Enhancement of stratum corneum lipid structure improves skin barrier function and protects against irritation in adults with dry, eczema-prone skin*

Simon G. Danby iD,1 Paul V. Andrew,1 Linda J. Kay,1 Abigail Pinnock,1 John Chittock iD,1 Kirsty Brown,1 Samuel F. Williams1 and Michael J. Cork iD1,2,3

1Sheffield Dermatology Research, Department of Infection, Immunity & Cardiovascular Disease, University of Sheffield Medical School, Sheffield, UK
2Sheffield Children’s NHS Foundation Trust, Sheffield Children’s Hospital, Western Bank, Sheffield, UK
3Sheffield Teaching Hospitals NHS Foundation Trust, The Royal Hallamshire Hospital, Sheffield, UK

Linked Comment: A.C. Kendall and A. Nicolaou. Br J Dermatol 2022; 186:764–765.

Summary

Background The skin of patients with atopic dermatitis is characterized by abnormal stratum corneum lipid levels. Consequently, the lamellar matrices are disrupted and skin barrier function is diminished, increasing skin sensitivity to irritants and allergens.

Objectives To determine whether a cream containing ceramides, triglycerides and cholesterol in a multivesicular emulsion can reinforce the skin barrier and protect against skin irritation.

Methods A randomized observer-blind intrapatient-controlled study in 34 adults with dry, eczema-prone skin was conducted. Each participant underwent 4 weeks of treatment with the test cream on one forearm and lower leg and a reference emollient cream on the other. Skin properties were determined before and after treatment. Lipid structure was assessed by Fourier-transform infrared spectroscopy using a novel interface.

Results Skin barrier integrity was greater at sites treated with the test cream [effect size for area under the transepidermal water loss curve C0 162, 95% confidence interval (CI) 206 to 118]. Skin sensitivity to sodium lauryl sulfate was reduced (C0 0 5 points visual redness, 97.57% CI 1 00 to 0 25), as was transepidermal water loss (C0 15 3 g m⁻² h⁻¹, 95% CI 20 3 to 10 4) compared with the reference. Sites treated with the test cream displayed enhanced lipid chain ordering, which was significantly associated with skin barrier integrity (r = 0.61). Compared with the reference, treatment with the test cream increased hydration (8.61 capacitance units, 95% CI 6.61–10.6) and decreased signs of dryness.

Conclusions The test cream facilitates skin barrier restoration and protects the skin from dryness and irritation. Compared with a commonly prescribed emollient in the UK, the test cream is highly suited to the management of dry, sensitive skin.

*Plain language summary available online

DOI 10.1111/bjd.20955

The skin barrier (SB) is a physical permeability barrier that prevents the loss of moisture and protects the body from the external environment. It is formed of the stratum corneum (SC), a defensive structure comprising corneocytes interwoven...
Enhancement of SC lipid structure improves skin barrier function, S.G. Danby

Enhancement of SC lipid structure improves skin barrier function, S.G. Danby

within a complex mixture of lipids (the lipid lamellae). These lipids include equimolar concentrations of cholesterol, ceramides and free fatty acids, arranged in multilamellar membrane sheets. The composition and structure of the lipid lamellae are vitally important for permeability barrier function.

In atopic dermatitis/eczema (AD) there are significant changes in the composition of lipids in the SC, including reduced levels of ceramides EOS, EOH and NP, increased ceramide AS at the species level and a general shift towards ceramides with short chain lengths at the subspecies level. These changes result in defective formation of the lipid lamellae and the corneocyte lipid envelope, and were found to correlate with xerosis. Reductions in skin-surface lipids, particularly key ceramides, in healthy infants have been correlated with decreased skin hydration. Furthermore, lipid levels are reduced in people over the age of 60 years compared with young adults, and can be associated with an increased incidence of skin dryness.

Topical supplementation with physiological lipids is a potentially important mechanism for supporting healthy skin. However, it is unclear whether topically applied lipids are able to enter the lipid matrices and bring about structural changes that alter permeability barrier function. The aim of this study was to compare the effects of a moisturizer containing skin lipids (ceramides, triglycerides and cholesterol) and humectants in a multivesicular emulsion (the test cream) on SB structure against those of a reference emollient cream (not containing these additives) in people with dry, eczema-prone skin. Previously we demonstrated that the test cream imparts sustained moisturization over a 24-h period. We hypothesized that the controlled delivery of skin lipids and humectants to the skin will support optimum SB structure and function, and consequently protect against dryness and irritation.

