerin, sodium hydroxide. pH 7.0
A	Atopiclair® cream, Menarini	Aqua, ethylhexyl palmitate, butyrospermum parkii butter, pentylene glycol, arachidyl alcohol, behenyl alcohol, arachidyl glucoside, butylene glycol, glyceryl stearate, glycyrrhetinic acid, capryloyl glycine, bisabol, tocopheryl acetate, PEG-100 stearate, carbomer, ethylhexylglycerin, piroctone olamine, sodium hydroxide, allantoin, DMDM hydantoin, sodium hyaluronate, vitis vinifera seed extract, disodium EDTA, ascorbyl tetraisopalmitate, propyl gallate, telmesteine
B	Basic Aqua cream, ICM Pharma	Purified water, white soft paraffin, cetostearyl alcohol, ceteareth-20, liquid paraffin, phenoxyethanol
C	Cetaphil® Restoraderm body moisturiser, Galderma	Aqua, glycerin, caprylic/capric triglyceride, helianthus annus seed oil, pentylene glycol, butyrospermum parkii butter, cyclopentasiloxane, cetearyl alcohol, sorbitol, behenyl alcohol, glyceryl stearate, allantoin, arginine, caprylyl glycol, ceteareth-20, cetyl alcohol, citric acid, dimethiconol, disodium EDTA, disodium ethylene dicocamide PEG-15 disulfate, glyceryl stearate citrate, hydroxypalmitoyl sphinganine, niacinamide, panthenol, sodium hyaluronate, sodium PCA, sodium polyacrylate, tocopheryl acetate

Single Application Test

A cohort of 10 volunteers with AD was recruited in January 2019 (same eligibility criteria as described above without the minimum pH_{SS} criterion). The forearms (volar face) were divided into three test sites of 4 × 5 cm each. Each test site received a 100 µl application of a single product (Table 1), or no treatment (control). Randomised site allocation was utilised to minimise site-dependent effects using a randomisation list generated at www.randomization.com. Product identities were concealed from the investigator and participant by assigning each a letter code. The pH_{SS} of the test sites was determined before and at set timepoints after treatment application. Participants were asked to refrain from washing the test sites until completion of the study.

Biophysical Measurements and Visual Assessments

Skin assessment procedures were performed in a room maintained at 21 ± 2 °C and 35–55% relative humidity. All test sites were acclimatised to room conditions for 20 min before assessment. Test sites were assigned visual scores for dryness (5-point scale from 0 to 4) and redness (4-point scale from 0 to 3). Hydration was measured using a Corneometer CM825 (CK electronic GmbH, Cologne, Germany). TEWL was measured using an AquaFlux AF200 condensing chamber probe (Biox Systems Ltd, London, UK). pH_{SS} was determined using a Skin-pH-Meter 905 (CK electronic GmbH, Cologne, Germany).

To determine skin barrier integrity, TEWL measurements were collected after skin tape stripping (STS). This process involved application to the skin of 20 D-Squame discs (Clinical & Derm, Dallas, USA). Each disc was applied to the test site and an even pressure applied to the disc for 5 s using a D-Squame pressure instrument (Clinical & Derm, Dallas, USA). After application, D-Squame discs were collected and the quantity of protein adhering to the disc was measured by assessing light transmission through the disc using a D-Squame scan 850A (Clinical & Derm, Dallas, USA). The total amount of protein collected was used to assess skin cohesion and to normalise protease activity.

Skin Sensitivity Testing

Skin sensitivity was assessed by exposure of the skin to 1% sodium lauryl sulphate (SLS) under occlusion. Participants were instructed to remove SLS patches after 24 ± 2 h, rinse the sites briefly in water and then avoid washing before the skin sensitivity assessments at 48 ± 2 h after initial application. Erythema was assessed by visual redness scoring at the patch site, collection of absorbance measurements with the Mexameter MK18 (CK electronic GmbH, Cologne, Germany) and by image capture with a colour-calibrated CCube dermoscope (Pixience, Toulouse, France). Skin barrier disruption was assessed by measuring TEWL.

Caseinolytic and Chymotrypsin-Like Protease Activity

Caseinolytic and chymotrypsin-like protease activities were evaluated at three depths within the SC (discs 1–3, 4–6 and 7–9). For the caseinolytic assay, each sample was incubated for 2 h with 10 µg/ml of the quenched, fluorescent, protease substrate, Bodipy FL casein (Thermo Fisher Scientific, Waltham, MA, USA) in 0.5% Triton-X 100. A wide range of metallo-, serine, acid and sulfhydryl proteases are capable of digesting this substrate, releasing highly fluorescent BODIPY FL peptides. Samples for the chymotrypsin-like activity assay were incubated for 2 h in a 50 µM solution of the fluorogenic peptide MeO-Succ-Arg-Pro-Tyr-AMC (Peptide Protein Research Ltd, Southampton, UK). Reactions were stopped with 10% acetic acid and fluorescence measured at 535 nm (BODIPY FL) and 460 nm (AMC) using the Hidex Sense fluorimeter (LabLogic Systems, Sheffield, UK).

