



Effects of Locally Applied Glycerol and Xylitol on the Hydration, Barrier Function and Morphological Parameters of the Skin

Csilla KORPONYAI¹, Edit SZÉL¹, Zoltán BEHÁNYI¹, Erika VARGA¹, Gábor MOHOS¹, Ágnes DURA², Shabtay DIKSTEIN³, Lajos KEMÉNY^{1,4} and Gábor ERŐS^{1,5}

¹Department of Dermatology and Allergology, ²Central Pharmacy and ⁵Department of Pathology, University of Szeged, Szeged, Hungary, ³Unit of Cell Pharmacology, School of Pharmacy, The Hebrew University, Jerusalem, Israel, and ⁴MTA-SZTE Dermatological Research Group, Szeged, Hungary

Glycerol and xylitol hydrate the skin and improve its barrier function over a short period. We studied the effects of glycerol and xylitol on the physiological properties and morphology of the skin after longer-term application. Twelve volunteers with dry skin were examined. Three areas on the arms were determined. Area 1 served as untreated control. The vehicle was applied to area 2, while area 3 was treated twice daily with a formulation containing glycerol (5%) and xylitol (5%) for 14 days. Transepidermal water loss (TEWL), hydration and biomechanical properties of the skin were monitored. Biopsies were taken for routine histology and immunohistochemistry for filaggrin and matrix metalloproteinase-1 (MMP-1). The polyols increased the skin hydration and protein quantity of filaggrin, elevated the interdigitation index, decreased the TEWL and improved the biomechanical properties of the skin, but did not change the protein expression of MMP-1. A combination of glycerol and xylitol can be useful additional therapy for dry skin.

Key words: glycerol; xylitol; filaggrin; skin hydration; trans-epidermal water loss.

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Corr: Csilla Korponyai, Department of Dermatology and Allergology, Faculty of Medicine, University of Szeged, Korányi fasor 6., HU-6720, Szeged, Hungary. E-mail: csillakorponyai81@gmail.com

Ageing and various dermatological disorders (e.g. atopic and irritant dermatitis) are accompanied by dry skin. Hence, it is of great importance to identify and apply agents that hydrate the skin, maintain dermal homeostasis and retard skin ageing. With this goal, glycerol is frequently used in dermatological preparations. This polyol acts as a humectant (1), hydrates the skin and improves the barrier function through a complex mechanism (2). A structurally similar polyol, xylitol, can also be used as a humectant and moisturizer (3–5). A recent study has revealed that, in addition to their skin-hydrating properties, both glycerol and xylitol exert anti-irritant and anti-inflammatory effects in a dose-dependent manner (6). Despite their similar chemical structures, these polyols induce different gene expression changes in the keratinocytes. *In vitro* experiments have demonstrated that glycerol decreases the

expression of human leukocyte antigen DR (HLA-DR), thereby reducing inflammation, while xylitol increases the expression of filaggrin (7). As a source of natural moisturizing factor (NMF) and also in other ways, filaggrin contributes to hydration and homeostasis of the skin (8). A potential filaggrin-inducing effect of xylitol may raise other potential pathways. It has been described that, via the induction of filaggrin expression, hydration of the skin can be accompanied by the suppression of matrix metalloproteinase-1 (MMP-1) (9). This enzyme plays a pivotal role in photoageing (10). The different impacts of glycerol and xylitol on gene expression suggest a possible synergistic effect through which a combined application of these polyols may achieve better therapeutic and cosmetic results. We earlier reported the anti-bacterial and skin-hydrating effects of a formulation containing glycerol and xylitol (11). However, in that *in vivo* investigation the polyols were applied for at most 48 h. The impacts accompanying longer application are therefore not known. Thus, our present objective was to study the effects of a glycerol- and xylitol-containing combination product on hydration, barrier function, biomechanical parameters and morphology of the human skin after 14 days of application. A further aim was to identify the protein quantities of filaggrin and MMP-1 in order to establish an explanation for the beneficial effects of these polyols.

MATERIALS AND METHODS

Glycerol was obtained from Cognis (Dusseldorf, Germany) and xylitol and Carbopol Ultrez 10 from Sigma Aldrich Corp. (St Louis, MO, USA). The quality of all these compounds met the European Pharmacopoeia (Ph Eur 5) standards. The rabbit polyclonal anti-human filaggrin antibody (catalogue no: AB24584) and the rabbit monoclonal anti-human MMP-1 antibody (clone EP 1247Y, catalogue no: AB52631) were from Abcam Ltd (Cambridge, UK).

