Effects of Glyceryl Glucoside on AQP3 Expression, Barrier Function and Hydration of Human Skin

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Key Words

Aquaporin 3 • Glyceryl glucoside • Water transport •
Transepidermal water loss measurement

Abstract

Background/Aim: Aquaporins (AQPs) present in the epidermis are essential hydration-regulating elements controlling cellular water and glycerol transport. In this study, the potential of glyceryl glucoside [GG; α -D-glucopyranosyl- α -(1 $\rightarrow$ 2)-glycerol], an enhanced glycerol derivative, to increase the expression of AQP3 in vitro and ex vivo was evaluated. **Methods:** In vitro studies with real-time RT-PCR and FACS measurements were performed to test the induction by GG (3% w/v) of AQP3 mRNA and protein in cultured human keratinocytes. GG-containing formulations were applied topically to volunteer subjects and suction blister biopsies were analyzed to assess whether GG (5%) could penetrate the epidermis of intact skin, and subsequently upregulate AQP3 mRNA expression and improve barrier function. **Results:** AQP3 mRNA and protein levels were significantly increased in cultured human keratinocytes. In the studies on volunteer subjects, GG significantly increased AQP3 mRNA levels in the skin and reduced transepidermal water loss compared with vehicle-controlled areas.

Conclusion: GG promotes AQP3 mRNA and protein upregulation and improves skin barrier function, and may thus offer an effective treatment option for dehydrated skin.

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Introduction

Water is vital for the correct physiological functioning, maintenance and appearance of healthy skin. To this extent, water loss from human skin is precisely regulated by the complex nature of the stratum corneum. Natural moisturizing factor present within the corneocytes binds water, holding it within the stratum corneum while intercellular lipids located around the corneocytes form a barrier to prevent transepidermal water loss (TEWL) [1].

In addition to the obvious need to prevent water loss from the skin to the environment, the skin itself must maintain a correct water balance in the viable cell layers. Aquaporins (AQPs) are a family of transmembrane channel proteins responsible in part for this role, transporting water and other small solutes between and into cells [for review, see 2]. The most abundant aquaporin in the epidermis is AQP3. This protein, which is mainly expressed in the plasma membrane of epidermal keratinocytes [3], belongs

to the so-called aquaglyceroporin group of AQP3s, transporting both water and the humectant glycerol and thereby playing an important role in maintaining hydration levels in the mammalian skin epidermis [4]. Phenotype analysis of AQP3-null mice has helped to explain the role of AQP3 in keratinocytes and epidermal tissues [5, 6]. Accordingly, AQP3-facilitated water and glycerol transport is involved in cell migration, accelerating the healing of cutaneous wounds, skin hydration state and skin elasticity. Moreover, AQP3 is also involved in cell proliferation, differentiation, lipid metabolism and barrier formation [4, 7].

Although AQP3 was found to be expressed in human skin and human epidermal skin models [8], understanding of its upregulation and downregulation in skin tissues is relatively limited. Importantly, data suggest that the level of activity of AQP3 in the epidermis is associated with the degree of skin hydration [4]. Decreased water transport by AQP3 in eczematous skin epidermis has been proposed as a cause for dry skin in this disease [9], while UV irradiation of human keratinocytes induced the downregulation of AQP3, resulting in dehydration [10]. In contrast, other studies have shown that AQP3 gene and protein expression can be upregulated in cultured human keratinocytes exposed to osmotic stress [11].

In our research aimed at improving the water balance of dehydrated skin, we investigated several novel compounds for their effects on skin hydration, including effects on AQP3 expression or function. We looked for naturally occurring examples and examined ways devised by cells to survive under conditions of variable ionic composition and salinity. For example, bacterial cells must maintain their internal osmotic pressure above that of the surrounding media. In extremely dry environments, salt-tolerant bacteria like *Pseudomonas mendocina* [12] or cyanobacteria *Synechocystis* species [13] respond to osmotic stress by synthesizing and accumulating endogenous solutes. These solutes, known as osmolytes, are typically low-molecular-weight compounds (such as glutamate) that exert their effect by increasing intracellular osmolarity, thereby reducing the loss of water to the external environment [14]. One such novel compound that we studied is a simple chemical derivative of one of the skin's endogenous humectants, glycerol. This compound, called glyceryl glucoside [GG; α -D-glucopyranosyl- α -(1 $\rightarrow$ 2)-glycerol], has been shown to play an important role in the survival of cyanobacteria in a dry, hostile environment [15].

