Arch Dermatol Res (2001) 293 : 191–199

ORIGINAL PAPER

A. Weerheim · M. Ponec

Determination of stratum corneum lipid profile by tape stripping in combination with high-performance thin-layer chromatography

Received: 2 August 2000 / Revised: 20 December 2000 / Accepted: 26 January 2001

Abstract **Intercellular lipids in the stratum corneum (SC) are responsible for the barrier function of mammalian skin. The main components of the SC lipids are ceramides, cholesterol, and free fatty acids, as established by thin-layer chromatographic analysis of lipids extracted from the human and mammalian SC. Up to now, for lipid analysis the extracts of the entire SC has been used and information on whether the lipid composition changes with the depth in the SC is scarce. Tape stripping is a technique which removes corneocyte layers step by step with an adhesive film. The use of this technique for lipid analysis was hampered by the contamination of lipid extracts with compounds co-extracted from the tape with organic solvents used for the extraction of SC lipids. The aim of the present study was to establish a suitable analytical method for the determination of the local SC lipid composition. For this purpose, the SC samples were collected by sequential stripping with Leukoplex tape in five healthy volunteers. The lipids were extracted with ethyl acetate:methanol mixture (20:80) and separated by means of HPTLC. The results of this study revealed that the free fatty acid level is highest and the cholesterol and ceramide levels lowest in the uppermost SC layers (about 4 strippings). The levels remained unchanged in the underlying SC layers. In these layers, the ceramide level was about 60 wt% and the free fatty acid and cholesterol levels were about 20 wt% each. Ceramides could be separated into seven different fractions and the relative amounts of individual ceramide fractions did not significantly change with the SC depth. Cholesterol sulfate levels were about 5% of total cholesterol and did not change with the SC depth, except for the for the first strip where the level was about 1%. The method developed makes it possible to study the differences in the SC lipid profile in healthy and diseased human skin with relation to the SC lipid organization and to the skin barrier function in vivo.**

Keywords Stratum corneum · Lipids · Tape stripping · Skin barrier

A. Weerheim · M. Ponec (✉)
Department of Dermatology, Leiden University Medical Center, bld. 3, Sylvius Laboratory, P.O.Box 9503, 2300 RA Leiden, The Netherlands
e-mail: ponec@mail.medfac.leidenuniv.nl,
Tel.: +31-71-5271900, Fax: +31-71-5271910

Introduction

The principal role of the skin is to protect the body against harmful agents and water loss. The main barrier resides in the uppermost layer of the skin: the stratum corneum (SC). Intercellular lipids that form the only continuous domain in the SC are required for a competent skin barrier. These lipids show a surprisingly high degree of organization and form lamellae that are oriented approximately parallel to the surface of the corneocytes (Madison et al. 1987; Hou et al. 1991). Based on electron microscopic and X-ray diffraction studies, it has been established that the lipids are arranged as lamellar structures, the organization of which is strongly dependent on lipid composition (Bouwstra et al. 1991; Bouwstra et al. 1998). The intercellular SC lipid domains consist mainly of free fatty acids (FFA), ceramides (CER), and cholesterol (CHOL). One can expect that within the SC the lipid profile changes, since the formation of the intercellular lipid structures is a result of a sequential series of events, including (a) synthesis of precursor lipids in stratum basale (SB), stratum spinosum (SS), and stratum granulosum (SG); (b) assembly of lipids in lamellar bodies in SS and SG; (c) exocytosis of lamellar bodies at the SG/SC interface; d) processing of lamellar body (LB) lipids to form characteristic lamellar structures. At the SG/SC interface the lipids undergo considerable metabolic changes: the phospholipids are degraded into glycerol and FFA and glucosylsphingolipids into CER (reviewed in Schürer and Elias 1991; Wertz and Downing 1991).

The classical method for extracting lipids in vitro was described by Bligh and Dyer (1959) and is based on the use

of a chloroform:methanol mixture. This approach is not suitable for direct use on the human skin surface since it induces skin necrosis.

Only a few studies have been performed using noninvasive in vivo lipid sampling methods. These approaches have been based on collecting SC samples with (a) a topical extraction procedure with solvent mixtures (Imokawa and Hattori 1985; Imokawa et al. 1986; Lavrijsen et al. 1994; 1995; Fulmer and Kramer 1986; Norlén et al. 1999; Saint Léger et al. 1988; Yamamoto et al. 1991; Bonté et al. 1995) – using this approach the information on the lipid composition in the upper SC can be gained; (b) a topical extraction procedure with solvent mixtures after removal of the upper SC with 5–15 consecutive tape strips (Norlén et al. 1999; Bonté et al. 1997); (c) collection of the upper SC by stripping with cyanoacrylate (Imokawa et al. 1986; di Nardo et al. 1998; Bleck et al. 1999); (d) collection of SC by six consecutive scrapings followed by extraction with a chloroform:methanol mixture (Lavrijsen et al. 1994, 1995); and (e) collection of upper SC with six consecutive tape strips followed by extraction with a chloroform:methanol mixture (Rogers et al. 1996). However, with these approaches the detailed information on whether the lipid composition changes with the SC depth remains scarce.

Tape stripping is a technique which removes corneocyte layers step by step with an adhesive film and up to now it has been the most frequently used method for investigation of the skin penetration process (Schaefer and Redelmeier 1996; Schwarb et al. 1999; Rougier et al. 1983, 1987; Weigmann et al. 1999). The use of this technique for lipid analysis has long been hampered by the contamination of lipid extracts with compounds co-extracted from the tape with organic solvents used for the extraction of SC lipids. The aim of the present study was to establish a suitable analytical method to determine the local SC lipid composition using the tape stripping approach. For this purpose, the method used for collection, extraction and separation of SC lipids has been developed.

The results of the present study show that tape stripping with Leukoflex with subsequent extraction in an ethyl acetate:methanol mixture and separation of extracted lipids by HPTLC makes it possible to establish the lipid profile within the SC. The method developed makes it possible to study the inter- and intra-individual differences in the SC lipid profile in healthy and diseased human skin with relation to the SC lipid organization and to the skin barrier function in vivo.

