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Urea analysis of extracts from stratum corneum and the role of urea-supplemented cosmetics

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Synopsis

This investigation was undertaken to quantify the amount of urea in extracts from stratum corneum of normal skin in comparison to extracts from skin after cleansing, or from skin after a prolonged topical application of urea-supplemented emulsions. We measured a dramatic decrease in the amount of extractable urea from stratum corneum after skin cleansing. This loss of urea can be partially compensated by a cleansing formula supplemented with urea. On the other hand, a skin care emulsion with urea supplementation increases significantly the amount of urea that can be solubilized from stratum corneum. From these results we conclude that the urea content of stratum corneum varies in a wide range, limited at the lower end by a reduced status that can be observed when skin cleansing had been performed, and at the higher end by an increased level that can be obtained after prolonged application of urea-containing emulsions. These findings might have important implications for therapeutic compensation of urea deficiency in pathological skin diseases and also for cosmetic compensation for a lack of water-retaining substances in dry skin.

INTRODUCTION

Urea is one of the most important soluble substances of the stratum corneum. In recent years this substance has become more and more important in dermatological therapy and cosmetics. Many diseases have been described that are characterized by a deficiency of urea, such as atopic dermatitis or clinical dry skin (1). The urea content of normal skin is nearly 1% (2). It contributes in a significant manner to the hydration of the stratum corneum. Besides amino acids, lactate, and other substances, urea contributes approximately 3–7% to the natural moisturizing factor (NMF) (3). The NMF appears to be responsible for the hydration status of stratum corneum. Otherwise urea is known for its keratolytic and pruritus-easing properties (4), and it is a very potent humectant in moisturizing creams (5,6). Its sources in the epidermis are sweat (7) and the decomposition of arginine by arginase during the process of keratinization (8).

The high relevance of urea prompted us to look for a rapid method for determination of the urea content of stratum corneum. Here we describe a noninvasive method of deter-

mining the water-soluble urea in the stratum corneum. First we determined the homogeneity of urea distribution on the volar forearms of volunteers. Then we investigated the urea loss of stratum corneum following a washing procedure and the partial supplementation of lost urea by addition of urea to a cleansing product. Because of the high loss of urea in consequence of the cleansing procedure, we measured to what extent an external application of urea influences the extractable urea and the hydration of stratum corneum.

EXPERIMENTAL

SUBJECTS

The experimental subjects were healthy female and male volunteers (age 18 to 60 years) without a history of dermatological disease and lacking visible hairs on the volar forearm. The skin area treated and examined was the volar forearm. The tests revealed no pathological findings. All subjects gave their informed consent. The number of subjects and the subjects themselves varied between the different tests.

STRATUM CORNEUM EXTRACTS

Extraction of water-soluble urea from stratum corneum was carried out using a plastic cylinder, 2.5 cm in diameter. The cylinder was fixed in position on the skin area by the volunteers. The urea was extracted with 1.5 ml distilled water for two minutes. The volunteers were asked to gently move the water within the cylinder by a movement of the forearm. Immediately after extraction, 75 µl of a sodium azide solution (4 g/100 ml distilled water) was added to each extract.

