

Hany Farwanah · Klaus Raith · Reinhard H. H. Neubert
Johannes Wohlrab

Ceramide profiles of the uninvolved skin in atopic dermatitis and psoriasis are comparable to those of healthy skin

Received: 17 November 2004 / Revised: 3 February 2005 / Accepted: 14 February 2005 / Published online: 1 April 2005
© Springer-Verlag 2005

Abstract Ceramides are sphingolipids consisting of sphingoidbases, which are amide-linked to fatty acids. In the stratum corneum, they represent the major constituent of the free extractable intercellular lipids and play a significant role in maintaining and structuring the water permeability barrier of the skin. Using thin layer chromatography, which represents the method of the first choice in analyzing the stratum corneum ceramides, at least seven classes can be distinguished. Each ceramide class contains various species, which have the same head group and different chain lengths. As in many other skin disorders, atopic dermatitis and psoriasis show derangements in content and profile of the ceramides. Such derangements were reported for both the lesional involved as well as for the normal-appearing uninvolved skin. In this study, we focused on investigating the stratum corneum ceramides of the uninvolved skin in atopic dermatitis and psoriasis patients compared to healthy skin. The aim of the investigations was to explore possible significant and specific differences which can be accomplished for purposes of early diagnostics. The skin lipids were collected by means of an *in vivo* topical extraction procedure using an extraction mixture consisting of *n*-hexane and ethanol, (2:1). An automated multiple development-high performance thin layer chromatography (AMD-HPTLC) method with photodensitometric detection were applied to separate the ceramides and to estimate their contents. For studying their molecular profile within each ceramide class, a new method of normal phase HPLC with atmospheric pressure chemical ionization mass spectrometry were

used. The results obtained by AMD-HPTLC exposed no significant alterations regarding the relative composition of the major stratum corneum lipids and primarily the ceramides. In addition, the mass spectrometric profiles within each ceramide class were similar in the patients and the healthy control subjects. In conclusion, this study revealed that the normal-appearing uninvolved skin of atopic dermatitis and psoriasis patients does not prove significant or specific deficiencies with respect to the free extractable major stratum corneum lipids and mainly the ceramides, when compared to healthy skin. Thus, they cannot be used for diagnostic purposes. Furthermore, our data are not consistent with the concept that impairments in the ceramide composition represent an obligate etiologic factor for both diseases.

Keywords Ceramides · Atopic dermatitis · Psoriasis

Introduction

The outermost layer of the skin, the stratum corneum, consists of corneocytes embedded in a complex matrix of multilamellar organized lipids containing mainly ceramides, cholesterol and free fatty acids [10, 11, 16].

The corneocytes have a peripheral rigid protein envelope, the so-called cornified envelope [25], to which a lipid monolayer (the cornified lipid envelope) is covalently attached [45]. While the intercellular lipids can be easily extracted by organic solvents, the covalently bound lipids need first to be hydrolyzed under alkaline conditions [45].

Ceramides, which comprise the major constituents of the free extractable stratum corneum lipids, are known to play a key role in structuring and maintaining the epidermal barrier function of human skin [7, 20]. They consist of sphingoid bases which are amide-linked to α hydroxylated, ω hydroxylated or non-hydroxylated fatty acids. Separation on high

H. Farwanah · K. Raith · R. H. H. Neubert
Institute of Pharmaceutics and Biopharmaceutics, Martin Luther University, W.-Langenbeck-Str. 4, 06120 Halle (S.), Germany

J. Wohlrab (✉)
Department of Dermatology and Venereology, Martin Luther University, Ernst-Kromayer-Straße 5, 06097, Halle (S.), Germany
E-mail: johannes.wohlab@medizin.uni-halle.de
Tel.: +49-345-5573911
Fax: +49-345-5573944

performance thin layer chromatography (HPTLC) plates shows seven ceramide classes. According to a nomenclature proposed previously [30, 38], they were recognized as Cer [EOS], [NS], [NP], [EOH], [AS], [AP], and Cer [AH]. The ceramide [AS] fraction, however, was shown to overlap with another fraction Cer [NH] with Cer [NH] being the major component [41]. Both Cer [AS] and Cer [NH] can only be separated after acetylation [41]. Most recently, a new ceramide class Cer [EOP] was identified [35]. Up to date, the function of each ceramide class is not known. However, the more lipophilic ceramides with ω esterified fatty acids and predominantly Cer [EOS] are reported to be of considerable importance [5].

The stratum corneum ceramides originate mainly from the deglycosylation of glucosylated ceramide precursors [17] catalyzed by the β -glucocerebrosidase [21]. Another pathway is the hydrolysis of sphingomyelin by means of the acid sphingomyelinase [17, 42]. Different skin diseases such as atopic dermatitis and psoriasis were reported to affect the qualitative and quantitative pattern of stratum corneum lipids, particularly the ceramides [9, 22, 30, 31, 47], both in the involved as well as in the uninvolved skin. However, a direct relationship between both diseases and the impaired lipid profiles could not be established. The reason for that might lay in the complexity and the diversity of factors influencing the stratum corneum lipid composition such as interindividual variations [32], season [39], age [15], gender [8] and not at least the variety of the different extraction- and analytical methods used by the different investigators. On the other hand, many molecular aspects of the mentioned diseases are still unclear and need to be explored in more detail, which is best done by means of powerful analytical methods.

