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Regular paper

Fatty acid profiles in various lipid fractions in the female epidermis. Does the body site and age matter?

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Background: The epidermis forms the barrier between an organism and its external environment. Although one of the major functional elements of the epidermis is the lipid-enriched extracellular matrix, containing mainly ceramides, cholesterol (CHOL) and free fatty acids, the data are limited regarding the lipid profile in the epidermis. The aim of the study was to determine the whole profile of fatty acids (FAs) in the epidermis and to examine any dependence according to the age of the subject and the site on the epidermis. Materials and methods: Epidermis extracts obtained from 10 adults and 6 children were analyzed by gas chromatography-mass spectrometry. Results: In total, 74 FAs in the human epidermis were identified. We observed the highest amounts of neutral lipids (including CHOL) compared to other lipid fractions in the epidermis, regardless of age. However, we detected an age-dependent content of the major lipid fractions, where the main difference was in the levels of polyunsaturated fatty acids. There were also differences in the lipid profile between various sites of the body, e.g. samples from the breast and abdomen were enriched with very long-chain fatty acids compared to the limb. Conclusion: Our research provides novel data concerning the lipid profile in the epidermis, gives further insight into skin biology and proves that the epidermis is a highly dynamic structure.

Keywords: ceramides, cholesterol, epidermis, mass spectrometry, polyunsaturated fatty acids, very long-chain fatty acids

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Abbreviations: AdA, adrenic acid; ALA, α-linolenic acid; ARA, arachidonic acid; BCFAs, branched-chain fatty acids; BMI, body mass index; C atoms, carbon atoms; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; ELOVL4, fatty acid elongase 4; EPA, eicosapentaenoic acid; FAs, fatty acids, FFAs, free fatty acids; FAME, fatty acid methyl esters; CERs, ceramides; CHOL, cholesterol; DGLA, dihomo-γ-linolenic acid; GSPL, glycosphingolipid; HCl, hydrochloric acid; KOH, potassium hydroxide; LA, linoleic acid; MUFAs, monounsaturated FAs; NL, neutral lipid; OCFAs, odd-chain FAs; PUFAs, polyunsaturated FAs; SM, sphingomyelin; SPLs, sphingolipids; SPE, solid-phase extraction; SB, stratum basale; SC, stratum corneum; SG, stratum granulosum; SS, stratum spinosum, UV, ultraviolet; VLCFAs, very long-chain FAs; VLC-MUFAs, very long-chain monounsaturated FAs

INTRODUCTION

The constantly renewing epidermis is the most external stratified part of the skin being known for high lipid content. The epidermis consists of four layers: stratum basale (SB), stratum spinosum (SS), stratum granulosum (SG) and stratum corneum (SC). The external layer – SC, is the most studied, being the actual barrier between the environment and the organism. SC is formed by corneocytes and the lipid-enriched extracellular matrix (Li *et al*., 2016). Analyses of the full-thickness epidermis are limited by the necessity of taking a deeper biopsy.

The content of lipids changes across the epidermis. In SB phospholipids make up 70% of lipids, followed by cholesterol (CHOL) and triacylglycerols (TAG). During the keratinocytes differentiation process, phospholipids are almost totally degraded. Its derivatives are further used in the synthesis of ceramides (CERs), which along with cholesterol (CHOL) and free fatty acids (FFAs) are the main lipid components of SC (Kihara, 2016). The lipid extracellular matrix has a unique, regular ultrastructure precisely controlled by lipid composition.

CERs are synthesized below the SC, in the stratum granulosum and, subsequently, quickly transformed into sphingomyelin (SM) and glucosylceramide converted into more complex glycosphingolipid (GSPL) in order to protect keratinocytes from the cytotoxic effects of CERs (Rabionet *et al*., 2014). SM, glucosylceramide and GSPL create a large group of sphingolipids (SPLs) (Fig. 1), which are present in large amounts in the stratum granulosum and SC layers (Holleran *et al*., 1991). In the epidermis, CERs form a dense bilayer phase and are responsible for hydration and barrier integrity (Assi *et al*., 2020; Ananthapadmanabhan *et al*., 2013). Importantly, CER play a role as a second messenger molecules in signalling pathways within the cells. The presence of FFAs in the more outer layers of the SC is associated with greater fluidity of the lipids close to the surface and lower cohesion, as well as the solubility of cholesterol in lamellar phases (Bonté & Pinguet, 1997; Sahle *et al*., 2015). They also contribute to the acidic pH at the surface of the SC, regulating permeability, inflammation, the antimicrobial barrier and desquamation (Khnykin *et al*., 2011). The last major lipid component of the epidermis is CHOL, which is of pivotal importance for the barrier permeability function. CHOL is abundantly secreted from lamellar bodies (Elias, 2005), and, additionally, is a product of cholesterol sulphate hydrolysis. Cholesterol