UREA DETERMINATION

Urea determination was carried out according to a method of Kerscher and Ziegenhorn (9), with modifications in concentrations of enzyme and NADH solutions. The enzyme solution I consisted of glutamate dehydrogenase (EC 1.4.1.3.) from bovine liver (about 15 U/mg lyophilisate; 40 kU/l), ADP-disodium salt (6.8 mmol/l), 2-oxoglutarate (41.7 mmol/l), bovine serum albumin (400 mg/l), Tris base (500 mmol/l), and succinate (200 mmol/l) adjusted to a pH of 8.0 at 25°C with NaOH. The enzyme solution II consisted of seven parts of urease (EC 3.5.1.5.) from jack bean (*ca.* 80 U/mg lyophilisate; 100 kU/l) in sodium-phosphate buffer (4 mmol/l) adjusted to a pH of 6.8 and one part of glycerine. The NADH solution consisted of β-NADH (5 mmol/l), ADP-disodium salt (6.8 mmol/l), 2-oxoglutarate (41.7 mmol/l), Tris base (500 mmol/l), and succinate (200 mmol/l) adjusted to a pH of 8.0 at 25°C with NaOH. One hundred forty microliters of standard or sample was mixed with 50 µl of enzyme solution I and 10 µl of NADH solution in 96-well plates. After ten minutes the absorption at 365 nm was measured (A_1) using the spectrophotometer Spectra Max 250 (Molecular Devices Corporation, Sunnyvale, California). Fifteen minutes after the initiation of the reaction by addition of 5 µl of enzyme solution II, the absorption at 365 nm was measured (A_2). Each solution was measured in quadruplicate. The urea concentration was calculated by determination of

the difference between A_1 and A_2 and by comparison with a standard calibration curve between 10 and 200 nmol/ml urea.

MEASUREMENT OF UREA CONCENTRATION ON THE VOLAR FOREARM

Four tests sites per volar forearm of 30 volunteers were extracted. The subjects included 15 women and 15 men between ages 21 and 73 (43.8 yr ± 17.3 yr).

MEASUREMENT OF UREA DISTRIBUTION ON THE VOLAR FOREARM

Three test sites per volar forearm of four volunteers (two women and two men of ages 27, 54, 41, and 47 yr) were extracted on two different non-consecutive days.

MEASUREMENT OF REPEATED EXTRACTION FROM THE SAME SKIN AREA

One skin site per volar forearm of 15 volunteers was extracted four times in quick succession. The subjects included eight women and seven men between ages 30 and 57 (45.1 yr ± 9.6 yr).

TREATMENT OF SKIN

Washing procedure. The experimental subjects were five volunteers (three women and two men of ages 29, 53, 26, 40, and 46 yr). A wet skin area of approximately 25 cm^2 was treated with 750 µl of cleansing product for 45 seconds. The product was rinsed off with water (30°C) for 30 seconds. Each volar forearm was separated into two areas: distal and proximal. Two areas of each volunteer were used as test areas, with one area used as a control. The particular allocation of each area was randomized. The skin extracts were taken immediately after the washing procedure.

Skin care procedure. Twelve volunteers were instructed to apply the skin care formulation twice a day, in the morning and the evening. The subjects included eight women and four men between ages 27 and 56 (35.1 yr ± 9.7 yr). Each test volunteer was instructed to apply a volume equivalent to 150 µl of product to each test area of 5 cm × 5 cm, estimated by themselves. Each test area had product applied for seven days. Each volar forearm was divided into distal and proximal sites. Two sites per volunteer were used as test areas, one as a control area. The fourth area was not used. The test area of the control product without urea, and the untreated area, were located on the same forearm. The localization of the test sites was randomized with this constraint. The volunteers were asked to avoid contact of test areas with water after the final product application on day 7. The skin extracts were taken 24 hours after the last product application.

Products. The cleansing products were sodium lauryl sulphate solution (4 g/100 ml distilled water), a standard cleansing product with and without a supplementation of 10 g urea/100 ml product, and water as a control. The skin care formulations were Laceran® Spezial Creme and Laceran® Spezial Creme 5% Urea.

ESTIMATION OF SKIN HYDRATION

The skin hydration was estimated (in arbitrary units) with the Corneometer CM 820 (Courage and Khazaka, Cologne, Germany) 24 hours after the last product application.

The probe was gently positioned onto the skin area. On each site, sixfold determinations were performed.

STATISTICS

Differences between products were calculated with the two-sided Wilcoxon U-test. Values with $p \leq 0.05$ were considered as significant.

RESULTS

UREA CONCENTRATION OF FOREARM SKIN EXTRACTS OF NORMAL SKIN

The urea concentrations of skin extracts of the volar forearms of 30 volunteers comprised a range of 10–1000 nmol/ml.