Previous analytical studies relied on thin-layer chromatography based on various manual procedures to separate the stratum corneum ceramides [34, 46]. Automated multiple development-HPTLC (AMD-HPTLC) has facilitated separation efficiency, quantification and reliability [4, 12, 48]. Further characterization of the ceramide classes with respect to the chain lengths of fatty acids and sphingoid bases became accessible with the use of mass spectrometry (MS). Offline coupling approaches of HPTLC and ESI-MS or ESI-MS/MS as well as reversed phase HPLC/MS were reported [36, 37, 43]. Recently, normal phase HPLC coupled to atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) was successfully used for the separation and the sensitive detection of all stratum corneum ceramides in one run [13]. Thus, the mass spectrometric profiles within each ceramide class could be directly and easily investigated. In this study, we used this new analytical approach as well as a previously developed AMD-HPTLC method [12] to investigate the extent of possible alterations regarding the ceramide profiles in the uninvolved skin by atopic dermatitis and psoriasis patients and to evaluate the feasibility of using such alterations for diagnostic purposes.

Materials and methods

Chemicals and reagents

Cer [NP] and Cer [AP] were provided by Cosmoferm (now Goldschmidt, Essen, Germany), Cer [NS] by Sederma (Le Perray en Yvelines, France), Cer [AS] (hydroxy fatty acid ceramide), cholesterol and palmitic acid were purchased from Sigma-Aldrich (Taufkirchen, Germany). These lipids were used as standards for AMD-HPTLC and HPLC/APCI-MS.

Solvents for extraction, HPTLC and HPLC/MS purposes were of HPLC grade and provided from Baker (Deventer, The Netherlands), Merck (Darmstadt, Germany) and Roth (Karlsruhe, Germany).

Subjects

The study design was permitted by the ethics committee of the medicine faculty at the Martin-Luther-University Halle-Wittenberg (Germany) and was carried out by an experienced dermatologist. After written approval was signed from all volunteers lipid extracts were obtained in vivo from 20 subjects (14 women and 6 men with an average age of 40 ± 13 years, distributed as following: seven healthy subjects, seven atopic dermatitis- and six psoriasis patients). The inclusion criteria were an eczema area and severity index (EASI) Score > 15 for atopic dermatitis patients [6, 18] and a psoriasis area and severity index (PASI) > 20 for psoriasis patients [14]. Systemic as well as topical application of drugs or skin care products were not allowed 8 and 4 weeks prior to investigations, respectively.

Extraction procedure

By the in vivo surface extraction, a cylindrical glass beaker with 4 cm ID (extraction area 12.56 cm^2) was filled with 10 ml *n*-hexane/ethanol 2:1 (v/v). The open side was pressed tightly to a skin area at the uninvolved or healthy inner forearm to prevent lateral leakage. The glass beaker was held by a PVC holder and an adjustable belt. The extraction time was exactly 5 min throughout. The extraction was carried out two times on each forearm resulting in 4 extracts per one volunteer. The extracts belonging to each volunteer were pooled and the solvents were evaporated at 50°C under a stream of nitrogen resulting in a dried residue, which thereafter was stored at -80°C . Before use, the lipid extracts were separated from hydrophilic substances and proteins as described elsewhere in detail [40]. Briefly, methanol, chloroform and distilled water were added to the extracts in the ratio 2:1:0.8 (v/v/v) leading to a homogenous solution. Upon changing the ratio to 2:2:1.8 (v/v/v), two phases were created with the stratum corneum lipids being in the lower

chloroform phase, which was used for both the AMD-HPTLC- as well as for the HPLC/APCI-MS analysis.

Ceramide separation by AMD-HPTLC

The HPTLC plates were washed thrice with chloroform/methanol 65:35 (v/v) before use. Sample application was carried out automatically using an Automatic TLC Sampler 4 (Camag, Muttenz, Switzerland) on a start line located 8 mm from the bottom of the plate and at a dosage speed of 100 μ l/s.

Sixteen samples were applied in lanes on each plate, including eight lanes for stratum corneum lipid extracts (each sample was analyzed in duplicate) as well as eight lanes for reference lipids of different quantities. The band length was 8 mm with 10.6 mm distance to the neighbouring lane. The development of the plates was carried out automatically using an AMD-2 apparatus (Camag, Muttenz, Switzerland) as described previously in detail [12]. Briefly, an AMD procedure including 17 steps and based on mixtures of chloroform, ethanol, acetone, *n*-hexane and ethylacetate under acidic conditions was used to separate the ceramide classes and the other lipids in the same run.

Densitometric profiling of the ceramides

After drying, the HPTLC plates were dipped into an aqueous solution of 10% CuSO₄, 8% H₃PO₄ (v/v), and 5% methanol for 20 s. Afterwards, the plates were charred in a drying oven at 160°C for 30 min.

The developed and visualized plates were scanned from 7 mm to front using a TLC Scanner 3 (Camag, Muttenz, Switzerland). The measurements were performed in reflectance mode at a wavelength of 546 nm. The slit dimensions were 4×0.2 mm at a scan speed of 20 mm/s and a data resolution of 100 μ m per step. Integration and quantification based on peak areas were performed using CATS software (Camag, Muttenz, Switzerland). To avoid experimental errors, individual curves were set up for each HPTLC plate. Quantitative results for all ceramides were related to Cer [NP] as a standard. The results were expressed as mean \pm SD. For statistical analysis Student's *t*-test was performed. A *P* value < 0.01 was considered significant.

Ceramide separation and their profiling by HPLC/APCI-MS

All separations were carried out using a silica column LichroCart (125–4, 5 μ m) filled with Si 60 Lichrospher particles (Merck, Darmstadt, Germany) in a normal-phase mode. The elutions were performed on a HPLC system consisting of a Spectra System P 4000 pump, an auto sampler AS 3000, and a controller SN 4000 (Thermo Separations, San Jose, CA, USA) at a flow

rate of 1 ml/min and with a gradient, which is described elsewhere in detail [13].

