Free Fatty Acids and Fatty Acids of Triacylglycerols in Normal and Hyperkeratotic Human Stratum Corneum*

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The content of the free fatty acids and the fatty acids of triacylglycerols has been measured in human plantar stratum corneum from normal and hyperkeratotic subjects with palmoplantar keratoderma. Fatty acids of triacylglycerols in normal tissues showed a characteristic pattern with a relative abundance of short-chain length and unsaturated fatty acids. Free fatty acid fraction was characterized by the predominance of saturated compounds. The relative amount of short-chain and monoene fatty acids in the hyperkeratotic stratum corneum was increased. These results seem to show a defect in the maturation of fatty acids in the living epidermis and present new evidence that the abnormality of lipid metabolism can influence the process of desquamation in stratum corneum. *J Invest Dermatol 87:68–71, 1986*

Important progress has been made in the study of the lipid composition of epidermis, particularly stratum corneum. Evidence for the involvement of lipids in stratum corneum cohesion and in a number of scaling skin disorders is well documented [1,2], particularly regarding the accumulation of cholesterol sulfate [3] in x-linked ichthyosis which is associated with a defect of steroid sulfatase [4]. Some of us have recently reported normal values of steroid-sulfatase activity in sole skin epidermis from postmenopausal women with palmoplantar keratoderma (PPK) [5]. It was concluded that abnormal desquamation in PPK was not related to modification of sterolsulfate hydrolysis.

Preliminary studies (Laurent, Nicollier, unpublished data) of epidermis from patients with various forms of PPK have frequently shown the presence of intracellular vacuoles. Such vacuoles with excess of neutral lipids, particularly triacylglycerols (TAG) have been reported in numerous defects of keratinization including psoriasis [6], lamellar ichthyosis [7], harlequin ichthyosis [8], and Refsum's disease [9]. The formation and accumulation of these droplets might be associated with a defect of fatty acid metabolism [1]. The literature is sparse as regards human stratum corneum free fatty acids (FFA) although they account for the major lipids of stratum corneum membrane complexes with cholesterol and ceramides [10,11]. Coon et al [12] have reported that the barrier zone lipids of epidermis include significant amounts of long-chain FFA but they attributed their findings to contaminants. Later on, the importance of long-chain fatty acids was reported in stratum corneum [13] and particularly in the plantar stratum corneum where C_{18} and C_{24} predominate [14].

In order to provide quantitative analytical information about the fatty acids of the human plantar stratum corneum and to further understand the pathogenesis of palmoplantar hyperkeratosis, the fatty acid composition of TAG and FFA was studied both in specimens of normal plantar stratum corneum and specimens from patients with PPK.

MATERIALS AND METHODS

Tissues Normal tissues were obtained from cadavers (7 males and 7 females) within 4 h following death. Hyperkeratotic tissues were obtained from 13 patients with PPK. The diagnoses of all patients in this study were established by clinical and histologic criteria [15]. All the biopsies were performed with a Davol dermatotome. Stratum corneum was isolated by heating the skin at 56°C for 3 min, and trypsinization: trypsin (DIFCO, 1:250) 0.3%, 45 min.

Lipid Extraction and Thin-Layer Chromatography Minced stratum corneum was homogenized in chloroform:methanol (2:1) (v:v) and lipids were extracted according to Bowyer and Janice [16]. The combined lipid extracts were washed with 0.2 vol of 0.1 M KCl and 0.2 vol of upper phase solvent containing KCl according to Folch et al [17]. The lower phase was evaporated in a rotary evaporator to dryness under vacuum at 35°C. The lipids were weighed and then redissolved in benzene and stored at −20°C.

The FFA and TAG were separated by thin-layer chromatography (TLC) according to the procedure of Nieminen et al [18]. Lipid classes were identified by cochromatography with the following standards: cholesterol, glycerol trioleate, and oleic acid (Sigma, St. Louis, Missouri). Standards were visualized by spraying with 50% H_2SO_4:ethanol (v:v) and heating at 110°C for 10 min. FFA and TAG were recovered from the silica gel 60 plates (Schleicher et Schull) by extraction with diethylether (2 × 3 ml).