REPRODUCIBILITY OF EXTRACTION

We observed no statistically significant differences when we compared samples taken from the same volar sites on the right and left forearms of the same individual. Minor fluctuations were observed for three volunteers, with only a little more fluctuation for one volunteer when comparing the results from a single individual (n = 4) taken on two different days (Figure 1).

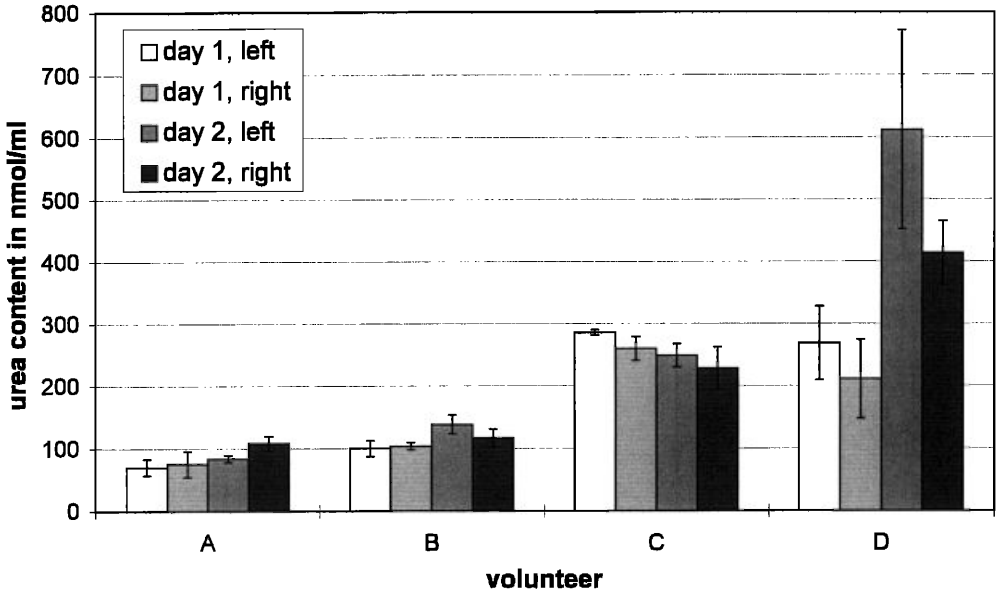

Figure 1. Comparison of the urea content of stratum corneum from the right and left forearm at two different days of extraction. Means and standard deviations were calculated from three extracts from each volar forearm. Subjects A–D.

REPEATED EXTRACTION FROM THE SAME SKIN AREA

To test the completeness of the extraction of urea from the stratum corneum pool of water-soluble substances by our method, we performed repeated extractions at a single skin area (n = 15). The urea concentrations of the second, third, and fourth extractions decreased from 15 to 8 percent of the initial extraction (Figure 2).

INFLUENCE OF SKIN CLEANSING ON UREA CONCENTRATION IN STRATUM CORNEUM EXTRACTS

The influence of skin cleansing on the stratum corneum urea amount of four volunteers is given in Figure 3. Skin cleansing was performed with water only or a solution (4%) of sodium lauryl sulphate (SDS) in water. Water only reduced the amount of extractable urea by 76% to 90%. Washing the skin area with a solution of SDS (4%) decreased the amount of extracted urea by 60% to 94% in a similar range. An SDS solution (4%) was compared with a standard shower gel to determine the influence of the detergent concentration (Figure 4). No significant difference was observed, although the concentration of detergents was about three times higher in the shower product. A further forearm skin area was treated to test the idea of possible restoration of the urea pool in stratum corneum by supplementation, using a shower product with 10% urea (Figure 4). When comparing data obtained after cleansing with a urea-supplemented product with data for skin treatment with the standard shower gel (see above), we observed a significantly higher amount of urea in the former skin extracts. The amounts varied in the individuals between 18% and 103%. However, a complete reconstitution of the urea pool by urea-containing products appears to be the exception.

