A FUNCTIONAL MECHANISTIC STUDY OF THE EFFECT OF EMOLLIENTS ON THE STRUCTURE AND FUNCTION OF THE SKIN BARRIER

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Running Head: Effect of emollients on the skin

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Key words

Eczema, skin barrier, emollient, transepidermal water loss, hydration, skin-surface-pH

What's already known about this topic?

- A skin barrier defect is a primary event in the development of atopic dermatitis (AD)
- Topical therapy to correct this skin barrier defect may prevent AD relapses
- Not all emollients exert a positive effect on the skin barrier. The use of Aqueous cream BP for example damages the skin barrier

What does this study add?

- We provide evidence that, in contrast to Aqueous cream BP, two commonly prescribed emollients exert no negative effects on the skin barrier, and are therefore suitable for further clinical testing in AD prevention trials
- We also highlight that while these ancillary treatments display clinically important ‘emollient’ properties they do little to actively improve skin barrier function

ABSTRACT

Background

Preventing relapses of atopic dermatitis (AD) through the regular use of topical products to repair the skin barrier defect is an emerging concept. It is still unclear if some commonly used emollients exert a positive effect on the skin barrier.

Objectives

To determine the skin barrier effects of emollients commonly prescribed in the UK.

Methods

Two cohorts of volunteers with quiescent AD undertook observer-blind forearm-controlled studies. The first (18 volunteers) treated the volar side of one forearm with 2 fingertip units of Doublebase™ gel twice daily for 4 weeks. The second cohort (19 volunteers) undertook the same regimen using Diprobase® cream. Transepidermal water loss (TEWL), stratum corneum integrity and hydration, skin-surface-pH and redness were determined at the test sites before and after treatment.

Results

Neither Diprobase® cream nor Doublebase™ gel significantly affected the underlying skin barrier function. Both emollients were associated with significantly increased skin-surface-pH immediately after application (by 0.8±0.19 and 1.0±0.18 units respectively), and no erythema. Diprobase® cream artificially and transiently (6 hours) improved permeability barrier function by 2.9-3.1 g/m^2/h TEWL and increased skin hydration by 6.0-6.2 units. Doublebase™ gel, containing humectants, was associated with a greater (between 10.1 and 13.0 units during the first 6 hours) and more sustained increase in hydration, lasting more than 12 hours following repeated use.

Conclusion

Diprobase® cream and Doublebase™ gel are not associated with skin barrier harm and appear to be appropriate for AD treatment. Whilst displaying emollient properties, neither formulation displayed an ability to actively improve sustained skin barrier function.

[250/250 words]

INTRODUCTION

Atopic dermatitis (AD) is a very common chronic inflammatory skin condition affecting up to 30% of children and up to 10% of adults worldwide and appears to be on the increase.[1–4] The early appearance of AD often heralds the development of other allergic diseases such as food allergy, asthma and allergic rhinitis.[5] Increasing evidence suggests that a skin barrier defect is the primary event in the development of AD (reviewed in Danby *et al* 2010).[6,7] This raises the possibility that correcting the skin barrier defect may prevent or delay the relapse or development of AD.[8]

Emollient therapy, to ameliorate established AD, is the first line treatment for AD.[9] Regular, liberal, use of routinely prescribed emollients significantly reduces the severity of established AD.[10] It has been suggested therefore that the routine use of emollients could reduce the risk of AD relapses, or its initial development, by ameliorating the skin barrier defect. In support of this a randomized controlled trial (RCT) in adult patients with established AD determined that the regular use of a barrier strengthening 'emollient' could prolong the period of remission compared to no treatment.[11] Furthermore the results of several pilot studies conducted in neonates at high risk of developing AD have indicated that the daily use of emollients from birth could significantly reduce the risk of developing AD.[8,12,13] Studies like these have renewed interest in emollients as potential skin barrier enhancing agents. By definition however emollients simply soothe, smooth and hydrate skin. To achieve AD prevention, topical therapy should not simply provide temporary relief, but ideally impart easily sustainable repair of the skin barrier defect in a way that would be acceptable to patients and their carers.[8]

We, and others, have demonstrated that emollients have very different effects on the skin barrier depending on their formulation.[14–17] In particular aqueous cream BP, containing the anionic surfactant sodium lauryl sulphate (SLS), was found to damage the skin barrier, with related adverse effects reported.[18–21] On the other hand some emollients, with purported skin barrier enhancing effects, were found to reduce the need for topical corticosteroids, reduce the severity of AD and delay relapse of the condition.[8,11,22] In a recent RCT in adult patients an emollient, previously found to strengthen the skin barrier, was shown to increase the period of AD remission compared to an emollient without

effects on the skin barrier.[23] These 'positive' emollients all have complex formulations and contain bioactive ingredients that potentially affect skin barrier homeostasis.[24,25] It is therefore important to recognize that not all emollients are the same. Just because one emollient reduces the severity of AD does not mean that all emollients will exhibit such a "class" effect. The heterogeneity of emollient performance has significant implications for the treatment of existing AD, its long-term control, and for prevention strategies in the future. Not surprisingly therefore the Eczema Priority Setting Partnership found that identifying the most effective and safe emollients for treating AD is a top priority shared by patients and healthcare professionals.[26] We therefore sought to conduct an independent academic study to determine the effects of commonly used / prescribed emollients in the UK on the skin barrier in order to provide independent evidence for healthcare professionals and to aid the design of future randomized controlled clinical trials focused on eczema control and prevention. In view of the absence of independent evidence on commonly used / prescribed emollients in either adults or children, we chose to conduct this initial mechanistic study in adult patients with quiescent AD, who display a skin barrier defect without visible inflammation.[27]