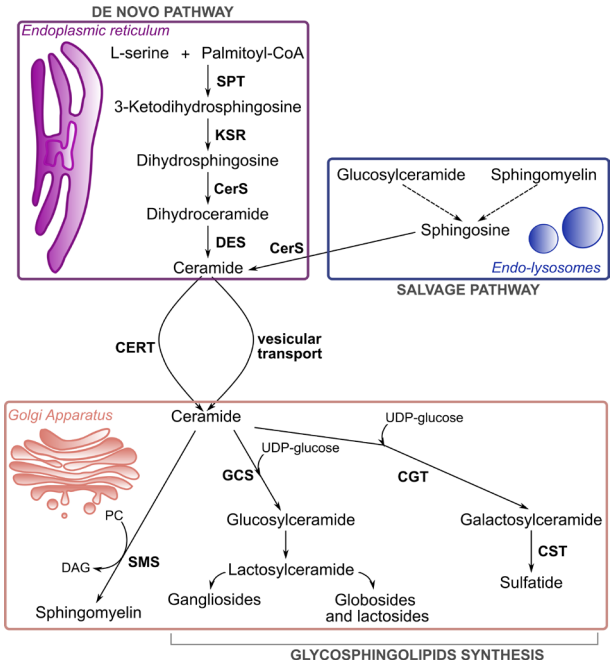

Figure 1. Ceramide – the main component of sphingolipids (modified from Jenkins and others (Jenkins *et al.*, 2009))
The diagram shows the overview of ceramide and sphingolipids synthesis and turnover. CerS, ceramide synthase; CERT, ceramide transfer protein; CGT, ceramide galactotransferase; CST, cerebroside sulfotransferase; DAG, diacylglycerol; DES, dihydroceramide desaturase; GCS, glucosylceramide synthase; KSR, 3-ketosphinganine reductase; PC, phosphatidylcholine; SMS, sphingomyelin synthase; SPT, serine palmitoyl-transferase.

sulphate plays the role of intercellular cement in the SC and must be hydrolyzed to free CHOL in order to enable the shedding of corneocytes (Nardo *et al.*, 1998). Small cholesterol particles are responsible for the protection and condensation of the bilayer (Ananthapadmanabhan *et al.*, 2013).

The major structural component of each complex lipid is a fatty acid (FA). Depending on the length of the aliphatic chain and the number of double bonds, FAs perform specific functions in the organism (Das & Olmsted, 2016; Ananthapadmanabhan *et al.*, 2013; Khnykin *et al.*, 2011; Assi *et al.*, 2020). According to the literature, very long-chain FAs (VLCFAs) and certain polyunsaturated FAs (PUFAs) are essential for the functioning of the epidermis (Chapkin *et al.*, 1986). However, data regarding the lipid profiles in the epidermis are limited. Therefore, the aim of the study was to determine the whole profile of FAs in the epidermis and to examine whether this is dependent on the age of the subject and the site on the epidermis.

MATERIALS AND METHODS

Reagents

Chemicals used for sample preparation: HPLC-grade chloroform (cat. no. 234429154), HPLC-grade methanol (cat. no. 621991154), HPLC-grade dichloromethane (cat. no. 628408152) and diisopropyl ether (cat. no. 384960112), hydrochloric acid (cat. no. 575283115), potassium hydroxide (cat. no. 746800113), acetic acid (cat no. 568733117), acetone (cat. no. 102480151), were purchased from Avantor Performance Materials Poland S.A. (Gliwice, Poland); hexane (cat. no. H/0406/17) was purchased from Fisher Chemicals, Thermo Fisher Scientific (Waltham, MA, USA). The internal standard for the GC-MS analysis 19-methylarachidic acid (cat. no. M5531) and the derivatizing agent 10% boron trifluoride-methanol solution (cat. no. 15716) were purchased from Sigma Aldrich (St. Louis, MO, USA).

