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INTRODUCTION

Atopic dermatitis (AD) affects millions worldwide and markedly impairs quality of life. AD is characterized by genetically determined and inflammation-driven skin **barrier dysfunction**. Barrier impairment increases transepidermal water loss (TEWL), xerosis, pruritus and scratching, and facilitates the penetration of allergens, pathogens and irritants, thereby promoting type 2 inflammation. Beyond the long-described ceramide deficiency in AD, **recent lipidomic profiling of atopic skin has shown reduced levels of additional lipids originating from both sebum and the *stratum corneum*¹**. In addition, **tight junctions in the *stratum granulosum* are impaired in AD**, facilitating allergen and irritant penetration; with reduced claudin-1 expression being inversely correlated with type 2 inflammation².

MATERIALS & METHODS

Objective: To assess two combinations of ingredients designed to reinforce barrier function, and a novel emollient balm for AD (AD balm) incorporating these actives.

➤ Ex vivo evaluation of a lipid blend on *stratum corneum*

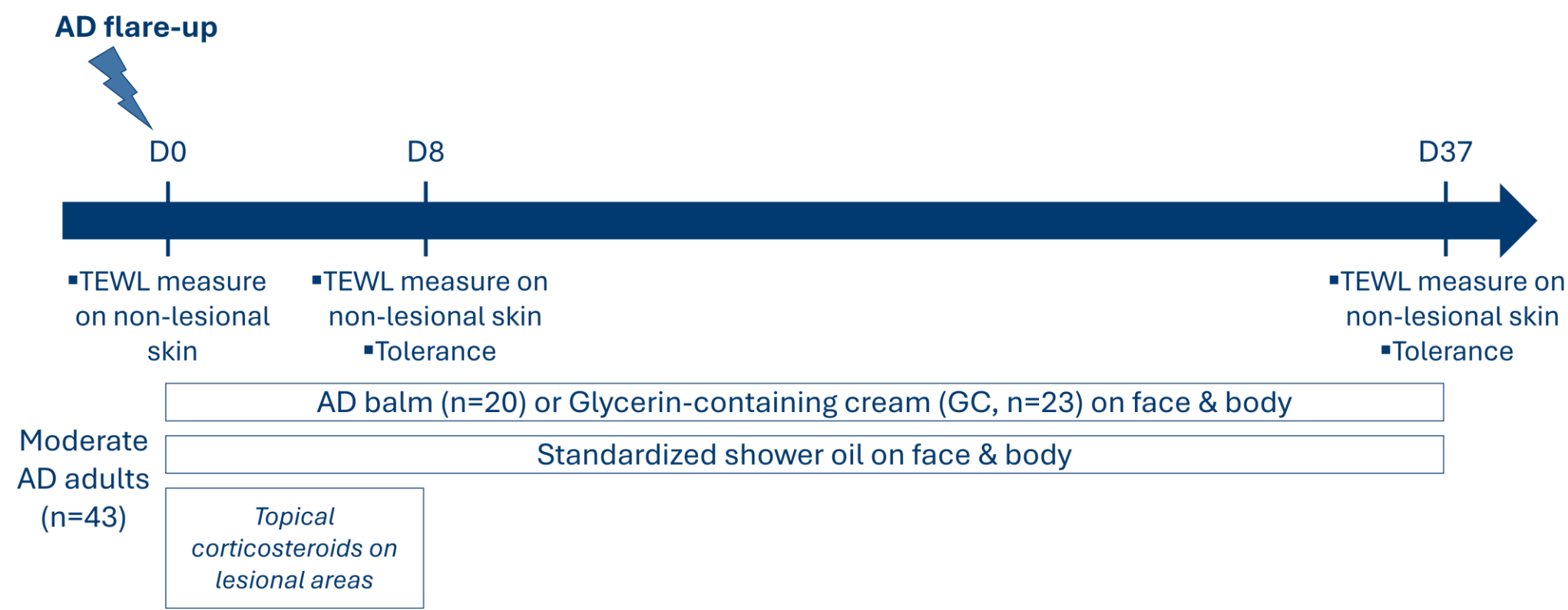
- 15-lipid blend composition: **15 different biomimetic lipid molecules reflecting those reduced in AD** (fatty acids C12:0, C14:0, C16:0, C18:0, C18:1, C18:2, C20:1, C22:1; fatty alcohol FOHC22:0; wax esters C40:2, C42:2; squalane; ceramides N18P18 and A18P18; and phytosphingosine)
- AD-like ex vivo model n°1:
 - Stripped human skin explants
 - Topical application of the lipid blend on D0, D1, D2, D3, D4
 - Ceramides** quantification by LC-MS on D5, normalized by total protein quantity.
- AD-like ex vivo model n°2:
 - IL-4/IL-5/IL-13/IL-22/IL-31-treated normal human skin explants
 - Topical application of the lipid blend on D0, D3 and D4
 - Filaggrin and loricrin** synthesis evaluated by immunostaining on D5. Ssurface of fluorescence, normalized by epidermis surface.