Patients and methods

Study design and setting

An observer-blind randomized intrapatient-controlled interventional study was undertaken, wherein each participant underwent 4 weeks of treatment with the test cream on one forearm (volar face) and lower leg (shin); and a reference cream, a simple paraffin-based cream not containing physiological skin lipids, on the other forearm and lower leg (right/left allocation randomized). The treatment areas included the whole volar face of the forearm from the cubital to the wrist and the whole forward-facing aspect of the lower leg from the knee down to the ankle. The condition of the skin was assessed before, during and after treatment as outlined below.

The University of Sheffield Research Ethics Committee approved the study (#018584). It was performed in accordance with the Declaration of Helsinki of 1964 and its later amendments, and all participants provided informed consent to participate.

Participants

A single cohort of adult volunteers with dry skin and a predisposition to eczema was recruited. The target for completion was 28 participants based upon a power calculation. Anticipating a 20% rate of loss to follow-up, the enrolment target was therefore 35 participants. The eligibility criteria are described in Appendix S1 (see Supporting Information).

Investigational products

Participants were asked to apply 2 fingertip units of product (Table 1) to one forearm and 2 fingertip units to one lower leg (on the same side) according to the allocation scheme (right/left) twice per day (separated by at least 6 h) for 28 days. All participants received product application training at the start of treatment, and undertook the first application under supervision (further details on randomization, masking and compliance in Appendix S1).

Outcomes

The primary outcomes were the differences in: (i) skin sensitivity following 28 days of treatment, measured as the change in transepidermal water loss (TEWL) and redness in response to the sodium lauryl sulfate (SLS) patches; (ii) SB integrity defined as the area under the TEWL curve (TEWL AUC) obtained during skin tape stripping (STS) post-treatment (day

| Product        | Name            | Manufacturer                        | Ingredients                                                                                                                                 |
|----------------|-----------------|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|
| Test cream     | CeraVe cream    | L’Oreal, Active Cosmetics Division  | Aqua/water, glycerin, cetearyl alcohol, caprylic/capric triglyceride, cetyl alcohol, ceteareth-20, petrolatum, dimethicone, phenoxyethanol, behentrimonium methosulfate, potassium phosphate, ethylhexylglycerin, sodium lauroyl lactylate, disodium ethylenediaminetetraacetic acid, dipotassium phosphate, ceramide NP, ceramide AP, phytosphingosine, cholesterol, xanthan gum, carbomer, sodium hyaluronate, tocopherol, ceramide EOP |
| Reference cream| Zerobase cream  | Thornton & Ross Ltd, Huddersfield, UK| Liquid paraffin (11%), chlororesol, white soft paraffin (10%), Cetomacrogol, ceteosteryl alcohol, phosphoric acid, sodium dihydrogen phosphate, purified water                                    |

Table 1 Investigational products
and (iii) TEWL measurements after 2 and 4 weeks of treatment between sites treated with the test cream and reference cream. Secondary outcomes included skin hydration (capacitance) and visual skin dryness score after 2 and 4 weeks. Skin acceptability based upon an exit questionnaire, and skin tolerability (visual redness scores) were tertiary outcomes. SC composition and structure based on attenuated total-reflection Fourier-transform infrared (FTIR) spectroscopy after 28 days of treatment was an exploratory analysis, with lipid structure identified as an outcome in the protocol. Further details can be found in Appendix S1.

**Statistical analysis**

All data were analysed using Prism 9 (GraphPad Software, La Jolla, CA, USA). The significance threshold was \( P < 0.05 \). Results are presented as the mean (SD) unless otherwise indicated. Further details are provided in Appendix S1.

**Results**

Recruitment took place between June 2018 and March 2019. In total, 67 volunteers consented to take part, of whom 36 were enrolled and randomized (Figure 1). The demographic characteristics of the study cohort are presented in Table 2. Product usage was similar, with participants using a mean 128 (83) g of the reference cream and 133 (52) g of the test cream over the 28-day treatment period (2.3 g and 2.4 g on average per application, respectively).

The primary outcomes for this study are presented in Figure 2. At the end of treatment, skin sensitivity to SLS applied under occlusion for 24 h was determined. The difference in reaction to SLS at sites previously treated with the test cream was significantly less compared with the reference, with effect sizes of \(-0.5 \text{ points (97.5% CI } -1.00 \text{ to } -0.25\) for visual redness, \(-25.1 \text{ AU (95% CI } -38.9 \text{ to } -11.4\) for objective redness and \(-15.3 \text{ g m}^{-2} \text{ h}^{-1} \text{ (95% CI } -20.3 \text{ to } -10.4\) for TEWL (Figure 2a–c).