Materials and Methods

Volunteers

The study was performed with five healthy volunteers with skin types II and III (according to Fitzpatrick's classification, 1988). All participants were judged to be devoid of skin disease and gave their informed consent prior to their inclusion in the study.

Collection of SC samples by tape stripping and extraction procedure

Tape strips were taken from the flexor forearm. The adhesive film Leukoflex tape (Beiersdorf, Hamburg, Germany) (1.5 × 5 cm) was removed with a pair of tweezers from the spool and fixed on a fat-free paper. The tape was transferred to the marked skin area and applied with a pair of tweezers with soft pressure and drawn off again. The tape stripping was repeated on the same skin area until the skin had a shiny appearance. On average, 18–20 strips per subject were collected. The tape was transferred into a tube containing 6 ml ethyl acetate:methanol (20:80) mixture and the lipids were extracted for 1 h at room temperature. Thereafter, the tape was discarded and the solvent evaporated at 50 °C under nitrogen. The extract was dissolved in 0.25 ml chloroform:methanol (2:1) mixture and stored at –20 °C until further analysis.

For comparison, the SC was isolated from the freshly excised human mammary skin by dispase treatment followed by proteinase K treatment, as described earlier (Ponec et al. 1997). The SC was then subjected to the chloroform:methanol extraction (Bligh and Dyer 1959) or to the ethyl acetate:methanol (20:80) mixture, as described above.

Collection of SC samples by scraping and extraction procedure

SC was collected by scraping of the skin with a single edged razorblade (GEM Scientific, American Safety Razor Co., Staunton, VA, USA) from a skin area of about 3 × 5 cm on the flexor side of the left forearm. The collected scrapes were subjected to chloroform:methanol extraction (Lavrijsen et al. 1994).

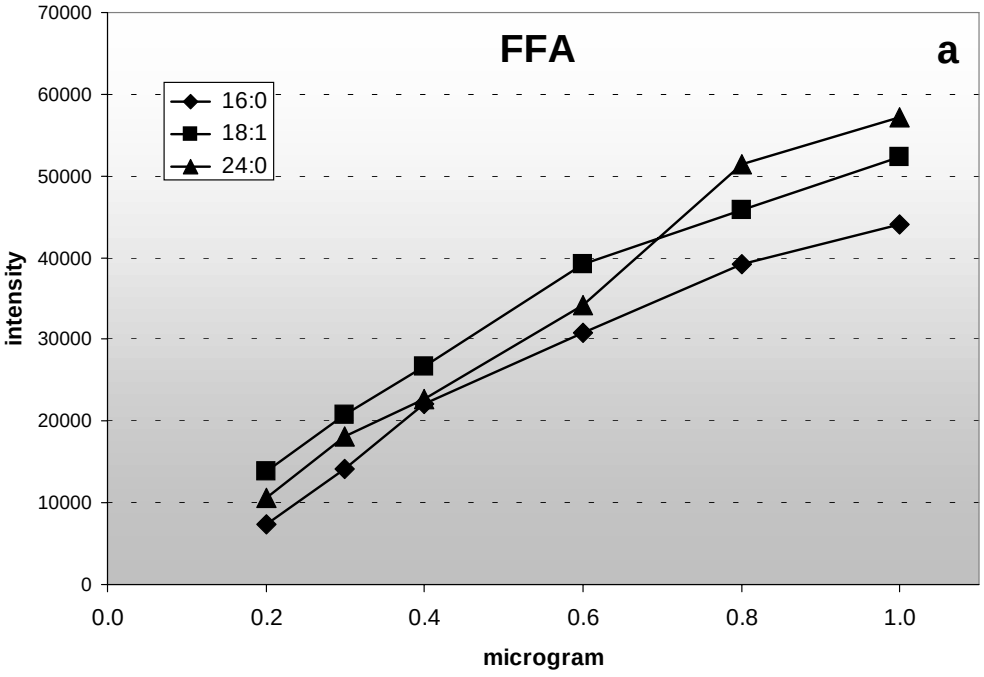
Isolation of human ceramides

Human epidermis was subjected to extensive extraction, as described earlier (Ponec et al. 2000). 90 mg of solvent extractable lipids were separated by column chromatography (4 × 4 cm, 20 g of Silicagel LiChroprep Si 60, 15–25 μm, Merck, Darmstad, Germany) by sequential elution with 100 ml of the following mixtures: hexane:chloroform:dioxane:methanol (55:40:4:1), hexane:chloroform:dioxane:ethyl acetate:methanol (51:40:2:6:1), hexane:chloroform:dioxan:ethyl acetate:ethylmethylketone:acetone:methanol (38:40:2:12:4:2:2), hexane:chloroform:dioxane:ethyl acetate:ethylmethylketone:acetone:methanol (20:56:2:6:8:4:4), chloroform:ethylmethylketone:acetone: methanol (72:16:8:4), chloroform: acetone:methanol (76:16:8), chloroform: acetone:methanol (68:16:16), chloroform:methanol (33:66). All solvents were purchased at Merck. Fractions containing individual CER were pooled and if necessary further purified using thin-layer chromatography.

High-performance thin-layer chromatography

Fifty microliters of lipid extracts were applied under a flow of nitrogen on the HPTLC plate (Merck) using Linomat IV (CAMAG, Muttenz, Switzerland) and separated by using the following sequential development system: (1) dichloromethane:ethyl acetate:acetone (80:16:4:), (2) chloroform:acetone:methanol (76:8:16), (3) hexane:chloroform:hexyl acetate:acetone:methanol (6:80:0.1:10:4).

