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Regional variation in the free amino acids in the stratum corneum

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Synopsis

Regional differences in water-binding free amino acids (FAAs) in the stratum corneum (SC) may be expected, since differences in skin biophysical properties are well known. The objective was to determine whether differences in skin hydration as a function of body site may arise from differences in the chemical makeup of the skin, specifically the FAAs. Levels were quantified from serial SC samples collected from the forearm, calf, back, torso, and jaw in two studies using HPLC methods. FAA levels were higher from the calf versus the forearm and lower from the jaw compared to torso and back skin. Body site variations in skin hydration could not be attributed to differences in FAA levels.

INTRODUCTION

Many factors are known to influence *in vivo* natural moisturizing factor (NMF) levels. These include ambient humidity (1), age (2–5), skin condition (6), exposure to solvents (7), and water (i.e., soaking) (7,8). Differences in NMF as a function of body site may be expected, as regional variations in skin biophysical properties and appearance are well documented. Variations in skin friction and hydration (9), and in roughness and scaliness across body sites (10), have been reported. SC thickness varies widely by location (11), with the genital area and facial skin the thinnest and the palms and soles the thickest. Anatomical site also affects water diffusion and the rate of barrier damage (12) and repair (13).

Water-soluble amino acids (FAA) constitute about 40% of the natural moisturizing factor (NMF) (1,14). Additional water-soluble materials, including lactates, urea, sugars, and ions, are also components of NMF. Recent work in our group has focused on the effects of various perturbations on free amino acids in the upper stratum corneum (SC) using an HPLC-based method from serial tapes (D-Squames®) (7,8). We have examined the effects of lipid extraction and warm water soaking on SC NMF (FAA) levels *in vivo*. These studies revealed a loss of NMF due to warm water soaking alone, which was intensified by acetone/ether extraction of the skin and which led to a regeneration of NMF over a four-hour time period, and proved

the existence of a gradient in NMF levels over the depth of the stratum corneum. Since the skin varies widely over the body surface in appearance, hydration, and biophysical properties (9), we hypothesized that some of these differences may arise from differences in the chemical makeup of the skin, such as FAA quantities. Our aim was to determine whether a relationship exists between FAA and skin hydration as a function of body site.

METHOD

Portions of this method have been published previously (7,8). Skin surface samples (using D-Squames® tapes) were collected, and biophysical measurements were made during two studies conducted during winter: forearm and calf (Study 1); and jaw, back, torso, and calf (Study 2). Figure 1 shows the locations of sample collection. Nineteen healthy female subjects (aged 23–60) were enrolled in Study 1 and fifteen in Study 2. Exclusion criteria included visually dry forearm skin and dermatological conditions such as psoriasis, rosacea, atopic dermatitis, and acne in the study areas. The Institutional Review Board of the University of Cincinnati Medical Center approved the protocols, and the subjects provided informed consent prior to inclusion in the study. No washing restrictions were imposed during Study 1, but moisturizer use was discontinued on the legs two weeks before measurement collection. During Study 2, the panelists did not use moisturizers on the day of sample collection. Fifteen tapes were serially collected from each site. The tapes were stored at −80°C until analysis.