SB function was measured indirectly as TEWL (before SLS patches were applied, Figure 2d). At baseline TEWL was similar between the forearm sites (16.5 ± 7.2 vs. 16.2 ± 7.1 g m\(^{-2}\) h\(^{-1}\)). After 2 and 4 weeks of treatment a progressively larger difference in TEWL between the treatment sites became apparent. After 4 weeks of treatment, TEWL was 1.21 g m\(^{-2}\) h\(^{-1}\) lower on the sites treated with the test cream compared with the reference (95% CI 0.34–2.1).

To determine the integrity of the SB, STS was performed in conjunction with TEWL measurements after 28 days of treatment (Figure 2e). TEWL increased at a significantly lower rate at sites treated with the test cream compared with the reference in response to the same physical challenge, suggesting greater SB integrity. The TEWL AUC was significantly different between the treatments (Figure 2f; effect size \(-16.2, 95\% \text{ CI } -20.6 \text{ to } -11.8\)). The cumulative amount of protein removed by STS (Figure 2g, h) was also significantly different between the sites (\(-65.9 \mu\text{g cm}^{-2}, 95\% \text{ CI } -87.4 \text{ to } -44.4\), indicating greater SC cohesion at sites treated with the test cream compared with the reference. However, the estimated total thickness of the SC was not affected by the treatment [9.4 (2.5) \(\mu\text{m}\) and 9.7 (2.0) \(\mu\text{m}\) for the sites treated with test and reference creams, respectively]. The TEWL AUC was positively associated with the sensitivity of the skin to SLS (\(r = 0.63\), Figure 2i), suggesting a relationship between improved SB integrity (lower TEWL AUC) and reduced sensitivity to irritants.

Figure 1 Study flow diagram.
Table 2 Cohort demographics

| Demographic                          | All completed participants | Group 1 Age 18–39 years | Group 2 Age 40–59 years | Group 3 Age ≥ 60 years |
|--------------------------------------|----------------------------|-------------------------|-------------------------|------------------------|
| n                                   | 34                         | 16                      | 12                      | 6                      |
| Age (years), mean (SD); range        | 43 (18); 20–89             | 28 (6); 20–38           | 50 (6); 40–59           | 72 (9); 66–89          |
| Sex, n (%)                           |                            |                         |                         |                        |
| Male                                 | 13                         | 3                       | 5                       | 5                      |
| Female                               | 21                         | 13                      | 7                       | 1                      |
| Ethnicity                            |                            |                         |                         |                        |
| White                                | 31                         | 13                      | 12                      | 6                      |
| Asian                                | 2                          | 2                       | 0                       | 0                      |
| Mixed                                | 1                          | 1                       | 0                       | 0                      |
| Fitzpatrick skin type, median (range)| 2 (1–5)                    | 2 (2–5)                 | 3 (1–3)                 | 2 (2–3)                |
| Eczema, n (%)a                        |                            |                         |                         |                        |
| Recently had eczema but it is fully  | 6                          | 2 (13)                  | 2 (17)                  | 2 (33)                 |
| resolved                             | Currenty have eczema, but  | 9 (26)                  | 1 (6)                   | 7 (58)                 | 1 (17)                 |
| my skin is currently clear           | Currently have eczema, and  | 19 (56)                 | 13 (81)                 | 3 (25)                 | 3 (50)                 |
| it is currently active/visibleb      | T(EWL on volar forearm at  | 16-4 (7.1)              | 17-8 (8.9)              | 16-2 (4.8)             | 12-9 (4.7)             |
| Hydration on lower leg at baseline   | (g m⁻² h⁻¹), mean (SD)     |                         |                         |                        |
| Dryness on lower leg at baseline, median (range) | 2-0 (0-4)               | 1 (0-3)                 | 2 (0-3)                 | 3 (3-4)                |

T(EWL, transepidermal water loss. aParticipants were asked to categorize their condition. bAt sites other than the test sites, which must be clear.

The secondary outcomes relating to skin moisturization are presented in Figure 3. The skin was significantly more hydrated on the lower legs treated with the test cream compared with the reference after 2 weeks (+10.1 AU, 95% CI 8.13–12.1) and 4 weeks (+8.61 AU, 95% CI 6.61–10.6). In agreement, dryness was reduced by a significantly greater extent at sites treated with the test cream. Images of the skin (Figure 3b, d) show the reduction in dryness observed over the 28-day treatment period. While the signs of dryness had resolved at sites treated with the test cream, they persisted at sites treated with the reference.