➤ In vitro evaluation of a duo of ingredients on tight junctions

- Duo of active ingredients: **Arginine + Glycosphingolipid**
- AD-like in vitro model:
 - IL-1β/IL-33/histamine-treated human primary keratinocytes
 - Ingredients added to the culture medium on D0.
 - Claudin-1 synthesis evaluated by immunostaining on D3. Intensity of fluorescence, normalized by cell number.

➤ Clinical evaluation of an emollient+ on barrier strengthening

- AD balm: an emollient+ containing the 15-lipid blend and the duo of active ingredients (among other active ingredients)
- Comparative, double-blind study clinical study:
 - 43 adults, 18-65 yo, with **moderate AD** (SCORAD 32.6 in average) and presenting with at least 1 lesional area at the beginning of the study
 - AD balm** (n=20) or **glycerin-containing cream** (GC cream, n=23) on face and body application for 37 days
 - Standardized hygiene with shower oil
 - Barrier assessment on non-lesional skin** (forearm or leg) measured by TEWL
 - Topical corticosteroids on lesional areas during the first 8 days
 - Tolerance** assessed by dermatologists and subjects



RESULTS

➤ Ex vivo evaluation of a lipid blend on *stratum corneum*

The biomimetic 15-lipid blend **significantly increased the components of the *stratum corneum*, thus demonstrated its effect on biological synthesis:**