The quantification was performed after staining (7.5% Cu-acetate and 2.5% $CuSO_4$ in 8% H_3PO_4 (w/w)) and charring at 80 ° or 160 °C. Fifty microliters of the tape extract (without lipids) were applied in parallel for corrections to be made for the background caused by solvent-soluble tape components. Quantification of lipid fractions was based on the known quantities of the co-migrated standards. For this purpose, CHOL (Sigma, Aldrich Chemie, Zwijndrecht, the Netherlands), cholesterol sulfate (Sigma), FFAs (C18:1, C16:0, C24:0), CER 1–7 (isolated as described above), CER GB3 (Gist Brocades, Delft, the Netherlands) were used.



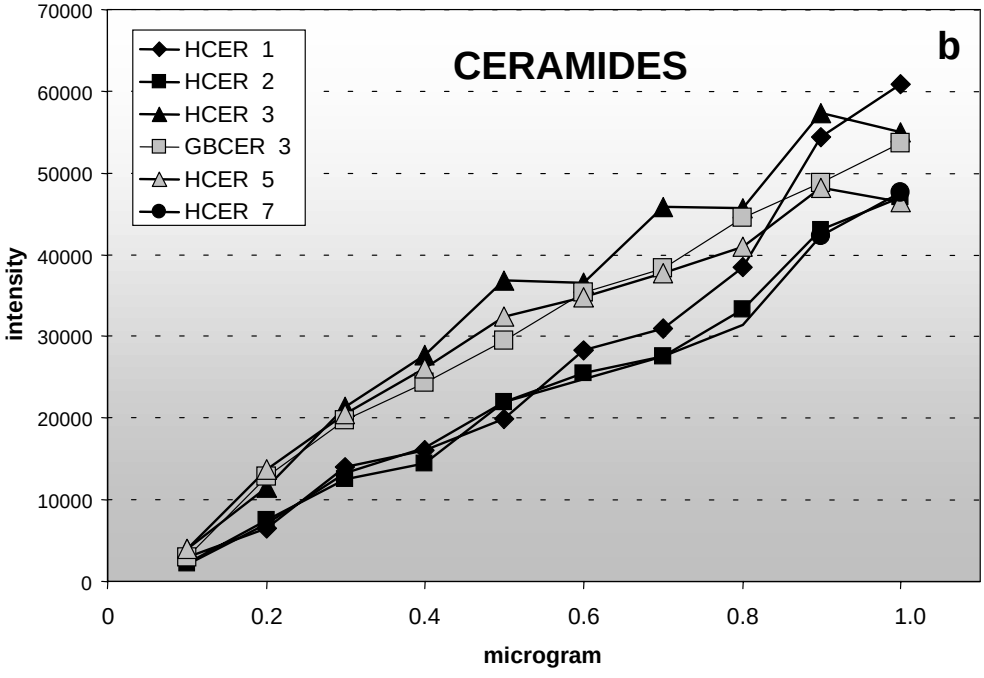


Fig. 1 a, b The choice of standards for quantification of stratum corneum (SC) lipids. Increasing concentrations of lipids (0–1 μg) were applied on the high-performance thin-layer chromatography (HPTLC) plate. The lipids were separated by HPTLC and for identification stained and charred. After densitometry, the area under the curve was determined and plotted against the concentration applied. **a** Free fatty acids (16:0, 18:1, or 24:0), **b** ceramides (natural human ceramides HCER 1, 2, 3, 5 and 7, and synthetic ceramide 3, GBCER3)

Preparation and analysis of fatty acid methyl esters

For the proper choice of the FFA, the total epidermal lipids were separated by HPTLC and the fraction of FFAs was scraped off from the plate and extracted with a mixture of chloroform:methanol:water (50:50:1). After evaporation of the solvents, the FFA fraction was dissolved in 100 μl toluene and subsequently transmethylated in 1 ml BCl_3/methanol (10%) using microwave irradiation, which was carried out at the lowest setting (85 W) for 4 h (Ponec et al. 2000). The fatty acid methyl esters (FAMEs) were separated and analyzed on a Vega GC 6000 gas chromatograph (Carlo Erba Instruments, Interscience, Breda, the Netherlands) using capillary column CP Wax 52 (Chrompack, Delft, the Netherlands). An initial temperature of 80 °C was increased to 160 °C with a rate 40 °C/min followed by a 2 °C/min increase to 250 °C, maintained until all peaks were eluted. The peaks were identified by comparisons with standards (Sigma). Integration of peak areas and calculation of relative percentage was performed by the Chromeleon (Chromatography Data Systems, Gynkotek, Separations, H.I. Ambacht, the Netherlands). Heptadecanoic acid was used as the internal standard.