Stratification of the study cohort by age revealed a greater effect size for each study outcome, except SC cohesion, in the group aged ≥60 years compared with the group aged 18–39 years; however, this difference between ages was not significant (Figure 4).

Analysis of skin molecular structure following treatment was conducted as an exploratory objective. Figure 5(a) presents the FTIR spectra of the test products. While the reference cream comprises a slightly higher total lipid level (in this case the nonphysiological lipids of paraffin) relative to water, the test cream contains glycerol and a range of physiological lipids (ceramides, fatty acids and cholesterol). The average spectra collected from the skin post-treatment reveal obvious differences in composition that align with the differences in the composition of the creams, with the exception of the higher water content associated with sites treated with the test cream.

Given that FTIR is a surface-based technique with a sampling depth of 1–0.1–1.5 μm, measurements were undertaken in combination with STS to profile the structure of the SC across its depth.

Due to the different cohesive properties, STS removed 5-59 (0.26) μm of the SC following treatment with the reference, and 6-83 (0.23) μm following treatment with the test cream. Accounting for an FTIR sampling depth of 1.0 μm, this means approximately 70% of the SC has been analysed. As can be seen in Figure 6, total lipid, glycerol and water levels were increased at sites treated with the test cream compared with the reference across the first ~5 μm (50%) of the SC. While the test cream was associated with increased lipid ester groups on the surface, found in triglyceride lipids like those in the test cream, these groups were absent from the inner layers of the SC, as expected.

As a key outcome related to skin moisturization, mean water levels across the first 5 μm of SC were calculated and show a significant difference between the treated sites (Figure 7). Moreover, the effect size steadily increased with increasing age (0.33, 0.44 and 0.54 for ages 18–39, 40–59 and ≥ 60 years, respectively). The relative level of water in the SC quantified by FTIR correlated significantly with capacitance measurements of SC hydration (r = 0.72, Figure 8), to a lesser extent with skin-surface dryness score (r = −0.36) and weakly with glycerol levels (r = 0.29).

Lipid chain conformational order and packing were assessed by quantifying the frequency of the lipid peak at 2850 cm⁻¹, corresponding to the symmetric stretching of the CH₂ group (Figure 6d). Throughout the depth of the SC the frequency of the lipid peak was lower at sites treated with the test cream, indicating a change in lipid arrangement. The mean frequency across the first 5 μm of SC was 0.23 cm⁻¹ (95% CI 0.17–0.29) lower at sites treated with the test cream compared with the reference (Figure 7). Upon stratification by age, this difference
Skin barrier (SB) primary outcomes assessed at forearm sites. (a–c) Skin sensitivity to sodium lauryl sulfate (SLS) following 28 days of treatment measured as the change (day 31, 24 h after patch removal, minus day 29, before patch application): (a) visual redness/erythema, (b) objective redness (arbitrary units, AU) and (c) transepidermal water loss (TEWL). (d) SB function measured as (resting) TEWL before, during (day 15) and after treatment (day 29). (e) TEWL in response to skin tape stripping (STS) (standardized physical skin challenge). (f) SB integrity determined as the area under the TEWL curve (AUC) in response to STS: higher TEWL AUC indicates weaker SB integrity. (g) The cumulative amount of protein removed by STS. (h) The total amount of protein removed by STS. (i) The relationship between TEWL AUC and skin sensitivity to SLS. Boxes indicate the median and 25th and 75th percentiles, with ‘+’ for the mean and whiskers showing 1.5 x interquartile range. Asterisks indicate the results of pairwise testing: ns, not significant; *p < 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.
was found to widen with advancing age. The frequency of the lipid peak was significantly correlated with SB integrity (TEWL AUC, $r = 0.61$; Figure 8), to a lesser extent with resting TEWL ($r = 0.48$) and weakly with SC glycerol levels ($r = -0.32$), but not with hydration ($r = -0.18$) or dryness ($r = 0.26$).

In total 28 adverse events were reported during the study, of which eight were possible adverse reactions (Table S1; see Supporting Information). Of these, seven were associated with the reference and one with the test cream. The possible reaction to the test cream was reported in a participant who also had a possible reaction to the reference.

On completing the study all participants were asked to complete a questionnaire on the cosmetic acceptability of the products. While both products were considered convenient to use and left the skin feeling moisturized, respondents found the test cream more pleasant to use overall, with a better texture and consistency, and reported that it spread more easily and absorbed more quickly (Figure S1; see Supporting Information).

