J. Cosmet. Sci., 54, 289–300 (May/June 2003)

Effect of soaking and natural moisturizing factor on stratum corneum water-handling properties

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Accepted for publication June 18, 2002. Presented as a poster at the Annual Scientific Seminar of the Society of Cosmetic Chemists, New Orleans, May 2001.

Synopsis

Stratum corneum (SC) hydration is partially regulated by water-soluble molecules, natural moisturizing factor (NMF) that is associated with the corneocytes. Routine water exposure, e.g., bathing, may deplete NMF and alter the SC water-handling properties. We determined the effects of bathing and solvent extraction on the volar forearm skin of eleven healthy volunteers. Acetone/ether (A/E) was used to remove surface and upper SC lipids. Adjacent sites were soaked for ten minutes or treated with the A/E–plus–soak combination. Subsequently, an NMF formulation was applied to the treated sites, and transepidermal water loss (TEWL), hydration, and moisture accumulation rate (MAT) were measured. A/E extraction increased TEWL, but did not effect MAT. Soaking produced a short-term increase in TEWL, followed by a decrease, and substantially reduced MAT, an effect that was maintained for five hours. NMF application significantly decreased TEWL and significantly increased MAT for all sites. The replacement experiment suggests that the MAT reduction occurred as a result of extraction of hygroscopic NMF components. The effects of soaking and NMF application are more readily detected by the MAT technique, whereas TEWL is more sensitive to A/E extraction. The results support the use of multiple assessments of barrier function and raise questions about the effects of cumulative repeated water exposure on SC function.

INTRODUCTION

The stratum corneum (SC) forms a dynamic interface between the environment and the internal milieu. Proper hydration of the SC is essential to provide an effective barrier to water loss and for optimum skin function (1). Plasticization of the SC imparts flexibility and prevents cracking, fissuring, barrier damage, and loss of integrity. SC moisturization occurs as a result of the inherent barrier to water loss provided by the corneocytes embedded in the lipid bilayer matrix and by the inherent humectancy of specific cellular components (2). Hydration is necessary for proper desquamation of the outer SC by facilitating degradation of desmosomal attachments (3). Topical moisturizers, used to treat dry, scaly skin conditions, provide humectancy and water-holding capability with

ingredients such as glycerin. Certain moisturizers impart a physical barrier to water loss with components such as petrolatum (4,5).

In contrast to the desirable effects of hydration, prolonged exposure of the SC to high levels of water causes maceration, barrier breakdown, and dermatoses, including inflammation, irritation, and urticaria (6–11). Warner *et al.* (12) described damage to the SC following prolonged exposure to water. Abnormalities included disruption of intercellular lamellar lipid bilayers, degradation of corneodesmosomes, and formation of amorphous regions within the intercellular lipid.

Repetitive exposure of the skin to water during routine bathing and hand washing is a common practice for many individuals. Imokawa *et al.* (13) and Jukura *et al.* (14) reported the extraction of soluble amino acids following *in vitro* exposure of isolated human stratum corneum to a 30-minute water soak or to an acetone/ether extraction (30 min) followed by a water soak (30 min). The extracted amino acids were constituents of natural moisturizing factor (NMF), the compounds that confer water-holding properties to the SC (15). Ramsing and Agner (16) described the effects of water soaking on irritated human skin in which subjects were exposed to water twice daily for 15 minutes over two weeks. A significant increase in blood flow was observed, but barrier function (TEWL) and baseline hydration were not significantly impacted.

Limited *in vivo* information has been reported about the effects of routine water exposure during bathing or showering on the water-handling properties of the SC, i.e., barrier function, hydration, and water-holding capacity. Since NMF consists of water-soluble materials, routine bathing might be expected to alter the NMF content of the stratum corneum. We hypothesized that short-term exposure to water *in vivo* removes NMF and alters the SC water-handling properties during the immediate post-bath period. In this study, we report the *in vivo* effects on SC water interactions of brief skin exposure to a water soak followed by the topical application of an NMF formulation. We discuss the implications of the results for current skin care practices.