Without postulating possible mechanisms or modes of action, we investigated the potential of GG as a hydrating agent. First, we assessed its effects on the *in vitro* regulation of AQP3 mRNA expression in a keratinocyte mono-

layer cell model. The positive outcome of these *in vitro* experiments led us to evaluate its capacity to modulate skin water balance *in vivo* by improving skin hydration and barrier function. Here we present results from both of these studies with GG, which confirm the validity of our approach.

Materials and Methods

In vitro Studies

Cell Culture

Neonatal normal human epidermal keratinocytes (Lonza, Walkersville, Md., USA) were grown in serum-free keratinocyte growth medium (Lonza) at 37°C and 5% CO₂. Cells from passage 3 were used in experiments. In the 24 h prior to harvest, cells were either left untreated (control) or prepared under experimental conditions by supplementing the culture medium with 3% w/v GG (Cognis, Düsseldorf, Germany) or 1% w/v glycerol (Emery Oleochemicals, Düsseldorf, Germany).

Isolation of Total RNA and Real-Time RT-PCR

Total RNA was obtained by cell lysis with 0.5 ml RNAzol B (WAK-Chemie, Steinbach, Germany) per dish (diameter 35 mm) followed by the addition of 50 μ l chloroform (Merck, Darmstadt, Germany). RNA was precipitated from the aqueous phase by the addition of equal amounts of 2-propanol (Merck) and washed twice with 70% ethanol (Merck). Total RNA content was then determined by UV spectrophotometry (NanoDrop ND-100; Thermo Scientific, Wilmington, Del., USA) at 260 nm.

Reverse transcription of isolated RNA and subsequent amplification of the target sequences were performed using the TaqMan EZ RT-PCR kit and ABI PRISM 7700 Sequence Detection System (PE Applied Biosystems, Weiterstadt, Germany) according to the manufacturer's instructions. Primer sequences and target-specific dual-labeled fluorogenic probes were developed using Primer Express software version 1.0 (Applied Biosystems). Sequences of probes and primers used for real-time RT-PCR are shown in table 1.

Quantification analysis of the TaqMan RT-PCR was carried out with SDS software version 1.6 (Applied Biosystems). The levels of AQP3 mRNA expression were normalized to the respective levels of the housekeeping gene 18S rRNA and the results expressed as change in AQP3 mRNA expression compared with untreated control cells. In this way, the threshold-cycle data were converted by using the $2^{-\Delta\Delta CT}$ calculation approach to express the data as a relative fold change.

Immunohistochemistry

Cells were stained as described previously [16]. Briefly, keratinocytes were fixed in 3% paraformaldehyde for 30 min at room temperature and permeabilized with 0.5% Triton X-100 (Sigma-Aldrich, St. Louis, Mo., USA) for 5 min. After washing with phosphate-buffered saline (PBS), cells were blocked with 3% bovine serum albumin (BSA; Sigma-Aldrich) for 30 min followed by incubation with primary antibody in 1% BSA for 1 h. The antibody for detecting AQP3, which was raised in rabbit and used in a 1:100 dilution, was kindly provided by Dr. Peter Deen (Radboud University Medical Centre, Nijmegen, The Netherlands).

Table 1. Sequences of probes and primers used for real-time RT-PCR

mRNA	Forward primer	Reverse primer	Probe
18S rRNA	GCATGGCCGTTCTTAGTTGG	TGAACGCCACTTGTCCCTCT	CCATAACGAACGAGACTGCATGC
AQP3	CCTCTGGACACTTGATATGATCA	ACGGTGAAGGCCTCCAGG	TTCTTTGACCAGTTCATAGCCTCC

The cells were washed with 0.05% Nonidet P-40 (Sigma-Aldrich) and PBS, incubated for 1 h with 1% BSA solution containing the fluorescently labeled secondary antibody (Alexa Fluor 488 chicken anti-rabbit IgG; Molecular Probes, Eugene, Oreg., USA) at 1:1,000 to visualize the target protein and DAPI (Molecular Probes, dilution 1:1,000) to stain nuclei.

After 3 washing steps with PBS, fluorescence images were recorded using a fluorescence microscope (Olympus, Hamburg, Germany).

Measurement of AQP3 Protein Levels by FACS

Flow cytometry was performed according to a previously described protocol [16]. Briefly, cells were centrifuged at 13,000 rpm and the pellet was washed twice with PBS followed by incubation in 3% paraformaldehyde (Sigma-Aldrich). After 2 washing steps the cells were permeabilized with 0.5% w/v Triton X-100 in PBS, washed again and blocked with 3% BSA in PBS for 30 min at room temperature.