**Discussion**

The test cream consistently displayed superior performance compared with the reference cream, by: (i) providing increased levels of skin hydration; (ii) treating dryness more effectively and more quickly; (iii) improving the structure and

---

**Figure 3** Skin moisturization outcomes measured at the lower legs. Skin hydration measured as electrical capacitance (a) and skin-surface dryness assessed visually (c) before, during (day 15) and after treatment (day 29). Boxes indicate the median and 25th and 75th percentiles, with ‘+’ for the mean and whiskers showing 1.5 x interquartile range. Asterisks indicate the results of pairwise testing: ns, not significant; **p < 0.01; ****p < 0.0001. (b, d) Representative colour images (16 x 12 mm) of the treatment sites, acquired before (day 1) and after (day 29) treatment: (b) images taken from a 75-year-old male participant; (d) images taken from a 37-year-old female participant.
function of the SB; and (iv) subsequently by reducing skin sensitivity to SLS. The reference was associated with a relatively high number of adverse events compared with the test cream, while providing only marginal changes in skin hydration. In addition, the test cream was widely considered more cosmetically acceptable, scoring positively in all assessed aspects compared with the reference, which had a number of negative characteristics.

At baseline the study cohort displayed a clear SB defect, with an average TEWL of 16.4 g m⁻² h⁻¹ compared with an average TEWL of 10.1 g m⁻² h⁻¹ in healthy controls, 12.9 g m⁻² h⁻¹ in quiescent AD and 17.9 g m⁻² h⁻¹ in patients with AD (nonlesional sites). Here we report a significant difference of 1.2 g m⁻² h⁻¹ after treatment with the test cream compared with the reference. In a similar study, the same formulation as the reference cream was found to have no significant effect on TEWL compared with untreated skin, suggesting that the difference observed here is due to a positive effect of the test cream.

Figure 4 Study outcomes following stratification by age. (a–c) Skin sensitivity to SLS following 28 days of treatment measured as the change in (a) visual redness, (b) objective redness and (c) transepidermal water loss (TEWL). (d–h) Resting TEWL (day 29 – day 1 change, d), SB integrity post-treatment (day 28, e), total amount of protein removed by STS (day 28, f), skin hydration (day 29 – day 1 change, g) and skin dryness (day 29 – day 1 change, h) in response to treatment for 28 days. Boxes indicate the median and 25th and 75th percentiles, with ‘+’ for the mean and whiskers showing 1.5 × interquartile range. Asterisks indicate the results of pairwise testing: ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.
associated with the skin of patients with AD at nonlesional sites on the forearms compared with healthy controls, depending on severity. Moreover, the true effect on SB function is likely masked by the confounding effect of humectant-containing emollients, like the test cream, on the skin – namely that hyperhydration of the skin can elevate water loss. For this reason, coupled with the low sensitivity of TEWL, we measured SB integrity. SB integrity, but not baseline TEWL, distinguishes the skin of patients with AD with and without food allergy, and differentiates the skin of filaggrin-associated and non-associated patients with AD. The significantly greater integrity of the SB following treatment with the test cream compared with the reference reported here was of a similar effect size to that reported between allergen-associated and non-associated AD, and was larger than the difference between nonatopic patients with AD and healthy controls.

The results of this study point to distinct effects of different emollients, and suggest that the test cream may help maintain SB integrity at levels associated with healthy skin, and potentially protect against skin changes associated with more severe and/or persistent forms of AD. In contrast, emollients similar to the reference are associated with marginally decreased SB integrity compared with healthy skin.

To explore whether changes in SB integrity alter susceptibility to environmental irritants we determined the effect of emollient pretreatment on cutaneous reactions to SLS. Pretreatment with some emollients can significantly enhance reactions to SLS, suggesting increased penetration of SLS into the skin. Here we show that compared with the reference, the test cream significantly reduces the response to SLS. Moreover, we show that the response to SLS is associated with SB integrity. In other studies, pretreatment with certain emollients has also been shown to reduce atopy patch test

Figure 5 Average Fourier-transform infrared spectrum of (a) the test products and (b) the skin sites post-treatment (after five tape strips and normalized to the amide II peak at ~1550 cm⁻¹). The letters indicate the peaks associated with water (W), lipids (L), triglycerides (T) and glycerol (G). (c) Focus on the lipid peak at ~2850 cm⁻¹ following baseline correction. The centre of gravity (COG) of this peak is associated with stratum corneum lipid.
Figure 6 Molecular properties of the stratum corneum (SC) determined by Fourier-transform infrared spectroscopy. (a) Total lipid, (b) lipid ester, (c) glycerol and (e) water levels relative to amide II and lipid peak centre of gravity (COG, d) by SC depth (inset graphs display mean SC or skin-surface levels). Asterisks indicate the results of pairwise testing: **p < 0.01, ****p < 0.0001.