Statistical Analysis

The primary outcomes were pH_{SS} and TEWL (skin barrier function). All statistical tests were

performed using Graphpad Prism v9.3.1 (Graphpad Software Inc.; La Jolla, CA, USA). Population means were compared using a paired *t*-test for continuous data; a Wilcoxon signed rank test for ordinal data (visual scores); and a two-way ANOVA with Šídák's post-test to evaluate the modulation of treatment and SC depth on protease activity. The significance threshold was set at $p = 0.05$.

RESULTS

A preliminary single-application test was performed to verify the capability of the experimental formulation to reduce pH_{SS} compared with a panel of widely used reference products. Two variants were included: CP, an emollient cream formulation with a novel zinc lactobionate buffering system (pH 3.2), and LCP cream, based on CP and including skin humectants (glycerin) and lipids (free fatty acids, ceramide and cholesterol) buffered to pH 4.0. Both test formulations markedly reduced pH_{SS}, bringing it down from an average of 4.49 ± 0.38 to 4.03 ± 0.09 and 3.58 ± 0.14 pH units for LCP and CP, respectively, 3 h post-application (Fig. 1). This was a significant reduction in pH_{SS} compared with the untreated site of −0.41 ($p = 0.019$) and −0.86 ($p = 0.0007$) for LCP and CP. In contrast, the reference emollient creams either had no effect (Product B, 0.13, $p = 0.59$) or significantly increased pH_{SS} (Product A, 0.72, $p < 0.0001$; Product C, 0.76, $p < 0.0001$) 3 h post-application. This trend was observed at both 6 h and 12 h after product application, highlighting the profound and contrasting effects of topical treatments on pH_{SS}. Although CP showed a greater decrease in pH, LCP was taken forward for further testing in a human interventional study to avoid excessive acidification of the SC.

For the main study, recruitment was open from October 2019 to March 2022, and 25 eligible participants were randomised and commenced treatment, of which 23 participants completed all study procedures and 21 were included in the analysis following blind review (Supplementary Materials Figure S1 and Table 2 for demographics).

Over the 56-day treatment period, average daily use of the test cream (LCP) was 2.11 ± 0.53 g (range 1.51–2.75 g) compared with 2.03 ± 0.53 g (range 1.42–2.76 g) of vehicle. Both treatments were well tolerated. There were eight adverse reactions of mild intensity possibly related to the interventions (four assigned to both treatments and four to the vehicle only, Supplementary Materials Table S1). Visual signs of erythema at baseline were assessed as mild/slight or less and were either unchanged or improved at the end of treatment at 93% of all sites, so no statistical analysis was undertaken (Supplementary Materials Table S2). Prior to treatment application there was no significant difference in pH_{SS} of the forearms randomised to treatment with either the test cream or vehicle (4.83 ± 0.35 versus 4.79 ± 0.37, respectively, $p = 0.75$, Fig. 2a). After 4 weeks of treatment (12 h after the last application of product) the pH_{SS} at sites treated with the test cream had decreased to 4.47 ± 0.31, whereas those treated with the vehicle increased to 5.26 ± 0.45 ($p < 0.0001$). Very similar results were reported after 8 weeks of treatment (Fig. 2a).

To assess the effects of the study treatments on skin barrier function, TEWL measurements were made at the same timepoints. At baseline there was no difference in TEWL between the two treatment areas (10.44 ± 3.18 versus 10.39 ± 2.74 g/m^2/h, $p = 0.99$, Fig. 2c). TEWL was correlated with pH_{SS} ($r = 0.46$ $p < 0.0001$, Fig. 2d). After 4 weeks of treatment with the test cream, TEWL decreased slightly (10.11 ± 2.56 g/m^2/h), whereas TEWL increased (11.61 ± 3.62 g/m^2/h) at sites treated with the vehicle (treatment comparison, $p = 0.008$). This difference in TEWL between treatment areas widened after a further 4 weeks of treatment (9.71 ± 2.47 versus 11.2 ± 3.62 g/m^2/h, $p = 0.0005$).

A corneometer was used to assess SC hydration. At baseline there were no differences in SC hydration between the treatment areas (34.09 ± 5.85 versus 35.25 ± 5.34 AU, respectively, $p = 0.29$, Fig. 2e). After 4 weeks of treatment, SC hydration had increased to 40.39 ± 6.38 AU on test-cream-treated sites and to 38.23 ± 6.97 AU on vehicle-treated sites