As detector, an ion trap mass spectrometer Finnigan LCQ classic (Thermo Electron, San Jose, CA, USA) equipped with an APCI source was used throughout. The APCI heater was set to 500°C and the heated capillary to 150°C. Nitrogen was used as both auxiliary and sheath gas at flow rates of 9 and 1.2 l/min.

In all experiments, the entire flow from the HPLC was directed to the APCI source. The injected ceramide amount was 5 μ g for each sample (including all ceramide classes). The spectra of ceramides were obtained in full scan mode between *m/z* 450 and 1500.

Results

Ceramide profile as obtained by AMD-HPTLC and densitometry

The extracted lipids were analyzed by AMD-HPTLC leading to the separation of the ceramides from other lipids and the ceramides themselves into seven fractions. After densitometric evaluation, the total ceramide amount, expressed in μ g/cm², as well as the percentage profile were determined for each sample. From Table 1, it can be observed that the ceramide amount in the uninvolved skin of atopic dermatitis (15.3 \pm 4.0 μ g/cm²) and psoriasis (11.4 \pm 3.5 μ g/cm²) patients is reduced when compared to healthy skin (16.2 \pm 3.5 μ g/cm²) with the reduction being more pronounced in the psoriasis patients. The differences were however not significant according to the performed Student's *t* test at *P* < 0.01. In Table 2 and Fig. 1, the percentage composition of the ceramide classes are presented. Again, no significant changes could be observed. These results indicate that the ceramide composition of the uninvolved skin in atopic dermatitis and psoriasis patients is not affected and comparable with that in healthy skin.

This study focuses clearly on ceramides. This is due to their utmost importance for the barrier function as well as because of their structural variability. In addition, Table 3 shows data for other lipid classes. Significant differences could not be obtained neither for cholesterol nor for free fatty acids.

Table 1 Amount of ceramides per extraction area

Skin type	Amount (μ g/cm ²)
Healthy	16.2 \pm 3.5
Dermatitis	15.3 \pm 4.0
Psoriasis	11.4 \pm 3.5

Amount in μ g/cm², mean \pm SD (*P* < 0.01, healthy: *n* = 7, dermatitis: *n* = 7 and psoriasis: *n* = 6)

Table 2 Ceramide profile of healthy controls versus uninvolved skin of atopic dermatitis and psoriasis patients as determined by AMD-HPTLC and densitometry

Skin type	Cer [AH]	Cer [AP]	Cer [NH/AS]	Cer [EOH]	Cer [NP]	Cer [NS]	Cer [EOS]
Healthy	14.1 ± 1.7	7.5 ± 1.3	20.7 ± 1.3	7.6 ± 1.3	16.5 ± 1.4	19.9 ± 1.5	13.7 ± 1.2
Dermatitis	15.0 ± 1.8	8.5 ± 1.0	20.5 ± 1.9	7.1 ± 1.4	14.8 ± 1.7	20.9 ± 1.4	13.3 ± 2.6
Psoriasis	16.7 ± 3.0	8.3 ± 1.2	20.4 ± 2.1	7.3 ± 1.9	13.1 ± 3.1	19.6 ± 1.2	14.7 ± 2.5

Percentage of the ceramide classes as related to the total ceramide sum; mean ± SD ($P < 0.01$, healthy: $n = 7$, dermatitis: $n = 7$ and psoriasis: $n = 6$)

Ceramide profile by means of normal phase HPLC/APCI-MS

In addition to the densitometric data, the chain length profile within each ceramide class is also of importance. It namely allows examining possible alterations at the molecular level.

This requires a specific MS detection. Therefore, a recently developed HPLC/APCI-MS [13] method for the separation and on line detection of all stratum corneum ceramides in one run was carried out. The mass spectra relating to the ceramides of the uninvolved regions of patients' skin were thereafter compared to those of healthy skin. As a result, no significant differences between healthy and uninvolved skin in both diseases could be obtained.

In Fig. 2, the ceramide separation chromatograms for the categories: healthy, atopic, and psoriatic volunteer is shown. In Figs. 3, 4 and 5 the mass spectrometric profiles of selected ceramides corresponding to the same samples presented in Fig. 2 are demonstrated. As can be observed, differences between healthy and diseased persons could not be obtained, neither regarding the sepa-

ration pattern nor concerning the mass spectrometric profiles of the presented ceramides [EOS], [NP], and [NH], which were reported to be altered by atopic dermatitis and psoriasis. The same result was also obtained by all other ceramide classes, which are not illustrated here.

Discussion

To achieve precise and reliable results, only patients fulfilling the inclusion criteria of an EASI > 15 or a PASI > 20 were considered. Such criteria would reveal significant results, if ceramide alterations were manifested in the uninvolved skin of atopic dermatitis or psoriasis patients. In addition, the extractions were performed by experienced dermatologists. By choosing the extraction mixture both the compliance of the patients as well as the extraction efficiency were considered. Although various extraction procedures may expose different results, it has to be taken into account that the relative amount of the ceramide classes do not change with stratum corneum depth [44]. The

Fig. 1 Ceramide profile after densitometric evaluation. No significant differences were obtained. ($P < 0.01$, healthy: $n = 7$, dermatitis: $n = 7$ and psoriasis: $n = 6$)