The AQP3 antibody described above was diluted 1:100 in 1% BSA and applied onto the cells for 1 h at room temperature. Secondary antibody labeled with fluorescein isothiocyanate (FITC; dilution 1:1,000, Sigma-Aldrich) in 1% BSA was added and cells incubated for 1 h at room temperature. After 3 washing steps with PBS, fluorescence was measured using BD FACSCanto (BD Biosciences, San Jose, Calif., USA) and analyzed using BD FACSDiva 4.0 software.

In vivo and ex vivo Studies

Studies with human subjects were performed according to International Conference on Harmonisation Good Clinical Practice guidelines and the ethical recommendations of the Declaration of Helsinki. All subjects provided written consent prior to participation.

AQP3 mRNA Levels in Preconditioned (Dry-Stressed) Skin Treated with GG

Study Population. Twenty female and male subjects aged 22–53 years participated in the study. Subjects had healthy, undamaged skin in the test areas and were classified as Fitzpatrick phototype I, II, III or IV.

Test Products. Two topical preparations were tested. The first was a PEG-40-stearate-based vehicle formulation containing 6.5% glycerol (vehicle; Eucerin Waterrich, Beiersdorf) and the second was the same formulation supplemented with 5% GG w/v (vehicle + GG).

Study Design. This was a double-blind study with respect to the product applied, and open to the subjects for the untreated control area. All subjects took part in a 14-day preconditioning period to dry-stress the skin. This process involved the twice-daily washing of the forearm (foaming of the moistened forearm

for 60 s before rinsing with tap water) with a commercially available standard neutral shower gel containing sodium laureth sulfate as the detergent and no added moisturizers. Additionally, during the 14-day preconditioning period and the subsequent study period, subjects were required to refrain from using skin care products on the arms, as well as special skin care cleansing products such as shower oils and exfoliating products. The preconditioning period was followed by a 7-day treatment period with test products.

Treatments. Test products (vehicle + GG, and vehicle only) were applied to designated test areas (each area was 5 × 5 cm) on the inner forearms twice daily. A third area was left untreated (control). The areas assigned to the test products and untreated control were randomized across participants. At the beginning of the application period volunteers were instructed by test center staff how to use the formulations in realistic amounts (i.e. approximately 2 mg/cm² according to COLIPA standards). The formulations were allowed to absorb into the skin before the areas were occluded by clothes. There was no further occlusion of the test areas. On day 7 of the study no product was applied in the morning prior to suction blister biopsy, ensuring that at least 15 h had elapsed since the last application of test formulations. Suction blisters (diameter 7 mm) were induced on each test site for each subject using a suction blister device (Festo, Esslingen-Berkheim, Germany) according to previously published techniques [17, 18]. Briefly, a negative pressure of 220 mbar was applied for 20 min followed by 320 mbar for approximately 2 h. Subsequently, fluid was removed from the blister with the aid of a sterile hypodermic needle, transferred into a tube and frozen at –80°C until further analysis. The GG content of the suction blister fluid was measured by liquid chromatography-mass spectrometry (LC-MS) analysis to determine evidence of its penetration into the epidermis. The roof of each suction blister was excised with sterile scissors. Skin samples were kept frozen subsequent to analysis of AQP3 mRNA levels.

Isolation of Total RNA and Real-Time RT-PCR. Standard procedures as described above for the *in vitro* studies were used for the isolation of total RNA and real-time RT-PCR from the skin samples.

Skin Hydration and Barrier Properties in Response to GG Treatment

Study Population. Twenty-four female subjects aged 50–70 years with dry skin (TEWL values 5–9 g/m²·h) were enrolled in the study.

Test Products. Two topical preparations were used. The first was a noncommercial vehicle formulation (based on PEG-40-stearate) containing 6.5% w/v glycerol. The second was the same vehicle formulation supplemented with 5% GG.