Our study involved 12 healthy human volunteers who were prone to dry skin: 7 women and 5 men aged 50–60 years. Inclusion criteria were low hydration values (≤ 25 AU) on the inner upper arms, measured with a Corneometer CM 825 (Courage+Khazaka electronic GmbH, Cologne, Germany). Exclusion criteria were: (i) major skin diseases; (ii) pregnancy or breast-feeding; (iii) systemic corticosteroid or cytostatic therapy within 30 days; (iv) any use of local drugs within 30 days that might influence the skin texture; and (v) any condition on the inner upper arms that could interfere with a clear-cut assessment of the skin; and (vi) current participation in any other clinical study.

The study was approved in advance by the local institutional ethics committee for human biomedical trials at the University of Szeged (No. 3353 2014-03-10, CSR/039/00426-5/2014). All subjects participated only after receiving detailed oral and written information and signing an informed consent agreement.

Experimental design

Three 2×2 cm areas were marked out on both lateral upper arms. Area 1 served as an untreated control. Area 2 received the vehicle (Carbopol Ultrez 10 0.4%, dissolved in purified water). A gel containing 5% xylitol and 5% glycerol (dissolved in the above-mentioned vehicle) was applied to area 3. Two treatments were applied daily for 14 days. Measurements were performed twice: the studied parameters were determined before the first application of the preparations and on day 14, 6 h after the last treatment. Finally, full-thickness skin biopsies were taken from each area with a 4-mm circular blade ("punch biopsy") under local anaesthesia. The wounds were then closed with a single suture.

In vivo measurements

The investigations were performed in the Cosmetological and Skin-Physiological Research Laboratory of the Department of Dermatology and Allergology at the University of Szeged. Room conditions were controlled; the relative humidity was 40–50%, and the ambient temperature was kept at 20–22°C. All measurements were performed after a 15–20 min relaxation period.

The extent of hydration of the skin surface was measured with a Corneometer CM 825. TEWL was determined with a Tewameter TM 300 (Courage+Khazaka electronic GmbH, Cologne, Germany). The skin friction was monitored with a Frictionmeter FR 700 (Courage+Khazaka electronic GmbH, Cologne, Germany). A Cutometer MPA 580 (Courage+Khazaka electronic GmbH, Cologne, Germany) was used to assess the skin elasticity. R-parameters, calculated by the software of the device, were registered and compared.

Furthermore, the skin was examined with a DUB®-USB high-frequency, high-resolution, ultrasound system (Taberna pro Medicum GmbH, Luneburg, Germany). A volume of 80 dB was used to take images which were evaluated off-line. Epidermal and dermal thicknesses and the echogenicity of the papillary dermis were measured by means of DUB-SkinScanner software.

Histology and immunohistochemistry

Tissue samples obtained with the biopsy were fixed in a buffered solution of formaldehyde (4%), embedded in paraffin and 3-µm thick coded slides were made. One slide was stained with haematoxylin-eosin (H&E), while the others were subjected to immunohistochemistry by means of a Leica BOND-MAX autostainer (Leica Biosystems, Nussloch, Germany). Retrieval was performed at pH=6 at 100°C for 20 min. Filaggrin antibody was used in 1:1000 dilution, and MMP-1 antibody in 1:50 dilution. Both antibodies were applied for 20 min. A Bond Polymer Refine Detection Kit (Leica Biosystems) was then used; the sections were exposed to 3,3'-diaminobenzidine (DAB) for 10 min, followed by counterstaining with haematoxylin.

All slides were scanned and analysed with Panoramic Viewer software (3D Histech, Budapest, Hungary). In the H&E-stained sections, the interdigitation index was determined, as described by Timár et al. (12). Briefly, the length of the line following the interdigitation between 2 points on the border between the epidermis and the dermis was divided by the length of a straight line between the same 2 points in order to calculate the interdigitation index. In the filaggrin-stained slides, the percentage of epidermal cells showing positive staining was determined. For the charac-

terization of the quantity of MMP-1 protein, a semi-quantitative scoring system was used: 1: mild, 2: moderate, 3: expressed positivity in the epidermis.