- +51% NP and NS long-chain ceramides
- +22% filaggrin, +180% loricrin

**+51%
LONG-CHAIN
CERAMIDES**

**+22%
FILAGGRIN**

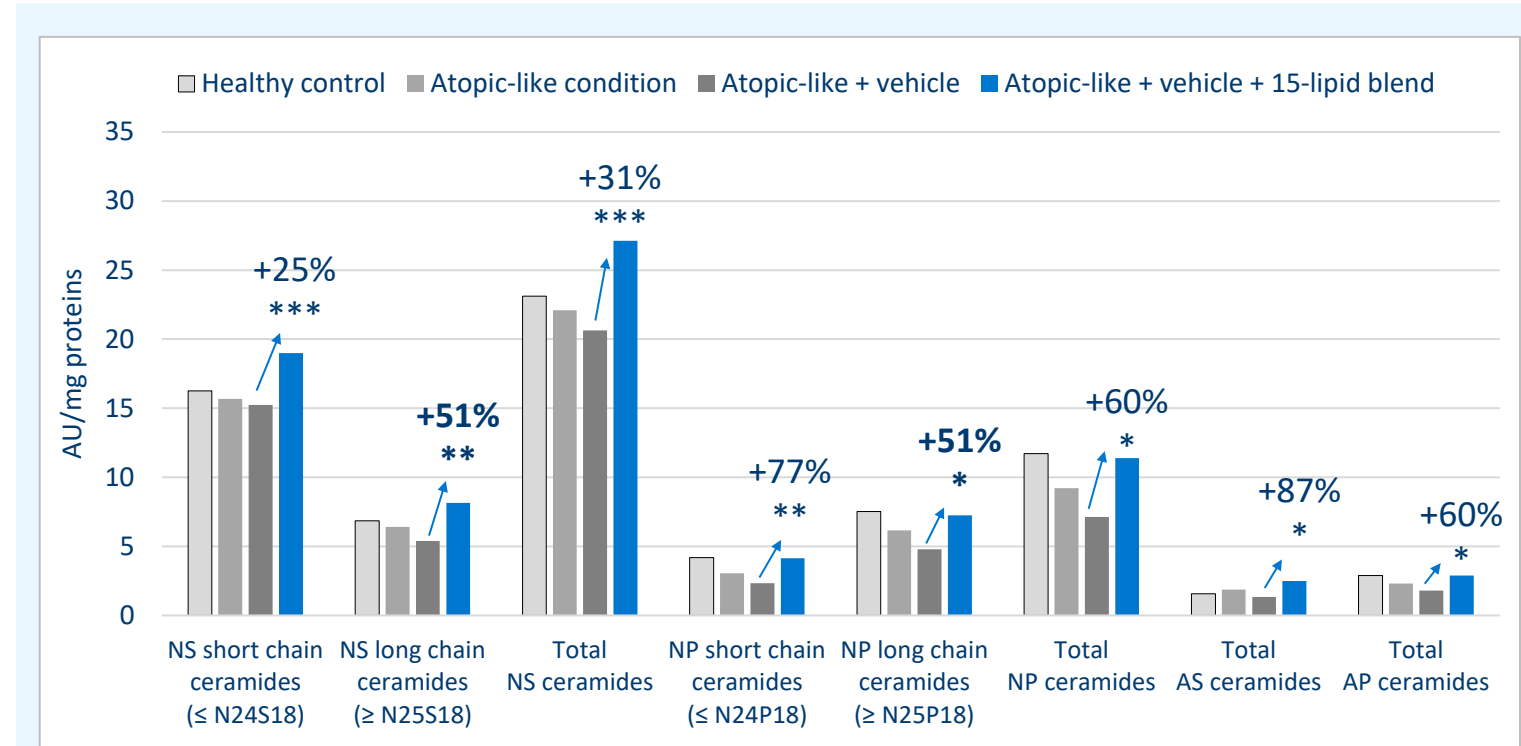


Figure 1. Ceramides synthesis by the 15-lipid blend
Quantification of ceramides synthesis by LC-MS on stripped human skin explants. *p<0.05, **p<0.01, ***p<0.001, Student t-test, versus "atopic-like + vehicle" condition
The 15-lipid blend significantly increased biological neo-synthesis of different ceramides on stripped ex vivo model, in particular some long-chain ceramides, not supplied by the blend, and known to be reduced in atopic skin³.

