J. Cosmet. Sci., 64, 165–174 (May/June 2013)

Effect of seasonal and geographical differences on skin and effect of treatment with an osmoprotectant: Sorbitol

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Accepted for publication August 6, 2012.

Synopsis

Human skin maintains an optimal permeability barrier function in a terrestrial environment that varies considerably in humidity. Cells cultured under hyperosmotic stress accumulate osmolytes including sorbitol. Epidermal keratinocytes experience similar high osmolality under dry environmental conditions because of increased transepidermal water loss (TEWL) and concomitant drying of the skin. This study was designed to determine if epidermal keratinocytes, *in vitro*, could be protected from high osmotic stress, with the exogenous addition of sorbitol. In addition, we evaluated the effect of a formulation containing topical sorbitol on skin barrier and moisturization of subjects living in arid and humid regions in summer as well as in winter. Results from *in vitro* experiments showed that 50 mM sorbitol protected epidermal keratinocytes from osmotic toxicity induced by sodium chloride. Clinical studies indicated that skin chronically exposed to hot, dry environment appeared to exhibit stronger skin barrier and a lower baseline TEWL. In addition, skin barrier was stronger in summer than in winter. Sorbitol exhibited significant improvement in both barrier repair and moisturization, especially in individuals subjected to arid environmental conditions.

INTRODUCTION

The skin is a large barrier organ that both protects the human body from environmental hazards and maintains optimal permeability barrier function in a terrestrial environment varying considerably in humidity (1). Functional and structural skin adaptation is a dynamic process, which starts immediately after birth in humans and mammalian skin in general (2). Although low external humidity increases transepidermal water loss (TEWL), prolonged hyperosmotic stress progressively upregulates cutaneous metabolic processes, including increased epidermal lipid and DNA synthesis, that eventually enhance permeability barrier function (3).

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With technological progress, people are now mostly exposed to multiple indoor and outdoor pollutants and environment parameters (air-conditioning, humidity) that can interact with each other and affect human health, particularly the skin. Thus, climate and geography can have a major impact on skin integrity (4). Exposure to dry environment has been shown to contribute to the exacerbation of cutaneous disorders such as epidermal hyperplasia, mast cell degranulation, and cytokine secretion (5).

There seem to be large differences between "acute" and long-term effects of dry environment on skin. Denda and Sato *et al.* (6) showed that a short period of exposure to a dry environment (48 h) induces desquamation of the stratum corneum and epidermal proliferation. Prolonged exposure of normal murine skin to a dry environment for 1–2 weeks leads to an increase in stratum corneum weight and thickness and a reduction in basal TEWL(3), suggesting that dryness first induces an epidermal proliferative response within 48 h followed by enhancement of the epidermal barrier function to adapt to the dry conditions. Chymotrypsin-like enzyme (stratum corneum chymotryptic enzyme [SCCE]) activity decreases in stratum corneum when the relatively humidity is lower (7,8) suggesting that people living in a hot and dry environment are adapted to upregulate the amount of enzyme to reach an *in situ* SCCE activity in the stratum corneum, which ensures a good desquamation in low humidity.

In the stratum corneum, water binds to the intracellular hygroscopic and hydrosoluble substances called "natural moisturizing factors" or NMFs. These NMFs contained in the corneocytes are formed during epidermal differentiation by degradation of the histidine-rich protein: filaggrin (9). NMF may represent up to 10% of the corneocyte mass. They are principally amino acids, carboxylic pyrrolidone acid, lactic acid, urea, sugars like glycerol, and mineral ions. Keratinization plays an important part in the formation of NMF that exhibit strong osmotic potential attracting the water molecules (10). The binding of water to NMF is the static aspect of cutaneous hydration. These processes, as well as the final step of corneodesmolysis that mediates exfoliation, are often disturbed on environmental challenge, resulting in dry, flaky skin conditions (9). The retention of water in the stratum corneum is also dependent on intercellular lipids orderly arranged to form a barrier to TEWL. Thus, the water content of the stratum corneum is necessary for its proper maturation and skin desquamation. Increased TEWL impairs enzymatic functions required for normal desquamation, resulting in the visible appearance of dry, flaky skin (11).