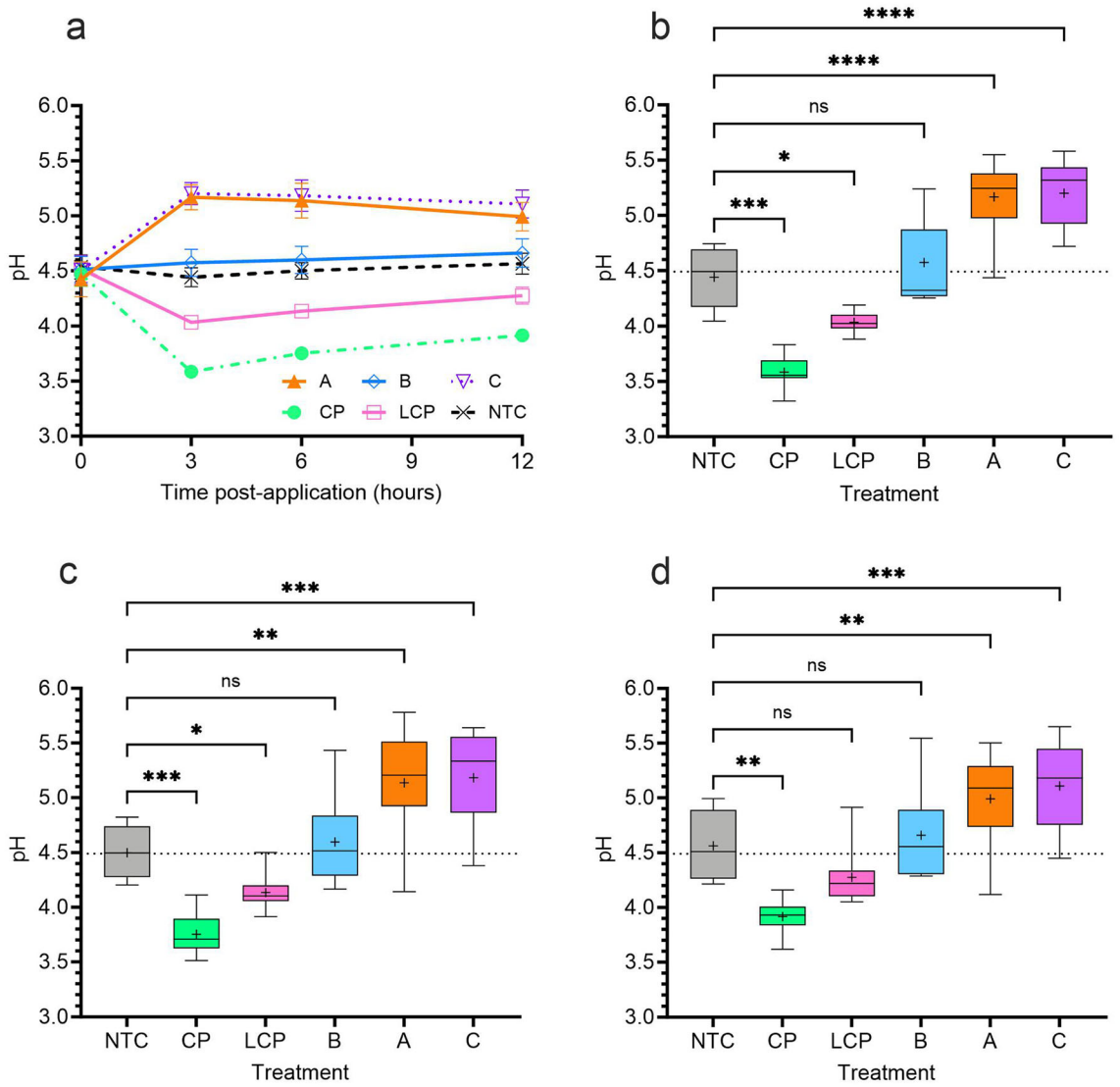


Fig. 1 The effect of a single application of the study products on pH_{SS}. **a** pH_{SS} up to 12 h post-application. The mean is shown with error bars indicating SEM. **b** pH_{SS} 3 h, **c** 6 h and **d** 12 h post-treatment. One-way ANOVA revealed a significant difference between treatments at all post-application timepoints ($p < 0.0001$). Significance values show the results of Dunnet's post-test for pairwise comparisons between treatments and the untreated control. Boxes represent the interquartile range, whiskers show the range, median is indicated as a horizontal line, '+' denotes the mean. *ns* not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. The dotted line indicates the baseline mean for all measurements. *NTC* no treatment control, *pHss* skin surface pH, *SEM* standard error of the mean, *ANOVA* analysis of variance

(treatment comparison, $p = 0.25$). Following 8 weeks of treatment, SC hydration was higher at sites treated with the test cream compared with sites treated with vehicle (40.97 ± 7.59 versus 38.01 ± 6.85 AU, respectively, $p = 0.024$).

To characterise the response to chemical irritants, the pre-treated test sites were exposed to SLS under occlusion for 24 h. Twenty four hours after patch removal, there was an increase in TEWL at all sites exposed to SLS (Fig. 3a). TEWL was lower at sites treated with the test

Table 2 Cohort demographics

Demographic	*n*
Sex	
Female, *n* (%)	14 (61%)
Male, *n* (%)	9 (39%)
Age	
Mean ± SD (min, max)	38 ± 15 (20, 69)
Ethnicity	
White British, *n* (%)	16 (70%)
White other, *n* (%)	2 (9%)
Asian-Indian, *n* (%)	2 (9%)
Asian-Bangladeshi, *n* (%)	1 (4%)
Chinese, *n* (%)	1 (4%)
Mixed-white and Black Caribbean, *n* (%)	1 (4%)
Eczema status[a]	
Current/clear, *n* (%)	8 (35%)
Current/visible, *n* (%)	15 (65%)

SD Standard deviation
[a]Self-reported

cream compared with those treated with vehicle (35.39 ± 14.09 versus 40.82 ± 12.43 g/m^2/h, respectively, $p = 0.0057$), suggesting a protective effect of the test cream. Furthermore, erythema was greater on the vehicle-treated sites (25/42 participants with median score of 2, moderate erythema) compared with the test cream (30/42, median score of 1, mild erythema, $p = 0.0094$, Fig. 3b). Objective measures of erythema (Erythema Index and Mexameter redness) confirmed this difference in SLS response (Fig. 3c).