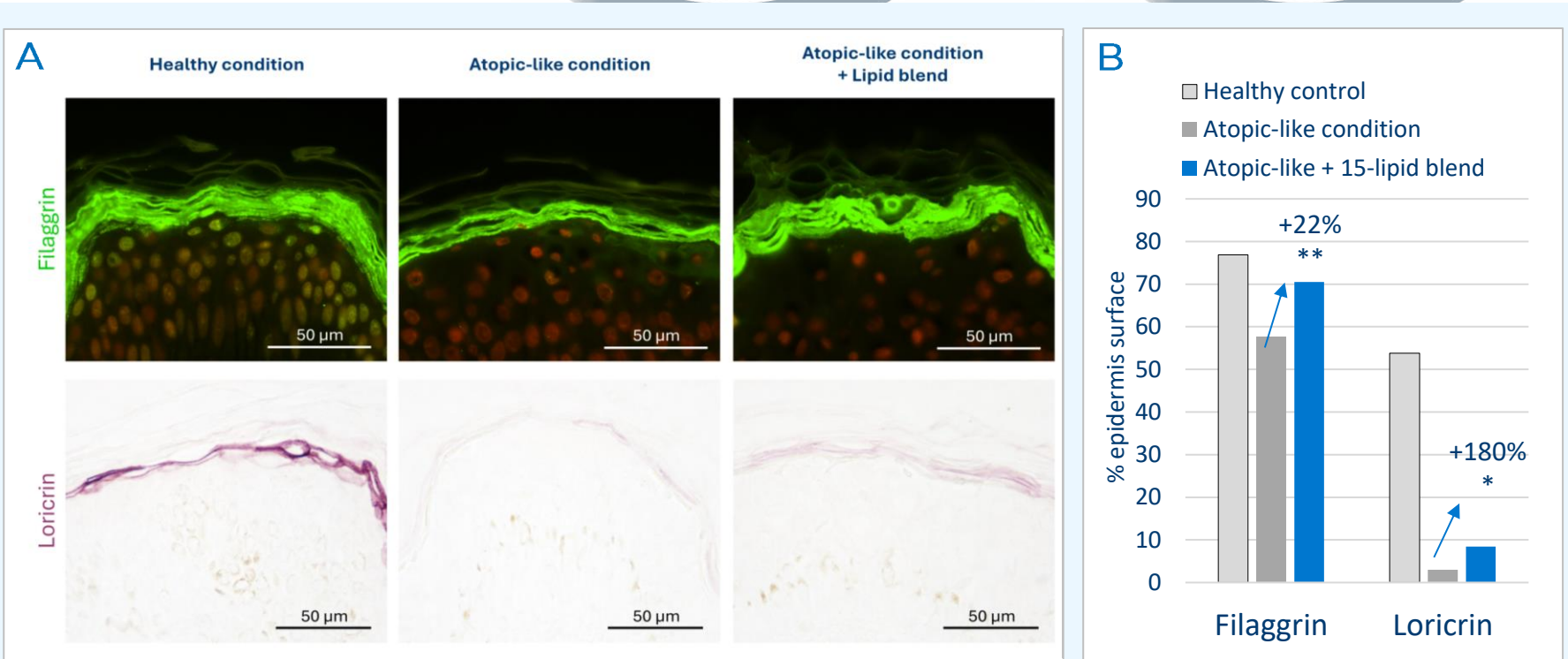


Figure 2. Filaggrin & loricrin synthesis by the 15-lipid blend
A/ Immunostaining on human skin explants (sections) of filaggrin and loricrin on normal (left), cytokines-stressed condition (middle) and cytokines-stressed condition with topical application of the 15-lipid blend (right). B/ Quantification of proteins synthesis by image analysis. *p<0.05, **p<0.01, Student t-test, versus "atopic-like" condition
The 15-lipid blend significantly increased biological neo-synthesis of filaggrin and loricrin proteins on cytokines-treated ex vivo model.

➤ In vitro evaluation of a duo of ingredients on tight junctions

The duo of biomimetic actives **synergistically increased claudin-1 by +250%**.