Cells experiencing long-term hyperosmotic stress accumulate sorbitol, a compatible organic osmolyte, and preferentially accumulate this sugar alcohol over inorganic ions (12,13). The renal medulla uses sorbitol and a host of other organic osmolytes, such as inositol, betaine, glycerophosphocholine, and taurine, to help cope with a routine exposure to high extracellular osmolality (14). Sorbitol is synthesized from glucose via the enzyme aldose reductase, which has been reported to increase in renal cells through an osmotically regulated process (15). These cells accumulate organic osmolytes consisting mainly of sorbitol, when cultured in high NaCl medium, and display normal cell volume as well as normal Na and K levels (15).

Studies were conducted to observe the effect of 1 month in-use treatment with sorbitol on improvement of barrier functions and moisturization of skin chronically exposed to hot and dry environment of Arizona versus the humid and cooler environment of New York in summer and winter (Table I).

Table I
Summer and Winter Weather of Long Island New York as a Representative of a Cold and Humid Region and Tucson Arizona that is Representative of a Hot and Arid Region (Weather Post, washingtonpost.com)

	Average RH		High Temperature		Low Temperature	
	NY	AZ	NY	AZ	NY	AZ
Winter	73.7	43.2	40	68	25.7	41.7
Summer	78.3	37.4	76	97.3	57.8	70.3

MATERIALS AND METHODS

IN VITRO STUDIES

Human epidermal keratinocytes (Cascade Biologics) were seeded into a 96-well plate and maintained in EpiLife media (Cascade Biologics, New York, NY) (16,17). Cells were incubated overnight at 37°C, either with or without 50 mM sorbitol (Sigma Aldrich, Buchs, Switzerland). Following incubation, media was removed, and cells were washed twice with phosphate buffered saline before the addition of fresh media containing 0, 100, 125, or 150 mM NaCl (Sigma S-9625). Cells were allowed to incubate overnight before viability determination using Promega's (Madison, WI) (G 3580) MTS cell viability assay.

The CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay is a colorimetric method for determining the number of viable cells in proliferation or chemosensitivity assays. The CellTiter 96® AQueous Assay is composed of solutions of a novel tetrazolium compound (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; MTS) and an electron coupling reagent (phenazine methosulfate; PMS). MTS is bioreduced by cells into a formazan product that is soluble in tissue culture medium. The absorbance of the formazan at 490 nm can be measured directly from 96-well assay plates without additional processing. The conversion of MTS into aqueous, soluble formazan is accomplished by dehydrogenase enzymes found in metabolically active cells. The quantity of formazan product as measured by the amount of 490 nm absorbance is directly proportional to the number of living cells in culture.

IN VIVO CLINICAL STUDIES

The following materials were used for the clinical studies:

Material A—Aqueous serum base containing sorbitol (Sigma S-6021) 0.5%.

Material B—Aqueous serum base (vehicle).

Clinical studies were designed to study the effect of 1 month in-use treatment with sorbitol on improvement of barrier functions and moisturization of skin chronically exposed to hot and dry environment of Arizona where average annual humidity is 23% (Weather Post, washingtonpost.com) as well as the relatively humid environment of New York (58% average annual humidity). Seasonal variations were observed by conducting the studies in summer (June–August) as well as in winter (December–February).

A total of 18 volunteers who had lived in Tucson Arizona for more than 7 years completed the study. Twenty-two, age- and ethnicity-matched volunteers were recruited from Long Island, New York, as a representative of a cold, humid geographical location.

The panelists were all Caucasian females between the ages of 18 and 45, who qualified based on the inclusion criteria specified in the protocol. The subjects reported to be of normal health with no evidence of acute or chronic disease including dermatologic or ophthalmologic problems.

