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Natural moisturizing factors (NMF) in the stratum corneum (SC). II. Regeneration of NMF over time after soaking

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Synopsis

The detrimental effects of prolonged water exposure on skin are well known. Information on the effects of short-term exposure, e.g., during bathing, showering, and hand washing, on NMF levels is limited. In an attempt to isolate the effects of soaking on the NMF, a time course for skin sample collection was devised in which adhesive tapes were applied 0.5 and four hours after soaking of the treated arm. Significant decreases in NMF levels, quantified by HPLC analysis of serial tape strips, were observed 0.5 hours after soaking, with a replacement of NMF occurring by the four-hour mark. This replacement corresponds to a parallel rebound in skin pH also observed at these times. Stratum corneum hydration values, measured instrumentally as the rate of moisture accumulation (MAT), however, were depressed 0.5 hours post-soaking and remained low four hours later. In addition, significant increases in skin pH were observed during the immediate post-soak period. These findings suggest that short-term exposure to water alone produces significant changes in the stratum corneum.

INTRODUCTION

The detrimental effects to the skin of long water exposure are well known. Extended water exposure causes maceration of the skin, disruption of the skin barrier and structure, and a dry flaky appearance once the excess water evaporates (1–6). The effects of ordinary water exposures, such as bathing or soaking, on the skin and its biophysical properties have been explored over years of research in many laboratories. Soak baths of relatively brief duration, up to 20 minutes in length, have been shown to temporarily increase skin hydration and reduce scaling (7). Studies of long-term exposures indicate that the skin looks well hydrated and scale-free immediately after the exposure, but that within two to three days, hyperplasia and inflammation occur (1). The boundary between the therapeutic and damaging effects of water exposure is unknown, as is the etiology of the resulting damage.

Increased stratum corneum (SC) hydration due to soaking has been shown to increase skin permeability to surfactants and other irritants (8,9). Even brief tap water washing increases

pH and skin dryness in infants (10). As has been recently shown, merely maintaining a low skin pH helps prevent chemically induced atopic dermatitis and can repair moderate age-related barrier defects (11,12). Increased SC pH has been shown to directly cause SC swelling and a change in lipid transition temperatures, increasing the reactivity of the skin (13). Surprisingly, experimental data on the effects of common practices such as bathing and relatively brief soaking on NMF levels is sparse. Earlier reports from our group (Visscher, Tolia, Fugitt, Hoath, Wickett) have shown that bathing/soaking reduces skin hydration and the rate of stratum corneum moisturization in both infants and adults (14,15). Topical application of NMF reversed the effects of soaking, but direct quantitative measures of NMF were not made. A direct connection between NMF levels, skin moisturization, and water handling properties in normal skin has not been well established experimentally.

Biophysical measurements such as TEWL or MAT are commonly used in cosmetic industry laboratories. Despite the frequency with which these tests are used, a complete understanding of what these instruments really measure in the skin is still lacking. We hypothesized that exposure to water (soaking) would reduce NMF levels in the outer stratum corneum relative to normal, non-exposed skin. We also hypothesized that there is a relationship between NMF levels and commonly used biophysical measurements such as MAT and skin pH.

We have also explored the relationship between NMF levels and biophysical instrument readings during the hours post-soak. The preceding paper (this issue) reported a new chromatographic method for quantitation of the free amino acid components of NMF and demonstrated its application for differentiating skin treatments that do not cause barrier damage. In this paper, we have used our method to quantify NMF following exposure to water alone, and to further explore the relationship between NMF and various commonly used biophysical measurements of skin moisture and acidity.

MATERIALS AND METHODS

SUBJECTS

Evaluations were performed on twenty-seven healthy female subjects aged 23-60 in two studies: Study 1 in September of 2003 (n = 8) and Study 2 in February of 2003 (n = 19). Exclusion criteria included visually dry forearm skin and dermatological conditions such as psoriasis and eczema on the study areas. The Institutional Review Board of the University of Cincinnati Medical Center approved the protocols. All subjects provided informed consent.

EXPERIMENTAL PROCEDURE

Prior to entry into the studies, subjects refrained from using moisturizer on their forearms for 72 hours. One 2 × 2-cm treatment site was marked on each volar forearm. Panelists acclimated to environmental conditions (temperature 21° ± 1°C and relative humidity 31% ± 5%) for 30 minutes before initial measurements were collected. Baseline measurements of MAT, sorption/desorption, and skin pH were made for all

sites. Biophysical measurements were made again at 0.25, 0.5, and 4 hours following treatment.