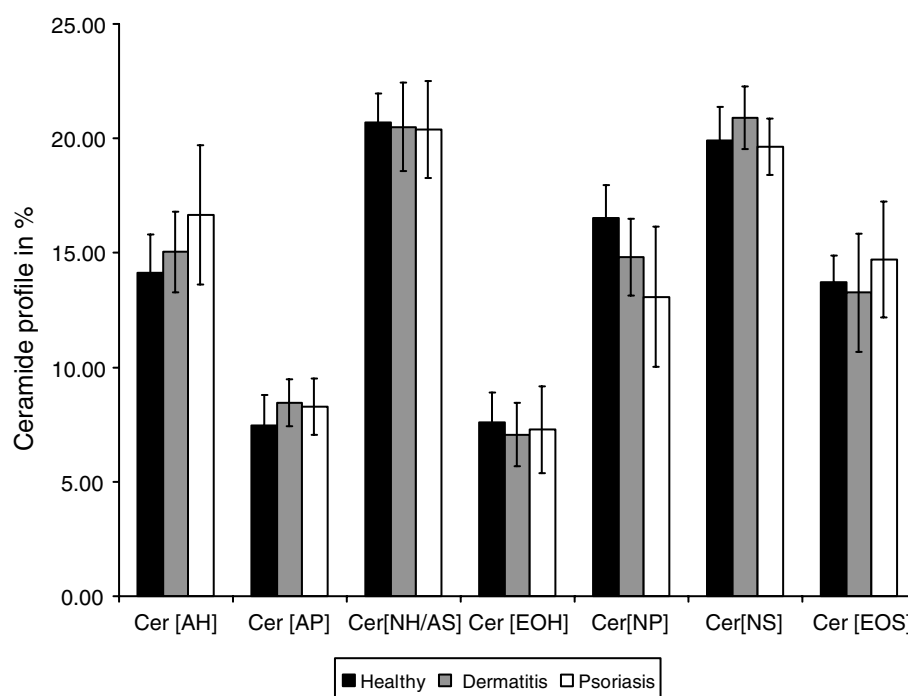


Table 3 Composition of stratum corneum main lipids in healthy skin compared to uninvolved skin in atopic dermatitis and psoriasis patients as analyzed by AMD-HPTLC and densitometry

Skin type	Ceramides	Cholesterol	FFA
Healthy	53.1 ± 6.3	17.0 ± 2.9	30.0 ± 5.1
Dermatitis	48.7 ± 5.5	19.2 ± 2.4	32.1 ± 5.4
Psoriasis	50.6 ± 10	18.4 ± 5.9	31.0 ± 11.7

Percentage of lipid classes as related to total lipid sum (ceramides + cholesterol + free fatty acids); Mean ± SD ($P < 0.01$, healthy: $n = 7$, dermatitis: $n = 7$ and psoriasis: $n = 6$)

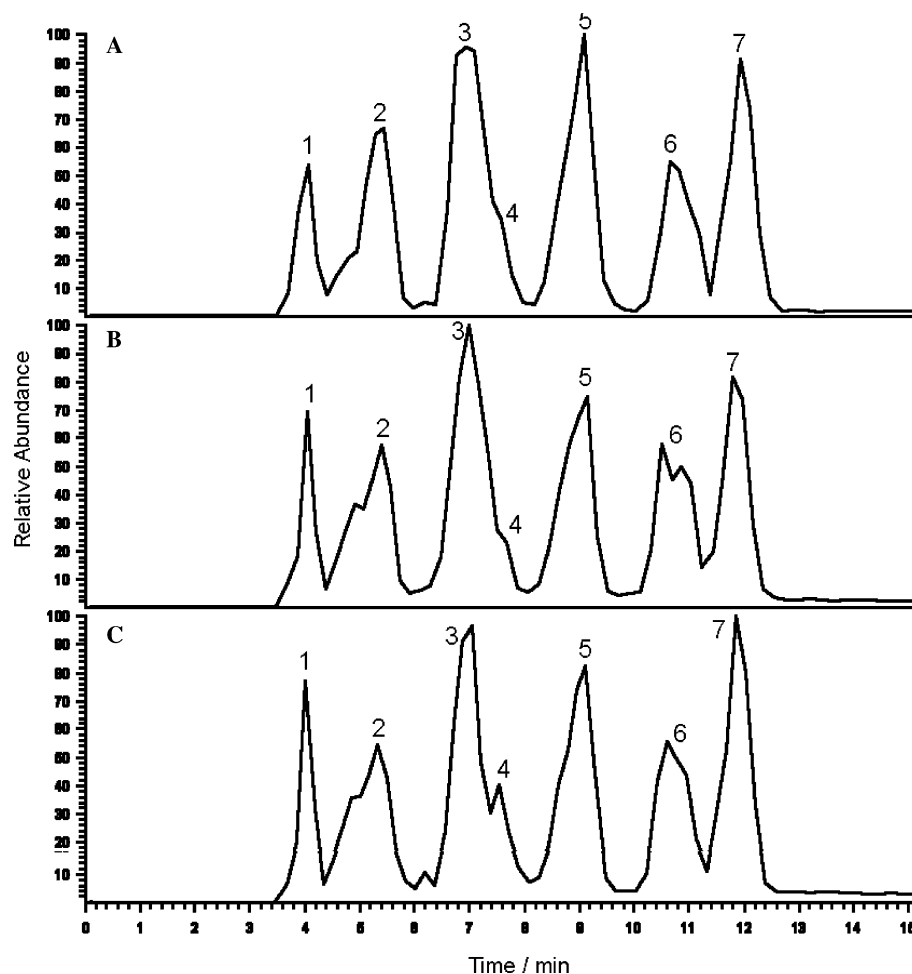
levels of ceramides, cholesterol and the free fatty acids were reported to be about 60%, 18% and 22%, respectively [44]. This finding is consistent with our results (Table 3).