Subjects exhibiting current sunburn, rashes, scratches, burn marks, and so on, which might interfere with evaluation of test results, were excluded from the study. Pregnant or lactating females were also excluded. The subjects were instructed to refrain from using any moisturizer a week before commencing the study in summer and winter; however, they were instructed to continue with their normal lifestyle and use of their makeup and sunscreen regimen and not change their activities and products during the course of the study. Written informed consent was obtained from each volunteer before entrance into the study.

The panel was divided in two equal groups, corresponding to each test material. The panelists were provided with the test material to be applied on full face twice a day for 4 weeks. Each treatment was recorded by the panelists on a daily diary. The panelists reported to the laboratory with a clean face devoid of moisturizer or makeup. Measurements were obtained before treatment and after 4-week treatment with the test materials. Before all measurements, the subjects were required to acclimate for 30 min in an environmentally controlled room with 70°F and 40% humidity.

Barrier Functions. A small area (1.5 × 1.5 inch) was marked on the cheek of the panelists. Baseline TEWL measurements were obtained from this test, site using the Servomed Evaporimeter (18–20). Tuck tape was used to cover the test area and after a firm stroke in both directions, the tape was peeled off. A total of three strippings were obtained. TEWL was recorded again. Strippings followed by TEWL measurements, were continued in groups of three. Skin barrier was considered disrupted when the TEWL reached about 18 $G/M^2/H$, and at this point the strippings were stopped and TEWL was obtained. The number of strippings to disrupt skin barrier was calculated using a linear equation (21–23).

Moisturization. Skin moisturization was measured with a Novameter DPM 9003 (NOVA Technology Corporation, Portsmouth, NH). The Nova measures skin moisturization as a function of increased skin surface water content. The instrument measures an output proportional to the skin's electrical capacitance in megahertz frequency range (24,25). Capacitance measurements were obtained by holding the probe firmly against the cheek area of the panelists. Three measurements were obtained from the upper cheek area at each time point (26,27).

RESULTS

IN VITRO STUDIES

As observed in Figure 1, NaCl yielded a dose-dependent decrease in cell viability that was inhibited by sorbitol, 44% and 81% at 125 and 150 mM NaCl, respectively. Data represent the mean of eight data points. Statistical significance was evaluated by analysis of

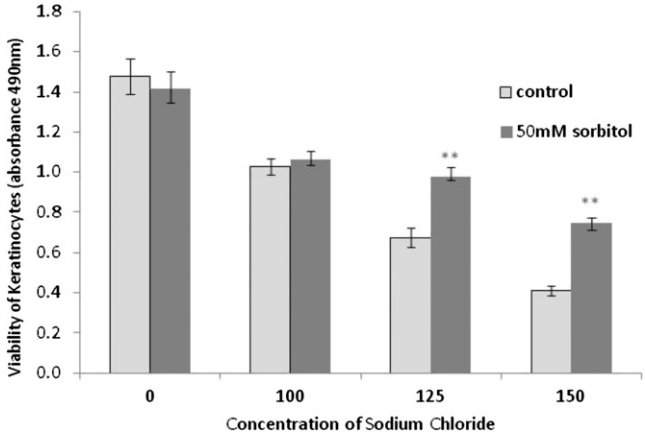

Figure 1. Effect of sorbitol on protection of keratinocytes from NaCl-induced toxicity. **Statistical significance $p < 0.01$.

variance using a Bonferroni post-test, comparing sorbitol treatments with its untreated control.

IN VIVO CLINICAL STUDIES

Barrier Functions. It is clear from Figure 2 that skin chronically exposed to a hot, dry environment contained a thicker stratum corneum. Consistent with the findings of Sato *et al*. (1998), there appeared to be a lot more layers of stratum corneum in the skin of people living in arid Arizona as compared to New York.

As observed in Figure 3, the baseline TEWL of the panel in arid Arizona was significantly lower than that living in New York indicating a hyperplasia of the stratum corneum of skin exposed to a hot dry environment (3). Reduction of ceramides (by 40%) has been reported in winter as opposed to (28,29) summer, so this could be a contributing factor.