Gas Liquid Chromatography (GLC) Methyl esters of FFA were prepared by the diazomethane method [19]. The TAG were

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Abbreviations:
FFA: free fatty acid(s)
GLC: gas liquid chromatography
PPK: palmoplantar keratoderma
TAG: triacylglycerol(s)
TLC: thin-layer chromatography

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Table I. Clinical Details of Patients with Palmoplantar Keratoderma (PPK)

Classes	Patient Number	Age (years)	Sex	Diagnosis	Age at Onset	Localization
Idiopathic	1	51	F	Keratoderma climactericum	50	
	2	49	F	Keratoderma climactericum	48	
	3	52	F	Keratoderma climactericum	51	
	4	56	M	Striate PPK (Brunauer-Fuhs)	20	Soles (circumscribed) Palms (bandlike)
	5	50	M	Idiopathic PPK	20	Soles and palms circumscribed
	6	39	M	Mechanical painful callosities	32	Soles
Symptomatic	7	70	F	Pityriasis rubra pilaris	69	Soles and plams; erythroderma
	8	58	F	PPK with eczema	57	Soles
	9	52	F	PPK with eczema	51	Soles and palms
	10	27	M	PPK with eczema	22	Soles and palms
Inherited	11	23	F	Meleda disease	3 months	Diffuse PPK of the soles and palms Extension of the lesions on the dorsal areas, ankles, and wrist
	12	31	M	Thost-Unna disease	Childhood	Diffuse PPK sharply limited to the soles and palms
	13	32	M	Epidermolytic PPK (Voerher)	Childhood	Diffuse PPK of the soles and palms

deacylated with 0.5 N KOH-methanol and fatty acids were esterified by 20% boron trifluoride in methanol using the procedure of Van Wijngaarden [20]. The methyl esters were chromatographed using a Girdel 30 gas chromatograph (Suresnes, France) with a flame ionization detector equipped with a SE 52 open glass capillary column (25 m × 0.2 mm) (Carlo Erba, Milano, Italy), using temperature programming: 100°–290°C at 2°C/min. Peaks were identified by comparison of retention time values to known standards (Applied Sciences, State College, Pennsylvania) and by GLC-mass spectrometry. In all cases, the gas-chromatograms were recorded and the peak areas were measured with a ICR-1B computing integrator (Intersmat, Lyon, France).

Mass Spectrometry The gas chromatographic-mass spectrometric analyses were performed with a Riber 10-10 B spectro meter (Rueil-Malmaison, France) on line to a Digital pdp 8/m data system (Westminster, Massachusetts). The SE 52 glass capillary column was coupled directly to the ion source of the mass spectrometer without any separator.

Statistical Analysis The data of all fatty acid quantifications in duplicate were statistically analyzed. Significant differences among groups were determined with the Mann Whitney-Wilcoxon test and the analysis of variance with an IBM 4341/2 computer system (Portsmouth, England).

RESULTS

The fatty acid content of plantar stratum corneum was measured in skin specimens from normal and abnormal subjects. A total of 27 skin specimens was studied including 7 specimens obtained from normal women and 7 specimens from normal men which constituted control specimens. Thirteen abnormal skin specimens were as shown in Table I: 6 from patients with idiopathic hyperkeratosis, 4 from patients with symptomatic hyperkeratosis, and 3 from patients with inherited hyperkeratosis. In all cases FFA and fatty acids of TAG were identified by their mass spectra after GLC-mass spectrometry.

In order to point out significant differences in the fatty acid composition between normal and pathologic stratum corneum, the relative abundance of each of them (in percentage of the total identified fatty acids) was compared (Tables II, III). It may be noticed that no hydroxy-fatty acid was identified in all tissues studied. The multivaried variance analysis showed no significant difference according to the sex, either in normal or pathologic tissues. Consequently, the variations were studied irrespective of this parameter. In normal stratum corneum, the relative abundance of C_{14}–C_{18} fatty acids was lower and that of C_{20}–C_{28} fatty acids was higher in the free fraction than in the TAG fraction. Regardless of their chain length, unsaturated (monoene) fatty acids were more abundant in the TAG than in the free fraction. The 2 most abundant monoene fatty acids of the TAG fraction were C_{18}:1 and C_{22}:1. The latter was not found in the free fraction. The major diene fatty acid was C_{18}:2 and no significant difference was observed between the free fraction and TAG fraction.