Study Design. This was a single-center, randomized, double-blind study for test products and open for the untreated control

Fig. 1. AQP3 mRNA expression in keratinocyte monolayers exposed for 24 h to 1% w/v glycerol (n = 9) or 3% w/v GG (n = 9) in the incubation medium. Harvested cells were subjected to quantitative real-time RT-PCR and measured AQP3 mRNA levels were normalized to the respective levels of 18S rRNA. Results are presented as mean $\pm$ SEM with respect to percentage AQP3 mRNA expression of untreated control cells (n = 6). The statistical significance of the results for GG with respect to control and glycerol-treated monolayers is shown.

area, performed under dermatological supervision. All subjects took part in a 10-day preconditioning period requiring twice-daily washing of the forearm with a standard shower gel (foaming of the moistened forearm for 90 s before rinsing with tap water) as described above. During the study the use of moisturizers and other skin care products was not allowed. The preconditioning period was followed by a 3-week treatment period with test products. Subjects were required to make 2 clinical visits over a 3-week period. This involved a baseline visit after the 10-day preconditioning period and a second visit following 3 weeks of product treatment.

Treatments. A stencil was used to delineate test areas (each measuring 5 $\times$ 5 cm) on the inner forearms. Two of these areas were treated with the test products and the remaining area was left untreated and used as a control. Subjects received similar instructions to those described earlier concerning the method of application of test formulations. The areas assigned to the test products and untreated control were randomized across participants. Subjects applied realistic amounts of the test formulations twice daily to the defined areas of the inner arms over the 3-week period. They were also provided with written usage instructions and a diary for recording test material application times and comments. No product was applied on the morning of the day on which measurements were taken.

Measurements. Skin hydration was measured using a Corneometer CM 825 instrument (Courage + Khazaka, Cologne, Germany). The mean result of 10 repeated measurements per area was taken at the end of the preconditioning period and compared with the mean result measured 3 weeks later upon completion of the product treatment period. For the measurement of skin barrier function, a Tewameter TM 300 (Courage + Khazaka) instrument was used to determine TEWL (g/m²·h), with lower values indicating less water loss and therefore improved barrier function, and vice versa. The measurements were carried out according to stan-

Fig. 2. Immunohistochemical localization of AQP3 in keratinocyte monolayers. Images of keratinocyte monolayers (**a**, control untreated, or **b**, treated with 3% GG) stained with a rabbit anti-human AQP3 antibody (1:100 dilution) and fluorescently labeled secondary antibody (Alexa Fluor 488 chicken anti-rabbit IgG; 1:1,000) to visualize the target protein (green). DAPI (1:1,000) was used to stain cell nuclei (blue). Fluorescence images were recorded using a fluorescence microscope (Olympus).

dard procedures [19]. The environmental conditions during the TEWL experiments were room temperature 21.5°C, relative humidity 45%. The mean result of 5 repeated measurements per area was taken as described earlier for the corneometry measurements.

Statistical Analysis

Results are presented as the mean $\pm$ standard error of the mean (SEM) where appropriate. One-way analysis of variance with post hoc comparisons using Tukey's test was used to compare data among groups. Values of $p < 0.05$ were considered to indicate statistical significance.

Fig. 3. FACS measurement of elevated AQP3 protein levels in cultured keratinocytes after GG 3% administration. Columns and FITC-A intensity plots: unfilled = control; filled = GG-treated. **a** The relative AQP3 signal (mean $\pm$ SEM) with respect to control (n = 10). **b** Forward versus side scatter plot of each cell population combined with the distribution of the AQP3-FITC signal (n = 10).

Results

AQP3 mRNA and Protein Expression Induced by GG in vitro

In vitro studies on keratinocyte monolayers were performed to determine if treatment with GG changes mRNA and protein expression levels with respect to control (untreated) cells.

Monolayers incubated with 3% w/v GG over a 24-h period showed a 215% increase on average in AQP3 mRNA expression compared with untreated cells (fig. 1). In contrast, when the bathing medium was supplemented with 1% glycerol, which results in a medium of similar osmolarity to that obtained with 3% GG, no significant differences in mRNA expression compared with control were observed. This finding suggests that GG exerts its action in a manner that cannot be explained by altered osmolarity.

Immunohistochemistry and FACS experiments were carried out to determine the cellular location and protein content of AQP3 in keratinocytes after treatment with GG (fig. 2). Fluorescence images of keratinocytes showed that AQP3 antibody was mainly located on the cell membrane of these cells (fig. 2a), and that treatment with 3% GG for 24 h upregulated AQP3 protein expression com-

pared with untreated cells (fig. 2b). In the same manner, fractions of cells that labeled positively for AQP3 antibody showed a 156% increase in AQP3 protein levels following treatment with 3% GG for 24 h compared with control (untreated cells, fig. 3a). Forward versus side scatter plots showed that the morphology of the cells was not altered by GG treatment (fig. 3b).