Research material

The skin samples were collected from healthy donors of Caucasian origin (with no clinical symptoms of cornification disturbances). 14 samples were obtained from 10 female adults (BMI 25–33, median age: 46.7±11.06) and 6 samples from children (3 males, 3 females, BMI 18.5–25, median age: 6.5±3.5).

In the adult group, the samples were taken from the abdomen (eight samples), breast (three samples) and limb (three samples), while in the children's group, from the limb (five samples) and abdomen (one sample).

The full skin biopsies were collected during surgery under general anaesthesia (in the case of adults: mainly bariatric surgery) and frozen at –80°C. The full-thickness epidermis was mechanically detached in a cryotome (Leica CM3050 S Cryostat, Leica Microsystems Inc, IL,). All steps involving the transportation of samples were performed in dry ice.

We obtained agreement to perform the study from the local Ethics Committee (Opinion number 17/2014 issued by the Ethics Committee at the Institute of Mother and Child in Warsaw).

Lipid analysis

Extraction of total lipids

Total lipids were extracted from epidermis samples with a chloroform-methanol mixture (2:1, v/v) according to Folch and others (Folch *et al.*, 1957). The obtained extract was divided into two parts, one for the total FA profile analysis and one for fractionation by solid-phase extraction (SPE) on a vacuum manifold (AH0-6023, Strata® Phenomenex®, Torrance, CA, USA) dried under a nitrogen stream and stored at –20°C.

Solid-phase extraction

Fractionation of total lipid samples followed the protocol established by Bodennec and others (Bodennec *et al.*, 2000). Extracts were reconstituted in chloroform and loaded onto aminopropyl columns (Strata® NH_2 500 mg, Phenomenex®, Torrance, CA, USA). Elution solvents and the expected composition of each collected fraction are given in Table 1. After elution, all the fractions were dried under a nitrogen stream.

Preparation of fatty acid methyl esters

The total lipid extracts and each fraction collected after SPE were subjected to 3 h of hydrolysis with 0.5 M KOH at 90°C. After incubation, the mixtures were acidified with 0.5 mL 6 M HCl. 1 mL of water was added, free fatty acids (FFAs) were extracted thrice with 1 mL of n-hexane and the organic phase was evaporated under a nitrogen stream. The extracts were then derivatized into fatty acid methyl esters (FAME) with 10% boron trifluoride in methanol solution at 55°C for 1.5 h. Then, 1 mL of water was added and the FAME were extracted with 3×1 mL n-hexane, dried under a nitrogen stream and stored at –20°C until analysis.

Table 1. Characterization of lipid fractions obtained using SPE – used elution solvents and expected lipid composition.

Fraction	Abbreviation	Elution solvent	Expected lipid species
Neutral lipids	NL	5 mL 15% ethyl acetate in n-hexane (v/v)	cholesteryl esters, triacylglycerols, methyl fatty acids, fatty alcohols, 1,2-diacylglycerols, 1,3-diacylglycerols, cholesterol and compounds with similar structure
Ceramides	Cer	4 mL chloroform-methanol (23:1, v/v)	ceramides type III, ceramides type IV, phyto-ceramides
Free fatty acids	FFA	3 mL 5% acetic acid in diisopropyl ether (v/v)	non-esterified normal fatty acids and α-hydroxylated fatty acids
Glycosphingolipids	GSPL	11 mL acetone-methanol (9:1.35, v/v)	neutral glycosphingolipids and globosides
Sphingomyelin	SM	4 mL chloroform-methanol (2:1, v/v)	sphingomyelin, trace amounts of sphingoid bases

GC-MS analysis

The FAME were analyzed with a GC-EI-MS QP-2010SE (Shimadzu, Kyoto, Japan) with chromatographic separation on a Zebron ZB-5MSi capillary column, 30 m×0.25 mm i.d. ×0.25 μm film thickness, (Phenomenex, Torrance, CA, USA). The samples were injected into dichloromethane. The separation parameters were set as follows: injector 310°C, column oven temperature 60–310°C (rate of 4°C/min and hold for 5 min at 310°C), total analysis run time of 67.5 min; helium was used as the carrier gas (column head pressure at 100 kPa). The MS analysis was conducted in full scan mode, with the mass scan range set at m/z 45–700. The electron impact source operated at 70 eV. FAs were identified using the reference standard mixture (37 FAME Mix, Sigma Aldrich, St. Louis, MO, USA). The fatty acids not included in the FAME Mix standard were accurately identified by individual standards purchased from Sigma Aldrich (St. Louis, MO, USA), Cayman Chemical (Michigan, USA) and the reference library NIST 11. The internal standard was 19-methylarachidic acid.