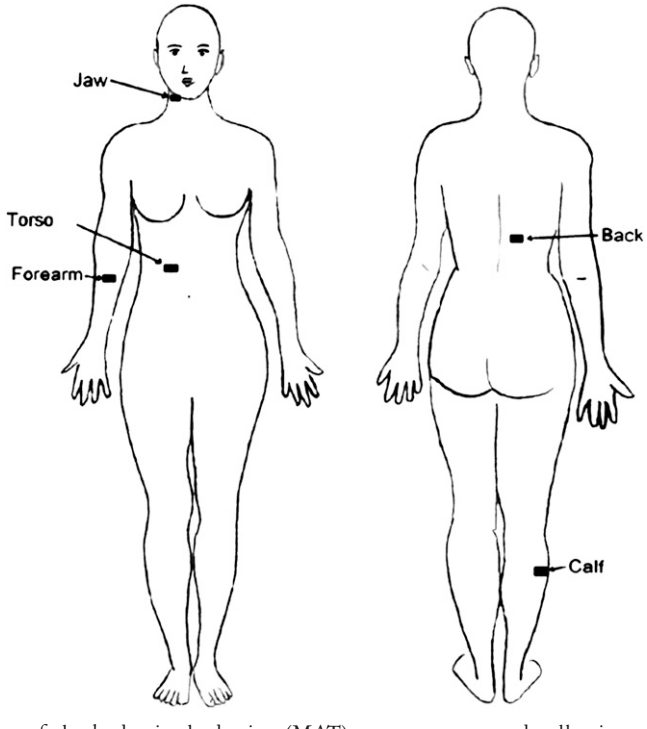

Figure 1. Locations of the body site hydration (MAT) measurements and collection of samples for FAA determination.

RATIONALE AND FAA ANALYSIS

Filaggrin deimination and proteolysis produces most of the SC free amino acids (15–17). Further processing converts histidine to urocanic acid (4) and arginine to citrulline (18,19). The presence of high urocanic acid and citrulline concentrations indicates NMF formation from filaggrin (4). Glutamic acid undergoes further reaction to pyrrolidone carboxylic acid (PCA) (20,21). Lactates, urea, sugars, and ions are also components of NMF. Based on available analytical techniques, we evaluated the free amino acid component of NMF. The tapes were extracted with 300 μl of 6mM perchloric acid spiked with 10 μl of 2 μmol/ml α-amino-n-butyric acid (AABA) for three hours. The tape was reserved for protein analysis. Samples were analyzed using the AccQ-Tag system (Waters Corp.) on a C-18 reverse-phase column (25-cm length, 4.6-mm internal diameter). The excitation wavelength was 250 nm with emission at 395 nm, and a column temperature of 40°C with a run time of 40 minutes. The HPLC results were standardized to the amount of protein removed by the individual tape using the Pierce BCA protein assay (22).

The rate of moisture accumulation (MAT) was measured with a NOVA® Dermal Phase Meter (NOVA® Technology, Portsmouth, NH). The method uses changes in skin capacitive resistance (the ratio of charge to potential on an electrically charged isolated conductor) under probe occlusion to determine skin hydration (23).

STATISTICS

General linear models (GLM) and analysis of variance (ANOVA) methods were used to evaluate the effects of body site on the protein removed, FAA, and hydration (SPSS and SigmaStat, SPSS, Chicago, IL). A value of $p < 0.05$ was considered statistically significant. Amino acid values are given as $\log_{10}$ values (pmoles) normalized to protein (μg), and proteins are given as μg/ml. Values are reported as estimates plus/minus standard error. To account for the high inter-individual variability, the subject was included as a factor/covariate in the statistical model (GLM). Site effects were assessed for tape 1 and for cumulative FAA normalized to cumulative protein for the sums as follows: sum 1+3, sum 1+3+5, sum 1+3+5+10, and sum 1+3+5+10+15.

RESULTS AND DISCUSSION

STUDY 1

The soluble protein from tape 1 was significantly higher for the calf versus the forearm ($p < 0.05$). The cumulative protein for tapes 1, 3, 5, 10, and 15 was not different. FAAs were higher for the calf for tape 1 and the cumulative values except for glutamic acid ($p < 0.05$). MAT was not different for the sites.

STUDY 2

Tape 1 protein was significantly lower for the calf versus the torso (ANOVA on ranks, Dunn's method post hoc versus torso control, $p = 0.02$). No site differences were noted