Statistical analysis

Statistical analysis was performed with the SigmaStat for Windows statistical software package (Jandel Scientific, Erkrath, Germany). The Shapiro-Wilk test was used to check normality. In terms of a few parameters, the values obtained varied significantly from the pattern expected if the data were drawn from a population with a normal distribution. Non-parametric methods were therefore used. The Wilcoxon signed-rank test was applied to compare data obtained before and after the treatments. The differences between the treatments were analysed with the Kruskal-Wallis one-way analysis of variance on ranks, followed by Dunn method for pairwise multiple comparison. In the Figures, median values with the 25th and 75th percentiles are given; $p < 0.05$ and $p < 0.001$ were considered statistically significant.

RESULTS

Statistical analysis did not reveal any difference between the day 0 values for the different areas. Corneometer CM 825 measurements revealed that the skin hydration did not display considerable changes in the control area. The vehicle appeared to exert some skin-hydrating effect. However, glycerol and xylitol led to more expressed increases in hydration (Fig. 1A).

The lack of treatment or exposure to the vehicle did not alter the TEWL by the end of the observation period. Application of glycerol and xylitol significantly reduced the TEWL. Furthermore, the TEWL values of the areas exposed to the polyols were found to be considerably lower than those of the control areas on day 14 (Fig. 1B).

The alterations in the biomechanical parameters are demonstrated in Fig. 1 C, D. Friction values measured after the use of vehicle were higher than the day 0 data, but did not differ from the appropriate values of the control area. Treatment with glycerol and xylitol resulted in much more expressed elevations in friction values, which were also higher than those determined in the control area (Fig. 1C). As concerns the R-parameters determined with the Cutometer MPA 580, only R0 exhibited noteworthy changes. R0 was significantly higher values after the application of glycerol and xylitol for 14 days, the other preparation not influencing this parameter (Fig. 1D).

Fig. 1 E–G. depicts the results obtained on the evaluation of the ultrasound images. Exposure to glycerol and xylitol led to a significant increase in epidermal thickness. The vehicle did not induce changes in this parameter that differed statistically from the day 0 values (Fig. 1E).

The dermal thickness was also enhanced by glycerol and xylitol. However, such changes were not found in the other areas (Fig. 1F). Both the vehicle and the preparation with glycerol and xylitol decreased the echogenicity of the papillary dermis. However, the polyols led to a more considerable reduction in this parameter and a difference was also found compared with the control area (Fig. 1G).