Statistical analysis

The data analysis was performed in SigmaPlot (Systat Software Inc., San Jose, CA, USA). Comparisons between the two groups were made with the Student's *t*-test (for parametric data) and the Mann-Whitney Rank Sum Test (for non-parametric data). For three or more groups, the one-way analysis of variance (ANOVA) was performed, followed by pairwise multiple comparison procedures (Tukey test) for parametric data. Non-parametric data were subjected to the Kruskal-Wallis ANOVA on ranks test followed by the Tukey test. All values are presented as mean ± S.D.

RESULTS

Differences in the fatty acid profile depending on the examined body sites

Research material from adults was collected from three sites of the body: the abdomen, breast and limb (Table 2). In the abdomen, the level of VLCFAs is high-

Table 2. Comparison of FA profile from selected sites of the adult body (% FA content of total lipids).

	Abdomen [mean±SD]	Breast [mean±SD]	Limb [mean±SD]	Abdomen *vs* breast	Abdomen *vs* limb	Breast *vs* limb
8:0	0.001±0.000	0.001±0.000	0.001±0.000	–	–	–
10:0	0.011±0.010	0.011±0.009	0.023±0.015	–	–	–
12:0	0.36±0.13	0.30±0.12	0.58±0.22	–	–	–
14:0	2.61±0.62	2.47±0.29	4.00±0.85	–	0.019	0.031
16:0	21.3±1.17	20.9±1.42	23.6±1.03	–	0.040	0.046
18:0	5.63±1.81	8.38±2.32	5.54±1.14	–	–	–
20:0	0.21±0.11	0.47±0.12	0.21±0.05	–	0.008	0.024
22:0	0.27±0.16	0.73±0.21	0.19±0.07	0.004	–	0.004
24:0	1.17±0.76	2.82±1.16	0.67±0.33	0.027	–	0.018
26:0	1.10±0.87	2.53±1.42	0.43±0.33	–	–	< 0.05†
28:0	0.41±0.34	1.40±1.07	0.14±0.12	–	–	< 0.05†
30:0	0.10±0.10	0.28±0.24	0.023±0.015	–	–	< 0.05†
32:0	0.009±0.009	0.023±0.012	0.001±0.000	–	–	0.028
Total ECFA	**33.2±4.66**	**40.4±5.05**	**35.4±2.33**	**–**	**–**	**–**
9:0	0.001±0.000	0.001±0.000	0.001±0.000	–	–	–
11:0	0.004±0.003	0.003±0.000	0.005±0.004	–	–	–
13:0	0.018±0.007	0.023±0.012	0.040±0.010	–	< 0.05†	–
15:0	0.47±0.15	0.63±0.17	0.99±0.45	–	< 0.05†	–