The comparison between normal and pathologic tissues showed that C_{14}–C_{18} FFA were more abundant in hyperkeratotic stratum corneum than in normal tissues whereas C_{20}–C_{28} were less abundant. The most striking features in the TAG fraction were the significantly higher values found for C_{18}:1, C_{18}:2 fatty acids and the lower values for C_{22}:1 in hyperkeratotic stratum corneum than in normal stratum corneum.

The differences observed between pathologic and normal tissues are summarized in Table III. The 2 most important findings were: (1) a relative increase of the short chain length fatty acids (C_{14}–C_{18}) and unsaturated (monoene) fatty acids in hyperkeratotic stratum corneum; these modifications of fatty acid composition are particularly obvious in the free fraction; and (2) no significant difference among the different groups of pathologic tissue except for symptomatic keratoderma; in this group, there was a significant increase of di- and triene unsaturated fatty acids.

DISCUSSION

The study of FFA and fatty acids of TAG in the plantar stratum corneum from normal and hyperkeratotic subjects, after identification by their mass spectra, emphasizes some findings. In normal tissues, fatty acids of TAG showed a characteristic pattern: a relative abundance of unsaturated fatty acids with short-chain length, particularly C_{18}:1 and C_{21}:1. This predominance of unsaturated acids is in good agreement with the results reported by Lampe et al [14]. The differences observed for FFA between our results and those of Lampe et al [14] are due to the fact that these authors have not reported the values for odd-chain fatty acids which represent nearly 20% of the free fraction and 11.4% of the total fatty acids according to Ansari et al [13]. However, it is possible to characterize FFA by the abundance of saturated compounds.

The striking feature in hyperkeratotic stratum corneum is the relative abundance of short-chain and monoene fatty acids. The stratum corneum fatty acids initially derive from the content of lamellar bodies which are synthesized primarily within the spinous cells. A comparison of the fatty acid composition of lipids from the living layers with that found in the stratum corneum

Table II. Fatty Acid Composition of Normal and Hyperkeratotic Stratum Corneum Lipids

	Percent Free Fatty Acids (means)[a]			Percent Fatty Acids of Triacylglycerols (means)[a]		
	Normal Stratum Corneum (n = 14)	Hyperkeratotic Stratum Corneum (n = 13)	P[b]	Normal Stratum Corneum (n = 13)	Hyperkeratotic Stratum Corneum (n = 13)	p[b]
C_{14}:0	0.7	2.0	0.001	3.9	2.2	0.044
C_{15}:0	0.8	2.2	0.000	2.9	2.7	NS
C_{16}:0	9.2	17.5	0.001	18.2	14.9	NS
C_{16}:1	0.2	0.4	0.001	2.8	2.6	NS
C_{16}:1	0.5	2.1	0.001	0.8	2.7	NS
C_{17}:0	1.7	2.6	0.041	2.6	2.5	NS
C_{17}:1	—	0.8	—	0.6	1.3	NS
C_{18}:0	11.9	16.8	0.002	14.8	12.2	NS
C_{18}:1	1.2	3.3	0.002	3.1	5.7	0.000
C_{18}:1	9.6	20.2	0.000	17.6	25.1	0.003
C_{18}:2	7.2	6.7	NS	7.9	11.6	NS
C_{19}:0	1.9	1.4	0.012	1.3	0.9	NS
C_{19}:1	—	—	—	—	0.2	—
C_{20}:0	5.1	2.9	0.000	2.2	1.4	NS
C_{20}:1	—	0.8	—	0.3	0.9	NS
C_{20}:1	—	0.9	—	1.0	2.3	NS
C_{20}:2	—	0.4	—	0.2	1.1	0.008
C_{20}:3	—	0.1	—	—	0.8	—
C_{21}:0	2.6	0.9	0.000	1.1	0.4	0.008
C_{22}:0	11.0	3.9	0.000	2.6	1.3	0.011
C_{22}:1	—	0.3	—	0.2	0.5	0.011
C_{22}:1	—	1.2	—	8.5	3.5	0.003
C_{23}:0	7.1	1.9	0.000	1.3	0.4	0.009
C_{24}:0	18.0	6.2	0.000	3.3	1.4	0.003
C_{24}:1	—	1.6	—	0.2	0.9	0.003
C_{25}:0	4.2	1.1	0.000	0.9	0.5	0.004
C_{26}:0	4.5	1.5	0.000	0.8	0.3	0.008
C_{27}:0	1.0	0.1	0.001	0.2	—	—
C_{28}:0	1.5	0.4	0.001	0.6	0.1	0.008