for the cumulative protein values (Table I). High inter-individual variability was found for protein levels from the torso (Table I). For tape 1, the jaw site had lower citrulline than the back, torso and calf, and lower serine than the calf. For the cumulative levels, the total FAA (log_{10} pmoles/μg protein) was lower for the jaw than for all other sites as follows: 3.12 ± 0.1, jaw; 3.41 ± 0.1 back; 3.26 ± 0.1 torso; 3.51 ± 0.1 calf ($F = 2.9$, $p = 0.05$). Citrulline was significantly lower for the jaw than for all other sites, and arginine was significantly lower for the calf (Figure 2). Cumulative citrulline (normalized to cumulative protein) for tape 1, sum 1+3, sum 1+3+5, sum 1+3+5+10, and the sum of all tapes is shown versus the respective cumulative protein amounts in Figure 3. Similar trends were observed for total FAA and individual amino acids. With SC depth, FAA first increased and then leveled off for the back and torso. The increase continued throughout the sampled region for the jaw. In contrast, the calf site remained relatively constant. MAT was significantly lower for the jaw than for either the torso or back ($p < 0.05$, Table I), but the jaw and calf were not different. There were no significant correlations between FAAs and MAT values for either study, as indicated in Figure 3.

At the outermost SC, the calf site had greater FAA than the forearm for comparable protein removed. FAAs were lowest for the jaw compared to the torso, back, and calf. The outcomes do not support the study hypothesis, i.e., body site variations in skin hydration (MAT) could not be attributed to differences in FAA levels. One important limitation in this study is that we examined only about 40% of the components of NMF (14). We did

Table I
Study 2 Protein Levels and Rate of Moisture Accumulation (MAT) for Back, Torso, Calf, and Jaw Sites

Site	Rate of moisture accumulation (MAT) (cru/sec)	Protein tape 1 (μg/ml)	Protein: Cumulative tapes 1,3,5,10,15 (μg/ml)
Back	2.5 ± 1.2	8.5 ± 2.3	49.2 ± 22.3
Torso	3.4 ± 2.0	22.3 ± 15.7	81.4 ± 23.0
Calf	1.6 ± 0.9	18.8 ± 3.9**	107.2 ± 22.0
Jaw	0.9 ± 0.7*	14.1 ± 3.1	66.2 ± 21.9

* Significant difference from back and torso ($F = 5.06$, $p = 0.01$).
** Calf and torso sites different by ANOVA on ranks, post hoc Dunn's versus torso contol ($p < 0.05$).

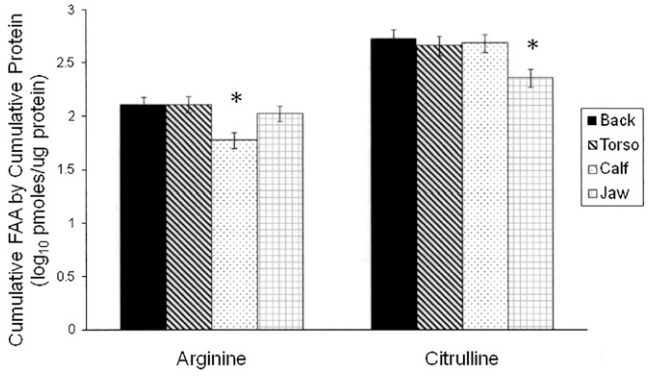

Figure 2. Cumulative FAA by cumulative protein is shown as log_{10} pmoles of amino acid normalized to μg protein. Citrulline was significantly lower for the jaw than for all other sites, and arginine was significantly lower for the calf (*$p < 0.05$).

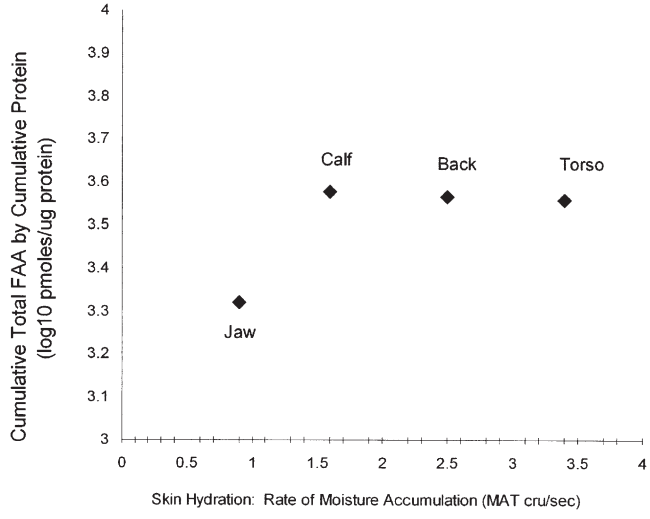

Figure 3. MAT was significantly lower for the jaw than for either the torso or back, but the jaw and calf were not different. The total FAA levels were not correlated with skin hydration, measured as the rate of moisture accumulation (MAT), for the body sites as indicated here for Study 2.