TREATMENT

These studies compared an untreated control arm to a treated arm that was soaked in warm water. One forearm was soaked in fresh water (temperature 40° ± 2°C) for ten minutes and blotted dry. The other, unsoaked arm served as control. Biophysical measurements were repeated on all test sites 15 minutes, 30 minutes, and four hours after soaking.

BIOPHYSICAL INSTRUMENTATION

The rates of moisture accumulation (MAT) and water sorption/desorption 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 by the probe to determine the extent of skin hydration (16). Transepidermal water accumulates under the sensor for twenty seconds and the value is calculated as the slope of the regression line (DPM). The MAT method provides a dynamic measure of SC water handling, as previously reported (17). Skin surface pH was measured using a Courage and Khazaka skin pH meter with a flat-surface glass electrode.

NMF MEASUREMENTS

NMF measurement studies followed the NMF collection and analysis protocols set forth in the preceding paper.

STATISTICS

The biophysical measurements were compared at baseline (prior to treatment), 0.5 hr post-soak and 4 hr post-soak using univariate GLM procedures in SPSS (SPSS, Inc), with time and subject included in the model. The NMF data were normalized (log10) prior to analysis and then compared using univariate GLM measures. The statistical design for the NMF data included tape strip number, treatment, treatment * tape (interaction) and treatment * subject (interaction). A value of ≤ 0.05 was considered statistically significant in all cases.

RESULTS AND DISCUSSION

For both studies, at tape 5 and for the sums of tapes 1, 3, and 5, as well as for the sums of tapes 1, 3, 5, and 10, the values at thirty minutes post-soak were significantly lower than both the control and four-hour post soak values for nearly every amino acid, as well as for the summed amino acids (Figure 1). Similar results were seen in Study 2 for tape 3 as for well. These studies show more significant differences between the soaked and control sites than found in our previous study, although directional differences were found in our

previous work. These data clearly show a regeneration or replacement of NMF in the middle layers of the SC four hours after soaking (Figure 1). Figure 2 shows the regeneration of citrulline over time over the various tape strip depths. Without the extraction used in previous studies in our laboratory, no significant treatment effects were found at

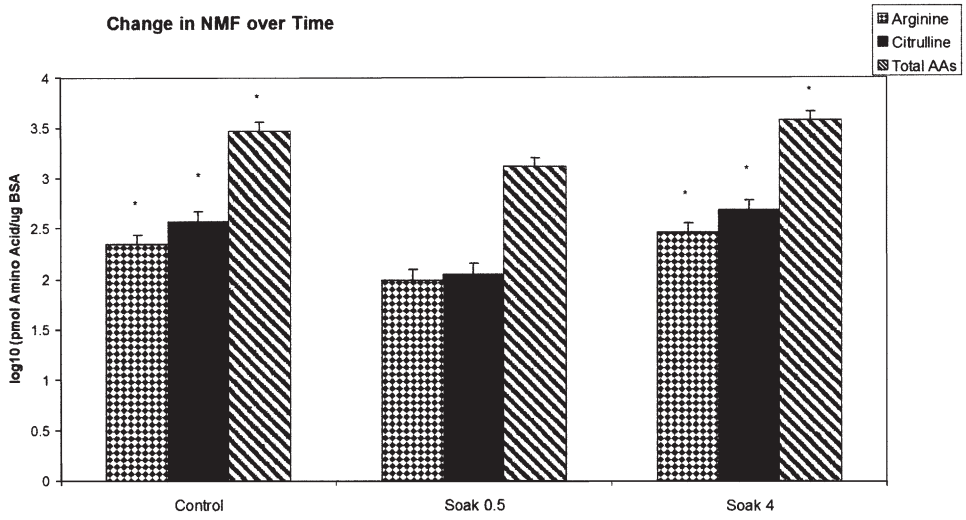

Figure 1. Significant reductions in NMF were observed in the soaked site 30 minutes post-soaking and rebounded to pre-soaking levels or slightly higher by the four-hour post-soak measurement. *Indicates significant difference from the 0.5-hour soak site, $p < 0.05$. Values are displayed as estimated means of the log10 normalized data with errors as ± standard error of the estimated means. These points are drawn from Study 2, tape strip 5.