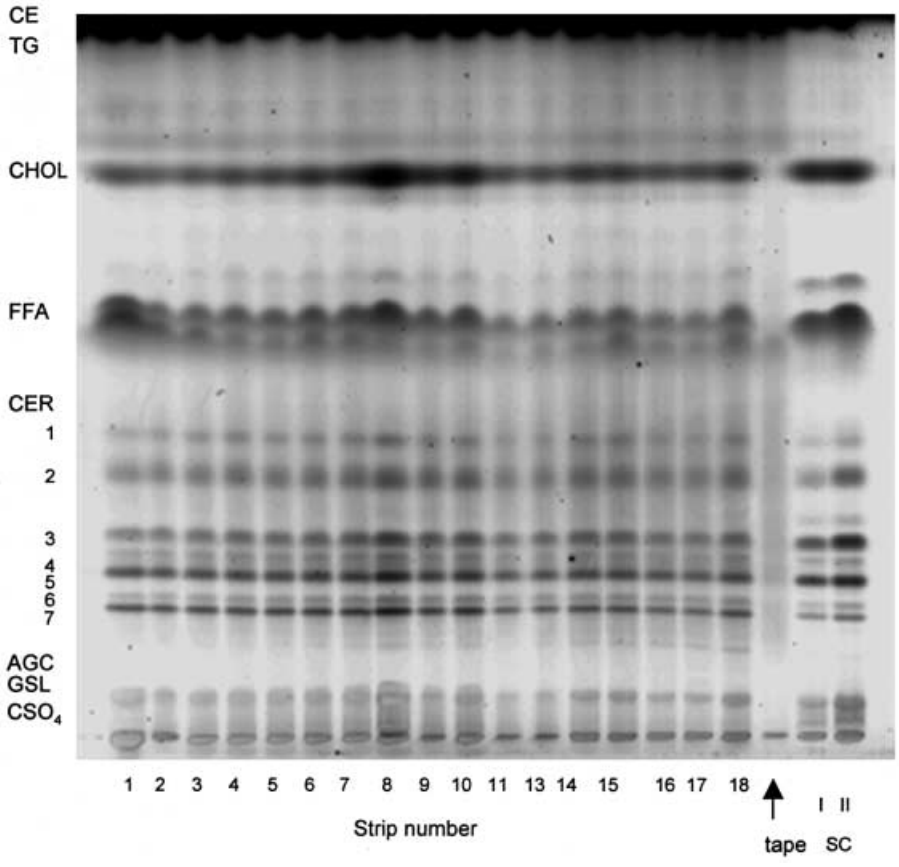


Fig. 2 Tape stripping in combination with high-performance thin-layer chromatography (HPTLC) to assess the stratum corneum (SC) lipid profile. SC samples were collected by consecutive tape strippings followed by extraction with an ethyl acetate:methanol mixture. One-fifth of extracted lipids was applied on the HPTLC plate and separated, as described in "Materials and methods". To establish the background, the same aliquot of extract obtained from a clean tape was run in parallel. For comparison, the lipids extracted from the entire SC were also applied (SC I and SC II)

Results

Choice of standards for lipid quantification

Human CERs consist of at least of eight different fractions that differ in their molecular structure (Wertz 1992; Robson et al. 1994; Stewart and Downing 1999). The differences in density of the bands after charring can be most probably ascribed to the differences in the number of hydroxyl groups (Fig. 1a). With all CER tested the dose-dependent increase in density was observed up to concentration of 1 μg/ml. For practical reasons, synthetic CER (GBCER3) was used for calibration purposes. The dose-dependent increase in density was similar to that seen with natural CER.

The FFA fraction in human SC is composed of fatty acids varying in chain length between C16 and C24, the major fractions being approximately C16:0 (7%), C18:0 (8%), C24:0 (31%), C25:0 (10%), C26:0 (25%). Since the long-chain fatty acids were the major fractions, C24:0 was used for quantification purposes. As shown in Fig. 1b, there is a linear increase in density up to concentration of 1 μg/ml. The linear dose-dependent increase of intensity was seen also for cholesterol sulfate (data not shown). In the latter case the charring has been performed at 80 °C.

Lipid profile in relation to the SC depth

For lipid analysis, the amount of lipids extracted with the ethyl acetate:methanol (20:80) mixture from the 1.5-cm^2 tape was sufficient for quantification of the major SC lipid classes in each individual tape strip (Fig. 2). The use of the ethyl acetate:methanol (20:80) mixture was justified by the finding that the lipid profiles obtained by the extraction of the entire SC with the ethyl acetate:methanol (20:80) mixture or with the chloroform:methanol mixture (Bligh and Dyer 1959) were comparable (Fig. 3). Modifiying the previously used development system (Ponec and Weerheim 1990) by using dichloromethane:ethyl acetate:acetone as the first solvent system allowed us to separate the tape strip components and the SC lipids. As can be seen in Fig. 2, applying the same aliquot of the tape extract did not perturb the lipid analysis. However, it was not possible to obtain the information on the total amount of lipids collected by individual strips by weighing, since during the extraction procedure the tape components are co-extracted with the SC lipids. All major lipid SC lipid classes including CHOL, FFAs and CER can be separated (Fig. 2).

The SC was removed by 18–20 consecutive tape strips. At the end of the stripping procedure, the epidermis revealed a shiny appearance indicating that most of the SC had been removed by this procedure. Using Leukoflex tape did not allow us to perform the lipid extraction and the quantification of corneal material simultaneously (such as

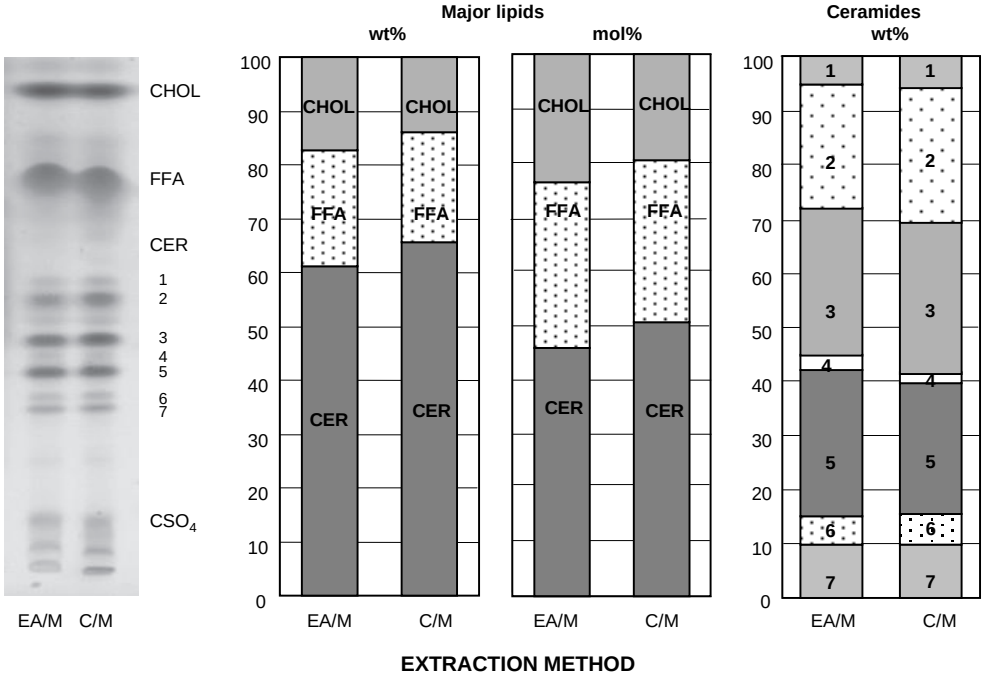


Fig. 3 Extraction procedure does not affect the stratum corneum (SC) lipid profile. The SC sheets were subjected to classic chloroform:methanol extraction (Bligh and Dyer 1959) or to the extraction procedure used for extraction of lipids from the collected tapes (ethyl acetate:methanol = 20:80). The levels of individual lipids were established after high-performance thin-layer chromatography separation and charring