MATERIALS AND METHODS

SUBJECTS

Eleven healthy adult female subjects, aged 22–53 years (mean 30 years), were enrolled during June of 2000. Exclusion criteria included visually dry forearm skin and/or low rates of moisture accumulation under probe occlusion (<0.28 capacitive reactance units/sec), dermatological conditions (psoriasis, eczema, irritant dermatitis, etc.), or steroid or insulin therapy. The Institutional Review Board of the University of Cincinnati Medical Center approved the protocol. All subjects provided informed consent prior to their inclusion in the study.

BIOPHYSICAL INSTRUMENTATION

Transepidermal water loss (TEWL) was determined using a DermaLab evaporimeter (Cortex Technology, Denmark). Baseline hydration and rate of moisture accumulation (MAT) were measured with a NOVA® Dermal Phase Meter (NOVA® Technology, Portsmouth, NH). The MAT uses changes in skin capacitive reactance (the ratio of

charge to potential on an electrically charged isolated conductor) under occlusion to determine the extent of skin hydration (17). Transepidermal water accumulates under the sensor for twenty seconds, and the value is calculated as the slope of the regression line (cru/sec). The MAT methodology provides a dynamic measure of SC water-handling ability, as previously reported (18).

NATURAL MOISTURIZING FACTOR AND VEHICLE FORMULATIONS

The NMF components (Table I) were added to a vehicle of hydroxyethyl cellulose (Natrosol) in distilled water, and the viscosity was adjusted to 300 cps to produce a formulation suitable for application to the skin. The formula was based on reported compositions of NMF (14,19,20). The levels of pyrrolidone carboxylic acid (PCA), urea, citrate, chloride, and total amino acids were taken from the NMF composition reported by Cler and Fourtanier (19). The relative ratios of neutral, basic, and acidic amino acids were formulated to match the composition of amino acids extracted from the skin after treatment with acetone/ether followed by a water soak (14). The relative amounts of the neutral amino acids approximated the ratios found in guinea pig epidermis (20). The formula pH was 5.6. A vehicle control was prepared in a similar fashion and adjusted to a viscosity of 300 cps and a pH of 5.6.

SITES AND INITIAL MEASUREMENTS

Six 2 × 2-cm treatment sites areas were marked on each volar forearm. The areas were randomized for left versus right and for position along the arm, and treatments were assigned as shown in Table II. Prior to entry into the study, subjects refrained from using a moisturizer on their volar forearms for 72 hours. Measurements were performed after

Table I
Natural Moisturizing Formulation

Component	Percent (% by wt)	mg/cm^2 applied to site
Pyrrolidone carboxylic acid	12	0.24
Urea	7	0.14
Sodium chloride	5	0.10
Sodium lactate	5	0.10
Potassium citrate	0.5	0.01
Serine	18.2	0.36
Glycine	9.1	0.18
Arganine	3.2	0.064
Glutamic acid	2.3	0.04
Tyrosine	0.5	0.01
Alanine	6.6	0.13
Hydroxyethyl cellulose[1]		
Deionized water[2]		

[1] Sufficient quantity to provide a viscosity of 300 cps.
[2] Added as necessary to provide a total of 100% by weight.
The natural moisturizing factor components were added to a vehicle of hydroxyethyl cellulose and the viscosity was adjusted to 300 cps for application to the skin. The formula was based on reported compositions of NMF (14,19,20).

Table II
Skin Treatments

Site	Arm A	Site	Arm B
1	Untreated	7	Soak
2	Untreated + vehicle	8	Soak + vehicle
3	Untreated + NMF	9	Soak + NMF
4	Acetone/ether extraction (A/E)	10	A/E + soak
5	A/E + vehicle	11	A/E + soak + vehicle
6	A/E + NMF	12	A/E + soak + NMF

The twelve 2 × 2-cm treatment sites were randomized for left vs right and for position along the arm. Both arms were extracted with acetone/ether as shown. One arm was then soaked in water for ten minutes. The other arm served as the control. Following the soak procedure, the NMF formulation was applied to the specified sites. Soak indicates water soak, and vehicle indicates the hydroxyethyl cellulose-containing control for the NMF formulation.

equilibration to environmental conditions (temperature 21° ± 1°C and relative humidity 31 ± 5%) for 30 minutes. Baseline skin measurements of TEWL, skin hydration, and MAT were made for each of the twelve treatment sites (time = 0 min).