To investigate the structural integrity and cohesiveness of the SC after 8 weeks of treatment, STS was combined with TEWL and quantification of protein removed, respectively. After the removal of 20 discs, TEWL was lower at sites treated with the test cream compared with sites treated with vehicle (31.55 ± 18.34 versus 36.9 ± 18.24 g/m^2/h, respectively, $p = 0.014$, Fig. 3d). The amount of protein removed was also lower at sites treated with the test cream compared with vehicle (343.6 ± 67.23 versus 359.3 ± 63.02 ug/cm^2, respectively, $p = 0.045$, Fig. 3e). Higher SC integrity and cohesion suggest an increased resistance to physical damage at sites treated with the test cream.

The activity of proteases at the skin surface contributes to desquamation and turnover of the healthy skin barrier. We sought to characterise the effect of treatment on caseinolytic and chymotrypsin-like protease activity at increasing depths within the SC. After 8 weeks of treatment, there was no difference in the caseinolytic activity of samples from the different treatment areas (Fig. 4a). Chymotrypsin-like protease activity was lower at sites treated with the test cream compared with vehicle ($p = 0.031$, Fig. 4b). A significant pairwise difference between the treatments was present in the most superficial sample (STS discs 1–3: test cream, 1.75 ± 1.58 versus vehicle, 2.66 ± 1.92 nU/µg, $p = 0.017$).

A subset of participants were asked to complete a supervised treatment application at the end of the 4-week assessment visit and invited to return for a repeat of the measurements after 6 h. At 6 h post-application, the pH_{SS} of the skin treated with the test cream was 4.18 ± 0.40, significantly lower than the vehicle-treated sites (5.65 ± 0.20, $p < 0.0001$, Fig. 2b). Hydration was significantly greater at sites treated with the test cream (44.14 ± 8.15 versus 37.09 ± 8.12 AU, $p = 0.0084$, Fig. 2f).

DISCUSSION

pH_{SC} is an important regulator of skin barrier function. Here we show that a new zinc lactobionate formulation (pH 4.0) containing skin lipids helps maintain an optimum pH and strengthen the skin barrier compared with a vehicle control (pH 7.0). Treatment with the new formulation also resulted in a more hydrated SC and a reduced sensitivity to irritation.

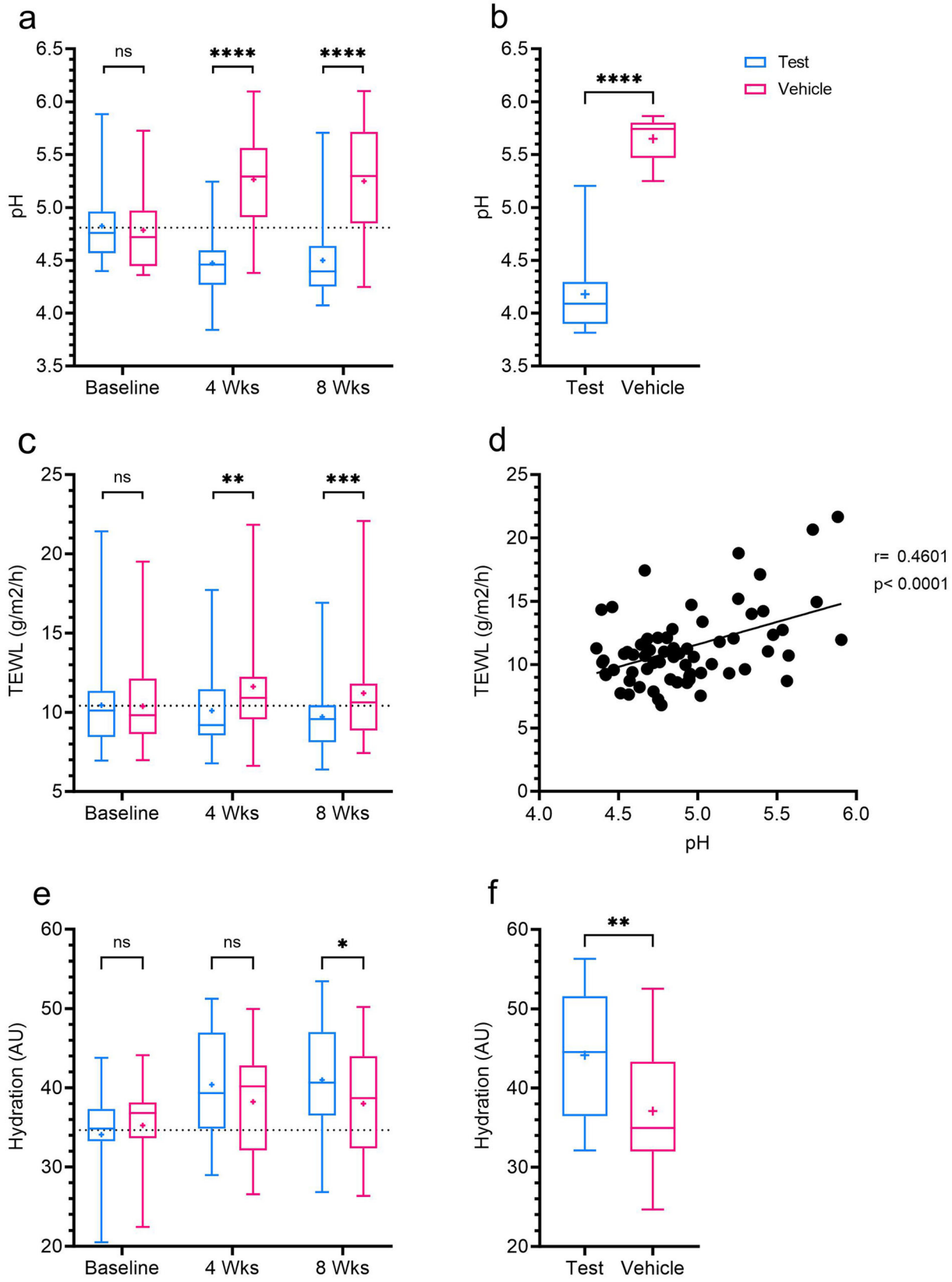
Topical Acidification of the Skin Barrier