Table 1 Relative amount of major stratum corneum lipids: literature review and new data. *CER* ceramide, *CHOL* cholesterol, *FFA* free fatty acid

Anatomical site	Major stratum corneum lipids (wt.%)			Extraction procedure	Reference
	CER	CHOL	FFA		
Forearm	41.9	13.1	41.9	Topical (hexane:methanol (2:3))	Bonté et al. 1997
Forearm	55.8	26.7	17.6	Topical (acetone:diethylether (1:1))	Lavrijsen et al. 1994
Forearm	43.0	19.0	35.0	Topical (hexane:isopropanol (3:2))	Norlén et al.1999
Forearm	33.0	37.0	23.1	Topical (hexane:ethanol 95:5))	Bleck et al. 1999
Forearm	66.7	15.3	18.1	Topical (hexane:methanol (2:1))	Deffond et al. 1986
Forearm	58.2	21.7	20.1	Topical (chloroform:methanol (2:1))	Deffond et al. 1986
Forearm	42.3	37.5	20.2	Topical (95% ethanol)	Yamamoto et al.1991
Forearm	15.6	20.3	64.1	Chloroform:methanol (2:1)	Motta et al. 1994
Forearm	60.2	17.9	21.9	Scraping and chloroform:methanol (2:1)	Present study
Leg	38.2	25.0	36.8	Chloroform:methanol (2:1)	Rogers et al. 1996
Leg	46.0	26.1	27.9	Chloroform:methanol (2:1)	Fulmer and Kramer 1986
Leg	43.2	33.6	23.2	Chloroform:methanol (2:1)	Lampe et al. 1983
Leg	58.7	19.6	21.7	Topical (hexane:methanol (2:3))	Saint Léger et al. 1988
Face	41.7	27.2	31.0	Chloroform:methanol (2:1)	Deffond et al. 1986
Plantar	70.0	18.1	11.9	Chloroform:methanol (2:1)	Deffond et al. 1986
Plantar	24.1	51.9	24.0	Chloroform:methanol (2:1)	Zellmer and Lasch 1997
Plantar	42.1	34.8	23.1	Chloroform:methanol (2:1)	Melnik et al. 1989
Palm	36.1	32.0	51.8	Topical (hexane:methanol (2:3))	Deffond et al. 1986
Mamma	50.3	32.6	17.0	Chloroform:methanol (2:1)	Lavrijsen et al. 1994
Mamma	61.1	17.3	21.6	Chloroform:methanol (2:1)	Present study

the protein content or the number of removed cells) removed by each strip. Analysis of SC lipids from collected tape strips revealed that the levels of FFA are highest in the uppermost SC layers (up to strip 4). In the following SC layers, the relative amount reaches values ranging from 15% to 20% of major SC lipid fractions (Fig. 4a). CHOL levels are low in the uppermost SC layers (10–15%) and increase to 15–20% in the lower layers (Fig. 4b). The CER fraction is the major SC lipid fraction and as with CHOL, the CER levels are lower in the uppermost two to three SC layers. In the lower SC layers the CER levels reach values ranging between 60–70% (Fig. 4c). A similar SC