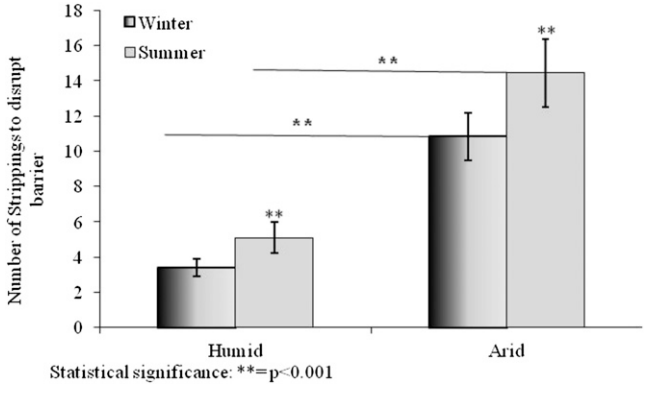

Figure 2. Skin barrier integrity of subjects living in arid and humid habitat, in summer and winter.

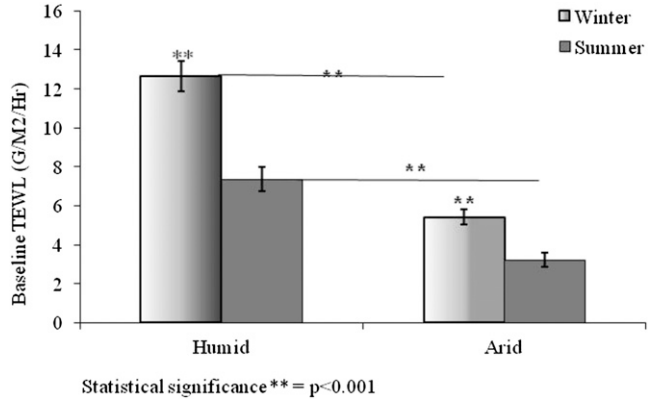

Figure 3. Skin barrier (TEWL) of subjects living in arid and humid habitat, in summer and winter.

Consistent with the barrier strength data in Figure 2, it appears that the baseline TEWL was lower in summer for all geographical situations.

The osmotic protectant appeared to be effective in improving skin barrier functions (Figures 4 and 5). Generally, better improvement was observed in the humid regions of New York, possibly because the baseline barrier of these panelists was not very strong as compared to the panel in Arizona. Product treatment did not appear to improve the barrier much for the panel in Arizona, possibly since they exhibited a strong barrier from the start (Figure 5).

Moisturization. Comparison of moisturization from humid and arid regions in winter and summer exhibited in Figure 6 shows no significant difference in skin moisturization in the different seasons from the two geographical situations. However, there appears to be a slightly better moisturization in summer than in winter on both geographical situations.

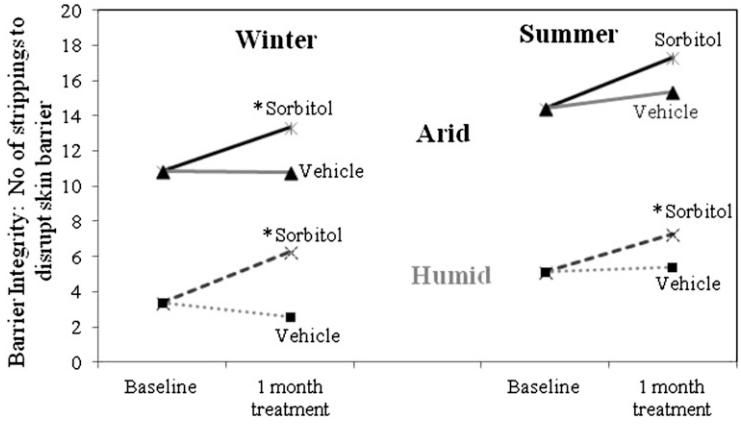

Figure 4. Effect of osmotic protectant on skin barrier integrity in humid and arid geographical region in winter and summer. More strippings are required for a strong barrier. *Statistical significance $p < 0.05$ as compared to baseline.