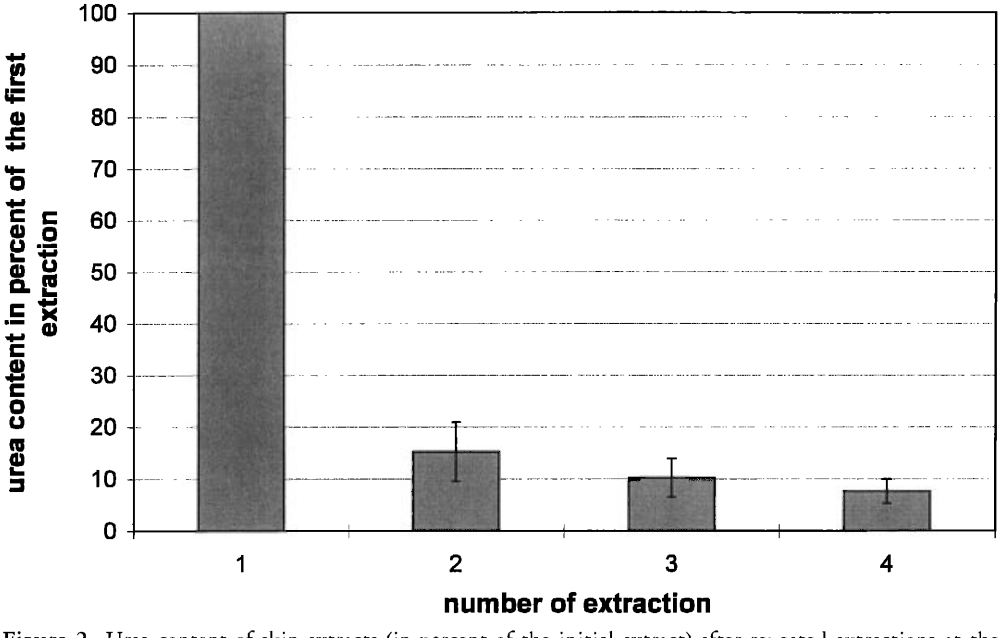

Figure 2. Urea content of skin extracts (in percent of the initial extract) after repeated extractions at the same skin site. Means and standard deviations were calculated from fifteen extracts from different individuals.