[a]Compositions are expressed in weight percentages of identified fatty acid methyl esters.
[b]Significant differences for $p < 0.05$ Mann-Whitney Wilcoxon test. NS = Nonsignificant, $p > 0.05$. — = Not detected.

indicates that the normal stratum corneum lipids are strikingly enriched in long-chain fatty acid, fully saturated acyl groups [2]. We can presume that such maturation of fatty acids in hyperkeratotic skin does not occur. Two clusters of fatty acids in epidermis can be distinguished [13]: one of these clusters ranged from C_{12}–C_{18} and the other one ranged from C_{19}–C_{28}. The particular fatty acid composition of the hyperkeratotic stratum corneum may be due either to a decreased oxidation of fatty acids of the first cluster which supplies cell energy [13] or to a defect in chain elongation leading to the fatty acids of the second cluster [21] and to an increase in desaturase activity [13]. The hypothesis of unspecific perturbation in the fatty acid metabolism of the hyperkeratotic stratum corneum is supported by the fact that the shift toward short-chain and monoene fatty acids was constantly found in PPK from various causes. Hence, it is not disease-specific. However, the molecular mechanism(s) of the metabolism perturbation remains unknown. How can these fatty acid modifications influence desquamation in the hyperkeratotic stratum

Table III. Relative Amounts of the Different Fatty Acid Types in Normal and Hyperkeratotic Stratum Corneum and Ratio of Fatty Acids Proportionate to Their Chain Length

Fatty Acid Type	Normal Stratum Corneum (n = 14)	Hyperkeratotic Stratum Corneum			
		Total (n = 13)	1 → 6[a] (n = 6)	7 → 10[a] (n = 4)	11 → 13[a] (n = 3)
Free fatty acid					
Saturated	81.2	62.5	59.5	57.7	69.4
Monoene	11.5	31.0	35.7	30.0	24.7
di and triene	7.2	6.6	4.7	12.3	6.0
$\frac{C_{14} \rightarrow C_{18}}{\text{above } C_{18}}$	0.8	2.7	3.2	4.2	1.5
Triacylglycerol					
Saturated	56.7	41.6	41.1	39.8	42.3
Monoene	35.1	45.6	50.3	39.4	44.5
di and triene	8.1	12.8	8.5	20.8	13.2
$\frac{C_{14} \rightarrow C_{18}}{\text{above } C_{18}}$	3.0	4.9	4.8	5.6	4.9

[a]Patients as numbered in Table I, were gathered in relation with the pathology.

corneum? We have to consider the role of fatty acids in membrane fluidity. Elias [2] has proposed a heterogeneous, 2-compartment model of the stratum corneum that ascribes a special role to intercellular lipids in the regulation of both barrier function and desquamation. For the latter he concluded that neutral lipids can influence the process of normal desquamation by their mass impact in stratum corneum lipid thermal transition. It is well known that the phase transition of lipid bilayers decreases with increasing unsaturation or decreasing chain length of the fatty acid chains [22]. Consequently, because of the abundance of neutral lipids in stratum corneum [14] the phase transition of the intercellular lipids is lower in the hyperkeratotic stratum corneum than in the normal tissue and can explain its abnormal desquamation.

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