Figure 7 Mean lipid peak centre of gravity (COG) and water levels across the first 5-μm depth of the stratum corneum (SC) for the whole cohort (a, c) and stratified by age group (b, d). Asterisks indicate the results of pairwise testing: ***p < 0.001, ****p < 0.0001.
reactions, suggesting that barrier restoration may help protect against both irritant and allergen penetration through the skin.21

Using FTIR spectroscopy, evidence of the delivery of lipids (except triglycerides) and glycerol deep within the SC is provided. Intact triglycerides remain on the skin surface, where they are likely to provide a source of fatty acids and glycerol upon hydrolysis by resident bacteria.22 While previous studies have shown that some preparations can successfully deliver ceramide lipids to the SC,23 the effect of these lipids on the structure of the lipid lamellae has not been quantitatively assessed. To address this, we used FTIR spectroscopy to measure the chain conformational order of lipids in the SC based on the frequency of the lipid peak at 2850 cm$^{-1}$. Changes in the frequency of this peak are associated with the lipid composition of the skin, SB function and AD severity.6 The SC profile for lipid structure observed is similar to that reported previously, suggesting the majority of the SC was sampled.24

When comparing the average structure through the SC following treatment, a shift in the frequency, of between 0.20 and 0.36 cm$^{-1}$ depending on age, towards a lower wavenumber (indicating a more ordered orthorhombic structure) was observed at sites treated with the test cream compared with the reference. This is consistent with the lower wavenumber (by ~0.4 cm$^{-1}$) observed in healthy controls compared with patients with AD.6 Building on the work of Damien and Boncheva in healthy skin,4 the results associate lipid structure with SB condition in people with dry eczema-prone skin undergoing emollient therapy. SB integrity proved a more robust fit with lipid structure than resting TEWL. This suggests that the enhancement of SB integrity is at least in part attributable to positive changes in SC lipid structure. The increasing effect size observed with advancing age is also consistent with the age-associated changes in SC lipid content.

It is not possible to attribute the effects on lipid structure to a particular ingredient. Previous studies have demonstrated that physiologic lipids are sufficient to enhance lipid lamellae structure.25 The test cream also contains a significant percentage of glycerol, which has contradictory effects, appearing to promote SB repair while also acting as a penetration enhancer.26 We found a weak correlation between SC glycerol levels and lipid structure or SB integrity, suggesting a limited role. Another candidate ingredient of the test cream, tocopherol, is an antioxidant reported to protect against chemical irritation.27,28 Tocopherol scavenges free radicals, including those responsible for lipid peroxidation, which damage the structures of the SC.29

Overall, after 4 weeks of treatment, the test cream increased skin hydration on the legs by approximately 50%, whereas the reference increased hydration by <10%. The level of increased hydration imparted by the test cream was significant, taking the skin from a dry or very dry state to a sufficiently hydrated state.10 In contrast, the effect of the reference was limited, with skin dryness persisting for longer. While capacitance is an established technique, it is an indirect measure, and so the change in hydration was confirmed by directly quantifying the level of water (OH bonds) in the skin relative to protein by FTIR spectroscopy. As reported previously, we show strong agreement between capacitance measurements and SC water levels.31 Both measures of hydration were inversely correlated with surface dryness.

Glycerol is expected to contribute significantly to the hydrating effects of the test cream. A recent systematic review found evidence to support the superiority of glycerol-based emollients over emollients without humectants for skin moisturization.32 That said, the weak correlation between SC glycerol levels and SC water levels reported here suggests a role for the other ingredients, such as hyaluronic acid, triglycerides and ceramides, all with putative skin moisturizing effects. For example, the addition of just 0.02% ceramide EOP and NP to a 5% glycerol cream more than doubled its effect on skin hydration.33

This study was conducted in an adult population of mixed sex with broad age range, demonstrating that the test cream is suitable across the adult age spectrum. The ethnic diversity of the study was limited by the fact that we received fewer expressions of interest to take part in this study from people with darker skin types. While the study provides strong mechanistic data to support the positive effects of the test cream on
skin, further studies are now required to explore the impact of longer-term use on the management of dry skin conditions, to determine whether xerosis and episodes of skin inflammation can be prevented.