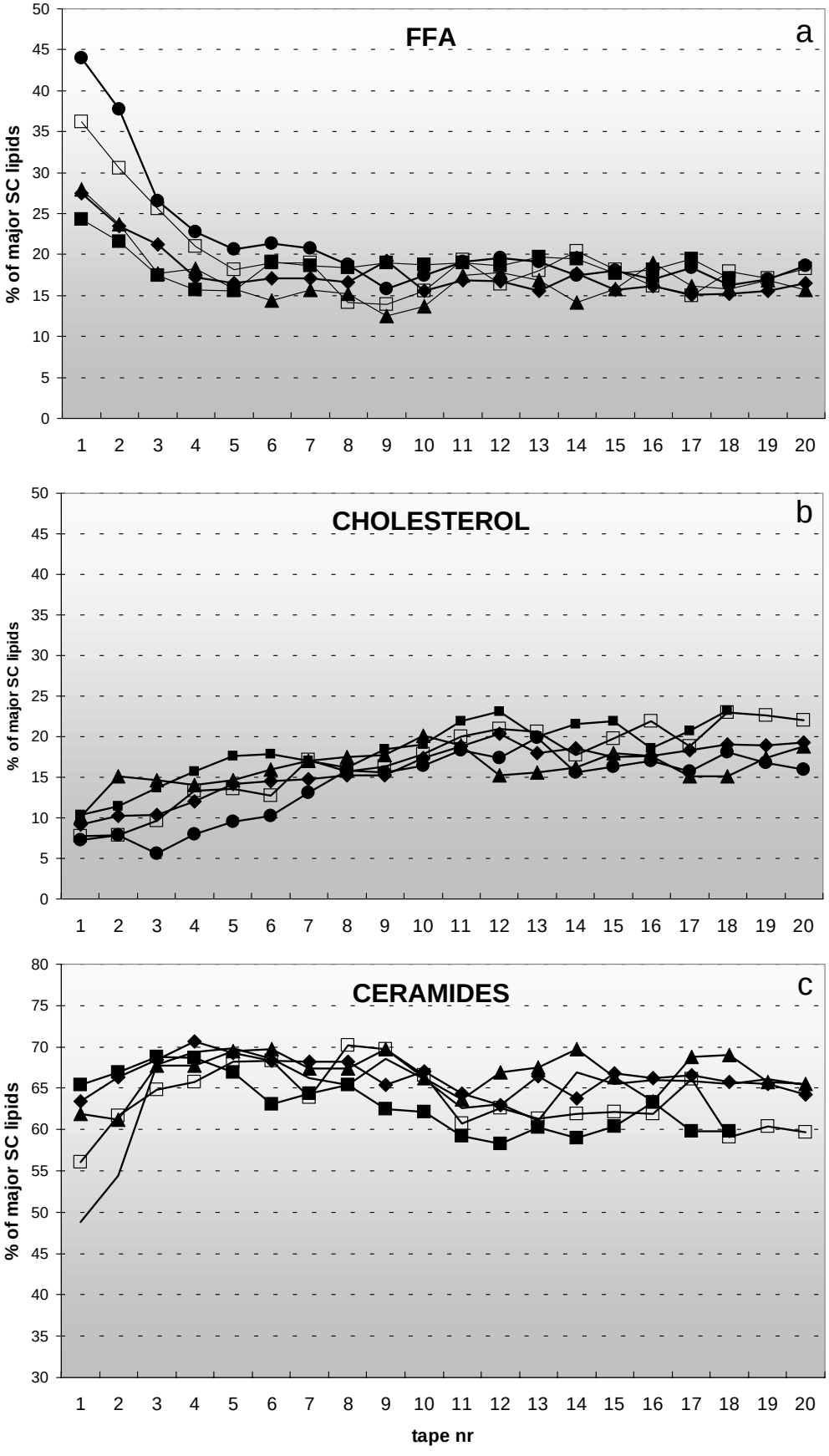


Fig. 4 a–c Inter-individual variation of free fatty acids (*FFA*; **a**), cholesterol (**b**), and ceramide (**c**) with stratum corneum (*SC*) depth. The relative amounts (%wt) of lipids in relation to SC depth are plotted for five different subjects

Table 2 Ceramide (*CER*) profile: literature review and new data

Anatomical site	Percent of total ceramides							Reference
	CER 1	CER 2	CER 3	CER 4	CER 5	CER 6	CER 7	
Leg	8.0	21.0	13.0	4.0	27.0	4.0	22.0	Robson et al. 1994
Leg	8.4	25.8	18.8	22.4[b]		24.6[c]		Paige et al. 1994
Forearm	7.4	18.5	27.8	22.2[b]		24.1[c]		Di Nardo et al. 1998
Forearm	8.7	21.0	19.8	26.6[b]		24.4[c]		Imokawa et al. 1991
Forearm	7.0	20.9	13.3	22.0[b]		9.7	14.2	Long et al. 1985
Forearm	7.0	21.0	13.4	22.2[b]		9.8	13.6	Wertz et al. 1985
Forearm	10.0	21.0	19.0	9.0	17.0	10.0	14.0	Bleck et al. 1999
Forearm	9.9	12.2	20.5	8.9	26.3	21.9[c]		Yamamoto et al. 1991
Mamma	10.0	24.0	21.0	7.0	20.0	6.0	12.0	Lavrijsen et al. 1994
Mamma	5.8	25.1	26.5	2.9	24.2	5.7	9.8	Present study
Forearm[a]	8.6	22.9	18.4	7.1	21.3	6.3	15.6	

[a] Stratum corneum was collected by scraping
[b] Values are the sum of CER 4 and CER 5
[c] Values are the sum of CER 6 and CER 7

lipid profile was found in samples collected by scraping (Table 1).

As shown in Fig. 5, the CER profile does not significantly change with SC corneum depth. The relative amounts of individual CER were similar in all subjects studied, and in samples collected by scraping, the level of CER 1 varied between 8% and 12%, CER 2 between 19% and 22%, CER 3 between 19% and 24%, CER 4 between 5% and 7%, CER 5 between 17% and 22%, CER 6 between 4% and 6%, and CER 7 between about 9% and 16%. The CER profile was similar to that found by other investigators (Table 2).

To determine cholesterol sulfate levels, the HPTLC plates had to be charred to 80 °C and scanned. At this temperature all sterol-containing fractions stain blue and from the densitometric data the amounts of cholesterol sulfate and CHOL could be determined. Using this approach it has been established that the CHOL:cholesterol sulfate ratio was about 95:5. This ratio did not significantly change with the SC depth except for the sample collected with the first strip in which cholesterol sulfate comprised about 1% of total CHOL. In scrapings as well as in lipids extracted from the entire SC with either chloroform:methanol or ethyl acetate:methanol extraction, the cholesterol sulfate amounted to 4–5%. The levels of cholesterol sulfate obtained with plates charred at 160 °were unrealistically high (20% of total CHOL), probably due to co-migration of some until now unidentified lipid fraction.

Discussion

The aim of the present study was to establish the lipid profile in relation to SC depth. Inspection of the literature revealed great variations in SC lipid profile data (Table 1). The relative amounts of CER varied from 16 to 70%, CHOL from 12 to 52%, and FFA from 12 to 64%. At present it is difficult to establish whether these differences can be ascribed to the differences in the method used for the collection of SC samples or for lipid extraction, separation, and quantification or due to differences in the anatomical site, age, skin type, etc. of the subjects participating in those studies. The results of the present study indicate that in SC at the forearm, the CER fraction is the most prominent SC lipid fraction amounting to about 60–70 wt%. The levels of CHOL and FFA were similar, ranging between 15 and 20 wt%. Similar findings have also been made by other investigators (Table 1). It has been suggested (Man-Qiang et al. 1993) that for the formation of a competent SC barrier, the CER, CHOL, and FFA should be present in an equimolar ratio. This means that an equimolar mixture of CER, CHOL, and FFA should contain about 47% CER, 27% CHOL, and 25% FFA, assuming that the average molecular weight of CER is about 700, of FFA 386 and of CHOL at 368. The levels of CER found in the present and a number of other studies (Table 1) are higher than the suggested optimal ones. Due to a great variation in the levels of SC lipids observed by various investigators, it is at present difficult to predict the ideal levels of major SC lipids required for the formation of a competent barrier.