In vivo and ex vivo Studies

AQP3 mRNA Levels in Preconditioned (Dry-Stressed) Skin Treated with GG

Based on the positive outcome of the in vitro experiments with GG, we sought to verify these results by determining if an increase in AQP3 expression could be induced in vivo by GG via the topical application of a 5% GG-containing formulation (see 'Materials and Methods').

Twenty subjects were enrolled and completed the study; the results of 1 subject were excluded due to incorrect application of the test products.

Analysis of extracted suction blister fluid by LC-MS demonstrated that GG penetrated the epidermis (on average 683 ng/ml, n = 19). Analysis of the excised blister epidermis revealed that the vehicle formulation con-

Fig. 4. Suction blister results highlighting upregulation of mRNA expression in preconditioned, dry-stressed skin treated for 7 days with a vehicle formulation based on PEG-40-stearate (vehicle) or the same vehicle formulation supplemented with 5% GG (vehicle + GG). The results for each case are presented as the mean $\pm$ SEM ($n = 19$) with respect to the untreated (control) area. * $p < 0.05$ with respect to vehicle only.

taining 5% GG induced a significant increase ($p < 0.05$) in AQP3 mRNA expression levels in the deroofed blister epidermis compared with vehicle-only treated skin (fig. 4). Taken together, these results demonstrate that, in response to a 7-day treatment period involving GG application to dry-stressed skin, GG penetrated the stratum corneum and induced AQP3 expression in the viable cell layers of the epidermis.

Skin Hydration and Barrier Properties in Response to GG Treatment

Corneometry and TEWL measurements were conducted to measure skin moisture and barrier function, respectively, in 10-day dry-stressed (preconditioned) skin and following a 3-week treatment period with GG.

Twenty-four subjects were enrolled in the study, with 1 patient dropping out for personal reasons. The efficacy of a vehicle formulation containing 6.5% glycerol supplemented with 5% GG applied under controlled, normal usage conditions was compared with that of the vehicle product containing 6.5% glycerol alone, and with a control (untreated) area.

Corneometry results showed that the mean level of hydration of GG-treated skin was significantly increased ($p < 0.05$) compared with the control area that was left untreated (data not shown). When results for the vehicle plus GG-treated skin were compared with those for skin

Fig. 5. Average differences in TEWL values ($\text{g/m}^2\cdot\text{h}$) measured at baseline and following a 3-week treatment period for 23 volunteer subjects. Baseline values were recorded from 10-day dry-stressed (preconditioned) skin. In the treatment period, skin was either left untreated or received a twice-daily topical application of a vehicle formulation (based on PEG-40-stearate) containing 6.5% glycerol, or the same vehicle formulation supplemented with 5% GG. Differences are expressed as the mean $\pm$ SEM. * $p < 0.05$ with respect to untreated and vehicle-only treated skin.

treated with the vehicle alone, no significant differences in hydration levels were observed.

In view of the in vitro results showing that GG upregulates AQP3 expression in keratinocytes, we considered a priori that corneometry measurements might not reveal the full extent of the biological action of GG in the viable layers of the epidermis compared with the physicochemical action of the topically applied glycerol in the vehicle. For this reason, TEWL measurements were also conducted to determine if GG improved barrier function properties.

Before treatment, the average Tewameter value in the 23 subjects was $7.88 \text{ g/m}^2\cdot\text{h}$, which is indicative of dehydrated skin ($5\text{--}9 \text{ g/m}^2\cdot\text{h}$). Compared with the untreated control area and the area treated with the vehicle formulation containing 6.5% glycerol, the GG-containing formulation induced a significant decrease in TEWL (i.e. decreased water loss), demonstrating improved skin barrier function after 3 weeks of treatment (fig. 5).

Discussion

The correct hydration of viable layers of the epidermis plays an important role in maintaining the integrity of the stratum corneum and thus the barrier function of the skin. Dysregulation of the water balance in the epidermis is thought to play a causative role in the onset of dehydra-

tion-related skin conditions. In recent years, numerous members of the AQP family of proteins have been identified in various body tissues and their properties and functions have been reported. Of these, AQP3 in particular has been shown to play a major role in the transport and distribution of epidermal water and glycerol in skin [11]. AQP3 is involved in the regulation of keratinocyte differentiation [20, 21] and numerous other processes in skin hydration, barrier function, wound healing and cell migration [4, 22–24]. Skin hydration was demonstrated to be decreased in AQP3 knockout mice, where both water and glycerol levels in the stratum corneum were diminished compared with wild-type skin [25, 26]. Decreased AQP3 expression has also been demonstrated in skin diseases associated with elevated TEWL and reduced stratum corneum hydration [22]. Moreover, recent reports show AQP3 to be decreased in several other skin disorders, such as psoriasis [27] or that associated with diabetes mellitus [28]. The AQP3 content in human skin has also been reported to decrease with age and chronic sun exposure [29]. Taken together, AQP3 appears to be a key player in epidermal biology and thus a potential target for therapeutic interventions [7].

