Effects of Aging and Xerosis on the Amino Acid Composition of Human Skin

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Amino acid compositions of skin samples from young and old subjects and from age-matched donors with dry skin syndrome (xerosis) were examined. The amino acid contents of the free amino acid (FAA) fraction, soluble hydrolysate (SH) fraction, and whole cell hydrolysate (WCH) were determined. The greatest differences were observed between the FAA compositions of the young and old normal subjects. Xerosis did not appear to affect the amino acid compositions of samples from young subjects as much as old subjects. Overall, the effect of aging on the amino acid contents was more pronounced than the effect of xerosis. The amino acid composition of the FAA showed a high degree of similarity to filaggrin, whereas the WCH showed a similarity to keratin. *J Invest Dermatol 95:296–300, 1990*

Spontaneous dry skin (xerosis) occurs predominantly on the dorsal and lateral surfaces of the extremities [1], is dependent on environmental conditions [2], and worsens during the dry winter months. The susceptibility and severity increase with age [3, 4]. The hallmarks of xerosis are the aggregated desquamating corneocytes [5] appearing as fine white scales and the decreased mechanical flexibility of the stratum corneum [6]. These conditions appear to be the result of changes in the biophysical properties of the stratum corneum consistent with a decreased water content*. Protein elements, capable of binding and organizing water, have been implicated as part of the molecular mechanism responsible for regulating stratum corneum water content. For example, the stratum corneum is unique in containing a high concentration of free amino acids [7, 8] and their deaminated derivatives [9–11], such as urocanic and pyrrolidone carboxylic acids. These derivatives and various domains of keratin have been implicated in water binding [12]. The interaction of these hygroscopic elements, including both proteins and free amino acids with water, could be responsible for the semi-crystalline-like state [13] of water in the stratum corneum.

It is thus possible that altered expression, modification, or proteolytic processing of the proteins of the stratum corneum are involved in the etiology of xerosis. Altered stratum corneum levels of free amino acids have been reported to be associated with environmentally-induced hyperkeratinization [14, 15] and psoriasis [16]. The histidine-rich filaggrin molecule has been proposed to be the source of 70–100% of the free amino acids of the stratum corneum [7, 8], and decreased filaggrin synthesis occurs in laminar ichthyosis [17]. Whether a similar alterationof stratum corneum proteins occurs in spontaneous xerosis remains to be determined.

Therefore, this study was designed to quantitate the amino acid composition of three fractions isolated from desquamated scales of the stratum corneum from individuals with or without xerosis and with respect to age. The three fractions were the free amino acids (FAA), soluble hydrolysate (SH), and whole cell hydrolysate (WCH). These studies sought to determine if significant differences in the amino acid compositions of these fractions exist in the normal aging skin, as well as in xerosis.

MATERIALS AND METHODS

Donors All donors were white women from the Rochester, New York area and judged to be free of any skin disease. Samples were obtained from the shins of both legs of each volunteer on two separate occasions 1 month apart. Donors did not use lotions or topical medications during the study, and legs were shaved 48–72 hours prior to skin sampling. Donors were divided into four groups: old/non-dry [n = 7], old/dry [n = 13], young/non-dry [n = 18], and young/dry [n = 8]. "Old" refers to subjects 60 years of age or older, while "young" subjects were 30 years of age or younger. "Dry" refers to subjects diagnosed as having typical dry skin syndrome (xerosis), and "non-dry" refers to controls with skin judged to be normal.

Skin Sampling Samples were obtained in late February and early March for the first sampling period, and in late March and early April for the second sampling period. An 8 × 8 cm area of the skin of each leg was scraped with a glass microscope slide; the cells were

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Abbreviations:
- Asx: aspartic acid plus asparagine
- FAA: free amino acids
- Glx: glutamic acid plus glutamine
- OPA: ortho-phthaldialdehyde
- SH: soluble hydrolysate
- WCH: whole-cell hydrolysate

*Brink PB, Walczak V, Gordon JS: Voltage clamp measurement of stratum corneum hydration (manuscript in preparation); Jacques SL, Gordon JS: Assessment of dry skin using focussed microwave (manuscript in preparation).

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collected directly on weighing paper. Samples were packed in dry ice for shipping and stored at −20°C.

Preparation of Samples The samples were prepared as outlined in Fig 1. After extraction of the skin samples in water, the supernatant solution was directly subjected to amino acid analysis without hydrolysis (FAA), or analyzed after total peptide bond hydrolysis in vacuo in 6 N HCl at 110°C for 24 h (SH). The WCH were also analyzed after total peptide bond hydrolysis under the same conditions. Thus, the difference between the SH and the FAA represents the overall amino acid composition of the water soluble proteins and peptides of the skin. Similarly, the difference between the WCH and the SH represents the overall amino acid composition of the insoluble proteins of the skin.