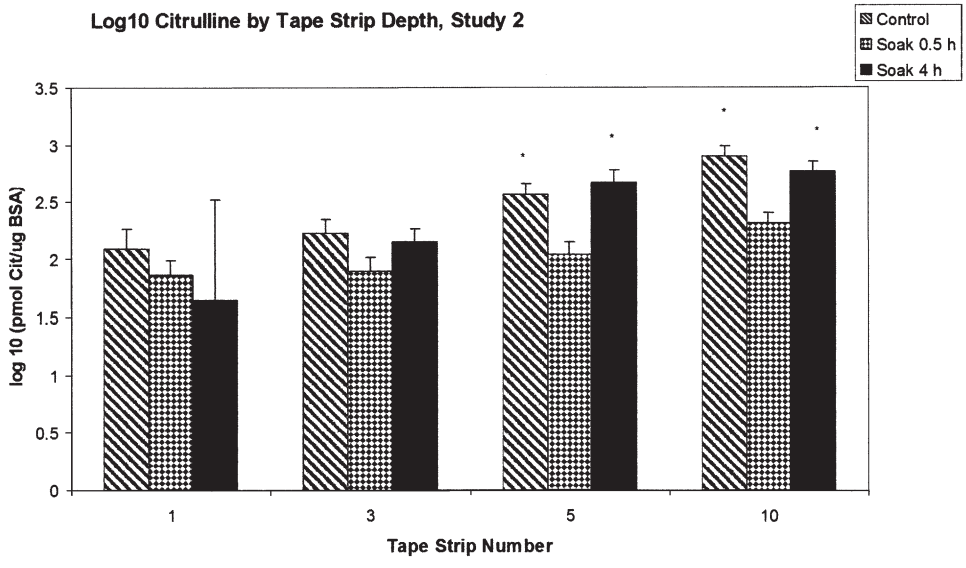

Figure 2. Significant reduction in NMF levels was caused by soaking as measured 30 minutes after the soak, but significant replacement of NMF was only observed in the lower levels of the SC as measured by tape strip depth. *Indicates significant difference from the 0.5-hour soak site, $p < 0.05$ Values are displayed as estimated means of the log 10 normalized data with errors as ± standard error of the estimated means.

tape 15 in Studies 1 and 2. Tables I and II provide the statistical results in detail. One potential clinical implication of the observed initial decrease in NMF followed by its regeneration is that it is related to the initial sensation of tightness often reported after washing, which generally subsides spontaneously within the first hours afterwards. However, the effects on tightness and other sensory parameters were not tested.

In an attempt to further elucidate the changes caused in the SC by the fresh-water soak and their evolution over time, we added pH measurements to our previously published methodology. Skin pH was significantly elevated immediately after soaking but returned to normal within four hours (Table III). Maintenance of the native SC pH has been shown to repair moderate barrier defects (12), and so a natural return to

Table I
Significant NMF Results, Study 1

Comparison	Significant differences
By depth, tape 3	Soak at 0.5 versus control; soak at 4 hr for Arg only
By depth, tape 5	Soak at 0.5 versus control; soak at 4 hr for total amino acids, Ser, Gly, His, Arg Cit, Phe
Sum of tapes 1, 3, 5	Same as tape 5
Sum of tapes 1, 3, 5, 10	Same as tape 5
Control	1 vs all other tapes for total amino acids, Ser, Gly, His, Arg, Cit, Phe 3 vs all for total, Ser, Gly, His, Phe 3 vs 10 for Arg
Soaked, time 0.5	1 vs all other tapes for total amino acids, Ser, Gly, His, Arg, Cit, Phe
Soaked, time 4	1 vs all other tapes for total amino acids, Ser, Gly, His, Arg, Cit, Phe 10 vs all other tapes for all amino acids *except* Cit

Time 0.5 indicates the tape strips collected from the soaked sites 0.5 hours after the soak. Time 4 indicates tape strips collected four hours after the soak. Tape strip numbers are listed as numbers only, e.g., 1 for tape strip 1. All comparisons listed are significant at $p < 0.05$. No significant differences were found at strip 1.