MATERIALS AND METHODS

Participants

One cohort of volunteers with healthy skin (cohort 1) and two cohorts of volunteers with quiescent AD (cohorts 2 and 3) were recruited. Recruitment to all cohorts was open to male and female volunteers, who were recruited on a first come first served basis, assuming they met the specified criteria. Inclusion criteria for cohort 1 included having no current or past signs or symptoms of any chronic skin condition; a Fitzpatrick skin-type of I-IV; and being aged between 18 years and over. Inclusion criteria for cohorts 2 and 3 included: a self-reported history of AD, but without any signs or symptoms within the 6 months leading up to participation in the study; a Fitzpatrick skin-type of I-IV; and being aged between 18 and 50 years old. Exclusion criteria for all cohorts included: having a skin condition other than AD, pregnancy, breast-feeding, the use of systemic corticosteroids in the past 12 months, the use of topical anti-inflammatories in the past 6 months, and a known allergy/ hypersensitivity to any of the excipients of the trial preparations. Informed consent was obtained from each participant. All participants received remuneration appropriate for their involvement. The NRES committee East Midlands - Derby formally known as Trent Multicentre Research Ethics Committee (MREC) approved the study, under the project reference 04/MREC/70.

Rationale for emollients selected for testing

To select the emollients used in this study we were guided by patient feedback collected as part of the Barrier Enhancement for Eczema Prevention (BEEP) eczema prevention feasibility study conducted in the UK and US.[8] In this study participants were able to choose the emollient intervention from a panel comprising of a topical oil, an ointment, and a cream/gel. In the UK these were: sunflower seed oil, liquid paraffin 50% in white soft paraffin and Doublebase™ gel. In the USA these comprised sunflower seed oil, Aquaphor® healing ointment, and Cetaphil® cream. Of the participants, 67.2% selected Doublebase™ gel or Cetaphil® cream indicating a preference for cream/gel formulations, and so we concentrated on this class of emollient. When the parents of infants enrolled onto the trial were offered a selection of emollients creams/gels, they said that they preferred Doublebase™ gel and Diprobase® cream. In the UK, based upon the 2013

prescription cost analysis for the NHS, Doublebase™ gel and Diprobase® cream were the most frequently prescribed emollients (including ointments) jointly accounting for 33% (16% and 15% respectively).[28] To date no independent evaluation of the effects of these emollients on the structure and function of the skin barrier has been conducted in adults or children. Given their distinct formulations we assessed the effects of these two emollients on the skin barrier in adult patients with quiescent AD.

Single application test

The forearms (volar face) of the volunteers in cohort 1 were divided into 4 test sites (4x3 cm) each. Each test site received a single 100µl application of Aqueous cream BP, Diprobase® cream, Doublebase™ gel (see Table 1 for details) or no treatment so that each treatment was repeated twice per subject (randomized allocation within each forearm using a randomization list generated at www.randomisation.com - product identities were concealed from the investigator and participant using unlabeled packaging). The biophysical properties of the test sites were determined before and at set time points after treatment application. Participates were asked to refrain from washing the test sites until completion of the study.

Treatment regimen for mechanistic studies

Cohorts 2 and 3 undertook 28-day forearm-controlled observer-blind studies involving self-treatment with Diprobase® cream or Doublebase™ gel (Table 1). There were two test sites per volunteer, one on each forearm (volar side, 3cm below elbow flexure to 3cm above the wrist). Each participant was asked to apply 2 finger-tip units of the respective emollient to one forearm and no treatment to the other (randomised allocation) twice daily (at least 6 hours apart) for 28-days. Prior to, and (12-20 hours) following the cessation of, treatment the biophysical properties of the test sites were assessed to determine the effect of the treatment on the underlying condition of the skin barrier. Care was taken to ensure that no emollient residues remained on the skin prior to testing by dry wiping with cotton wool as required.