In the present study we sought to identify compounds capable of increasing AQP3 expression in human skin. One such compound that we identified in our investigations was GG, a derivative of the endogenous humectant glycerol. This compound had been shown to be actively transported into cyanobacterium *Synechocystis* species as a mechanism of survival in increasingly saline environments [15]. We first tested this compound in vitro on human keratinocytes, where we observed a significant increase in AQP3 mRNA expression in cells treated with 3% GG compared with control (untreated) cells. Taking into account that this GG-induced upregulation of AQP3 mRNA expression might be due simply to an altered osmotic environment, similar experiments were conducted in which 1% glycerol was used to provide an equivalent hyperosmotic stimulus, and revealed no stimulatory effect on AQP3 mRNA expression (fig. 1). The actual mechanism by which GG upregulates AQP3 mRNA and protein expression has yet to be elucidated and warrants further investigation.

In subsequent immunohistochemistry and FACS experiments in which AQP3 protein was identified with a fluorescently labeled antibody, we were able to show that AQP3 protein expression was significantly upregulated (an increase of 156%) in human keratinocytes after GG treatment. A clear difference in membrane staining was evident, which lends support to the role of AQP3 as a plas-

ma membrane-bound channel that mediates the flow of water and glycerol into and out of cells.

Suction blister analysis was used to determine whether effects similar to the in vitro results could be observed following topical application of a GG-containing formulation. Blisters are formed by the application of low-level pressure to the skin over several hours, separating the epidermis from the dermis without inducing an inflammatory response. When the vehicle formulation containing 5% GG was applied topically to preconditioned, dry-stressed skin of human volunteers for 7 days, analysis by LC-MS of the fluid extracted from the blister showed that GG was capable of penetrating the epidermis. Further, AQP3 mRNA levels in the skin were significantly upregulated compared with untreated and vehicle-treated controls.

Subsequent studies were carried out to assess the hydration and barrier strengthening properties provided by 5% GG applied topically to 23 subjects for 3 weeks in a vehicle formulation containing 6.5% glycerol. While corneometry results for GG were similar to those afforded by glycerol, measurements of TEWL values in 10-day dry-stressed (preconditioned) skin and following the 3-week treatment period with GG demonstrated a significant decrease in TEWL values in response to the GG treatment, indicating an improvement of the skin's barrier function. Moreover, as the vehicle in both cases contained 6.5% glycerol, these results prove the potency of GG to augment barrier restoration and fortification properties beyond that provided by glycerol alone [30, 31]. This is an interesting and important finding because it demonstrates the capacity of GG to augment the skin's barrier function over and above that achieved by glycerol, which is a widely accepted hydrating agent that is included in most moisturizing product formulations. The use of formulations containing GG could therefore be contemplated as an additional step to preventing water loss from the skin. This would be relevant clinically, given that AQP3 levels decrease both with age and as a consequence of sun exposure [29].

Our TEWL findings suggest there may be a physiological association between TEWL and AQP3 expression. Previous reports on AQP3 knockout mice have not found a clear association between AQP3 expression and water loss by evaporation [for review, see 4]. Various knockout mice studies have shown that a lack of AQP3 did not significantly affect TEWL, and appeared to decrease the normal physiological response to dry air exposure or occlusion compared with controls [4]. This apparent discrepancy between the animal studies and our clinical

study could be due to the fact that mice have a different AQP3 expression profile and location within the epidermis compared with humans. Moreover, Hara and Verkman [32] demonstrated that topical or systemic administration of glycerol had an effect on barrier repair (quantitatively measured by TEWL) in AQP3 null mice. So there is some divergence on this point in mouse studies. Further work is required to clarify this as findings in

knockout mice cannot necessarily be directly extrapolated to humans.

We propose that AQP3 rebalancing by GG could play an important role in skin health by improving epidermal water supply and barrier function, thereby helping to regulate water homeostasis in viable layers of the epidermis. These findings warrant further investigation and analysis in clinical trials.

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