In terms of relating the effects observed to the current standard of care for dry skin, the reference represents a broad category of simple emollient creams (without humectants). In the UK this category represented 37% of leave-on emollient prescriptions in 2018, of which the reference cream was the most prescribed. Based on this, the inferiority of the reference can be generalized to this class of emollient. In contrast, the test cream has a unique formulation comprising a number of ingredients with known skin effects (including glycerol, ceramides, phytosphingosine, cholesterol, sodium hyaluronate and tocopherol) delivered in a unique multivesicular emulsion delivery system. Extensive research has already established the importance of the relative concentrations of exogenously applied skin lipids and humectants on skin function, and so it is not possible to relate the effects of the test cream with other products.

In conclusion, by enhancing the SC lipid structure, treatment for 4 weeks with the test cream facilitated improved SB function, and consequently provided protection from skin irritation. Compared with a commonly prescribed emollient, the test cream brought about superior levels of skin moisturization that more effectively reduced the signs of skin dryness. The results have significant implications for clinical practice given the very common use of simple emollient creams, like the reference used here, for the management of xerotic skin conditions. Studies suggest that very few patients and caregivers currently feel they can adequately manage AD symptoms, including chronic skin dryness and a propensity for inflammatory lesions. Given that irritants like SLS are important triggers for AD lesions, the effects of the test cream on the SB could potentially help maintain healthy skin and reduce the burden of managing this condition. In support of this, Draelos reported that the addition of the test cream to topical corticosteroid treatment could help improve outcomes of mild-to-moderate AD.

Acknowledgments

We are very grateful to L’Oreal for providing the funding to undertake this investigator-led study, and to all of our volunteers who have given up their time to take part in this study; thank you. We would also like to thank Leung Tang, Graham Miller and Alex Harvey at Agilent Technologies for generously offering their support and sharing their expertise in FTIR analysis.