In this study, we have used both AMD-HPTLC [12] with densitometric detection and normal phase HPLC/APCI-MS [13] to analyze the extracted stratum corneum lipids, and particularly the ceramides. The used AMD-HPTLC procedure has the advantages not only to enable the separations of all stratum corneum lipids in one run in a relatively short time but also to reveal consistent quantifications due to compressed thin bands resulting from the several gradient steps. With the

utilized HPLC/APCI-MS method, it was possible to separate and profile the stratum corneum ceramide classes simultaneously and thus to facilitate studying probable ceramide alterations at the molecular level. Furthermore, it is to our knowledge the first time, in which a direct mass spectrometric analysis of the distinctive lipophilic and for ESI-MS not accessible ceramides such as Cer [EOS] was performed.

Regarding atopic dermatitis, it was reported that alterations in the total and relative amounts of the ceramides represent an etiologic factor for the disease, Cer [EOS] being most affected both in the involved and the uninvolved skin [22]. Bleck et al. showed that in non-lesional (uninvolved) skin of atopic dermatitis patients, Cer [AS] consists of two subfractions, which can be separated by HPTLC [3]. In addition, a reduction of Cer [NP] was found to correlate with an increased transepidermal water loss (TEWL) in both involved as well as uninvolved skin [9]. A more recent work showed a decrease of Cer [EOH] by atopic dermatitis patients [27]. On the biochemical level, the enzymes sphingomyelin deacylase [19], glucosylceramide deacylase [23] as well as a bacterial derived ceramidase [33] were recently reported to be possible causes for the ceramide deficiency. Conversely, the activities of the sphingolipid biosynthesis

Fig. 2 Separation of stratum corneum ceramides by means of normal phase HPLC/APCI-MS; **a**, **b** and **c** correspond to the ceramide separation by a healthy volunteer, an atopic dermatitis and a psoriasis patient, respectively. In all chromatograms, 1 Cer [EOS], 2 Cer [NS], 3 Cer [NP], 4 Cer [EOH], 5 Cer [NH] and Cer [AS], 6 Cer [AP], 7 Cer [AH]



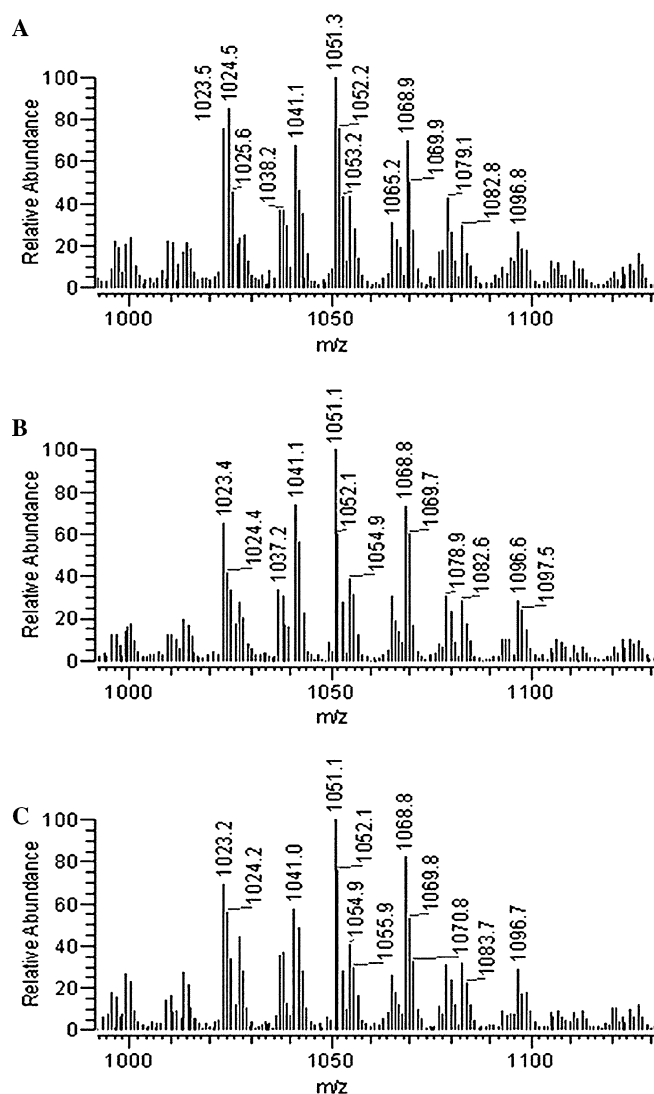


Fig. 3 Mass spectra $[M - H_2O + H]^+$ of Cer [EOS] by **a** healthy volunteer, **b** atopic dermatitis patient and **c** psoriasis patient. The spectra correspond to peak 1 in Fig. 2

enzymes β -glucocerebrosidase [24] and acid sphingomyelinase [26], were found to be not essentially impaired.

Di Nardo et al. [9] have defined a “clinically and functionally in-between population” of atopic dermatitis patients, who show intermediate levels of lipids. A study performed by Matsumoto et al. revealed that the decrease in the total ceramide amount and in Cer [EOS] is restricted to the involved atopic skin and is not extended to the uninvolved areas [29]. Furthermore, patients free from atopic dermatitis symptoms for more than 5 years showed normal TEWL and water content values [28]. Our data are consistent with these findings. Neither the total absolute (Table 1) or the relative (Table 2, Fig. 1) ceramide amounts nor the mass spectrometric profiles of Cer [EOS] (Fig. 3), Cer [NP] (Fig. 4), Cer [NH] (Fig. 5) in the uninvolved skin of atopic dermatitis patients prove any significant differences compared to healthy controls. Similar results regarding the mass spectra of