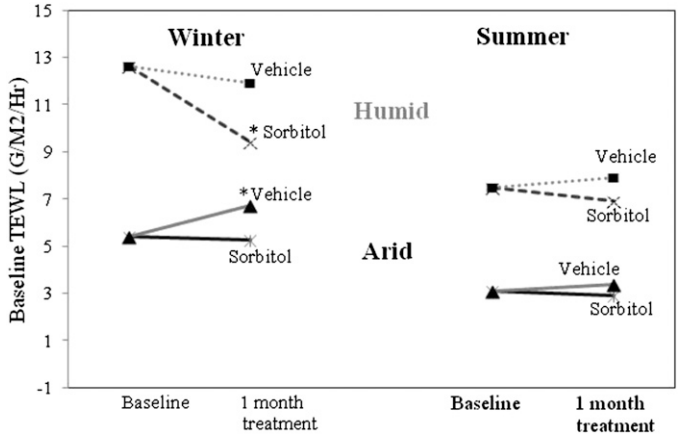

Figure 5. Effect of osmotic protectant on basal TEWL in humid and arid geographical region in winter and summer. Lower TEWL is indicative of improved barrier. *Statistical significance $p < 0.05$ as compared to baseline.

As observed in Figure 7, there was a significant improvement of 29% in skin moisturization with the osmotic protectant ($p > 0.01$) in New York in summer. The vehicle also appeared to improve skin moisturization by 14% ($p = 0.007$). In winter, the vehicle was extremely drying for all the panelists, and there was a visible flakiness of skin observed for all the panelists in Arizona. The formulation containing sorbitol was only 5% effective compared to its baseline, clearly indicating that this vehicle was too drying on skin to change skin moisturization response. It is also possible that the severely arid environment of the Arizona winter confounded any moisturizing effect of the formulations tested.

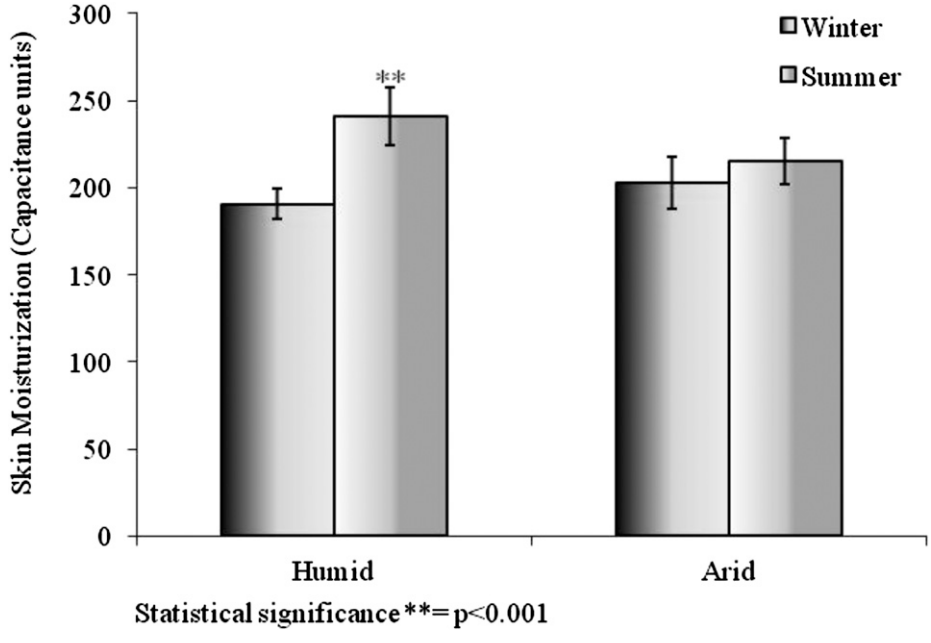

Figure 6. Moisturization of subjects from humid (New York) and arid (Arizona) regions in winter and summer.