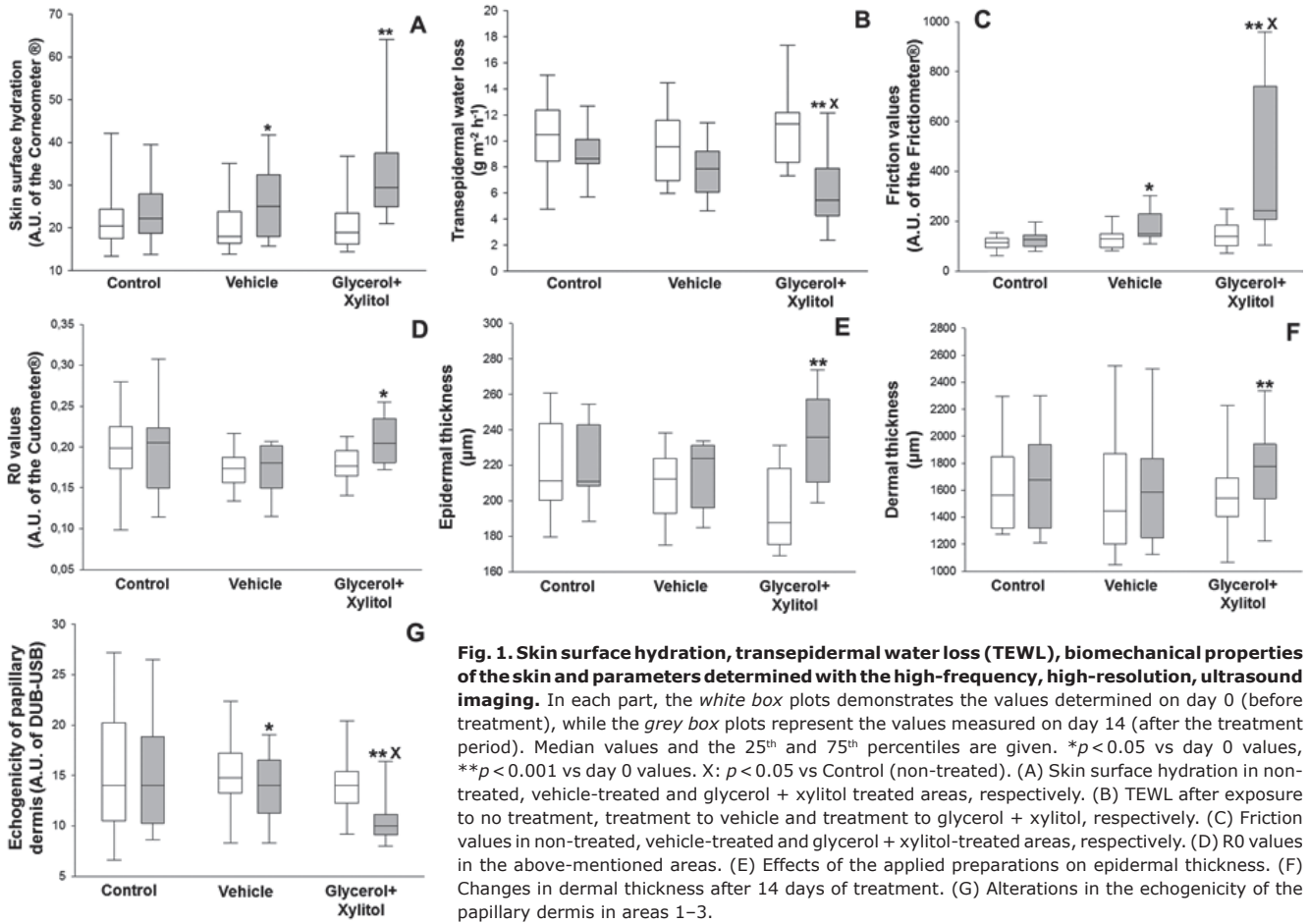


Fig. 1. Skin surface hydration, transepidermal water loss (TEWL), biomechanical properties of the skin and parameters determined with the high-frequency, high-resolution, ultrasound imaging. In each part, the *white box* plots demonstrates the values determined on day 0 (before treatment), while the *grey box* plots represent the values measured on day 14 (after the treatment period). Median values and the 25th and 75th percentiles are given. * $p < 0.05$ vs day 0 values, ** $p < 0.001$ vs day 0 values. X: $p < 0.05$ vs Control (non-treated). (A) Skin surface hydration in non-treated, vehicle-treated and glycerol + xylitol treated areas, respectively. (B) TEWL after exposure to no treatment, treatment to vehicle and treatment to glycerol + xylitol treated areas, respectively. (C) Friction values in non-treated, vehicle-treated and glycerol + xylitol-treated areas, respectively. (D) R0 values in the above-mentioned areas. (E) Effects of the applied preparations on epidermal thickness. (F) Changes in dermal thickness after 14 days of treatment. (G) Alterations in the echogenicity of the papillary dermis in areas 1–3.

As demonstrated by the histological images, the interdigitation index displayed relatively low values in the control area. Fourteen days of use of glycerol and xylitol resulted in a more expressed interdigitation between the epidermis and the dermis compared with the untreated control. The vehicle did not appear to influence the interdigitation (Fig. 2).

In the control areas, approximately 25% of the epidermal cells showed positivity to filaggrin. Exposure to xylitol and glycerol increased this ratio considerably. Application of the vehicle alone was not accompanied by changes in the expression of filaggrin (Fig. 3).

MMP-1 protein was also found to be present in the epidermis. However, the applied formulations did not change the quantity of MMP-1. No significant difference was found between the control and the treated areas in terms of MMP-1 (data not shown).