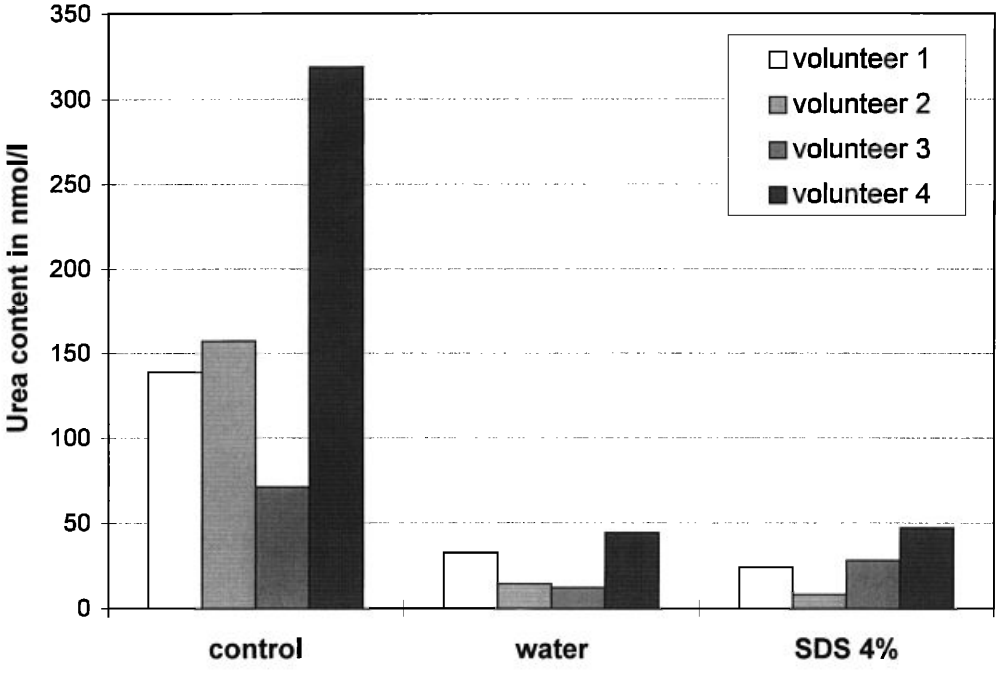

Figure 3. Urea content of stratum corneum skin extracts (nmol/ml) after washing procedure with water and with a 4% sodium lauryl sulphate solution in comparison with an unwashed skin area.

EFFECTS OF UREA-CONTAINING SKIN CARE FORMULATIONS

The skin care and stratum corneum urea-content-influencing effect of prolonged application of the urea-containing cream Laceran® Spezial Creme 5% Urea and of the cream Laceran® Spezial Creme without urea as a control were compared. The skin care efficacy of both products was inferred from measurements of skin hydration. Treatment with both care products apparently caused a significant increase of skin hydration (Figure 5a). The skin hydration efficacy indicated for Laceran® Spezial Creme 5% Urea is significantly higher than that indicated for Laceran® Spezial Creme.

An only slightly decreased amount of urea in skin extracts after treatment with Laceran® Spezial Creme was detected. The urea content of stratum corneum after a one-week application of Laceran® Spezial Creme 5% Urea is significantly higher than that following the application of Laceran® Spezial Creme and that of the untreated area (Figure 5b).

DISCUSSION

Because of the high relevance of urea for skin physiology, a rapid method of urea determination is of great value. Therefore, we established a noninvasive method for determination of the urea content of stratum corneum. The concentration of urea in skin extracts was measured by a rapid enzymatic assay. This method allows the simultaneous determination of up to 20 skin extracts by using 96-well microtiter plates. Our extraction method has no obvious influence on stratum corneum integrity, in contrast to the

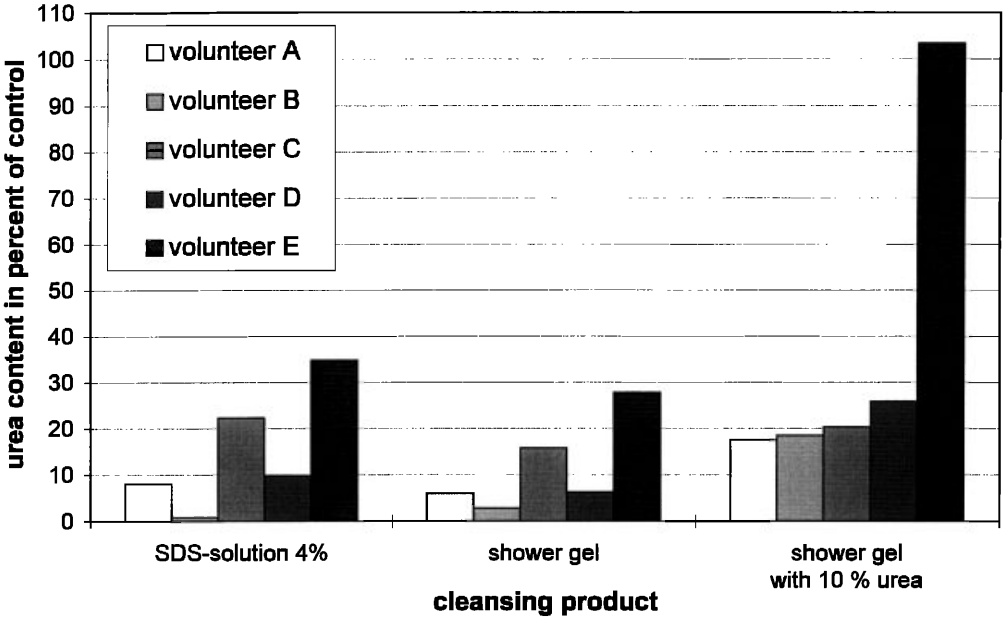

Figure 4. Comparison of urea content (in percent of control) of stratum corneum after treatment with different cleansing products.