17:0	0.30±0.10	0.41±0.05	0.36±0.09	–	–	–
19:0	0.026±0.014	0.067±0.029	0.030±0.010	< 0.05†	–	–
21:0	0.020±0.008	0.050±0.026	0.010±0.000	–	–	< 0.05†
23:0	0.081±0.055	0.29±0.12	0.050±0.026	0.002	–	0.003
25:0	0.29±0.24	0.78±0.31	0.18±0.07	0.027	–	0.025
27:0	0.089±0.089	0.35±0.24	0.040±0.026	–	–	–
29:0	0.035±0.033	0.14±0.12	0.017±0.012	–	–	–
31:0	0.004±0.007	0.013±0.006	0.001±0.000	–	–	–
Total OCFA	**1.34±0.65**	**2.75±0.90**	**1.73±0.39**	**< 0.05†**	**–**	**–**
iso 10–M–12:0	0.004±0.005	0.017±0.006	0.023±0.006	–	< 0.05†	–
iso 12–M–13:0	0.016±0.007	0.037±0.025	0.033±0.015	–	–	–
iso 13–M–14:0	0.024±0.009	0.050±0.035	0.063±0.032	–	< 0.05†	–
iso 14–M–15:0	0.054±0.019	0.070±0.017	0.077±0.015	–	–	–
iso 15–M–16:0	0.051±0.014	0.063±0.006	0.060±0.017	–	–	–
iso 16–M–17:0	0.006±0.004	0.015±0.021	0.003±0.000	–	–	–
iso 17–M–18:0	0.058±0.012	0.073±0.015	0.11±0.03	–	0.002	0.046
iso 18–M–19:0	0.003±0.000	0.009±0.010	0.003±0.000	–	–	–
iso 20–M–21:0	0.005±0.003	0.008±0.004	0.005±0.004	–	–	–
iso 22–M–23:0	0.005±0.003	0.013±0.006	0.010±0.000	–	–	–
iso 24–M–25:0	0.015±0.009	0.033±0.025	0.027±0.015	–	–	–
iso 26–M–27:0	0.007±0.004	0.030±0.035	0.008±0.004	–	–	–
***iso* BCFA**	**0.25±0.06**	**0.42±0.15**	**0.43±0.11**	**–**	**–**	**–**
anteiso 11–M–12:0	0.006±0.004	0.010±0.000	0.017±0.006	–	0.003	–
anteiso 12–M–14:0	0.076±0.032	0.15±0.06	0.21±0.11	–	< 0.05†	–
anteiso 13–M–15:0	0.004±0.002	0.010±0.000	0.014±0.014	–	–	–
anteiso 14–M–16:0	0.063±0.017	0.070±0.010	0.093±0.012	–	0.027	–
anteiso 20–M–22:0	0.004±0.002	0.010±0.000	0.005±0.004	–	–	–
anteiso 22–M–24:0	0.014±0.005	0.023±0.006	0.013±0.006	–	–	–
anteiso 24–M–26:0	0.009±0.006	0.020±0.010	0.005±0.005	–	–	0.039
***anteiso* BCFA**	**0.17±0.06**	**0.29±0.08**	**0.36±0.14**	**–**	**–**	**–**
2.6.10–triM–12:0	0.015±0.009	0.050±0.036	0.083±0.055	–	< 0.05†	–
4.8.12–triM–13:0	0.004±0.005	0.017±0.012	0.023±0.015	–	–	–
4.8.12–triM–14:0	0.010±0.005	0.040±0.010	0.060±0.053	< 0.05†	–	–
Tri–methyl–BCFA	**0.030±0.014**	**0.11±0.06**	**0.17±0.12**	**–**	**< 0.05†**	**–**
8–M–16:0	0.006±0.004	0.010±0.000	0.020±0.017	–	–	–
10–M–16:0	0.011±0.006	0.033±0.012	0.060±0.036	–	< 0.05†	–
18–M–24:0	0.005±0.003	0.008±0.004	0.009±0.010	–	–	–
20–M–26:0	0.004±0.002	0.010±0.000	0.002±0.001	–	–	–
Other BCFA	**0.025±0.011**	**0.061±0.016**	**0.091±0.061**	**< 0.05†**	**< 0.05†**	**–**
Total BCFA	**0.48±0.13**	**0.88±0.26**	**1.04±0.43**	**–**	**< 0.05†**	**–**
Total SFA	**35.0±5.34**	**44.0±6.04**	**38.2±2.95**	**–**	**–**	**–**
10:1	0.002±0.003	0.004±0.005	0.004±0.006	–	–	–
12:1	0.008±0.004	0.010±0.000	0.007±0.005	–	–	–
9–14:1	0.30±0.09	0.21±0.07	0.29±0.21	–	–	–
13–14:1	0.084±0.049	0.17±0.09	0.44±0.17	–	< 0.05†	–