Biophysical measurements

Transepidermal Water Loss (TEWL) measurements were performed using an AquaFlux AF200 condensing chamber probe (Biox Systems Ltd., London, UK). Redness, skin

surface pH and capacitance were measured using a Mexameter MX18, a Skin-Surface-pH Meter PH905, and a Corneometer CM825 respectively (CK electronic GmbH, Cologne, Germany). All assessments were performed in a room maintained at 21±2°C and 38-50% relative humidity according to published guidelines.[29] All test sites were acclimatised to room conditions for 20 minutes before assessment. Tape-stripping, to experimentally disrupt the SC, was performed as previously described.[27] Determination of the amount of protein removed by tape-stripping was based on the IR absorbance (SquameScan 850A, CuDerm, Dallas, USA) of tape-strips in accordance with published methodology.[30] Total stratum corneum (SC) thickness (H) was estimated, from the relationship between the cumulative amount of protein removed and TEWL based on Fick's first law.[31]

SC protease activity

Assessment of protease activity was made on samples comprising three consecutive tape-strips as previously described.[30] Caseinolytic, chymotrypsin-like and trypsin-like activities were determined using EnzCheck® (Life Technologies Ltd., Paisley, UK), MeOSuc-Arg-Pro-Tyr-AMC (Peptide Protein Research Ltd, Funtley, UK), and Boc-Phe-Ser-Arg-AMC (Bachem, Bubendorf, Switzerland) substrates respectively.

Data analysis

The results were analysed in Prism v6.01 (Graphpad Software Inc., CA, USA). The significance threshold was $p<0.05$. Results are presented as mean ± standard error of the mean (SEM).

RESULTS

The effect of a single application of three different emollients on the biophysical properties of the skin

Single applications (100 µl) of Aqueous cream, Diprobase® cream, Doublebase™ gel, and no treatment as a control, were made to separate sites on each forearm of 4 participants with healthy skin (cohort 1, mean age 38±5 years, 3 male). Figure 1 illustrates the change in the biophysical properties of the skin over 24 hours following application of the treatments. Both Aqueous cream and Diprobase® cream significantly reduced TEWL by approximately 3 g/m^2/h for at least 6 hours. After 6 hours the effect diminished (TEWL reduced by 2.070 and 1.968 g/m^2/h respectively) but remained significant. Doublebase™ gel lowered TEWL by a significantly reduced extent, reaching a decrease of 1.850 g/m^2/h only after 24 hours. All treatment applications elevated skin hydration, however there were significant differences between the effects of the emollients. Doublebase™ gel significantly elevated skin hydration by between 10.083±2.329 and 13.043±2.313 capacitance units during the first 6 hours. By comparison the effects of Diprobase® cream and Aqueous cream were less pronounced, with the increase in hydration reaching 6.196±1.002 and 7.836±1.667 capacitance units respectively at their peak during the first 6 hours. After 24 hours the hydrating effects of all three emollients were similar. All three treatments significantly elevated skin surface pH compared to the control, with the highest increase observed for Doublebase™ gel of 0.976±0.179 units (on average) compared to 0.844±0.186 units for Diprobase® cream and 0.823±0.135 units for Aqueous cream 10 minutes after application. The pH of the skin reduced steadily over the following 24 hours, at which time only the sites treated with Diprobase cream were statistically different from the untreated control.

The effect of 28-days treatment with Diprobase® cream on the skin barrier

Nineteen volunteers with quiescent AD (cohort 2, mean age 33±2 years, 14 female) applied an average of 0.96±0.07g Diprobase® cream to one forearm twice per day for 28 days. The other forearm was left untreated as a control. Figure 2 illustrates the biophysical properties of the test sites 12-20 hours following the last application of emollient. No emollient residues were evident on the test sites at the point of testing. At

this point the transient occlusive effects of the emollients on TEWL have passed and TEWL values reflect the effect of the treatments on the underlying properties of the barrier. No effect of the treatment on TEWL was observed indicating that treatment with Diprobase® cream does not adversely affect skin barrier function. Upon challenge by tape-stripping to assess the integrity of the SC, TEWL was found to be marginally elevated on sites treated with Diprobase® compared to the untreated control sites (Fig 2b), suggesting that the underlying condition of the SC has been adversely affected, albeit to a small degree. There was only a small decrease in SC cohesion (increased protein removed after 3 tape-strips) and no change in estimated SC thickness on the treated compared to the control sites. In agreement with the effects of a single application of Diprobase® cream, hydration was not affected, but skin surface pH was significantly higher on the treated sites (4.97±0.0697 units) compared to the untreated control sites (4.79±0.0561). There were no significant differences in skin redness indicating an absence of visible erythema. The effect of treatment with Diprobase® cream on 3 types of SC protease activities associated with skin barrier breakdown and cutaneous inflammation was determined *ex vivo*, and revealed a significant effect of chymotrypsin-like protease activity, but not trypsin-like or broad-spectrum protease activity.