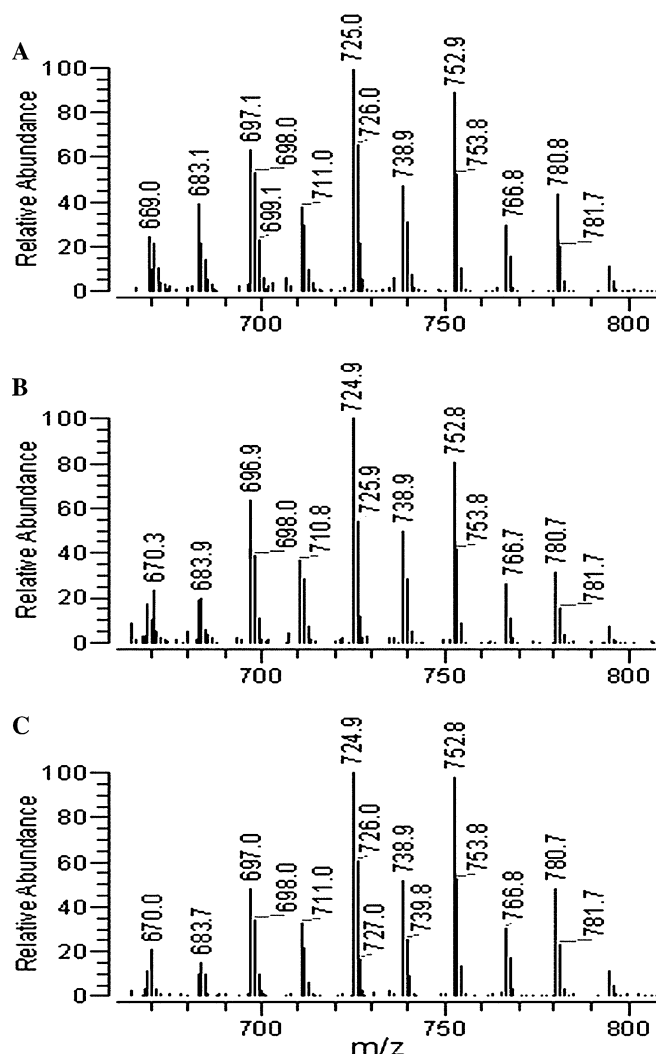


Fig. 4 Mass spectra $[M + H]^+$ of Cer [NP] by **a** healthy volunteer, **b** atopic dermatitis patient and **c** psoriasis patient. The spectra correspond to peak 3 in Fig. 2

Cer [NS], [EOH], [AP], [AH] were obtained (Data not shown).

In psoriatic skin Cer [EOS], [NP], [EOH], [AS], [AP] were reported to be decreased and Cer [NS] to be increased. The decrease of Cer [EOS] and Cer [EOH] were suggested to be responsible for the defective barrier function [30, 31]. Others [1] have found that the level of prosaposin, an activator protein in the sphingolipid pathway, is decreased in psoriasis patients, the decrease being more pronounced in the involved skin compared to the uninvolved skin. On the other hand, it was previously found that TEWL and water content do not differ in nonlesional (uninvolved) skin of psoriatic patients when compared to control subjects [2] indicating an intact barrier function. The results obtained here support this finding.

From this study, we conclude that the uninvolved skin of atopic dermatitis or psoriasis patients can be considered to be “healthy” regarding the composition of

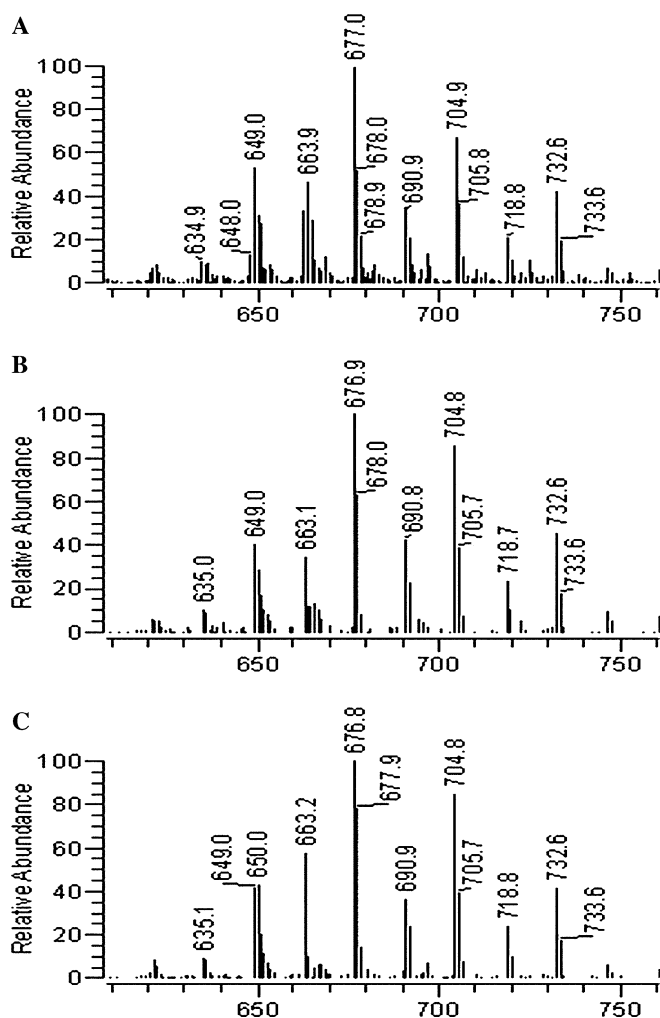


Fig. 5 Mass spectra $[M - H_2O + H]^+$ of Cer $[NH]$ by **a** healthy volunteer, **b** atopic dermatitis patient and **c** psoriasis patient. The spectra correspond to peak 5 in Fig. 2