DISCUSSION

Moisturizers of different types are cornerstones for the treatment of dry skin. Humectant-based formulations, which attract and bind water from the deep epidermis and environment, effectively hydrate the skin and provide

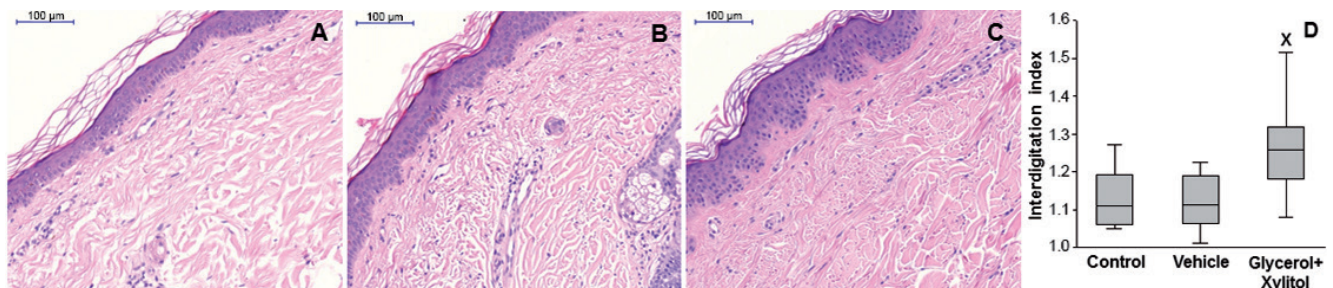


Fig. 2. Photomicrographs of the skin from different areas (haematoxylin-eosin staining, scale bar 100 µm). (A) Control, (B) Vehicle-treated skin, (C) Skin exposed to glycerol + xylitol, (D) Interdigitation index of the above-mentioned areas. Median values and 25th and 75th percentiles are given. X: $p < 0.05$ vs Control.

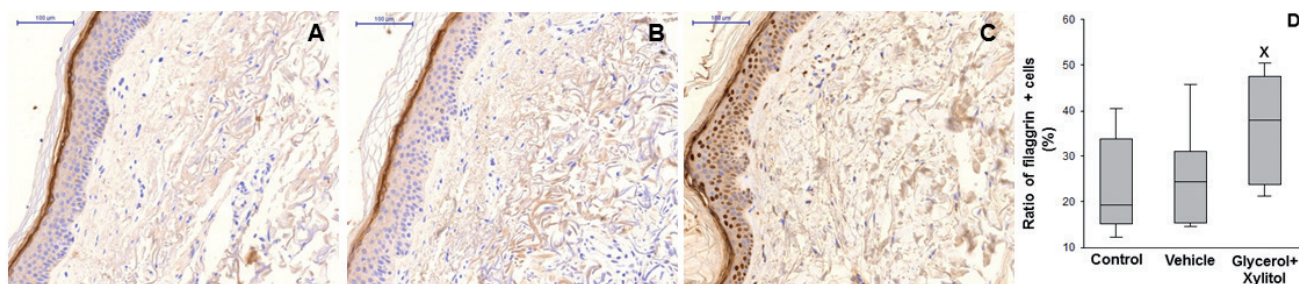


Fig. 3. Presence of filaggrin in the epidermis (immunohistochemistry for filaggrin, counterstaining with haematoxylin; filaggrin-positive cells appear brown; scale bar 100 μ m). (A) Control, (B) vehicle-treated skin, (C) skin exposed to glycerol + xylitol, (D) ratio of filaggrin-positive cells expressed as percentage. Median values and 25th and 75th percentiles are given. X: $p < 0.05$ vs Control.

better patient compliance than other preparations (13). Glycerol belongs among the most frequent moisturizers; its beneficial effects have been utilized for several decades. Many studies have confirmed that glycerol effectively hydrates the stratum corneum (SC) (2). The skin-hydrating effect of xylitol has also been described in atopic dermatitis (14) and in a murine model of irritant contact dermatitis (6). Our previous study revealed that the combined application of glycerol and xylitol significantly elevated the hydration of the SC in healthy probands within 2 h (11). Our present investigation has shown that a longer application of these polyols increases not only the water content of the SC, but also that of the deeper layers. Ultrasound imaging has revealed an increase in both the epidermal and dermal thickness after exposure to glycerol and xylitol. Thickening of the skin can be regarded as a marker of hydration (15). Nevertheless, different skin diseases (e.g. inflammation) may also lead to swelling, but no sign of such disorders was detected in this study. Moreover, the echogenicity of the papillary dermis was measured to be lower and can be explained in terms of the binding of a larger amount of water due to the moisturizing effect (16). Improvement of the studied biomechanical parameters may also originate in the hydrating effect of glycerol and xylitol. Since the friction coefficient correlates positively with the hydration of the SC (17), the higher friction values, i.e. the smoother skin, detected in our investigation may be explained in terms of a higher water content of the skin. The parameter R0, which reflects the passive behaviour of the skin to force, was also found to be higher after the application of polyols. R0 demonstrates a positive correlation with hydration (18) and can be used as a marker in the study of hydrating agents (19). However, another study has suggested that the effects of glycerol on the mechanical parameters may be independent of its hydrating ability (20). Thus, the plasticity and distensibility of the skin may be influenced not only by hydration, but also by other mechanisms. The effects of glycerol on the epidermal lipid structure may explain its beneficial impact on its mechanical properties (21). Nevertheless, we have confirmed by means of different methods that glycerol and xylitol considerably increase the water