Average pH_{SS} for the cohorts presented here, comprising participants with a recent history of AD (uninvolved areas), was below 5.0. A number of studies have reported pH_{SS} substantially above 5.0 in similar cohorts [10, 11, 19, 22]. Nevertheless, our observations, undertaken in accordance with published methodology [23], are consistent with our earlier work [24] and may reflect differences in cohort demographics and/or regional differences such as tap water pH or washing practices. Despite the relatively low pH_{SS} at baseline, treatment with the test cream and vehicle induced a significant and sustained difference in pH_{SS} between the treatment sites. The vehicle was formulated to match the pH of many widely available emollients used for eczema treatment [21] and exhibited similar effects on pH_{SS} to the reference products included here in the single application test. Importantly, the average pH_{SS} of vehicle-treated skin after 8 weeks (pH 5.25) was within the expected range for patients with AD. Relative to the vehicle, the test cream maintained a significantly reduced pH_{SS} (after 4 weeks of treatment we observed a 1.47 unit reduction 6 h post-application reducing to a 0.75 unit difference 12 h post-application, whereupon the next application was due). The difference of 0.75 represents > fivefold increase in the concentration of H^+ ions, which is expected to have a significant impact on the biochemical activity and functional performance of the SC [1, 25]. The scale of change in pH was greater than previously reported for topical preparations with a low pH [26–30] and the final pH reached was substantially lower (with a much greater decrease relative to the reported pH for AD-affected skin), suggesting a greater potential to normalise skin barrier function.

Maintenance of an Acidic SC Improves Skin Barrier Function

TEWL reflects the ability of the permeability barrier formed by the intact SC to prevent excessive water loss from the skin and correlates with the severity of AD [31, 32]. Remarkably, we observed a significant difference in TEWL of 1.49 $g/m^2/h$ between skin treated with the test cream and vehicle. A difference in basal TEWL was not observed under similar usage conditions with previously reported 'acidic' topical emollient creams [26, 28, 30], most likely reflecting the greater capacity for the formulation tested here to maintain a lower pH_{SC}. The scale of change in TEWL is comparable to previously reported differences (1.5–3.92 gm^2h) observed between patients with AD and controls [11, 32–35]. Although basal TEWL is a useful biomarker of skin health, challenge to the barrier by physical or chemical disruption is required to fully characterise its integrity and function.

Differences in barrier integrity measured with tape stripping have been identified in comparisons of patients with AD with and without filaggrin mutations [35] and between patients with AD with co-morbid food allergy and those without food allergy [36]. In response to tape stripping, the increased barrier integrity and greater cohesion of the skin barrier observed in this study at the more acidic sites is consistent with other studies in which pH_{SS} has been manipulated [4, 5]. A reported difference of 0.4 pH units in lightly pigmented skin compared with darkly pigmented skin correlated with improved skin structure and function in the group with a lower pH [37]. Similarly, a 0.35 pH unit difference between groups of elderly care home residents induced by 7 weeks of treatment with skincare products adjusted to either pH 4 or pH 6 resulted in significantly greater barrier integrity, cohesion and recovery following STS in participants in the low pH treatment group compared with the high pH treatment group [28]. These findings were confirmed in a similar healthy, aged cohort using a different formulation, yet neither preparation reached a pH_{SS} below 5.0 despite a formulation pH of 4 [26]. A lower buffering capacity of these formulations would explain why we see a stronger response to the test formulation under investigation here [38]. Consistent with improved barrier function, these interventions to reduce skin surface pH also improve the hydration status of the skin [28, 29] in


a
ns
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pH
6.5
6.0
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TEWL (g/m2/h)
25
20
15
10
5
Baseline
4 Wks
8 Wks
d
TEWL (g/m2/h)
r= 0.4601
p< 0.0001
4.0
4.5
5.0
5.5
6.0
pH
e
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*
Hydration (AU)
60
50
40
30
20
Baseline
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8 Wks
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Hydration (AU)
Test
Vehicle