the ceramides and the other investigated lipids. Consequently, the free extractable stratum corneum lipids, and particularly the ceramides in the uninvolved skin of the mentioned diseases cannot be used for diagnostic purposes. In addition, our findings imply that impairments in the composition of the stratum corneum lipids and thus in their biosynthesis do not represent an obligate etiologic factor neither for atopic dermatitis nor for psoriasis. However, Ceramide profiles of different extraction procedures and from different anatomical sites are to be compared. Moreover, further investigations by means of tandem MS are to be carried out. In Addition, one has to bear in mind that the barrier function is not restricted to the intercellular lipids and primarily ceramides but also includes other components such as the cornified envelope, the lipid cornified envelope, the corneocytes and the lipid organization, which can be affected in skin diseases and therefore have also to be taken into account.

Acknowledgements The authors gratefully acknowledge financial support from the Kultusministerium Sachsen-Anhalt, project no.

3364A/0021L. The authors thank Manuela Woigk for excellent laboratory assistance.

References

- Alessandrini F, Stachowitz S, Ring J, Behrendt H (2001) The level of prosaposin is decreased in the skin of patients with psoriasis vulgaris. *J Invest Dermatol* 116:394–400
- Berardesca E, Fideli D, Borroni G, Rabbiosi G, Maibach H (1990) In vivo hydration and water-retention capacity of stratum corneum in clinically uninvolved skin in atopic and psoriatic patients. *Acta Derm Venereol* 70:400–404
- Bleck O, Abeck D, Ring J, Hoppe U, Vietzke JP, Wolber R, Brandt O, Schreiner V (1999) Two ceramide subfractions detectable in Cer(AS) position by HPTLC in skin surface lipids of non-lesional skin of atopic eczema. *J Invest Dermatol* 113:894–900
- Bonte F, Pinguet P, Chevalier JM, Meybeck A (1995) Analysis of all stratum corneum lipids by automated multiple development high-performance thin-layer chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 664:311–316
- Bouwstra JA, Gooris GS, Dubbelaar FE, Weerheim AM, Ijzerman AP, Ponc M (1998) Role of ceramide 1 in the molecular organization of the stratum corneum lipids. *J Lipid Res* 39:186–196
- Charman C, Williams H (2000) Outcome measures of disease severity in atopic dermatitis. *Arch Dermatol* 136:763–769
- Coderch L, Lopez O, de la Maza A, Parra JL (2003) Ceramides and skin function. *Am J Clin Dermatol* 4:107–129
- De Paepe K, Weerheim A, Houben E, Roseeuw D, Ponc M, Rogiers V (2004) Analysis of epidermal lipids of the healthy human skin: factors affecting the design of a control population. *Skin Pharmacol Physiol* 17:23–30
- Di Nardo A, Wertz P, Giannetti A, Seidenari S (1998) Ceramide and cholesterol composition of the skin of patients with atopic dermatitis. *Acta Derm Venereol* 78:27–30
- Downing DT, Stewart ME, Wertz PW, Colton SW, Abraham W, Strauss JS (1987) Skin lipids: an update. *J Invest Dermatol* 88:2s–6s
- Elias PM (1981) Epidermal lipids, membranes, and keratinization. *Int J Dermatol* 20:1–19
- Farwanah H, Neubert R, Zellmer S, Raith K (2002) Improved procedure for the separation of major stratum corneum lipids by means of automated multiple development thin-layer chromatography. *J Chromatogr B Analyt Technol Biomed Life Sci* 780:443–450
- Farwanah H, Nuhn P, Neubert R, Raith K (2003) Normal-phase liquid chromatographic separation of stratum corneum ceramides with detection by evaporative light scattering and atmospheric pressure chemical ionization mass spectrometry. *Anal Chim Acta* 492:233–239
- Fredriksson T, Petterson U (1978) Severe psoriasis - oral therapy with a new retinoid. *Dermatologica* 157:238–244
- Ghadially R, Brown BE, Sequeira-Martin SM, Feingold KR, Elias PM (1995) The aged epidermal permeability barrier. Structural, functional, and lipid biochemical abnormalities in humans and a senescent murine model. *J Clin Invest* 95:2281–2290
- Grubauer G, Feingold KR, Harris RM, Elias PM (1989) Lipid content and lipid type as determinants of the epidermal permeability barrier. *J Lipid Res* 30:89–96
- Hamanaka S, Hara M, Nishio H, Otsuka F, Suzuki A, Uchida Y (2002) Human epidermal glucosylceramides are major precursors of stratum corneum ceramides. *J Invest Dermatol* 119:416–423
- Hanifin JM, Thurston M, Omoto M, Cherill R, Tofte SJ, Graeber M, Group TEE (2001) The eczema area and severity index (EASI): assessment of reliability in atopic dermatitis. *Exp Dermatol* 10:11–18