As far as the change in CER content is concerned, Bonté et al. (1997) found a slight decrease with the SC depth (from 31.7% to 29% after 5 strippings). In the present study the levels of CER were found to be lower in the uppermost two to three SC layers but remained unchanged in the lower SC layers, reaching values ranging between 60% and 70%. A similar SC lipid profile was found in samples collected by scraping.

In human SC the lipids present in the intercellular space form a lamellar structure in which the lipids are organized in two lamellar units, with repeat distances of about 13 nm and 6 nm, respectively. Since the long-phase lamellar phase is present in various species (reviewed in Bouwstra et al. 2000) and its presence is unique for the SC, it is important to know which lipids are required for the formation of this phase. From the studies performed with isolated SC lipid mixtures, it became evident that the long phase is formed over a wide range of CER/CHOL/FFA molar ratios provided that CER 1 is present (Bouwstra et al. 1998, 2000). The data of the present study clearly indicate no significant change in the CER profile throughout the entire SC, the relative amounts of various CER fractions being close to levels found by other investigators (Fig. 5, Table 2). In

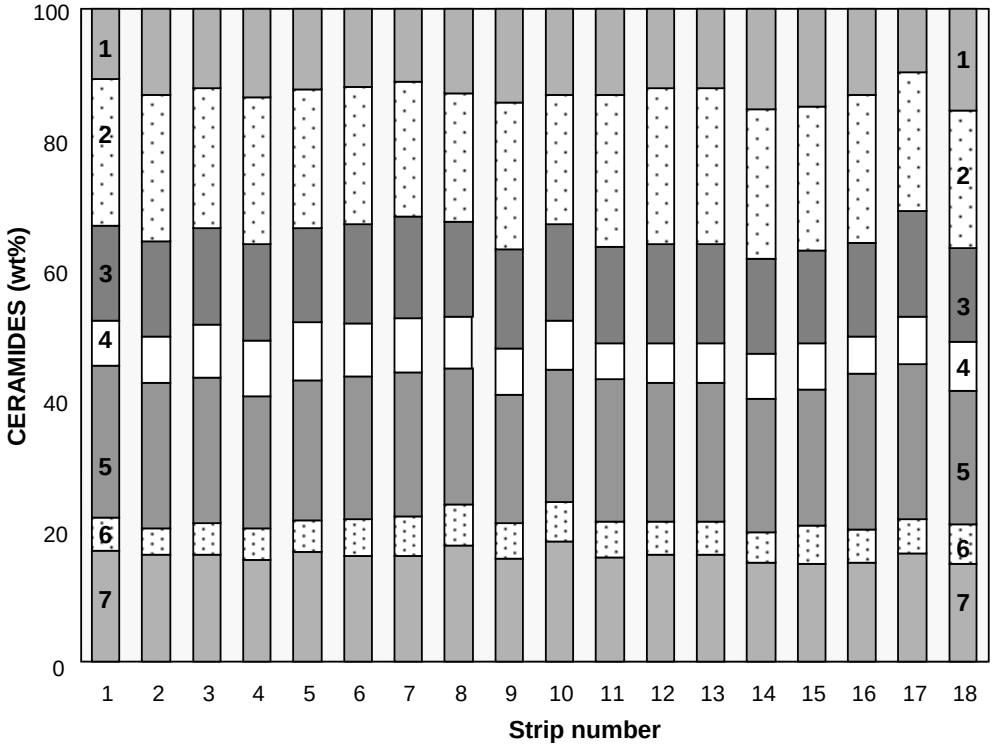


Fig. 5 The ceramide profile does not significantly change with the stratum corneum (*SC*) depth. The lipids collected by sequential stripping were extracted and separated by means of high-performance thin-layer chromatography and the relative amounts of individual ceramide (%wt) are plotted

addition, the relatively small variation in CER profiles found by various investigators (Table 2) can provide an explanation for the consistent finding of the long-phase lamellar structure in a great variety of SC samples.

The increased levels of FFA in the uppermost SC layers have also been found in studies conducted by Lavrijsen at al. (1994) in which the SC samples were collected by sequential scraping or in other studies (Bleck et al. 1999; Bonté et al.1997) in which the SC lipids were collected by topical extraction of SC before and after removal of the uppermost SC layers by stripping. It is not clear whether the higher FFA levels are due to the presence of surface lipids or to an increased degradation of CHOL and CER in the uppermost SC layers.

Cholesterol sulfate that belongs to the minor SC fraction has been shown to affect the SC lateral packing and the appearance of crystalline CHOL in separate domains (reviewed in Bouwstra et al. 2000). In the presence of cholesterol sulfate, CHOL can easily incorporate into the lamellar structure and in addition, the formation of the liquid lamellar phase has also been noticed. The levels of cholesterol sulfate found in the present study are sufficient to assure the proper SC lamellar lipid organization.

In conclusion, the assessing lipid profiles with SC depth, as shown in the present study, can advance investigations on SC barrier function in healthy and diseased skin.

Acknowledgements The research was partially supported by Stiftung Forschung 3R, Switzerland, project no. 66/98.