References

1. Schurer NY, Elias PM. The biochemistry and function of stratum corneum lipids. Adv Lipid Res 1991; 24:27–56.
2. Meguro S, Arai Y, Masukawa Y et al. Relationship between covalently bound ceramides and transepidermal water loss (TEWL). Arch Dermatol Res 2000; 292:463–8.
3. Damien F, Boncheva M. The extent of orthorhombic lipid phases in the stratum corneum determines the barrier efficiency of human skin in vivo. J Invest Dermatol 2010; 130:611–14.
4. Proksch E, Jensen JM, Elias PM. Skin lipids and epidermal differentiation in atopic dermatitis. Clin Dermatol 2003; 21:134–44.
5. Di Nardo A, Wertz P, Giannetti A, Seidenari S. Ceramide and cholesterol composition of the skin of patients with atopic dermatitis. Acta Derm Venereol 1998; 78:27–30.
6. Janssens M, van Smeden J, Gooris GS et al. Increase in short-chain ceramides correlates with an altered lipid organization and decreased barrier function in atopic eczema patients. J Lipid Res 2012; 53:2755–66.
7. Fluhler JW, Darlenksi R, Taieb A et al. Functional skin adaptation in infancy – almost complete but not fully competent. Exp Dermatol 2010; 19:483–92.
8. Ghdally R, Brown BE, Sequeira-Martin SM et al. The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. J Clin Invest 1995; 95:2281–90.
9. Wilhelm KP, Cua AB, Matbach HI. Skin aging. Effect on transepidermal water loss, stratum corneum hydration, skin surface pH, and casual sebum content. Arch Dermatol 1991; 127:1806–9.
10. Mischo M, von Kobyletzki LB, Bründermann E et al. Similar appearance, different mechanisms: xerosis in HIV, atopic dermatitis and aging. Exp Dermatol 2014; 23:446–8.
11. Sinikumpu SP, Jokelainen J, Haarala AK et al. The high prevalence of skin diseases in adults aged 70 and older. J Am Geriatr Soc 2020; 58:2565–71.
12. Danby SG, Andrew PV, Brown K et al. An investigation of the skin barrier restoring effects of a cream and lotion containing ceramides in a multi-vesicular emulsion in people with dry, eczema-prone, skin: the RESTORE study phase 1. Dermatol Ther (Heidelb) 2020; 10:1031–41.
13. Danby SG, Chittock J, Brown K et al. The effect of tacrolimus compared with betamethasone valerate on the skin barrier in volunteers with quiescent atopic dermatitis. Br J Dermatol 2014; 170:914–21.
14. Danby SG, Chalmers J, Brown K et al. A functional mechanistic study of the effect of emollients on the structure and function of the skin barrier. Br J Dermatol 2016; 175:1011–19.
15. Jungersetd JM, Scheer H, Mempel M et al. Stratum corneum lipids, skin barrier function and filaggrin mutations in patients with atopic eczema. Allergy 2010; 65:911–18.
16. Flöhr C, England K, Radulovic S et al. Filaggrin loss-of-function mutations are associated with early-onset eczema, eczema severity and transepidermal water loss at 3 months of age. Br J Dermatol 2010; 163:1333–6.
17. Loden M. Effect of moisturizers on epidermal barrier function. Clin Dermatol 2012; 30:286–96.
18. Leung DYM, Calatroni A, Zaramela LS et al. The nonlesional skin surface distinguishes atopic dermatitis with food allergy as a unique endotype. Scand J Med Sci 2019; 11:eaa2685.
19. Angelova-Fischer I, Mannheimer AC, Hinder A et al. Distinct barrier integrity phenotypes in filaggrin-related atopic eczema following sequential tape stripping and lipid profiling. Exp Dermatol 2011; 20:351–6.
20. Glooor M, Hauth A, Gehring W. O/W emulsions compromise the stratum corneum barrier and improve drug penetration. Pharmazie 2003; 58:709–15.
21. Billmman-Eberwein C, Rippke F, Ruzicka T, Krutmann J. Modulation of atopy patch test reactions by topical treatment of human skin with a fatty acid-rich emollient. Skin Pharmacol Appl Skin Physiol 2002; 15:100–4.
22 Wertz PW. Lipids and the permeability and antimicrobial barriers of the skin. J Lipids 2018; 2018:5954034.
23 Draelos ZD, Baalbaki NH, Raab S, Colon G. The effect of a ceramide-containing product on stratum corneum lipid levels in dry legs. J Drug Dermatol 2020; 19:372–6.
24 Mendelsohn R, Flach CR, Moore DJ. Determination of molecular conformation and permeation in skin via IR spectroscopy, microscopy, and imaging. Biochim Biophys Acta 2006; 1758:923–33.
25 Man MQ, Feingold KR, Elias PM. Exogenous lipids influence permeability barrier recovery in acetone-treated murine skin. Arch Dermatol 1993; 129:728–38.
26 Bettinger J, Gloor M, Peter C et al. Opposing effects of glycerol on the protective function of the horny layer against irritants and on the penetration of hexyl nicotinate. Dermatitis 1998; 197:18–24.
27 Scheppp CM, Meinken MC, Lademann J et al. Topical antioxidants protect the skin from chemical-induced irritation in the repetitive washing test: a placebo-controlled, double-blind study. Contact Dermatitis 2012; 67:234–7.
28 Casari A, Farnetani F, De Pace B et al. In vivo assessment of cytological changes by means of reflectance confocal microscopy — demonstration of the effect of topical vitamin E on skin irritation caused by sodium lauryl sulfate. Conta Dermatitis 2017; 76:131–7.
29 Thiele JJ, Hsieh SN, Ekanayake-Mudiyanselage S. Vitamin E: critical review of its current use in cosmetic and clinical dermatology. Dermatol Surg 2005; 31:805–13.
30 Heinrich U, Koop U, Leneuve-Duchemin MC et al. Multicentre comparison of skin hydration in terms of physical-, physiological- and product-dependent parameters by the capacitive method (Corneometer CM 825). Int J Cosmet Sci 2003; 25:45–53.
31 Machado M, Hadgraft J, Lane ME. Assessment of the variation of skin barrier function with anatomic site, age, gender and ethnicity. Int J Cosmet Sci 2010; 32:397–409.
32 van Zuuren EJ, Fedorowicz Z, Christensen R et al. Emollients and moisturisers for eczema. Cochrane Database Syst Rev 2017; 2:CD012119.
33 Huang HC, Chang TM. Ceramide 1 and ceramide 3 act synergistically on skin hydration and the transepidermal water loss of sodium lauryl sulfate-irritated skin. Int J Dermatol 2008; 47:812–19.
34 National Health Service. Prescription cost analysis – England 2018. Available at: https://digital.nhs.uk/data-and-information/publications/statistical/prescription-cost-analysis/2018 (last accessed 12 January 2022).
35 Bikowski J, Shroot B. Multivesicular emulsion: a novel, controlled-release delivery system for topical dermatological agents. J Drug Dermatol 2006; 5:942–6.
36 Zuberbier T, Orlov SJ, Paller AS et al. Patient perspectives on the management of atopic dermatitis. J Allergy Clin Immunol 2006; 118:326–32.
37 Draelos ZD. The effect of ceramide-containing skin care products on eczema resolution duration. Cutis 2008; 81:87–91.

Supporting Information
Additional Supporting Information may be found in the online version of this article at the publisher’s website:

Appendix S1 Supplementary methods and results
Figure S1 Summary of participant responses to statements on cosmetic acceptability
Table S1 Summary of adverse events.
Video S1 Author video.