method described by Schwarz (10–12) and Kloss and Schwarz (13). Schwarz examined the urea content of stratum corneum in samples scraped from the skin surface. A similar extraction method was introduced by Koyama *et al.* (14). However, in our protocol, water is the only extraction solvent instead of a sequence of an organic and an inorganic solvent. The rapidity and the safety of our method offers the possibility of using it routinely on human volunteers when testing skin care formulations and cleansing products. We observed a good reproducibility of the urea concentration in different skin extracts taken from the volar forearms of an individual volunteer even when taken on separate days. Therefore, it appears possible to compare different volar forearm skin sites when the testing of a number of products is required. Repeated extractions from an identical skin area resulted in a tenfold lower urea concentration in extracts when compared to the initial extraction. The extraction procedure causes a swelling of stratum corneum that might cause a slightly enhanced mobilization of urea in repeated extracts. The extension of extraction time will therefore produce only a slight increase of urea extracted from stratum corneum. From these results we conclude that the amount of extracted urea by the given method is representative of the urea content of stratum corneum.

Treatment of skin with water or cleansing products dramatically decreases the urea content and, therefore, one important component of the NMF of stratum corneum. This might be a possible factor in the development of a dry and scaly skin after prolonged skin cleansing with water exclusively (15). In our experiments neither the presence nor the actual concentration of detergents in cleansing products had any influence on the decrease of urea content in stratum corneum. However, a supplementation of cleansing product with 10% urea has a measurable positive effect on the urea amount that can be extracted from the skin surface. This positive effect on extractable urea might be related