The effect of 28-days treatment with Doublebase™ gel on the skin barrier

Eighteen volunteers with quiescent AD (cohort 3, mean age 29±8 years, 14 female) applied an average of 1.64±0.14g Doublebase™ gel to one forearm twice per day for 28 days. The other forearm was left untreated as a control. The amount of Doublebase™ gel applied was significantly greater than the amount of Diprobase® cream applied despite the same instructions being issued to the volunteers, and indicates that the container type and consistency of the emollient play a role in the amount of product applied even when the same directions are issued. Figure 3 illustrates the biophysical properties of the test sites 12-20 hours following the last application of emollient. No emollient residues were evident on the test sites at the point of testing. Doublebase™ gel did not significantly affect basal TEWL, indicating that treatment has no negative effects on skin barrier function. Upon tape-stripping of the skin however, TEWL increased at a significantly slower rate on the sites treated with Doublebase™ gel compared to the control sites. The amount of protein removed by tape-stripping was also significantly and consistently

lower on treated sites compared to the control across the depth of the SC, indicating that the skin is more resistant to disruption by tape-stripping. SC thickness was unaffected by the treatment. Skin hydration was significantly increased from 33.5±1.44 units before treatment to 46.67±1.51 units 12-20 hours after cessation of treatment with Doublebase™ gel. There was no significant change in skin hydration on the control site. Skin surface pH was also significantly increased from 5.00 to 5.234 units on the treated sites, but not on the control sites. There were no significant differences between the Doublebase™ gel treated sites and the controls for skin redness or any of the *ex vivo* protease activities tested for.

DISCUSSION

Main Findings

In individuals with healthy skin, a single application of Diprobase® cream, Aqueous cream BP, and to a lesser extent Doublebase™ gel reduced TEWL demonstrating that they transiently occlude the skin, thereby creating an artificial barrier. The degree of restoration was greatest immediately after application, and declined after 6 hours highlighting the need for regular application. The findings support current guidance to apply emollients 2-4 times daily.[9] Adherence to this guidance is associated with a greater treatment effect.[10] All emollients tested also transiently (between 6 and 24 hours) increased stratum corneum hydration, an effect associated with improved permeability barrier function.[32] Doublebase™ gel was associated with a 2-fold greater increase in hydration compared to Diprobase® and Aqueous cream, an effect most likely related to the glycerol in Doublebase™ gel.[32] Glycerol is a humectant, previously found to hydrate the stratum corneum. All three emollients transiently elevated skin surface pH by more than 0.5 units for at least 3 hours.

Following treatment twice daily for 28-days in patients with quiescent AD, neither Diprobase® cream nor Doublebase™ gel significantly affected the underlying barrier function of the skin (indicated by no change in baseline TEWL), assessed 12-24 hours following the last application. This demonstrates that the restoration of permeability barrier function seen for these emollients is both artificial and transient. Both emollients were associated with significantly increased skin surface pH for a sustained period, but induced no erythema under the conditions tested. The use of Doublebase™ gel, but not Diprobase® cream, led to a sustained increase in SC hydration, in agreement with the findings after a single application. The effects of Aqueous cream BP have been reported previously.[18,20]

How this study fits in with existing evidence

The emollient Aqueous cream was recently found to elevate TEWL by an average of 2.44 $g/m^2/h$ (24%) following a similar treatment regimen in a similar population (quiescent AD).[20] Following topical treatment with Aqueous cream, the SC was also found to be thinner, which was attributed to an increased rate of desquamation.[18] In support of this, *ex*

vivo activities of proteases engaged in desquamation were found to be elevated following treatment with Aqueous cream, and the surface area and maturity of the uppermost corneocytes was reduced suggesting premature shedding.[19] These negative effects of Aqueous cream are linked to the presence of SLS as a surfactant/emulsifier in the formulation. SLS is a standard skin irritant used in patch testing, and is known to increase protease-mediated degradation of the skin.[33] The fact that treatment with neither Diprobase® cream nor Doublebase™ gel reduced skin barrier function or SC thickness in this study strongly suggests that the SLS in Aqueous cream is responsible for its negative effects, and that the emulsifiers in Diprobase® cream and Doublebase™ gel are significantly less damaging.

It is notable that while neither Diprobase® cream or Doublebase™ gel damaged the skin barrier, they also did not improve the underlying condition of the skin barrier. Moreover skin surface pH was increased significantly following treatment with Diprobase® cream and Doublebase™ gel, by almost 1 unit immediately following a single application in healthy patients, and by approximately 0.2 units 12-20 hours following the last application of a 28-day regimen in patients with quiescent AD. Diprobase® cream and Doublebase™ gel have a pH of 4.9 and 7.1 respectively. The elevation of skin surface pH after application of these emollients reflects an adaption of the skin surface properties to those of the topical product. Despite the difference in pH between the products the effects on skin surface pH were similar. The persistence of product components on the skin, such as acids or buffers, likely accounts for the affects observed after 12-20 hours following application, although no surface residue of emollient was evident at this time-point. These changes are similar in scale to the differences between healthy and AD skin, between non-lesional and lesional AD skin, and between the skin of filaggrin loss-of-function mutation associated and non-associated AD skin.[34,35] The pH of the SC plays an important central role in skin barrier homeostasis and AD pathophysiology. Elevated SC serine protease activity, including chymotrypsin-like and trypsin-like activities is just one effect of elevated SC pH. What is not clear from the data presented here is the depth of the SC to which pH is disrupted and what the clinical consequences of this are. In the case of Diprobase® cream, a marginal reduction in SC integrity was seen upon tape stripping (elevated TEWL in response to tape-stripping), albeit not to the same extent as