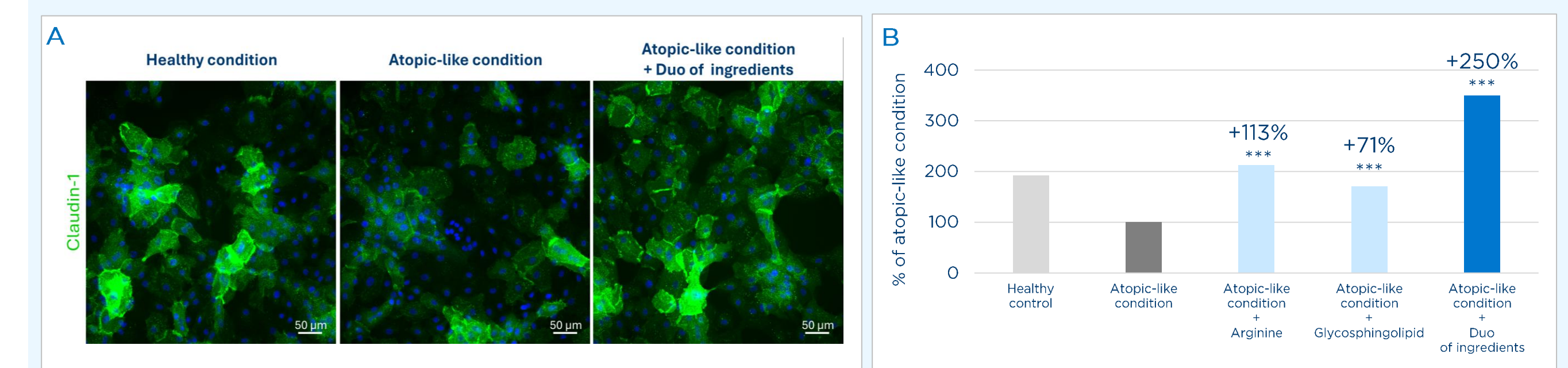


Figure 3. Claudin-1 synthesis on keratinocytes by the duo of ingredients
A/ Immunostaining on human keratinocytes of claudin-1 (green) and nucleus (blue) on normal (left), cytokines-stressed condition (middle) and cytokines-stressed condition with addition of the duo of active ingredients (right). B/ Quantification of proteins synthesis by image analysis. *p<0.05, **p<0.01, Student t-test, versus "atopic-like" condition.
Arginine alone and glycosphingolipid alone increase claudin-1 synthesis by keratinocytes, while the combination of these two ingredients enhanced synergically its synthesis.
In intestinal epithelial cells, where tight junctions also assure cell-cell cohesion, arginine was shown to stimulate Claudin-1 expression⁴ by the activation of AMPK signalling pathway⁵, while glycosphingolipid demonstrated to help stabilizing tight junctions⁶.

**+250%
CLAUDIN-1**

CONCLUSION/DISCUSSION

Replenishing with 15 lipids reduced in AD and biologically supporting both the *stratum corneum* and tight junctions in the *stratum granulosum*, this novel emollient balm robustly improved skin barrier function in adults with moderate AD from 8 days of use. These effects may help enhance hydration and provide sustained protection against penetration of flare triggers such as allergens and irritants.

➤ Clinical evaluation of an emollient+ on barrier strengthening

- TEWL decreased significantly in the AD balm group from D8 and further to D37.
- In the GC group, TEWL only decreased significantly on D37.
- Barrier improvement was significantly greater with AD balm than with GC.