◀**Fig. 2** Biophysical properties of the skin surface are modified by use of the study products. pH_{SS} (**a**) TEWL (**c**) and hydration(**e**) at baseline, after 4 weeks and after 8 weeks of study treatment, 12 h post-application. A significant interaction between treatment and time was established in a two-way ANOVA ($n = 21$); **a**, $p < 0.0001$; **c**, $p = 0.0025$; **e**, $p = 0.005$. Significance values show the results of Šídák's post-test for pairwise comparisons between treatments at each timepoint. pH_{SS} (**b**) and hydration (**f**) after 4 weeks of treatment, 6 h post application. Significance values show the result of a paired *t*-test, $n = 10$. **d** Pre-treatment correlation between pH_{SS} and TEWL, r indicates Pearson correlation coefficient. Boxes represent the interquartile range, whiskers show the range, median is indicated as a horizontal line, '+' denotes the mean. *ns* not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. The dotted line indicates the baseline mean for all measurements. *wks* weeks, *AU* arbitrary units, *pHss* skin surface pH, *TEWL* transepidermal water loss; *ANOVA* analysis of variance

agreement with the increase in hydration observed in this study.

The composition and structural integrity of the lipid lamellae is a critical factor determining permeability barrier function [4, 39, 40]. Kilic et al. [30] reported that a 0.5-unit decrease in pH between skin sites treated for 4 weeks with water-in-oil emulsions was sufficient to increase the abundance of ceramides and enhance intercellular lipid organisation. We see a greater pH change here, suggesting significant changes to lipid structure within the SC. Improved barrier function (reduction in TEWL) seen here with the test formulation supports improved lipid lamellae structure. To support optimum lipid metabolism, the test product also contains a range of skin lipids to contribute to improved skin barrier function.

pH-Mediated Suppression of Protease Activity

Consistent with the changes in structural integrity, cohesion between corneocytes in the SC appears to weaken as pH becomes more neutral, resulting in larger amounts of protein being removed by tape stripping. Analysis of corneodesmosome density by electron microscopy and quantification of corneodesmesomal protein DSG-1 suggests a decrease in these structures at higher pH [4]. These cell-to-cell junctions are degraded by proteases – such as the desquamatory serine proteases of the kallikrein (KLK) family – which exhibit optimal activity at neutral pH [6, 41, 42]. Accordingly, superbases have been used experimentally to neutralise the skin surface in mice, achieving a pH_{SC} of 7 [3] and resulting in robust protease activation. pH_{SC} affects both the enzymatic protease activity and the abundance of catalytically active protease [5]. The ex vivo assay used here reflects catalytically active protease abundance and revealed a significant difference in the chymotrypsin-like activity, attributed to KLK7, of the most superficial layers of the SC dependent on treatment, despite relatively low activity overall. This suggests that SC acidification brought about by the test cream suppresses desquamatory activity, leading to the retention of corneocytes and greater structural integrity of the SC. Suppression of KLK7 activity and abundance is therefore likely to contribute to the positive effects of skin acidification on the skin barrier.

Protection from Irritation

Beyond water permeability, a key function of the healthy skin barrier is to prevent the entry of exogenous allergens, irritants and pathogens into the skin. We used exposure to the known irritant SLS to test this function of the skin barrier. Treatment areas pre-treated with the test cream showed less visible signs of inflammation and reduced barrier perturbation compared with areas treated with the vehicle. These findings are commensurate with earlier work showing that topically applied alpha-hydroxy acids (AHA) can reduce skin sensitivity to SLS [43]. Unfortunately, the application of AHA such as lactic acid can cause stinging, an adverse effect that we did not observe here using polyhydroxy acid lactobionic acid [44]. This further supports the conclusion that the permeability barrier is strengthened by the test cream at reduced pH. Contact with irritants is a well-known trigger for dermatitis, suggesting that