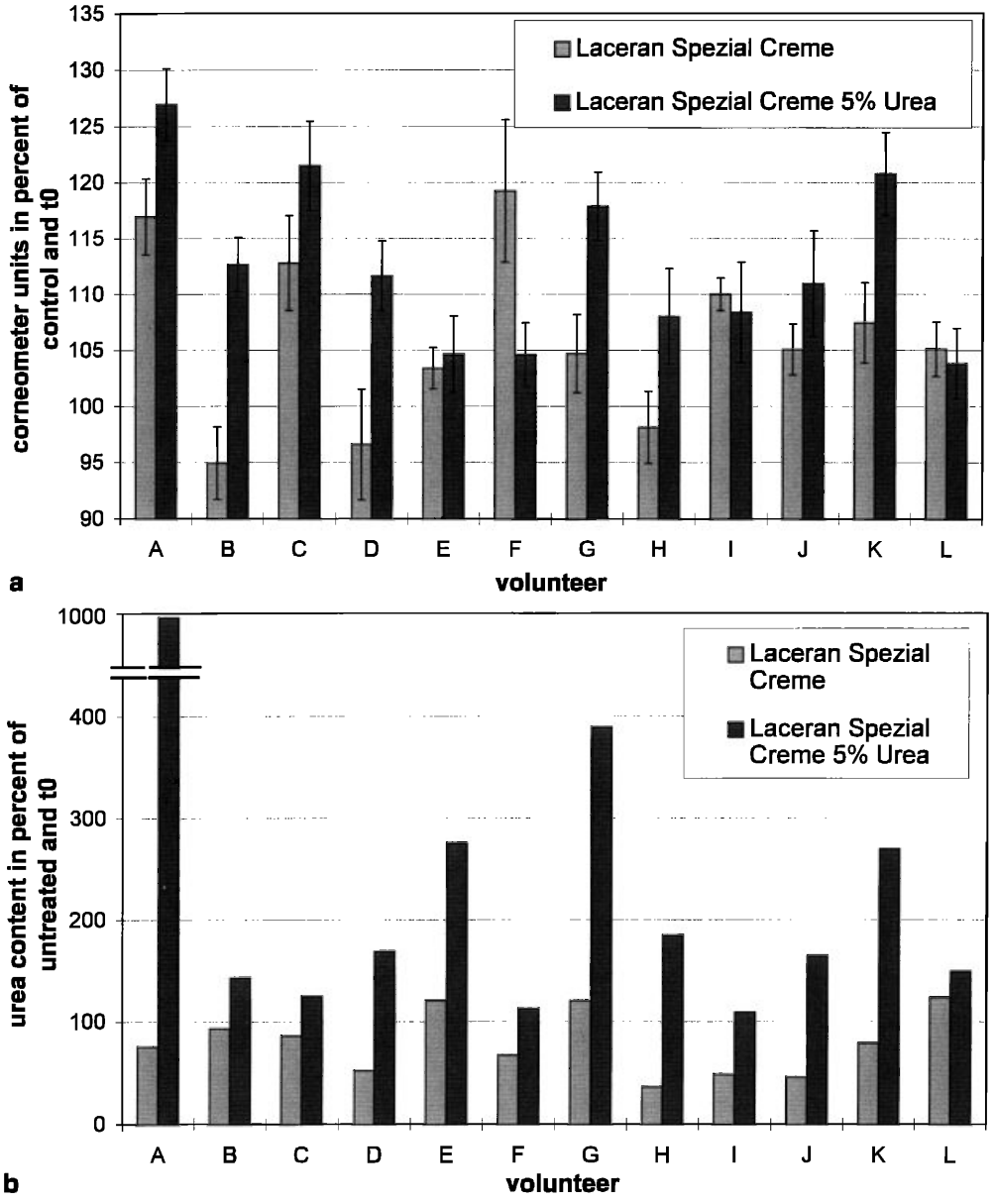

Figure 5. Comparison of Laceran® Spezial Creme and Laceran® Spezial Creme 5% Urea in relation to their hydration (Figure 5a) and the increasing urea content of stratum corneum (Figure 5b). Means and standard deviations of the estimation of skin hydration were calculated from six measurements at each test site. The corneometer units of the test site after the treatment were related to the value before treatment (tO) and the corneometer units of the control site.

to a reduced concentration gradient of urea between stratum corneum and wash solution. Only a limited compensation of urea loss during the washing procedure can be achieved with urea supplementation of cleansing products. On the other hand, a considerable supply of urea to stratum corneum can be achieved with urea-containing skin care formulations. For example, a prolonged treatment of skin with Laceran® Spezial Creme

5% Urea increases significantly the urea content of stratum corneum compared to an untreated area and a treatment with Laceran® Spezial Creme, respectively. The increased urea amount remains at a high level for at least 24 hours after final application. This lasting effect is a possible factor in the increased hydration of skin surface following treatment with the urea-containing product compared with the non-urea-containing control product. We would propose that the reduced amount of urea in skin extracts of skin areas treated with Laceran® Spezial Creme is caused by reduced extractability of urea as a consequence of a visible residual lipid layer even after 24 hours following final application. This, however, is not found for the Laceran® Spezial Creme 5% Urea, which differs in basis formulation from the Laceran® Spezial Creme.

Clearly, urea treatment of skin causes an increase of skin hydration as measured by corneometry. This enhancement results in an improved clinical appearance, i.e., smoothness, scaliness, or erythema, of patients with dry or xerotic skin (5, 6, 16, 17). Our results support the concept of treatment of skin diseases, e.g., atopic dermatitis, or conditions of clinical dry skin with urea-containing products to compensate for the reduced amount of urea in stratum corneum (1).

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