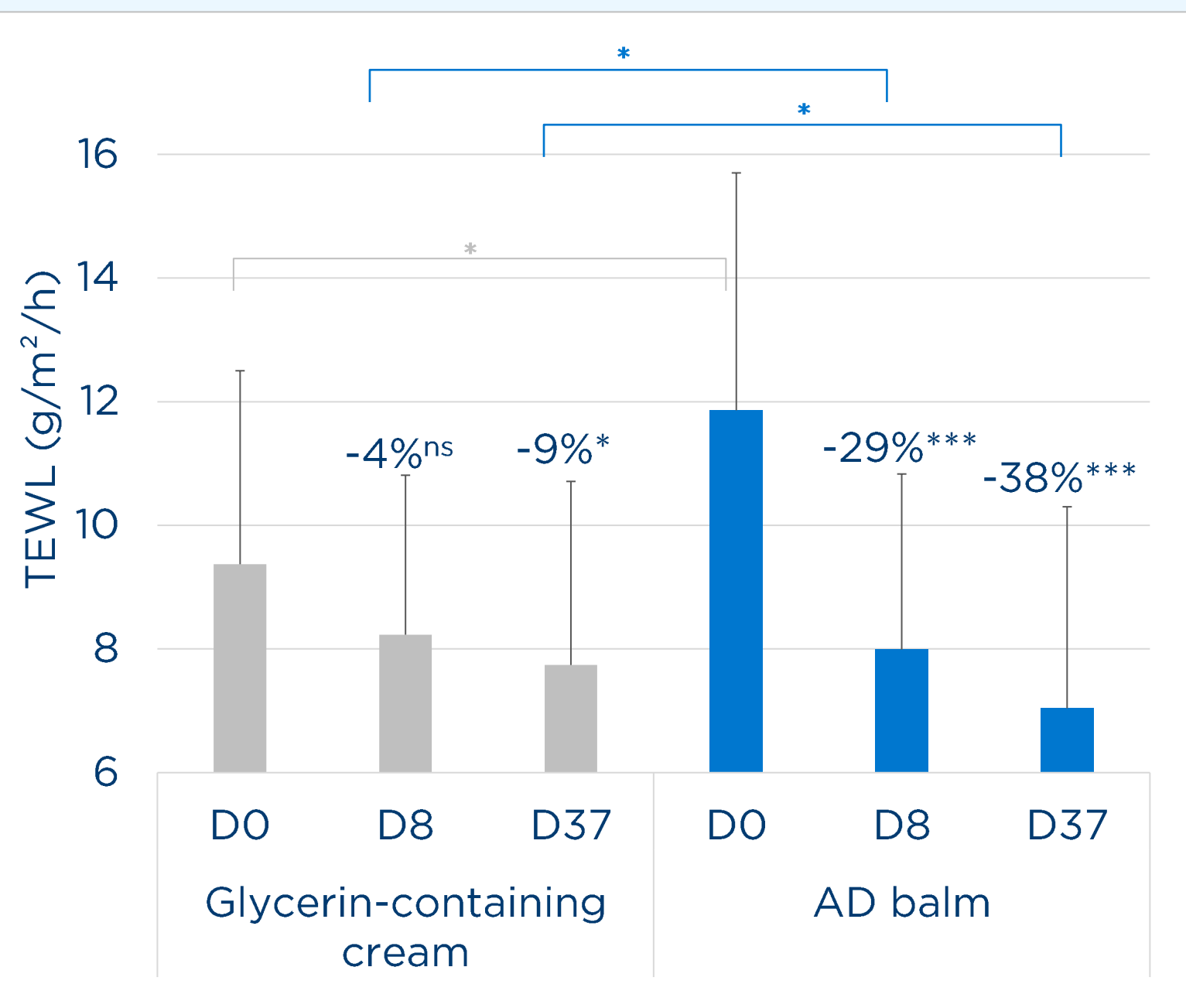


Figure 4. Skin barrier function assesement on non-lesional skin of moderate AD
Average +/-SD. n.s. non significant, *p<0.05, ***p<0.001, Student or Friedmann or Wilcoxon test, versus D0 or between groups

After 8 days of application, the AD balm showed a statistically significant improve in skin barrier function (-29% TEWL), while control group showed a non-significant reduction after 8 days and a slight statistical significant effect after 37 days.

Considering that the known average TEWL difference on forearm between healthy adults and non-lesional skin of atopic adults is 23%, the AD balm may be considered as rebuilding the barrier to a degree comparable to that of healthy skin.
Furthermore, the data available in the literature with the same type of protocol (moderate atopic adults with adapted cleanser) shows that a ceramides-based cream strengthens the barrier by only -15% TEWL in 7 days⁷.

NB1: Randomisation between groups was done according to SCORAD, explaining TEWL difference on D0.
NB2: TEWL was also assessed in the affected areas. However, topical corticosteroids had a major impact on restoring the skin barrier function. Although the results tended to show better restoration with the AD balm than with the GC cream, no statistically significant difference was observed between groups.

**HIGH
TOLERANCE**

AD balm was very well tolerated, including on lesional areas when used in combination with topical corticosteroids.

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² De Benedetto A, et al. Tight junction defects in patients with atopic dermatitis. J Allergy Clin Immunol. 2011 Mar;127(3):775-86.e1-7.
³ Ishikawa J, et al. Changes in the ceramide profile of atopic dermatitis patients. J Invest Dermatol. 2010 Oct;130(10):2511-4.
⁴ Ren W, et al. Serum amino acids profile and the beneficial effects of L-arginine or L-glutamine supplementation in dextran sulfate sodium colitis. PLoS One. 2014 Feb 5;9(2):e88335.
⁵ Xia Z, et al. L-Arginine alleviates heat stress-induced intestinal epithelial barrier damage by promoting expression of tight junction proteins via the AMPK pathway. Mol Biol Rep. 2019 Dec;46(6):6435-6451.
⁶ Patterson L, et al. Glucosylceramide production maintains colon integrity in response to Bacteroides fragilis toxin-induced colon epithelial cell signaling. FASEB J. 2020 Dec;34(12):15922-15945.
⁷ Spada F, et al. A daily regimen of a ceramide-dominant moisturizing cream and cleanser restores the skin permeability barrier in adults with moderate eczema: A randomized trial. Dermatol Ther. 2021 Jul;34(4):e14970.