ACETONE/ETHER EXTRACTION

Three sites on each forearm (six total) were treated with a 1:1 mixture of acetone/ether (A/E) to remove surface lipids and intercellular lipids from the outer SC layers. The acetone/ether extraction procedure was expected to remove only very small quantities of water-soluble materials from the skin (21). The sites were exposed to A/E for five minutes using a glass extraction cup to hold the solvent. The areas were then wiped repeatedly with cotton pads dipped in the A/E mixture. This process was continued until the TEWL reading increased to approximately 2× the baseline value. Following extraction, the biophysical measurements were repeated for each of the six sites.

SOAKING

Once the A/E extraction was complete, one forearm was soaked in fresh water (temperature 40° ± 1.4°C) for ten minutes and blotted dry. The sites on the other (unsoaked) forearm served as control sites. Fifteen minutes after soaking, the biophysical measurements were repeated for all 12 sites, including the untreated control site.

NMF AND VEHICLE TREATMENT

The NMF formulation was applied (2 mg/cm^2) to four sites immediately following the post-soak measurements: untreated (no extraction, no soak), A/E-treated, soaked, and A/E plus soak. The vehicle control (2 mg/cm^2) was applied to another set of sites (untreated, A/E-treated, soaked, A/E plus soak). The biophysical measurements were made 30 minutes after application for all 12 sites (Table II), including the untreated control site. The test areas were left undisturbed for 3.5 hours. The subjects returned to the test facility and the measurements were made following the 30-minute equilibration.

STATISTICS

Analysis of variance was used to compare treatment groups. Repeated measure statistics were used to evaluate the changes in the treatment sites over time. The paired comparison *t*-test was used to evaluate the effects of treatment variables. To normalize the data for variations in skin condition along the forearm, the change from baseline was used in the paired comparison procedures (SigmaStat, Jandel Scientific). $p < 0.05$ was considered statistically significant.

RESULTS

The biophysical data (mean, ± SE) for all sites and time points are shown in Tables III, IV, and V. The changes and associated statistical significance are given in Table VI ($p < 0.05$ was considered statistically significant). Figure 1 illustrates the changes in TEWL relative to the changes in MAT for the water soak. Figure 1 also shows the effect of NMF on TEWL and MAT for the soaked site 30 minutes after NMF application. The values in the figure have been normalized for changes in the parallel untreated control site.

Relative to the initial skin condition, extraction of normal skin with 1:1 acetone/ether significantly increased TEWL but did not change MAT (Table VI) or baseline hydration (Table V). Exposure of normal skin to a fresh water soak significantly increased the TEWL at 15 minutes after the soak procedure. Thirty minutes later, the TEWL had decreased, presumably as a result of surface water evaporation. After four hours, TEWL had returned to the baseline value (Table VI). Soaking significantly decreased the MAT, a decrease that was sustained after four hours. Soaking significantly decreased the baseline hydration in the short term (15 min), but not after an additional thirty minutes. A/E

Table III
Summary of Effects of Skin Treatments on Transepidermal Water Loss (TEWL) Over Time

Site	Treatment	Baseline	Post-extraction	Post-soak (15 min after)	Post-formula application (30 min after)	Post-formula application (4.5 hr after)
1	Untreated	9.8 ± 2.5	—	9.8 ± 2.0	8.1 ± 1.9	9.5 ± 2.8
2	Acetone/ether extraction	9.1 ± 1.9	11.6 ± 2.7	—	8.5 ± 1.7	10.4 ± 1.9
3	Water soak	9.8 ± 2.0	—	11.2 ± 2.1	8.3 ± 2.2	9.6 ± 2.1
4	A/E + soak	9.3 ± 3.0	12.2 ± 4.3	12.5 ± 2.7	9.4 ± 2.5	10.5 ± 3.5
5	Untreated + vehicle	9.1 ± 2.4	—	—	7.7 ± 2.2	8.7 ± 2.4
6	Untreated + NMF	8.9 ± 2.4	—	—	6.9 ± 2.3	7.9 ± 2.4
7	A/E + vehicle	8.7 ± 1.9	10.9 ± 2.9	—	8.4 ± 2.3	9.9 ± 2.2
8	A/E + NMF	9.1 ± 2.2	10.9 ± 3.2	—	7.7 ± 2.1	9.0 ± 2.3
9	Soak + vehicle	9.4 ± 2.1	—	10.7 ± 2.0	7.9 ± 1.7	8.8 ± 1.8
10	Soak + NMF	9.0 ± 1.6	—	10.5 ± 2.0	6.5 ± 1.7	7.7 ± 2.2
11	A/E + soak + vehicle	8.8 ± 2.5	11.0 ± 3.1	12.1 ± 2.2	9.0 ± 2.2	10.2 ± 2.5
12	A/E + soak + NMF	9.1 ± 2.5	14.8 ± 7.1	12.2 ± 2.3	8.0 ± 2.4	9.2 ± 2.9