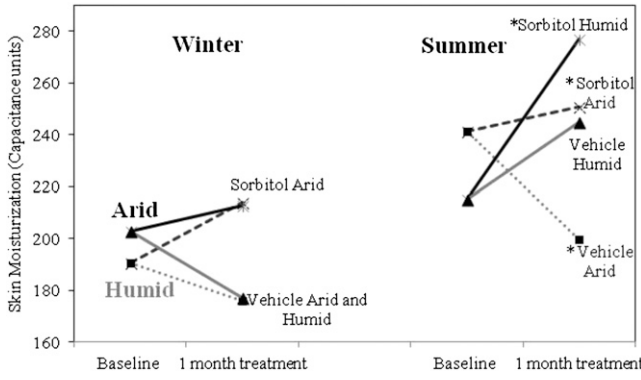

Figure 7. Effect of osmotic protectant on skin moisturization in humid and arid geographical region in winter and summer. *Statistical significance $p < 0.05$ as compared to baseline.

In New York in winter, there was a significant improvement of 12% in moisturization with the osmotic protectant ($p > 0.0002$) while the vehicle was drying on skin. For the summer months in New York, only 4% improvement was observed while the vehicle was extremely drying on skin. In this study also, visible drying of skin was noticed. It appears that this vehicle was too drying on skin to modulate skin moisturization response.

DISCUSSION

It is clear from this study that environmental conditions markedly influence epidermal structure and function. In this study, the subjects living in arid conditions exhibited lower TEWL and thicker stratum corneum. The number of lamellar bodies in stratum granulosum, the extent of lamellar body exocytosis, and the number of layers of stratum corneum have been observed to be increased in animals kept in a dry environment (3). The dry weight of the stratum corneum and the thickness of the epidermis have also been reported to be increased in a dry environment. In addition, an increase in total stratum corneum lipids has been observed in dry environment; however, lipid analysis has not revealed any significant differences in lipid distribution (3).

The level of relative humidity influences skin barrier development, with more rapid barrier formation at lower humidity (30,31). Dry environment decreases the water content of the stratum corneum which in turn perturbs desmosome degradation in intact stratum corneum (6,9), and the consequent impairment of desquamation in normal skin results in thicker stratum corneum (6). Dry and damaged skin is linked to depleted ceramide levels (32); a reduction of ceramides (by 40%) has been reported in winter as opposed to summer (28,29). Based on the observations of Mammone *et al*. (17), sorbitol induces a dehydration effect or "osmotic stress" on the keratinocytes, increasing the levels of messenger RNA for the differentiation markers involucrin, transglutaminase, and filaggrin, as well as Keratin K1 and K10 and involucrin protein levels. These observations suggest that keratinocytes in the epidermis may use dehydration as a sign to trigger the differentiation of the skin barrier.

The relative humidity in Tucson Arizona during the course of testing in winter did not exceed 12%. Most subjects in Arizona appeared to have oily skin, although they

complained of having dry skin. This is possibly a manifestation of adaptation to the hot environment; since ambient temperature influences the sebum excretion rate. This is related to an increased delivery of sebum to the surface of the skin without an increment in the number of active sebaceous follicles (33).

Although skin barrier appears to be effected by ambient humidity of the environment, there was not much difference in skin moisturization of subjects in New York and Arizona. Chou *et al*. (31) noticed no differences in skin capacitance between subjects exposed to ultralow humidity versus the control group exposed to normal relative humidity. Our results also did not exhibit a big change in skin moisturization between the arid and humid locations except in summer when the subjects in dry environment appeared to exhibit slightly drier skin. Since the amino acids of NMF play a major role on skin moisturization (34), it is possible that the decrease in humidity in winter reduces the total free amino acid generation and consequent dryness of the stratum corneum (35).

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