Amino Acid Analyses Amino acids were resolved on a high-performance liquid chromatography cation exchange column (AA-511; Interaction Chemicals Inc.) and detected spectroflurometrically after post-column reaction with ortho-phthaldialdehyde (OPA) [18]. As OPA does not react with secondary amines, values for proline and hydroxyproline were not measured. Furthermore, tryptophan (destroyed by acid hydrolysis) and cysteine (low fluorescence) were not quantitated. Additional amino acids or their derivatives accounted for less than 0.5% of the total OPA-positive material. The metabolites urocanic acid and pyrrolidone carboxylic acid were analyzed using an anion exchange column (Ultrasil Ax, 4.6 mm ID × 25 cm, 10 μm, Altex Inc.) and monitoring the eluate at 210 mm [8].

Statistical Analyses The different samples were grouped according to donor type and a Q test was performed to identify values falling out of range [19]. The means and standard deviations were determined for each group and subjected to a p test comparing matched groups [20] for all amino acids. Values with $p \leq 0.05$ were considered as significant.

RESULTS

Sample Reproducibility No statistically significant differences were observed when comparing samples taken from the right and left legs of the same individual, and only minor fluctuations were observed when comparing the same individual on the two dates of testing.

Distribution of Amino Acids and Proteins The relative amounts of OPA-positive material in the three fractions were not significantly different among the four groups of subjects. The FAA constituted approximately 69% of the SH, and the SH constituted approximately 25% of the WCH. Thus, the overall distribution of amino acids, peptides, and proteins was approximately 75% insoluble cellular material, 17% free amino acids, and 8% soluble proteins and peptides. These data were obtained both by weight loss and amino acid analysis.

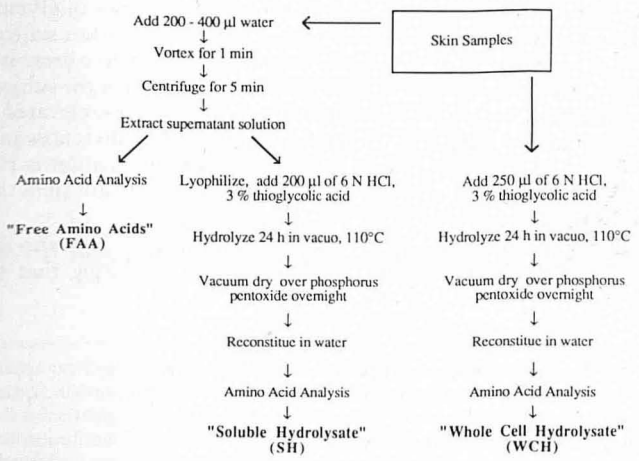

Figure 1. Preparation of samples for amino acid analysis.

Comparison of the Four Groups of Subjects Table I summarizes the statistically significant ($p \leq 0.05$) differences in the amino acid contents of the three fractions in the four donor groups. The largest number of statistically significant differences between groups was observed on comparison of the young and old-normal subjects. A lesser number of significant differences was found in the comparison of old-normal and old-xerotic subjects. A decreasing number of significant changes was observed in the comparison of old-xerotic and young-xerotic subjects, and even fewer in the comparison of young-normal and young-xerotic subjects. Cases involving the two variables (age and xerosis) simultaneously were not compared.

Effects of Aging In the case of the normal subjects, each of the three fractions showed significant differences ($p \leq 0.05$) in the amino acid composition as a function of age. The FAA showed an increase in the percentages of Ser, Glu, and Gly in the old subjects, but levels of Leu, Phe, Lys, Trp, and Orn were decreased (Fig 2, Table I). The changes in Glx, Leu, and Lys were paralleled in the WCH (Fig 3, Table I). The differences in the observed changes in the FAA from the SH or WCH can be attributed to the higher concentration of each amino acid following hydrolysis. The amino acid composition of the WCH revealed seven amino acids with significant age-related changes. The decreased Lys and Arg with the accompanying increase in Glx suggests an overall more acidic protein population with age. In view of the proposed involvement of filaggrin (histidine rich protein) as a major source of free amino acids in the stratum corneum, it is interesting to note that variations in histidine levels were not observed in any of the fractions.