19. Hara J, Higuchi K, Okamoto R, Kawashima M, Imokawa G (2000) High-expression of sphingomyelin deacylase is an important determinant of ceramide deficiency leading to barrier disruption in atopic dermatitis. *J Invest Dermatol* 115:406–413
20. Holleran WM, Man MQ, Gao WN, Menon GK, Elias PM, Feingold KR (1991) Sphingolipids are required for mammalian epidermal barrier function. Inhibition of sphingolipid synthesis delays barrier recovery after acute perturbation. *J Clin Invest* 88:1338–1345
21. Holleran WM, Takagi Y, Menon GK, Jackson SM, Lee JM, Feingold KR, Elias PM (1994) Permeability barrier requirements regulate epidermal beta-glucocerebrosidase. *J Lipid Res* 35:905–912
22. Imokawa G, Abe A, Jin K, Higaki Y, Kawashima M, Hidano A (1991) Decreased level of ceramides in stratum corneum of atopic dermatitis: an etiologic factor in atopic dry skin?. *J Invest Dermatol* 96:523–526
23. Ishibashi M, Arikawa J, Okamoto R, Kawashima M, Takagi Y, Ohguchi K, Imokawa G (2003) Abnormal expression of the novel epidermal enzyme, glucosylceramide deacylase, and the accumulation of its enzymatic reaction product, glucosylsphingosine, in the skin of patients with atopic dermatitis. *Lab Invest* 83:397–408
24. Jin K, Higaki Y, Takagi Y, Higuchi K, Yada Y, Kawashima M, Imokawa G (1994) Analysis of beta-glucocerebrosidase and ceramidase activities in atopic and aged dry skin. *Acta Derm Venereol* 74:337–340
25. Kalinin AE, Kajava AV, Steinert PM (2002) Epithelial barrier function: assembly and structural features of the cornified cell envelope. *Bioessays* 24:789–800
26. Kusuda S, Cui CY, Takahashi M, Tezuka T (1998) Localization of sphingomyelinase in lesional skin of atopic dermatitis patients. *J Invest Dermatol* 111:733–738
27. Macheleidt O, Kaiser HW, Sandhoff K (2002) Deficiency of epidermal protein-bound omega-hydroxyceramides in atopic dermatitis. *J Invest Dermatol* 119:166–173
28. Matsumoto M, Sugiura H, Uehara M (2000) Skin barrier function in patients with completely healed atopic dermatitis. *J Dermatol Sci* 23:178–182
29. Matsumoto M, Umemoto N, Sugiura H, Uehara M (1999) Difference in ceramide composition between “dry” and “normal” skin in patients with atopic dermatitis. *Acta Derm Venereol* 79:246–247
30. Motta S, Monti M, Sesana S, Caputo R, Carelli S, Ghidoni R (1993) Ceramide composition of the psoriatic scale. *Biochim Biophys Acta* 1182:147–151
31. Motta S, Monti M, Sesana S, Mellesi L, Ghidoni R, Caputo R (1994) Abnormality of water barrier function in psoriasis. Role of ceramide fractions. *Arch Dermatol* 130:452–456
32. Norlén L, Nicander I, Lundh Rozell B, Ollmar S, Forslind B (1999) Inter- and intraindividual differences in human stratum corneum lipid content related to physical parameters of skin barrier function in vivo. *J Invest Dermatol* 112:72–77
33. Ohnishi Y, Okino N, Ito M, Imayama S (1999) Ceramidase activity in bacterial skin flora as a possible cause of ceramide deficiency in atopic dermatitis. *Clin Diagn Lab Immunol* 6:101–104
34. Ponec M, Weerheim A (1990) Retinoids and lipid changes in keratinocytes. *Methods Enzymol* 190:30–41
35. Ponec M, Weerheim A, Lankhorst P, Wertz P (2003) New acylceramide in native and reconstructed epidermis. *J Invest Dermatol* 120:581–588
36. Raith K, Neubert R (2000) Liquid chromatography-electrospray mass spectrometry and tandem mass spectrometry of ceramides. *Anal Chim Acta* 403:295–303
37. Raith K, Zellmer S, Lasch J, Neubert R (2000) Profiling of human stratum corneum ceramides by liquid chromatography-electrospray mass spectrometry. *Anal Chim Acta* 418:167–173
38. Robson KJ, Stewart ME, Michelsen S, Lazo ND, Downing DT (1994) 6-Hydroxy-4-sphingenine in human epidermal ceramides. *J Lipid Res* 35:2060–2068
39. Rogers J, Harding C, Mayo A, Banks J, Rawlings A (1996) Stratum corneum lipids: the effect of ageing and the seasons. *Arch Dermatol Res* 288:765–770
40. Signorelli P, Hannun YA (2002) Analysis and quantitation of ceramide. *Methods Enzymol* 345:275–294
41. Stewart ME, Downing DT (1999) A new 6-hydroxy-4-sphingenine-containing ceramide in human skin. *J Lipid Res* 40:1434–1439
42. Uchida Y, Hara M, Nishio H, Sidransky E, Inoue S, Otsuka F, Suzuki A, Elias PM, Holleran WM, Hamanaka S (2000) Epidermal sphingomyelins are precursors for selected stratum corneum ceramides. *J Lipid Res* 41:2071–2082
43. Vietzke JP, Brandt O, Abeck D, Rapp C, Strassner M, Schreiner V, Hintze U (2001) Comparative investigation of human stratum corneum ceramides. *Lipids* 36:299–304
44. Weerheim A, Ponec M (2001) Determination of stratum corneum lipid profile by tape stripping in combination with high-performance thin-layer chromatography. *Arch Dermatol Res* 293:191–199
45. Wertz PW, Madison KC, Downing DT (1989) Covalently bound lipids of human stratum corneum. *J Invest Dermatol* 92:109–111
46. Wertz PW, Miethke MC, Long SA, Strauss JS, Downing DT (1985) The composition of the ceramides from human stratum corneum and from comedones. *J Invest Dermatol* 84:410–412
47. Yamamoto A, Serizawa S, Ito M, Sato Y (1991) Stratum corneum lipid abnormalities in atopic dermatitis. *Arch Dermatol Res* 283:219–223
48. Zellmer S, Lasch J (1997) Individual variation of human plantar stratum corneum lipids, determined by automated multiple development of high-performance thin-layer chromatography plates. *J Chromatogr B Biomed Sci Appl* 691:321–329