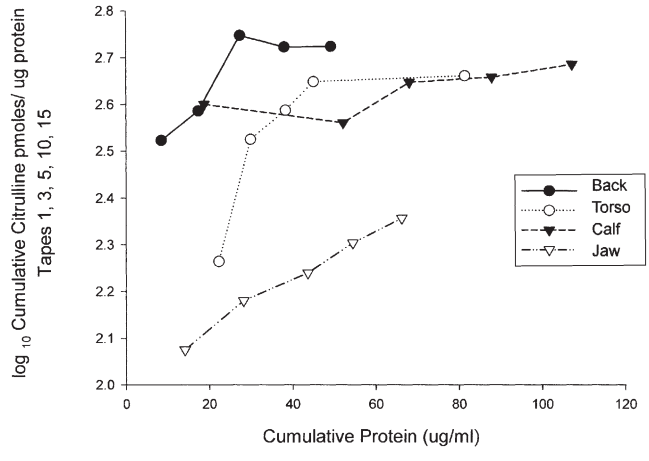

Figure 4. Cumulative citrulline (normalized to cumulative protein) for tape 1, sum 1+3, sum 1+3+5, sum 1+3+5+10, and the sum of all tapes is shown versus the respective cumulative protein amounts for the four sites. Similar trends were observed for total FAA and individual amino acids. With SC depth, FAA first increased and then leveled off for the back and torso. The increase continued for the jaw but remained relatively constant for the calf.

not measure PCA, urocanic acid, lactates, urea, sugars, or ions. Other NMF components such as lactates are positively correlated to SC hydration (24) and omission of lactate levels could account in part for the lack of correlation between putative NMF and MAT. Another potential source of protein that could account for higher citrulline levels in the samples is keratin 1. Peptidylarginine deiminase isoform 1 (PAD1) is responsible for deimination of K1 in the SC (25). Keratin 1 is expected to be present at higher levels in dry

skin (26). If conditions were favorable for proteolysis to individual amino acids, keratin 1 may have been an additional source of citrulline for the sites with lower SC hydration.

SC cohesiveness, measured as protein removed by the tape strip, showed significant differences between body sites, and these differences persist through the SC (Figure 4). This confirms recent work by Breternitz *et al.* (27), showing significant differences in protein amounts removed from varying body sites.

Koyama *et al.* (6) found lower levels of citrulline and serine in the cheek versus the back and higher arginine and histidine. Similarly, Egawa and Tagami (5) reported lower NMF for the cheek versus the forearm. These accounts are consistent with our result of lower citrulline in "face" versus back, although we evaluated the jaw rather than the cheek. The outcomes are not consistent with those of Horii *et al*. (2), who found significantly lower FAA in the lower leg along with reduced skin hydration and increased xerosis. The higher FAA in the calf is somewhat consistent with the findings of Takahashi and Tezuka (4), who reported higher FAA in the lower leg of older subjects with xerosis versus younger normal controls, but some of the increase was attributed to age (4).

Proteolysis of filaggrin to free amino acids and to further conversion products takes place when filaggrin-containing coenocytes move up into the drier regions of the stratum corneum. Thus, proteolysis may not occur until the outermost layers need moisture for SC plasticization of the outermost layers (28). While we did not quantify dryness, calf skin is often visibly scaly, suggestive of aberrant desquamation. Greater amounts of FAA in the calf may indicate an up-regulation in order to subsequently increase hydration. Reduced SC turnover may also explain higher levels of FAA (29). Assessments of SC thickness and turnover rates are needed to evaluate this explanation. Additional experiments including the analysis of PCA, urocanic acid, and lactates by other analytical methods are warranted.

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