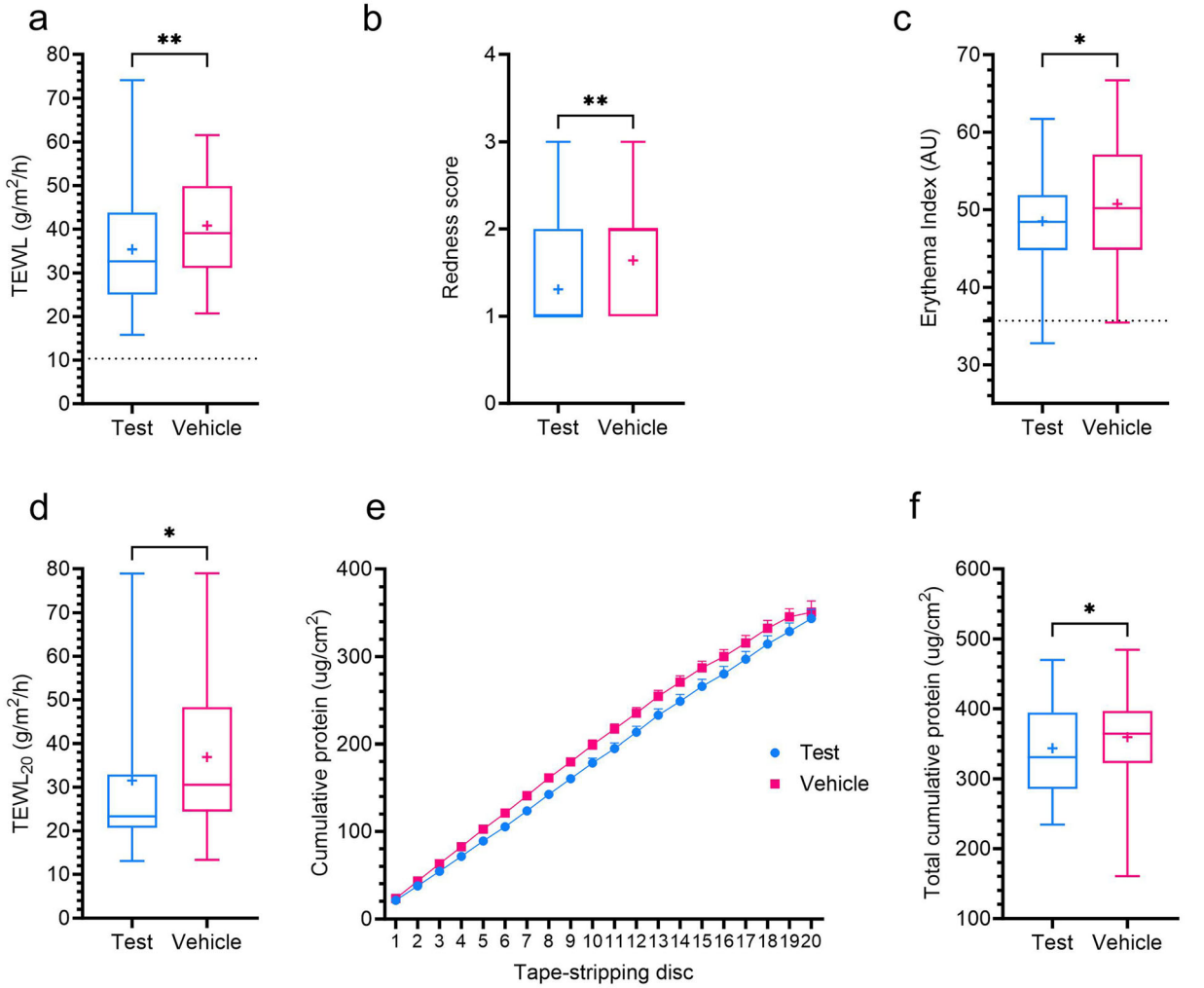

Fig. 3 After 8 weeks of treatment with the study products, skin sensitivity, skin barrier integrity and skin barrier cohesion differ depending on treatment. Following 24 h exposure to SLS, differences between treatment sites were observed in **a** TEWL (g/m^2/h), $n = 42$, **b** visual redness score (subjective score from 0 to 3), $n = 42$ and **c** Erythema Index (AU), $n = 40$. STS reveals treatment site differences in **d** $TEWL_{20}$ (barrier integrity), $n = 42$. **e** Cumulative amount of protein removed, mean ± SEM, $n = 42$. **f** Total amount of protein removed (skin barrier cohesion), $n = 41$. Significance values show the results of a paired *t*-test (panels **a**, **c**, **d**, and **f**) or Wilcoxon signed rank test (panel **b**). Boxes represent the interquartile range, whiskers show the range, median is indicated as a horizontal line, '+' denotes the mean. *ns* not significant, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. The dotted line indicates the pre-test mean for all measurements. *AU* arbitrary unit, *TEWL* transepidermal water loss, *SLS* sodium lauryl sulphate; *STS* skin tape stripping, $TEWL_{20}$, TEWL after 20 consecutive tape strips, *SEM* standard error of the mean

prophylactic use of a low pH barrier strengthening treatment could be a useful strategy for preventing flares.

Limitations and Future Work

The participant cohort completing this study is relatively small in number, which impacts upon the generalisability of the study conclusions. However, the study cohort reflects the