Effects of Xerosis When the effects of xerosis were examined in the old patients, the picture was quite different from that seen in aging alone (Fig 2–4, Table I). The FAA or samples from old xerotic subjects exhibited elevated levels of Gly, Leu, Tyr, Phe, and Lys as compared to that from the normal old subjects. When comparing the SH from the old-normal and old-xerotic subjects, a de-

Table I. Changes in Amino Acid Compositions of Defined Groups ($p \leq 0.05$)

Parameter	FAA	SH	WCH
Old/normal vs young/normal			
Elevated with age[a]	Ser	Ser	Orn
	Glu		Glx
	Gly		
Decreased with age[b]	Leu	Thr	Leu
	Phe		Met
	Lys		Lys
	Trp		Val
	Orn		Arg
Old/normal vs old/dry			
Elevated in xerosis	Gly	None	Asx
	Leu		Val
	Tyr		
	Phe		
	Lys		
Decreased in xerosis	Thr	His	His
			Orn
Old/dry vs young/dry			
Elevated with age	Gly	Glx	None
	Ala	Ala	
Decreased with age	Orn	Orn	None
	Thr		
Young/normal vs young/dry			
Elevated in xerosis	None	None	None
Decreased in xerosis	None	None	Met
			Lys

[a] Designates a/b > 1.
[b] Designates a/b < 1.

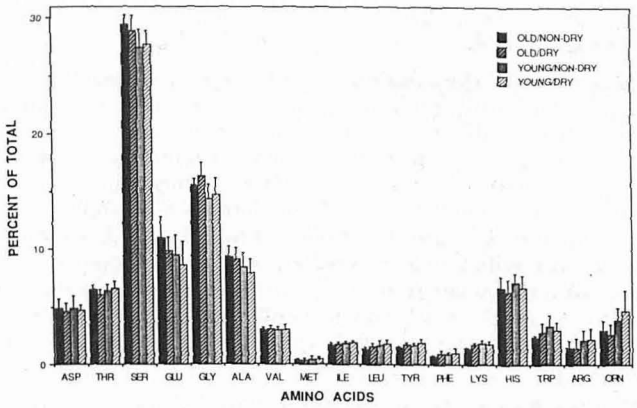

Figure 2. Comparison of free amino acids (FAA) from human stratum corneum from young and old subjects with or without xerosis. The FAA values were obtained after analysis of the samples as described in *Materials and Methods*. The ratios of amino acids were calculated for each sample and means and SE were determined for each amino acid of every group. Q tests were performed on the individual samples before group means were calculated.

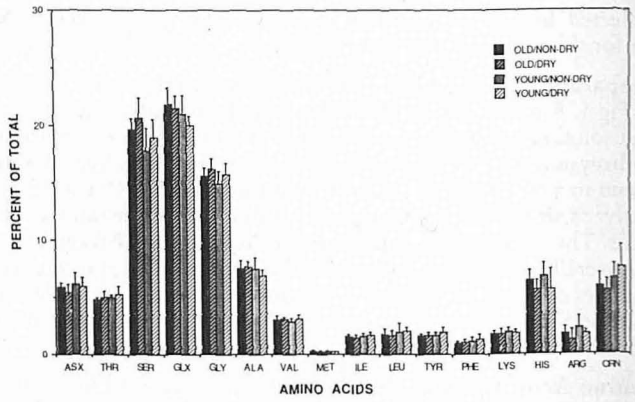

Figure 4. Comparison of soluble hydrolysate (SH) from human stratum corneum from young and old subjects with or without xerosis. Samples were obtained, prepared, and analyzed as described in *Materials and Methods*. The data were calculated and presented as described for Fig 2.

crease in the level of His was observed in the xerotic subjects. This change was paralleled in the WCH. In evaluating the old-normal vs old-xerotic subjects (Table I), parallels with both age (Gly, Orn) and xerosis (Gly, Thr) were observed in FAA. The changes in the SH did not show any similarity to the SH changes of any other groups, and no changes were seen in the WCH.

Finally, a comparison of the young normal and the young xerotic subjects showed no parallel to the effects of xerosis in the older subjects (old-normal vs old-xerotic). The decrease in Met and Lys in WCH due to xerosis in the young subjects was also seen as an effect of age in the normal subjects.