TEWL ($g/m^2/hr$) was measured for each site prior to any treatment or application procedure (baseline reading), following the A/E extraction, 15 minutes after the water soak, 30 minutes after NMF application, and 4.5 hours following NMF treatment. The values are mean ± SEM, n = 11. A/E indicates acetone/ether extraction, soak indicates water soak, and vehicle indicates the hydroxyethyl cellulose-containing control for the NMF formulation.

Table IV
Summary of Effects of Skin Treatments on Rate of Moisture Accumulation (MAT) Over Time

Site	Treatment	Baseline	Post-extraction	Post-soak (15 min after)	Post-formula application (30 min after)	Post-formula application (4.5 hr after)
1	Untreated	1.6 ± 1.5	—	0.8 ± 0.5	1.3 ± 1.2	1.3 ± 1.0
2	Acetone/ether extraction	1.1 ± 0.8	1.2 ± 0.9	—	1.1 ± 0.8	1.2 ± 0.8
3	Water soak	1.1 ± 1.0	—	0.5 ± 0.5	0.5 ± 0.3	0.7 ± 0.7
4	A/E + soak	1.1 ± 0.8	1.0 ± 0.9	0.3 ± 0.3	0.4 ± 0.3	0.5 ± 0.4
5	Untreated + vehicle	1.4 ± 1.4	—	—	0.8 ± 0.7	1.3 ± 1.3
6	Untreated + NMF	1.4 ± 1.1	—	—	2.6 ± 2.0	1.5 ± 1.3
7	A/E + vehicle	0.9 ± 0.6	0.9 ± 0.6	—	0.7 ± 0.4	1.0 ± 0.8
8	A/E + NMF	1.0 ± 1.3	1.1 ± 1.1	—	1.9 ± 1.2	1.4 ± 1.3
9	Soak + vehicle	1.0 ± 0.5	—	0.5 ± 0.3	0.6 ± 0.3	0.5 ± 0.2
10	Soak + NMF	1.4 ± 1.0	—	0.6 ± 0.3	1.7 ± 1.1	0.7 ± 0.4
11	A/E + soak + vehicle	1.0 ± 1.0	1.6 ± 1.5	0.3 ± 0.1	0.4 ± 0.3	0.6 ± 0.4
12	A/E + soak + NMF	1.0 ± 0.9	1.4 ± 1.2	0.2 ± 0.2	1.5 ± 1.3	0.9 ± 0.5

The rate of moisture accumulation (MAT) (cru/sec) was measured for each site prior to any treatment or application procedure (baseline reading), following the A/E extraction, 15 minutes after the water soak, 30 minutes after NMF application, and 4.5 hours following NMF treatment. The values are reported as slope of the regression line and are mean ± SEM, n = 11. (For abbreviations, see footnote to Table III.)

Table V
Summary of Effects of Skin Treatments on Baseline Hydration at Various Times

Site	Treatment	Baseline	Post-extraction	Post-soak (15 min after)	Post-formula application (30 min after)	Post-formula application (4.5 hr after)
1	Untreated	12.7 ± 3.5	—	9.25 ± 2.1	10.7 ± 3.0	12.3 ± 3.0
2	Acetone/ether extraction	10.6 ± 2.1	14.5 ± 2.1	—	13.7 ± 3.0	11.3 ± 2.6
3	Water soak	10.4 ± 3.0	—	6.4 ± 1.4	7.2 ± 1.5	6.1 ± 1.8
4	A/E + soak	10.8 ± 3.1	11.7 ± 2.9	4.2 ± 1.4	5.1 ± 1.5	4.3 ± 1.0
5	Untreated + vehicle	10.7 ± 2.7	—	—	12.6 ± 2.9	12.0 ± 3.9
6	Untreated + NMF	12.4 ± 2.6	—	—	21.8 ± 5.9	12.7 ± 3.9
7	A/E + vehicle	10.0 ± 1.5	13.8 ± 1.9	—	11.5 ± 3.4	9.1 ± 1.6
8	A/E + NMF	12.4 ± 2.1	15.7 ± 4.0	—	19.9 ± 4.7	12.8 ± 2.5
9	Soak + vehicle	9.9 ± 1.6	—	5.78 ± 1.2	7.3 ± 1.9	8.33 ± 2.2
10	Soak + NMF	13.4 ± 2.4	—	6.78 ± 1.0	15.1 ± 2.6	9.3 ± 1.7
11	A/E + soak + vehicle	13.2 ± 2.3	16.3 ± 3.6	3.7 ± 1.0	6.0 ± 1.0	5.8 ± 1.5
12	A/E + soak + NMF	12.2 ± 3.5	10.6 ± 2.8	7.3 ± 1.5	16.6 ± 5.9	9.9 ± 3.2