observed for treatment with Aqueous cream. This coincided with the increase in chymotrypsin-like protease activity. It is reasonable to speculate therefore that the reduction in SC integrity results from pH-induced changes in the rate of desquamation/proteolytic degradation of the corneodesmosomal junctions between corneocytes. Protease activity was broadly increased after treatment with Doublebase™ gel, but this wasn't found to be significant and skin barrier integrity appeared to be improved. A functional consequence of elevated pH was therefore not observed for Doublebase™ gel, yet this could have been concealed. The reduction of skin surface pH and SC serine protease activity, through the use of buffers and some emollients (comprising buffers and acids), has been shown to elicit positive effects on the skin, such as reduced TEWL.[36,37] This suggests that the inability of the emollients tested here to control SC pH limits theirs beneficial effects on the skin.

The role of humectants in emollients

Humectants, like glycerol found in Doublebase™ gel, are added to emollients to improve their capacity to hydrate the skin. In line with this, skin hydration was 30% higher 12-20 hours following the last application of a 28-day treatment regimen with Doublebase™ gel; achieving a level of hydration sufficient to restore xerotic AD skin to healthy levels of hydration.[34] The degree of hydration was dependent on the length of time since the last application and the number of treatments made, based on the increased level of hydration observed following the 28-day treatment regimen compared to the single application of gel. Care is required when interpreting the hydration data obtained using the capacitance method because surface contaminants can affect the measurements. Whilst challenging to avoid following short treatment times, every care was taken to ensure surface residues were not present 12-20 hour post-treatment time-point following the 28-day treatment regimen. Similar findings have been reported for other emollients formulated with different humectants.[38,39] The role of the humectant is highlighted by the absence of increased hydration 12-20 hours following a similar treatment regimen with Diprobase® cream, which does not contain a humectant. However it should be noted that increased amounts of Doublebase™ gel compared to Diprobase® cream tended to be applied by participants who were self-treating. Under controlled conditions, a single application of 100µl Diprobase® cream in healthy individuals was observed to hydrate the skin

transiently, but to a significantly reduced extent compared to the application of an identical amount of Doublebase™ gel. Skin occlusion is the mechanism by which non-humectant emollients hydrate the skin. Evidencing this, a transient (lasting between 6 and 24 hours) reduction in TEWL by approximately 3 $g/m^2/h$ following application of Diprobase® cream was observed in conjunction with the elevation of skin hydration. The occlusivity of Doublebase™ gel was not as marked; whether this is due to reduced skin occlusion by Doublebase™ gel and/or the presence of added glycerol requires further investigation.[40] Humectants such as glycerol are hygroscopic, and so increase water levels within the SC. Following increases in SC humectant levels TEWL can increase as a result of increased evaporation from the larger store of water.

Conclusion

The finding that topical treatment with Aqueous cream adversely affects the skin barrier, and in doing so could potentially prolong or exacerbate AD, highlighted the need to determine the safety and appropriateness of other emollients as treatments for AD. We conclude that, unlike Aqueous cream, both Diprobase® cream and Doublebase™ gel are safe and appropriate for AD treatment as topical leave-on therapy in adults displaying a skin barrier defect. At birth the skin barrier is sub-optimal, and takes a number of months to years to reach adult-like status.[41] With this in mind the safety of these emollients should also be assessed in neonatal and infant skin. Notably the emollients tested did not display the barrier-strengthening properties reported for some other emollients in the literature. Given that the broad differences observed between infant and adult skin are similar to the differences observed between adults with and without eczema, it is unlikely that a treatment unable to strengthen the barrier in adults could strengthen it in infants and neonates.[41] This finding highlights that not all emollients are the same, and that effects on the underlying skin barrier represent a key distinguishing factor. A direct comparison of a barrier-strengthening topical product with an emollient without skin barrier effects revealed a significant difference in their ability to control AD.[23] The humectant glycerol, which differentiates Doublebase™ gel from Diprobase® cream, appears to significantly enhance the hydrating effects of Doublebase™ gel. Further clinical testing is required to determine whether Doublebase™ gel and Diprobase®

cream differ in their clinical efficacy and to assess the clinical benefit of added humectants per se beyond improved SC hydration.

TABLES

Table 1: Emollients used in this study

	Aqueous cream BP	*Diprobase® cream*	*Doublebase™ gel*
Container:	500g tub	50g tube	500g pump dispenser
Manufacturer:	Ecolab, UK	Merck Sharp & Dohme Limited, UK	Dermal Laboratories, UK
Occlusives:	Liquid paraffin White soft paraffin	White soft paraffin Liquid paraffin	Liquid paraffin
Humectants:			Glycerol
Surfactants/emulsifiers:*	Cetostearyl alcohol Sodium lauryl sulphate	Cetostearyl alcohol Macrogol cetostearyl ether	Isopropyl myristate Sorbitan laurate Triethanolamine
Other ingredients: (including stabilizers and preservatives)	Phenoxyethanol Purified water	Chlorocresol Sodium dihydrogen phosphate Sodium hydroxide Phosphoric acid Purified water	Carbomer Phenoxyethanol Purified water
Product pH	7.34±0.009	4.92±0.003	7.13±0.002