DISCUSSION

Although xerosis can have both genetic [4] and environmental [2,4,21] causes, the underlying molecular changes are unclear. Free amino acids are believed to play a central role in stratum corneum water binding. Experimentally induced [14,15] as well as disease-related hyperkeratinization [16,17] have been shown to be associated with decreases in the stratum corneum concentration of these amino acids or their precursors. For this reason, we have sought to determine if similar changes exist in xerosis and aging. Although we have confirmed that the free amino acid compositions of desquamating human corneocytes are consistent with a filaggrin origin, there were no changes in FAA to total scale protein ratio with xerosis or aging. In addition, the changes in FAA composition identified with aging cannot simply be explained by changes in the proteolysis of filaggrin. The decreased level of histidine in the protein hydrolysates from old dry skin samples vs old normal samples is consistent with decreased filaggrin levels, but only minor changes were seen in young subjects. These data must be interpreted with caution because the deaminated amino acids were not quantitated in this study. Only desquamating scales, which represent an endpoint of differentiation were analyzed. It is possible that filaggrin proteolysis could have reached the same point in both normal and dry scales, but the rates of proteolysis could be different. This would be apparent only by analyzing the deeper layers of the stratum corneum†.

Whereas significant changes in the amino acid composition of the free amino acids and the proteins/peptides of desquamated scales were seen with age, the effects of xerosis were only observed in the elderly population. The physiologic significance of these changes is not obvious. Amino acids constitute the largest portion of the water-soluble, dialyzable fraction of the epidermis and experimentally induced hyperkeratinization results in a lowering of these substances. Rossmiller and Hoekstra [14] reported that in chemically induced xerosis of guinea pig skin the nutritionally non-essential amino acids were reduced to one-third normal levels, whereas the concentration of the essential amino acids was unchanged. They attributed these to changes in rates of metabolism within the epidermis rather than to reduced levels of tissue proteolysis or systemic delivery. In the above study with chemically induced xerosis, the dry animals were reported to exhibit marked decreases in glycine, alanine, histidine and arginine. In our study with human subjects and in spontaneous (inherent) xerosis, we did not observe decreased levels of any of these amino acids in the FAA fraction of the subjects with xerosis. In fact, of these amino acids, glycine was elevated in the old xerotic subjects. Although species-specific differences may account for these differences, the more likely explanation is that chemically induced xerosis has a different molecular basis than that of spontaneous xerosis.

Some correlation can be drawn between the pattern of amino acid changes seen here and our previous results, suggesting that the

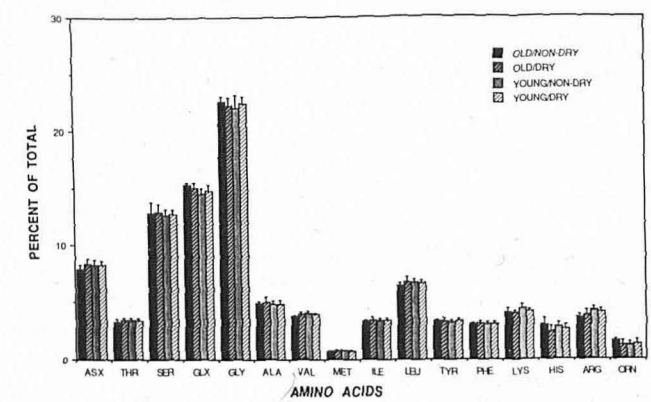

Figure 3. Comparison of whole cell hydrolysate (WCH) from human stratum corneum from young and old subjects with or without xerosis. Samples were obtained, prepared, and analyzed as described in *Materials and Methods*. The data were calculated and presented as described for Fig 2.

†Filaggrin can be detected using immunologic assays, but direct application of antibody to skin slices or cell homogenates does not provide a quantitative measurement because filaggrin, as well as peptides generated from filaggrin during its breakdown, reacts with the antibody. Two-dimensional electrophoretic separation of proteins from different skin layers, followed by Western blotting and immunochemical staining, is required.

Table II. Hydropathy Index of Amino Acids of Human Skin[a]

Subjects	FAA	SH	WCH
Young/normal	67.0	64.5	69.7
Young/xerosis	66.3	64.0	69.7
Old/normal	68.1	65.1	69.5
Old/xerosis	68.2	65.2	69.9

[a] Hydropathy indices were calculated by multiplying the hydropathy values with the amino acid compositions of the respective fractions (see Ref. [29]).

underlying mechanisms of dry skin in the old and young are different (Geesin et al, unpublished observations). For example, old normals and young xerotics exhibit seasonal variation in the total protein extracted from a single tape stripping, whereas old xerotics and young normals do not. The changes in the amino acid composition of the protein-derived fractions of desquamating scales with age seen in the present study might explain these differences in the properties between young and old. The decrease in the histidine content of the soluble protein fraction seen in old dry skin could underlie the differences in responsiveness to environmental conditions. Furthermore, the increase in the overall hydrophobicity of the FAA and SH fractions from the elderly (Table II) could reflect a decreased potential to interact with water in these individuals.