Table II
Significant NMF Results, Study 2

Comparison	Significant differences
By depth, tape 3	Soak at 0.5 versus control; soak at 4 hr for total amino acids, Ser, Gly, His, Arg
By depth, tape 5	Soak at 0.5 versus control; soak at 4 hr for total amino acids, Ser, Gly, His, Arg Cit, Phe
Sum of tapes 1, 3, 5	Soak at 0.5 versus control; soak at 4 hr for all *except* Phe
Sum of tapes 1, 3, 5,10	Same as sum of tapes 1, 3, 5
Control	1 vs all for total, Ser, Gly, His, Arg, Cit 10 vs all for total, Ser, Gly, His 1 vs 5, 10 for Phe 3 vs 10 for Arg, Phe
Soaked, time 0.5	1 and 3 vs 10 for Ser, Gly, His, Arg 1 vs 5 for Ser, Cit 3 vs 10 for Ser 3 vs all for Cit
Soaked, time 4	1 and 3 for total amino acids, Ser, Gly, His, Arg Cit, Phe

All comparisons listed are significant at $p < 0.05$. Time 0.5 indicates the tape strips collected from the soaked sites 0.5 hours after the soak. Time 4 indicates tape strips collected four hours after the soak.

pre-perturbation levels within a relatively short time frame is necessary for basic skin health. Some studies hypothesize that NMF components are directly responsible for acid mantle formation (18,19). We found a negative correlation between pH and NMF levels in Study 1, which supports this conclusion. Clearly, a sequence of events has been initiated in the SC by this brief soak, perhaps due to removal of NMF and leading to NMF generation and a rebalancing of SC pH.

A significant reduction in MAT due to soaking (Table IV) was found to persist until the four-hour point in Study 1. MAT was not significant 15 minutes post-soak, possibly due to residual moisture from the soak itself. The control site was significantly different from the soaked site both 30 minutes and four hours post-soak. These conclusions were not duplicated in our second study; however, the first study took place in September and the second in February. Differences in environmental conditions (average relative humidity in Cincinnati in September is 88%; in February, 71%) led to extremely low MAT values for all times in Study 2, possibly leading to difficulty resolving differences between treatments and times due to use of the extremes of the measurement range of the device. The data from both studies were combined for analysis, but the difference between the first and second studies was directional (p=0.07, t-test). TEWL is the generally accepted method of measuring barrier damage (20). In our previous studies involving soaking of the skin (14), consistently non-significant differences in TEWL validated the lack of barrier damage due to soaking of the skin. For this reason, TEWL measurements were discontinued and pH measurements were added (Table III).

Table III
Effect of Soaking on Skin Surface pH

	Baseline	.25 Hours post-soak	4 Hours post-soak
Study 1	5.46 ± 0.13	6.14 ± 0.14*	5.53 ± 0.13*
Study 2[a]	5.68 ± 0.07	6.02 ± 0.08	5.79 ± 0.08

Soaking caused a significant elevation in SC pH in Study 1, which returned to normal after four hours. pH was measured for each site prior to any treatment (baseline), and 15 minutes and four hours after the fresh-water soak. The values are mean ± standard error (Study 1, Study 2).
[a]Covariates appearing in the model for Study 2 (the model contained time, subject, logTotal, and SDT) are evaluated at the following values: logTotal = 3.0 SDT = 7740.0 (no covariates were used in analysis of Study 1).
*Indicates significant difference from baseline, $p \leq 0.05$.

Table IV
Moisture Accumulation Test (MAT)

	Control site 4 hours post-soak	Soaked site at baseline	Soaked site 0.5 hours post-soak	Soaked site 4 hours post-soak
Study 1	0.60 ± 0.08	0.65 ± 0.47	0.24 ± 0.09*	0.31 ± 0.09*

Soaking produced a significant decrease in MAT values that persisted for the study duration. The rate of moisture accumulation (MAT) (cru/sec) was measured for each site prior to any treatment (baseline), and 15 minutes, 30 minutes and four hours after the fresh-water soak. The data collected 15 minutes after the soak are omitted, as the arms were still wet. The values are reported as estimated slope of the regression line as generated by univariate GLM procedures and are mean ± standard error (Study 1).
*Indicates significant difference from control, $p \leq 0.05$.

These studies confirm the conclusions previously drawn (14) as to the effects of soaking on the NMF; that is to say, a simple ten-minute fresh-water soak can remove significant amounts of the free amino acid components of the stratum corneum. No clear correlation was observed between NMF levels and skin hydration as measured by the MAT, but it appears likely that NMF (as free amino acid levels) relates to SC pH. These results are consistent with studies showing that hydration measurements relate mainly to extremely superficial layers of the SC (21), from which the NMF has already been almost completely lost and cannot be replaced without shedding of the uppermost cells. Our results confirm that SC recovery to its baseline condition after soaking is relatively slow and complex in its kinetics.

ADDITIONAL INFORMATION

Complete numerical NMF data are available by correspondence.

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