content in the superficial and deeper layers of the skin. The water-binding capacity of the polyols, originating in their chemical structure, is a possible, but not the only, explanation of the hydrating effect. An important new finding of our study is that the application of these polyols increases the quantity of filaggrin at the protein level in the skin. The preliminary *in vitro* data suggest that xylitol leads to an elevated protein expression of filaggrin (7). Since the application of glycerol alone does not seem to influence the expression of filaggrin *in vivo* (22), it can be assumed that xylitol is responsible for the increased quantity of filaggrin. However, the exact mechanism via which this polyol elevates the expression of filaggrin demands further investigation. Although the vehicle alone (as an aqueous solution) influences few parameters, which may suggest some hydrating effect of this preparation, the application of the glycerol- and xylitol-containing gel was accompanied by a more expressed hydration of the skin, the filaggrin expression was not altered by the vehicle. The polyols therefore seem to be responsible for the real hydrating ability of the preparation.

Besides hydration, glycerol and xylitol effectively improve barrier function of the skin, as indicated by reduced TEWL values. Our earlier examination revealed that exposure to glycerol and xylitol for 24 h increased SC hydration, which was not accompanied by a change in TEWL (11). Thus, a longer application of polyols appears to be needed for an improvement of the barrier function. This finding is in accordance with those of previous studies, which concluded that the use of glycerol for at least 3 days reduces TEWL significantly (23, 24). Xylitol tends to decrease TEWL in patients with atopic dermatitis after 7 days of use, but this change is not significant (14). However, in our recent animal experiments glycerol and xylitol separately were able to prevent the elevation of TEWL in irritation (6). The hydrating effect of the polyols may be a potential explanation for the improvement of TEWL. An inverse relationship between TEWL and SC hydration is well known and moisturizers often improve barrier function. The mechanism of this interplay has not yet been fully clarified (25). However, the filaggrin expression and NMF level appear to contribute to the

skin barrier integrity. An age-related decline in barrier function has been described (26) and it may be connected with a lower NMF level in aged skin (27). A recent study revealed that exposure to irritants, which results in barrier disruption, decreases the levels of NMF (28). Thus, influencing the NMF level via the filaggrin expression may contribute to the anti-irritant effect of the polyols.

Histological analysis has revealed that the morphology of the dermal-epidermal junction (DEJ) is also influenced by the polyol treatment. It is known that skin ageing is accompanied by the flattening of the DEJ, and the rate of ridge height increase decreases with age (29). The interdigitation index is an appropriate indicator for the characterization of this alteration (12). Morphological changes of ageing may originate in dermal atrophy, decreased collagen biogenesis and loss of elastic fibres (30, 31). An elevation in interdigitation index after exposure to polyols might indicate some rejuvenation effect of glycerol and xylitol. Since ultrasound images have shown that polyol-induced hydration affects not only the epidermis, but also the dermis, these compounds may interfere with age-related dermal alterations. However, it should be mentioned that polyols influenced neither the gross elasticity (parameter R2 of the Cutometer) nor the net elasticity (parameter R5 of the Cutometer) in the present study. Hence, no considerable restoration of the elastic fibres is to be expected. Moreover, glycerol and xylitol do not seem to decrease the quantity of MMP-1, which might have contributed to their potential anti-ageing effect. Thus, a rejuvenation effect of the polyols and its mechanism requires further examination.

In conclusion, the combined application of glycerol and xylitol effectively hydrates the skin, and improves its barrier function and mechanical properties. These beneficial effects may originate in the increased protein quantity of filaggrin induced by these polyols. Hence, the combination of glycerol and xylitol may be useful additional therapy for dry skin and may also soothe the age-associated changes in the skin.

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Conflict of interest: SD is an owner of a patent for a glycerol- and xylitol-containing formulation. The other authors declare no conflict of interest.

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