In the case of the WCH, the changes may represent differential expression or proteolysis of stratum corneum proteins, but also may represent differential modification of amino acids. In this respect, it is particularly noteworthy that covalent modifications as the oxidation of lysine, histidine, and arginine are known to occur, and the resultant products accumulate during aging. For example, His can be oxidized to Asn/Asp and Arg into Gln/Glu [22]. Indeed, in studying the effect of age (i.e., old-normal versus young-normal), there was a decrease in Arg with an increase in Glx in the WCH. Similarly, in comparing the effect of xerosis (old-normal versus old-dry), we observed a decrease in His with a concurrent increase in Asx. Other covalently modified amino acids are known to accumulate in aging as a result of other postsynthetic processes, including glycation [23], deamidation [24], and racemization [25]. In skin, however, it must be remembered that the abundant proteins of the stratum corneum are synthesized in the upper layers of the epidermis, with its rapid turnover of cells. Thus, these modifications would have to occur at a substantially accelerated rate in the epidermis than they do in the tissues in which they have been previously observed. Given the more environmentally exposed position of skin, such an acceleration is possible and should be tested.

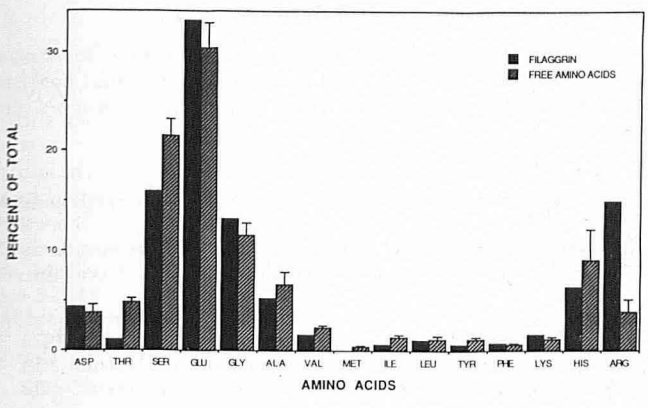

Figure 5. Comparison of amino acid composition of filaggrin with free amino acids (FAA) from human stratum corneum. The FAA from 16 subjects (4 from each group) were compared to the amino acid composition of filaggrin from guinea pig [7]. In accordance with the calculations of Scott et al, the values of histidine and urocanic acid; glutamic acid, glutamine, and pyrrolidone carboxylic acid; and ornithine and arginine were combined, respectively (due to interconversions of these species in the skin).

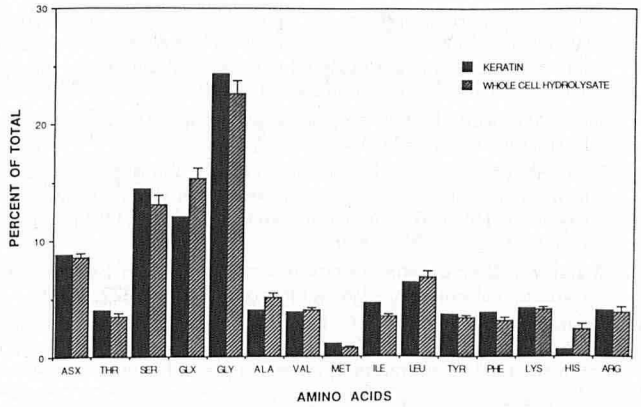

Figure 6. Comparison of amino acid composition of keratin with whole cell hydrolysate (WCH) from human stratum corneum. The WCH from the old-xerotic group was compared to the amino acid composition of keratin [30]. Sample preparation, analyses, and calculations were as described in *Materials and Methods* and in the legend to Fig 2.

The methods developed and utilized here are amendable to further studies on environmentally induced aging of skin. The methods for obtaining skin samples, as well as analysis, are highly sensitive and reproducible, and can be of use in establishing biochemical bases for changes in amino acid composition with respect to age and/or xerosis.

In conclusion, whether the changes in the amino acid composition of scale observed in this study are involved in generating the various classes of dry skin will depend on identification of the proteins involved and a determination of their function. As pointed out above, the overall composition of the FAA and WCH fractions are essentially identical to filaggrin and keratins, respectively (Fig 5 and 6), but the changes observed with age or xerosis cannot be ascribed to either of these classes of protein. Nor can they be identified with that of another abundant set of stratum corneum proteins, those making up the corneocyte envelope [26]. It should be noted, however, that the three amino acids which increase with age are the same three predominant amino acids seen in isolated keratohyalin granules [27], of which filaggrin is only one component. If, as our data suggest, these amino acids are not derived from filaggrin, their source could be the cysteine-rich proteins [28]. The function of these other keratohyalin proteins is not known.

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