*These may also be used as skin softening agents

FIGURE LEGENDS

Figure 1: The effect of a single application of Aqueous cream (open circles), Diprobase® cream (closed squares) and Doublebase™ gel (open diamonds) on skin barrier function (TEWL, panel a), skin hydration (capacitance, panel b), and skin surface pH (panel c). Results are presented as the change compared to untreated skin. There are 8 repeats of each treatment in 4 subjects. For all three measured parameters a significant effect of the treatment and the interaction between the treatment and time-post washing was found ($p<0.05$, 2-way repeated measures ANOVA). Symbols (α, β, γ) indicate the results of a Dunnett post-test comparing all treatments to the untreated control.

Figure 2: The effect of 28-days treatment with Diprobase® cream, compared to no treatment (NTC), on the biophysical and biological properties of the skin in people with quiescent AD. (a) Skin barrier function, (b) skin hydration, (c) skin surface pH, (d) objective erythema, (e) SC integrity measured as TEWL in conjunction with tape-stripping, (f) SC cohesion (protein removed by tape-stripping), (g) estimated SC thickness, (h) *ex vivo* SC broad-spectrum caseinolytic protease activity, (g) chymotrypsin-like protease activity, (h) and trypsin-like activity. TEWL, capacitance, skin surface pH and redness were compared with ANCOVA using baseline (Day 0) values as the covariant. SC integrity and cohesion were assessed using a repeated measures 2-way ANOVA. Both the treatment ($p<0.0001$) and the number of tape-strips significantly ($p=0.0002$) affected SC integrity, but not the interaction between the two (n.s.). The number of tape-strips ($p<0.0001$) and the interaction between tape-strip number and treatment ($p=0.0009$) affected SC cohesion, but not the treatment independently (n.s.). SC thickness and caseinolytic, chymotrypsin-like and trypsin-like activities were compared using a paired t-test. *Significant differences using either a t-test (a-d and g-j) or Bonferroni post-test (e and f). n.s., not significant.

Figure 3: The effect of 28-days treatment with Doublebase™ gel, compared to no treatment (NTC), on the biophysical and biological properties of the skin in people with quiescent AD. (a) Skin barrier function, (b) skin hydration, (c) skin surface pH, (d) objective erythema, (e) SC integrity measured as TEWL in conjunction with tape-stripping, (f) SC cohesion (protein removed by tape-stripping), (g) estimated SC

thickness, (h) *ex vivo* SC broad-spectrum caseinolytic protease activity, (g) chymotrypsin-like protease activity, (h) and trypsin-like activity. TEWL, capacitance, skin surface pH and redness were compared with ANCOVA using baseline (Day 0) values as the covariant. SC integrity and cohesion were assessed using a repeated measures 2-way ANOVA. The treatment (p=0.0003), the number of tape-strips (p<0.0001) and the interaction between the 2 factors significantly (p<0.0001) affected SC integrity. The treatment (p<0.0001), the number of tape-strips (p<0.0001) and the interaction between the 2 factors significantly (p<0.0001) affected SC cohesion. SC thickness and caseinolytic, chymotrypsin-like and trypsin-like activities were compared using a paired t-test. *Significant differences using either a t-test (a-d and g-j) or Bonferroni post-test (e and f). n.s., not significant.

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FIGURES

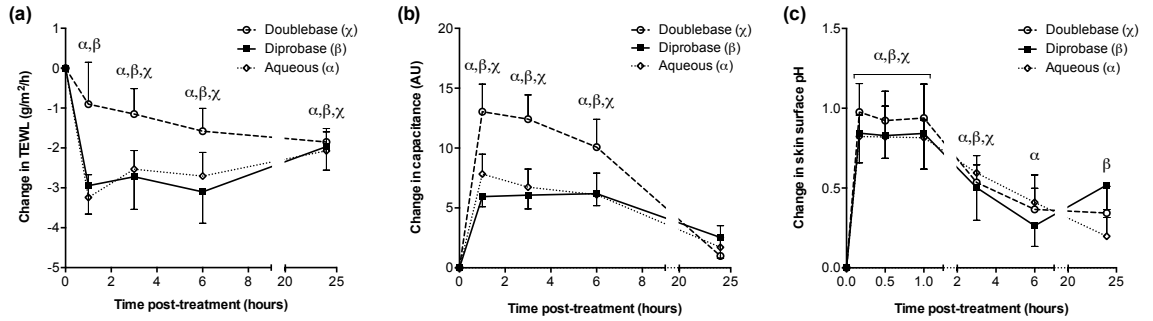

Figure 1: The effect of a single application of Aqueous cream (open circles), Diprobase® cream (closed squares) and Doublebase^{TM} gel (open diamonds) on skin barrier function (TEWL, panel a), skin hydration (capacitance, panel b), and skin surface pH (panel c). Results are presented as the change compared to untreated skin. There are 8 repeats of each treatment in 4 subjects. For all three measured parameters a significant effect of the treatment and the interaction between the treatment and time-post washing was found ($p<0.05$, 2-way repeated measures ANOVA). Symbols (α, β, γ) indicate the results of a Dunnett post-test comparing all treatments to the untreated control.