The skin hydration (cru) was measured for each site prior to any treatment or application procedure (baseline reading), following the A/E extraction, 15 minutes after the water soak, 30 minutes after NMF application, and 4.5 hours following NMF treatment. The values are mean ± SEM, n = 11. (For abbreviations, see footnote to Table III.)

extraction followed by a water soak resulted in TEWL, MAT, and baseline hydration profiles similar to those of the individual A/E and soak treatments (Table VI).

Application of the NMF resulted in significantly lower TEWL for the untreated control, the A/E extracted site, and the water-soaked site after thirty minutes (Table VI). The TEWL was directionally lower 30 minutes after NMF treatment for the A/E–plus–water-soak site. The significant decrease in TEWL for the A/E site and the directional

Table VI
Statistical Analysis of Effects of Skin Treatments on Instrumental Measurements

	TEWL 30 min after NMF application	MAT 30 min after NMF application	TEWL after 4 hr	MAT after 4 hr
Treatment				
Acetone/ether	↑	ND	↑	ND
Water soak	↓	↓	ND	↓
A/E + water soak	↓	↓	↓	↓
Effect of NMF				
Untreated + NMF	↓	↑	↓	ND
A/E + NMF	↓	↑	↓	ND
Soak + NMF	↓	↑	↓	↓ (d)
A/E + soak + NMF	↓ (d)	↑	↓ (d)	↑

The effects of the A/E extraction, water soak, and NMF treatment for the respective sites on barrier integrity (TEWL) and SC water-handling properties (MAT) were determined relative to the initial skin condition. The changes and associated statistical significance are shown. ↑ indicates a significant increase ($p \leq 0.05$), ↓ indicates a significant decrease, ND indicates no significant difference, and (d) is a difference at $p \leq 0.07$.

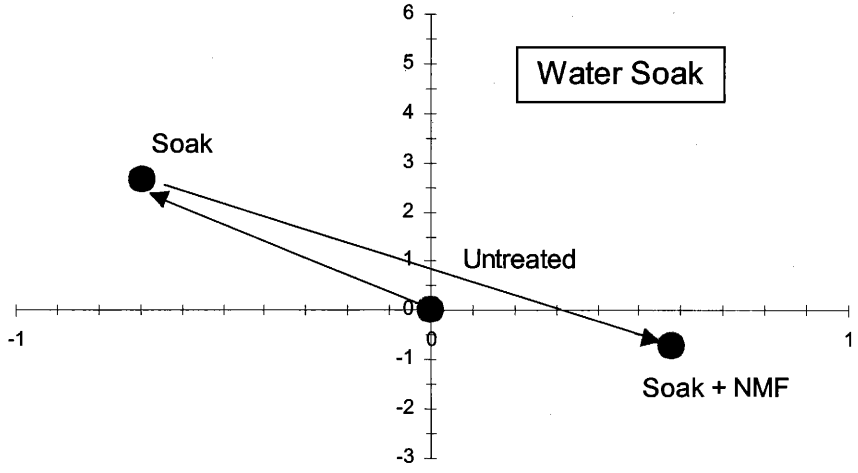

Figure 1. The changes from baseline skin condition in TEWL versus MAT are shown for one of the sites. The site was first soaked in water and then treated with an NMF formulation. The measurements were made 30 minutes after application. The numerical values shown in the figure have been normalized for changes in the parallel untreated control site.

decrease in TEWL for the extracted–plus–soaked site were maintained four hours later (Table VI). Direct comparison of the NMF treatments with the corresponding vehicle controls indicated that the decreases in TEWL were due to the NMF components and not to the vehicle itself (Table VI).