References

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

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917

Bonté 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 664:311–316

Bonté F, Saunois A, Pinguet P, Meybeck A (1997) Existence of a lipid gradient in the upper stratum corneum and its possible biological significance. Arch Dermatol Res 289:78–82

Bouwstra JA, Gooris GS, van der Spek JA, Bras W (1991) The structure of human stratum corneum as determined by small angle X-ray scattering. J Invest Dermatol 96:1006–1014

Bouwstra JA, Gooris GS, Dubbelaar FER, Weerheim A, Ijzerman AP, Ponec M (1998) The role of ceramide 1 in the molecular organization of the stratum corneum lipids. J Lipid Res 39:186–196

Bouwstra JA, Dubbelaar FER, Gooris GS, Ponec M (2000) The lipid organization in the skin barrier. Acta Derm Venereol Suppl (Stockh) 208:23–30

Deffond D, Saint Leger D, Leveque JL, Agache P (1986) In vivo measurement of epidermal lipids in man. Bioeng Skin 2:71–85

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

Fitzpatrick TB (1988) The validity and practicality of sun-reactive skin type I through VI. Arch Dermatol 124:869–871

Fulmer AW, Kramer J (1986) Stratum corneum lipid abnormalities in surfactant-induced dry scaly skin. J Invest Dermatol 86:598–602

Hou SY, Mitra AK, White SH, Menon GK, Ghadially R, Elias P (1991) Membrane structures in normal and essential fatty acid-deficient stratum: characterization by ruthenium tetroxide staining and X-ray diffraction. J Invest Dermatol 96:215–223

Imokawa G, Hattori M (1985) A possible function of structural lipids in water-holding properties of the stratum corneum. J Invest Dermatol 84:282–284

Imokawa G, Akasaki S, Hattori M, Yoshizuka N (1986) Selective recovery of deranged water-holding properties of the stratum corneum. J Invest Dermatol 87:758–761

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

Lampe MA, Burlingame AL, Whitney J, Williams ML, Brown BE, Roitman E, Elias PM (1983) Human stratum corneum lipids: characterization and regional variations. J Lipid Res 24:120–130

Lavrijsen APM, Higounenc IM, Weerheim A, Oestmann E, Tuinenburg EE, Boddé HE, Ponec M (1994) Validation of an in vivo extraction method for human stratum corneum ceramides. Arch Dermatol Res 286:495–503

Lavrijsen APM, Bouwstra JA, Gooris GS, Weerheim A, Boddé HE, and Ponec M (1995) Reduced skin barrier function parallels abnormal stratum corneum lipid organization in patients with lamellar ichthyosis. J Invest Dermatol 105:619–624

Long SA, Wertz PW, Strauss JS, Downing DT (1985) Human stratum corneum polar lipids and desquamation. Arch Dermatol Res 277:284–287

Madison KC, Swarzendruber DC, Wertz PW, Downing DT (1987) Presence of intact intercellular lamellae in the upper layers of the stratum corneum. J Invest Dermatol 88:714–718

Man-Qiang M, Feingold KR, Elias PM (1993) Exogenous lipids influence permeability barrier recovery in acetone treated murine skin. Arch Dermatol 129:728–738

Melnik BC, Hollmann J, Erkler E, Verhoeven B, Plewig G (1989) Microanalytical screening of all major stratum corneum lipids by sequential high-performance thin-layer chromatography. J Invest Dermatol 92:231–234

Motta S, Sesan S, Monti M, Giuliani A, Caputo R (1994) Interlamellar lipid differences between normal and psoriatic stratum corneum. Acta Derm Venereol Suppl (Stockh) 186:131–132

Norlén L, Nicander I, Rozell BL, Ollmar S, Forslind B (1999) Inter- and intra-individual differences in human stratum corneum lipid content related to physical parameters of skin barrier function in vivo. J Invest Dermatol 112:72–77

Paige DG, Morse-Fisher N, Harper JI (1994) Quantification of stratum corneum ceramides and lipid envelope ceramides in the hereditary ichthyosis. Br J Dermatol 131:23–27

Ponec M, Weerheim A (1990) Retinoids and lipid changes in keratinocytes. In: Packer L (ed) Methods in enzymology. Academic, San Diego, pp 30–41

Ponec M, Weerheim A, Kempenaar J, Mulder A, Gooris GS, Bouwstra J, Mommaas AM (1997) The formation of competent barrier lipids in reconstructed human epidermis requires the presence of vitamin C. J Invest Dermatol 109:348–355

Ponec M, Boelsma E, Weerheim A (2000) Covalently bound lipids in reconstructed human epithelia. Acta Derm Veneorol 80:89–93

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

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

Rougier A, Dupuis D, Lotte C, Roguet R, Schaefer H (1983) In vivo correlation between stratum corneum reservoir function and percutaneous absorption. J Invest Dermatol 81:275–278

Rougier A, Lotte C, Maibach HI (1987) In vivo percutaneous penetration of some organic compounds related to anatomic site in humans: predictive assessment by the stripping method. J Pharm Sci 76:451–454

Saint Léger D, François AM, Léveque JL, Stoudemayer TJ, Grove GL, Kligman AM (1988) Age-associated changes in stratum corneum lipids and their relation to dryness. Dermatologica 177: 159–164

Schaefer H, Redelmeier TE (1996) In vitro approach for predicting percutaneous absorption. In: Schaefer H, Redelmeier TE (eds) Skin barrier. Karger, Basel, pp 129–152

Schürer NY, Elias PM (1991) The biochemistry and function of stratum corneum lipids. In: Elias P (ed) Skin lipids: advances in lipid research, vol 24. Academic, San Diego, pp 27–56

Schwarb FP, Gabard B, Rufli T, Surber C (1999) Percutaneous absorption of salicylic acid in man after topical administration of three different formulations. Dermatology 198:44–51

Stewart ME, Downing DT (1999) A new 6-hydroxy-4-sphingenine-containing ceramide in human skin. J Lipid Res 40:1434–1439

Weigmann H, Lademann J, Meffert H, Schaefer H, Sterry W (1999) Determination of the horny layer profile by tape stripping in combination with optical spectroscopy in the visible range as a prerequisite to quantify percutaneous absorption. Skin Pharmacol Appl Skin Physiol 12:34–45

Wertz PW (1992) Epidermal lipids. Semin Dermatol 11:106–113

Wertz PW, Downing DT (1991) Epidermal lipids. In: Goldsmith LA (ed) Physiology and molecular biology of the skin.Oxford University Press, New York, pp 205–236

Wertz PH, Miethke M, 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

Yamamoto A, Serizawa S, Ito M, Sato Y (1991) Stratum corneum lipid abnormalities in atopic dermatitis. Arch Dermatol Res 283:219–223

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