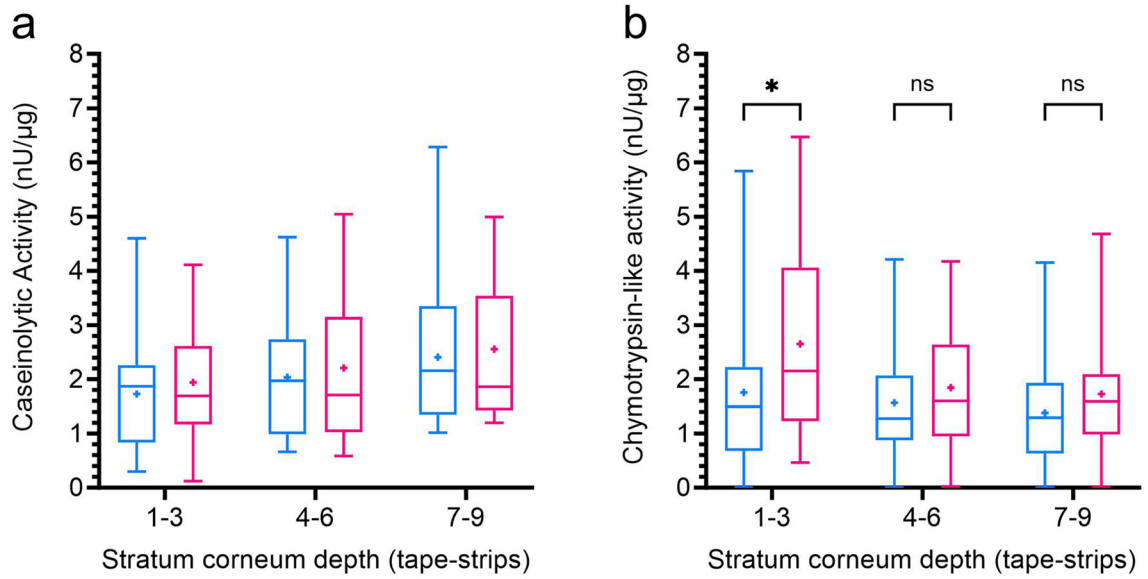

Fig. 4 Protease activity after 8 weeks of treatment. **a** Caseinolytic protease activity at three depths through the stratum corneum. Sampling depth ($p < 0.0001$) but not treatment ($p = 0.25$) was a significant factor in a two-way ANOVA ($n = 21$), the interaction between treatment and sampling depth was not significant ($p = 0.84$). No pairwise comparisons were made. **b** Chymotrypsin-like activity at three depths through the stratum corneum. Treatment ($p = 0.031$) and sampling depth ($p = 0.02$) were significant factors in a two-way ANOVA ($n = 21$), the interaction between treatment and sampling depth was not significant ($p = 0.3$). Significance values show the results of Šídák's post-test for pairwise comparisons between treatments at each sampling depth. Boxes represent the interquartile range, whiskers show the range, median is indicated as a horizontal line, '+' denotes the mean. *ns* not significant, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. *ANOVA* analysis of variance

demographic range of the wider UK population well; with a significant age range (20–69 years), near equal proportions of male and female participants and representation from a range of ethnic groups. None of the participants were receiving clinical care for AD at the time of the study and were not using 'active' drug treatments. Non-medical investigators confirmed that assessment sites were clear of visible inflammation and skin breakdown during screening. Further work should address the use of skin acidification in individuals with more severe eczema, particularly at lesional sites where baseline skin pH is likely to be higher. Given the impact of skin pH on the resident microbiome, it would be useful to examine whether use of this treatment in a high pH perilesional site can reduce the load of pathogenic bacterial species and normalise the broader microbiome, compared with a product used to treat AD with a higher pH.

The choice of comparator treatment for any study of a topical preparation is inherently affected by trade-offs, as it is rarely possible to study the effects of individual ingredients in isolation and the outcomes are dependent on the complete formulation. We chose to use a vehicle without key ingredients to show that delivery of these components at an acidic pH leads to improved skin properties. The observations we made are therefore consistent with an effect of barrier modification driven by a change in pH. However, we cannot quantify the contribution of other ingredients individually, such as the physiologic lipids, to the changes in barrier performance. Whilst evidence clearly supports a role for pH_{SS} in skin hydration, it is important to note that the test cream contains the humectant glycerin, which is a well-established skin moisturiser, in addition to other moisturisers (i.e. ceramide). Both the vehicle and test cream contain the moisturiser and

penetration enhancer propylene glycol, and so moisturisation is achieved here by multiple mechanisms.

CONCLUSION

The majority of clinical studies which have manipulated skin pH using an emollient have targeted a cohort of older participants [26–30] to address the increase in pH and xerosis that occurs in aged populations [45]. This study offers insight into the use of an acidifying emollient in patients with AD.

The regular application of the test cream used in this study brought about a physiologically relevant reduction in pH and improved skin barrier function compared with a vehicle. Improved hydration and decreased protease activity suggest that use of a skincare product containing humectants and physiologically relevant lipids at pH 4 can contribute positively to skin health and potentially help improve disease control for patients with AD. The pH and buffering capacity of a topical preparation are likely to have a significant impact on the biophysical effects of that treatment. Therefore, consideration should be given to the pH of a preparation when formulating and prescribing treatments to address skin conditions with a known barrier impairment. Recent evidence has linked KLK7 with chronic itch in a murine model of AD [46]. Reducing the activity of this protease through modification of skin pH is an attractive possibility for reducing the burden of pruritus in patients with AD.

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Data Availability. The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest. Simon G Danby, has received fees for giving lectures and/or attending advisory boards and research funding from Almirall, Astellas Pharma, Bayer Dermatology, Hyphens, Leo Pharma, L'Oreal, MSD, Pfizer, Rohto Pharma, Sanofi and Stiefel-GSK. Michael J Cork, has been/is a Clinical Trial Investigator for the following organisations: Atopix, Galapagos, Hyphens, Johnson & Johnson, Kymab, Leo, L'Oreal/La Roche Possay, Novartis, Pfizer, Regeneron, and Sanofi-Genzyme. He is an Advisory Board member, Consultant &/or invited lecturer for the following organisations: Abbvie, Amlar, Astellas, Atopix, Boots, Dermavant, Galapagos, Galderma, Hyphens, Johnson & Johnson, Kymab, Leo, L'Oreal/La Roche Possay, Menlo, Novartis, Oxagen, Pfizer, Procter & Gamble, Reckitt Benckiser, Regeneron, Sanofi-Genzyme. Paul V Andrew, Abigail Pinnock, Anna Poyner, Kirsty Brown, John Chittock, Linda J K Kay declare that they have no competing interests.

Ethical Approval. The University of Sheffield Research Ethics Committee approved the study, under the project reference 019255. It was performed in accordance with the Helsinki Declaration of 1964, and its later amendments, and all subjects provided informed consent to participate.

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