Application of NMF produced a significantly higher MAT for all four skin conditions at thirty minutes following treatment (Table VI). The MAT increase was maintained four hours later for the A/E-extraction–plus–water-soak site only. The changes in MAT following NMF treatment were not attributable to the vehicle.

The addition of NMF to the skin resulted in a significant increase in baseline hydration

for the untreated and the A/E-extracted–plus–water-soaked sites at thirty minutes (Table V). For the soaked site, NMF application increased hydration directionally (p = 0.07). NMF addition had no effect on the A/E-extracted site.

DISCUSSION

The human stratum corneum is a thin, flexible, mechanically tough membrane that is essential for cutaneous protection against environmental insults and loss of body water in a dry, terrestrial environment. The SC barrier properties are conferred by intercellular lipids arranged as lamellar sheets surrounding corneocytes tightly linked by desmosomal connections (22–24). The flexibility and water-holding properties of the SC are, in large part, attributable to hygroscopic, water-soluble compounds, collectively known as NMF (15,25). The primary constituents are high concentrations of amino acids, including derivatives such as pyrrolidone carboxylic acid and urocanic acid, that are found in the stratum corneum (26,27). NMF is generated in part by proteolysis of the SC protein filaggrin, which occurs under conditions of optimal water activity, i.e., in a relative humidity range of 70% to 95% (2).

Interference with either the composition or structure of the lamellar lipids or the production or accumulation of NMF can, presumably, disturb the water balance of the stratum corneum. Disruption of the intercellular lamellae can occur as a result of treatment with solvents and with water itself (12,28). In this study, we examined the effects of a common perturbation, exposure to water via soaking, on the SC permeability barrier and water-handling properties. We used a solvent system to extract the hydrophobic surface residue and SC lipids from the upper layers. Acetone/ether extraction has been shown to remove lipids without removing the water-soluble components (13). Extraction of the intercellular SC lipids was expected to increase access to the soluble components within the upper stratum corneum during the soaking phase. We anticipated that the combination of solvent extraction (barrier damage) followed by water exposure would model the effects of surfactant–water interactions with the SC and determine the relative contributions of changes in lipids and changes arising from removal of water-soluble materials. To test the hypothesis that water-soluble components significantly impact SC water-handling properties, we applied an NMF composition back to soaked or extracted skin sites and evaluated the water-handling properties using measurements of TEWL and hydration.

Acetone/ether extraction significantly increased TEWL for five hours (Table VI), suggesting that recovery from lipid depletion was not immediate. The finding that solvent treatment did not significantly change MAT is consistent with reports that very small amounts of water-soluble components are removed with acetone/ether extraction (21).

The MAT methodology has been used as a surrogate for TEWL in animal models (29,30) and cell culture systems (29), and for assessing the development of barrier function in premature human infants (31). In situations of extreme barrier compromise, e.g., tape stripping or premature birth, increased MAT paralleled an increase in TEWL (31,32). Decreased MAT has been observed to indicate barrier development or recovery (29,30,32). In this study, barrier compromise by A/E extraction, while producing a statistically significant increase in TEWL, was small and did not significantly alter MAT. We have observed that MAT decreases in dry scaly skin (e.g., due to low relative

humidity) and during the later stages of barrier recovery (33). Treffel and Gabard (18) reported TEWL and MAT values for skin sites treated with moisturizer or exposed to surfactant irritation. TEWL changed only after long-term exposure, whereas MAT differences were detected with shorter treatment times. In addition, control skin sites evaluated in both July and November were statistically different for MAT, but not for TEWL. Van Neste (34) used a dynamic measure of water movement in combination with TEWL to investigate surfactant effects. He found that surfactant-damaged skin had an increased TEWL and an increased rate of moisture accumulation. The two measurements correlated better for damaged skin than for normal skin.

Soaking the skin in water resulted in an immediate increase in TEWL, followed by a reduction, and produced a significant effect on MAT, yielding very low values (Table VI, Figure 1). While TEWL returned to baseline after five hours, MAT remained significantly lower than the pre-soak value (Table VI). This result indicates that recovery of the SC barrier from the effects of soaking was not immediate. The decreased MAT values suggest that the upper layers of the stratum corneum became significantly drier as a result of water exposure.