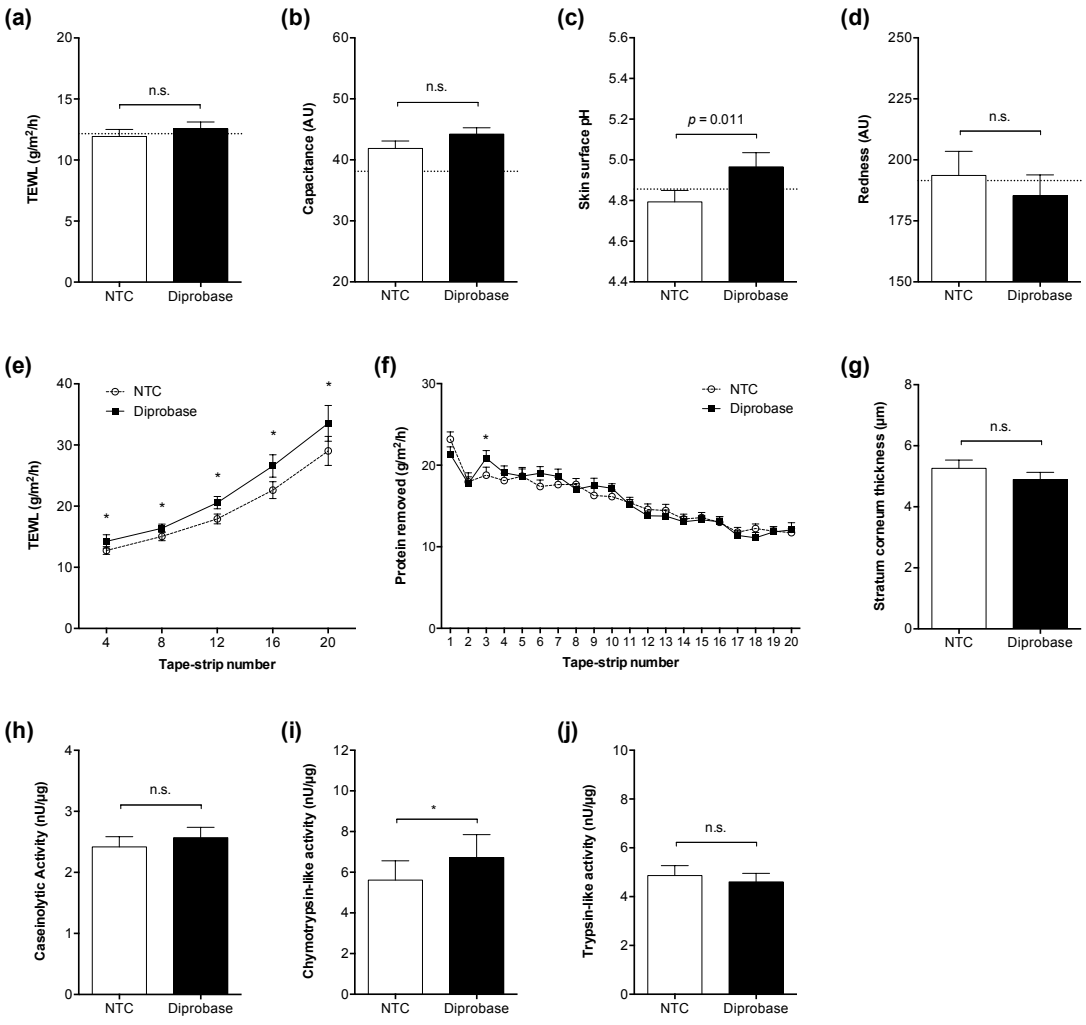

Figure 2: The effect of 28-days treatment with Diprobase® cream, compared to no treatment (NTC), on the biophysical and biological properties of the skin in people with quiescent AD. (a) Skin barrier function, (b) skin hydration, (c) skin surface pH, (d) objective erythema, (e) SC integrity measured as TEWL in conjunction with tape-stripping, (f) SC cohesion (protein removed by tape-stripping), (g) estimated SC thickness, (h) *ex vivo* SC broad-spectrum caseinolytic protease activity, (g) chymotrypsin-like protease activity, (h) and trypsin-like activity. TEWL, capacitance, skin surface pH and redness were compared with ANCOVA using baseline (Day 0) values as the covariant. SC integrity and cohesion were assessed using a repeated measures 2-way ANOVA. Both the treatment (p<0.0001) and the number of tape-strips significantly (p=0.0002) affected SC integrity, but not the interaction between the two (n.s.). The number of tape-strips (p<0.0001) and the interaction between tape-strip number and treatment (p=0.0009) affected SC cohesion, but not the treatment independently (n.s.). SC thickness and caseinolytic, chymotrypsin-like and trypsin-like activities were compared using a paired t-test. *Significant differences using either a t-test (a-d and g-j) or Bonferroni post-test (e and f). n.s., not significant.