Solvent extraction followed by a water soak increased TEWL and decreased MAT (Table VI). In an investigation of the molecular mechanisms responsible for SC elastic properties, Jukura *et al.* (14) reported that acetone/ether extraction did not change molecular mobility within the stratum corneum. However, treatment of the SC with water and the subsequent release of water-soluble materials significantly reduced molecular motion and eliminated the SC elasticity. Depletion of the water-soluble materials also decreased bound water and increased free water. Replacement of the neutral and basic amino acids increased the molecular mobility of the SC, whereas the addition of water alone had no effect. It was hypothesized that removal of water-soluble materials increased molecular interaction between keratin fibers. The water-soluble components of the SC, and not water alone, were responsible for molecular mobility and, therefore, for SC elasticity.

Application of NMF consistently lowered TEWL, following A/E extraction, soaking, and extraction–plus–soaking. These effects were seen after thirty minutes and were maintained for another four hours (Table VI). We hypothesize that hygroscopic NMF components bind associated water, including water within the SC, and thereby reduce the rate of evaporative loss, i.e., TEWL. In an investigation of the skin effects of urea-containing moisturizers, Serup (35) reported that the reduction in the rate of evaporative loss (TEWL) indicated an improvement or restoration of SC barrier function.

Addition of NMF to all skin sites resulted in a significant increase in the rate of moisture accumulation after thirty minutes (Table VI). For the site that was extracted, soaked, and treated with NMF, the MAT value was elevated four hours later (Table VI). In contrast, the MAT of the soak + NMF site was significantly lower after four hours than it had been 30 minutes after NMF application and significantly lower than the initial value (Table VI). The two sites behaved differently, and the findings suggest that the soaked site could not retain the water associated with the NMF amino acids.

Bulgin and Vinson (36) used calorimetric techniques to examine water in the SC and reported three types of water: tightly bound water (primary), readily releasable bound water (secondary) and free bulk water. Free water existed only in very highly hydrated skin states. Takenouchi *et al.* (37) investigated the bound water in scaly skin conditions. Primary bound water was specified as water not readily lost, even at 0% RH, and was

reported to be approximately 5 mg water/100 mg of dry SC. Secondary water, held loosely by molecular bonds to the SC components, was measured using DSC techniques after subtracting the primary component. Normal skin had a 20–30% higher level of secondary water than xerotic and psoriatic skin, due to its higher secondary water-holding capacity. Takenouchi *et al.* showed that normal skin had substantially increased levels of amino acids, i.e., hygroscopic NMF, and significantly higher water-holding capacity than scaly skin. Secondary bound water was associated with primary bound water through hydrogen-bonding interactions and exhibited rapid hydration and dehydration with environmental changes. Previous investigations in our laboratory have demonstrated that the water-holding capacity of normal skin, measured with the sorption-desorption technique of Tagami, was significantly reduced following fresh water bathing (38,39).

Based on the current study, we suggest that water soaking removes some water-soluble amino acids, i.e., NMF, from skin and thereby reduces the amount of secondary bound water. The decreased rate of moisture accumulation observed after soaking might be due to a decrease in the NMF-dependent bound water that gives rise to the higher capacitance reading prior to the soak. We further speculate that, in the presence of a normal SC barrier (i.e., normal TEWL, no damage) and the absence of eccrine sweating, the rate of moisture accumulation provides a dynamic, functional assessment of the loosely bound water ascribed to the hygroscopic NMF. In contrast to the single-point measurements of baseline hydration, the MAT technique differentiated the effects caused by soaking and NMF application (Tables IV, V). Therefore, MAT is preferred over single-point determinations.

The results of the water soaking experiment suggest that SC recovery to normal is relatively slow. The findings raise questions about the effects on the skin of cumulative repeated wet-dry-wet exposures, such as those encountered in the diapering environment or in multiple hand washing situations (for health care workers, parents, child care providers). Additional studies are required to examine the kinetics of the depletion/restoration and to confirm the removal of NMF by direct quantitation of the amino acids. Finally, investigations of the mechanisms by which the epidermis responds to common environmental effects (e.g., bathing) are warranted in order to develop appropriate skin care practices and products.

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