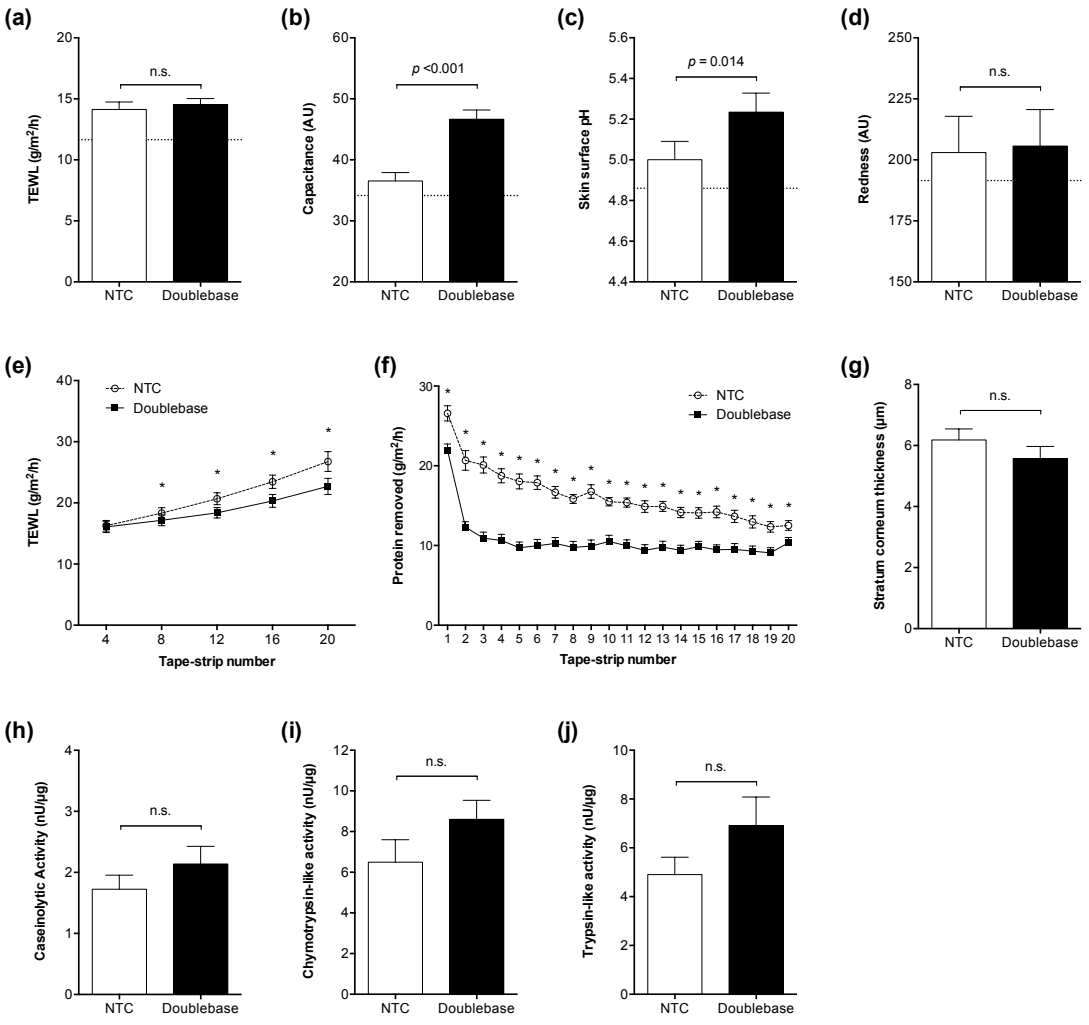

Figure 3: The effect of 28-days treatment with Doublebase™ gel, compared to no treatment (NTC), on the biophysical and biological properties of the skin in people with quiescent AD. (a) Skin barrier function, (b) skin hydration, (c) skin surface pH, (d) objective erythema, (e) SC integrity measured as TEWL in conjunction with tape-stripping, (f) SC cohesion (protein removed by tape-stripping), (g) estimated SC thickness, (h) *ex vivo* SC broad-spectrum caseinolytic protease activity, (g) chymotrypsin-like protease activity, (h) and trypsin-like activity. TEWL, capacitance, skin surface pH and redness were compared with ANCOVA using baseline (Day 0) values as the covariant. SC integrity and cohesion were assessed using a repeated measures 2-way ANOVA. The treatment (p=0.0003), the number of tape-strips (p<0.0001) and the interaction between the 2 factors significantly (p<0.0001) affected SC integrity. The treatment (p<0.0001), the number of tape-strips (p<0.0001) and the interaction between the 2 factors significantly (p<0.0001) affected SC cohesion. SC thickness and caseinolytic, chymotrypsin-like and trypsin-like activities were compared using a paired t-test. *Significant differences using either a t-test (a-d and g-j) or Bonferroni post-test (e and f). n.s., not significant.