16:1	6.24±0.97	5.53±1.52	9.22±3.18	–	–	–
18:1	41.8±5.45	34.8±2.98	39.3±2.99	–	–	–
19:1	0.020±0.008	0.017±0.006	0.023±0.006	–	–	–
20:1	0.62±0.18	0.49±0.09	0.44±0.29	–	–	–
22:1	0.055±0.023	0.080±0.010	0.027±0.015	–	–	0.020
24:1	0.11±0.06	0.23±0.09	0.040±0.030	0.043	–	0.010
26:1	0.005±0.003	0.010±0.000	0.005±0.004	–	–	–
Total MUFA	**49.2±6.23**	**41.5±4.38**	**49.8±3.14**	**–**	**–**	**–**
COCA	0.002±0.003	0.007±0.005	0.013±0.006	–	–	–
CPOA2H	0.19±0.03	0.19±0.03	0.26±0.09	–	–	–
CFA	**0.19±0.03**	**0.19±0.04**	**0.27±0.10**	**–**	**< 0.001**	**< 0.001**
16:2 n–6	0.015±0.005	0.013±0.006	0.008±0.004	–	–	–
18:2 n–6	12.4±1.08	9.83±2.44	9.63±1.10	–	0.041	–
ARA	1.72±0.70	2.80±0.48	1.12±0.08	–	–	< 0.05†
DGLA	0.34±0.10	0.50±0.07	0.24±0.19	–	–	–
20:2 n–6	0.17±0.08	0.13±0.02	0.13±0.10	–	–	–
AdA	0.23±0.06	0.27±0.07	0.17±0.16	–	–	–
Total PUFA n–6	**14.8±1.61**	**13.6±1.90**	**11.3±1.44**	**–**	**0.021**	**–**
18:3 n–3	0.041±0.017	0.030±0.000	0.033±0.006	–	–	–
EPA	0.10±0.03	0.13±0.02	0.087±0.045	–	–	–
DHA	0.35±0.17	0.33±0.04	0.14±0.09	–	–	–
DPA n–3	0.23±0.06	0.27±0.04	0.15±0.10	–	–	–
Total PUFA n–3	**0.72±0.23**	**0.77±0.09**	**0.41±0.23**	**–**	**–**	**–**

Value is mean ±S.D. Content of FA given as a percentage (%). *p*–value Student's *t*–test, † *p*–value Mann–Whitney Rank Sum Test. AdA, adrenic acid (22:4 n–6); ALA, α–linolenic acid (18:3 n–3); ARA, arachidonic acid (20:4 n–6); BCFA, branched–chain fatty acids; CFA, Cyclopropane fatty acids; COCA, Cyclooctanecarboxylic acid; CPOA2H, Cyclopropaneoctanoic A2–hexyl; DGLA, dihomo– γ –linolenic acid (20:3 n–6); DHA, docosahexaenoic acid (22:6 n–3); DPA, docosapentaenoic acid (22:5 n–3); ECFA, even–chain fatty acids; EPA, eicosapentaenoic acid (20:5 n–3); LA, linoleic acid (18:2 n–6); MUFA, monounsaturated fatty acids, OCFA, odd–chain fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Bold type represents the main groups of fatty acids.

er than in the limb, although their highest levels were in the breast. This relationship also applied to VLCFAs with an odd chain, such as 21:0, 23:0 and 25:0. Levels of total *iso*- and *anteiso*-branched-chain FAs (BCFAs) were very similar in the limb and breast, while in the abdomen they were lower, and levels of BCFAs with three methylene groups were higher in the epidermis of the limb. Very long-chain monounsaturated FAs (VLC-MUFAs) were several times higher in the breast. α-linolenic acid (ALA; 18:3 n-3) and linoleic acid (LA; 18:2 n-6) were detected at the highest levels in the abdomen. N-6 PUFAs – arachidonic acid (ARA; 20:4 n-6), dihomo-γ-linolenic acid (DGLA; 20:3 n-6) and adrenic acid (AdA; 22:4 n-6) were almost two times higher in the abdomen compared with the breast and limb, while n-3 PUFAs, eicosapentaenoic acid (EPA; 20:5n-3) and docosapentaenoic acid (DPA; 22:5 n-3) tended to be at higher levels in the breast. The total content of n-3 and n-6 PUFA was almost the same in both the abdomen and breast.

Age-dependent differences in the epidermis fatty acid profile

In the epidermis of adults, every VLCFA and all isoforms of BCFAs were significantly higher (Table 3). Among VLCFAs, we observed the biggest difference in the content of 28:0 (20-fold), 27:0 (17-fold), 30:0 (14-fold), 29:0 (11-fold), 23:0, 24:0 and 25:0 (about 10-fold higher than in children). Greater differences were observed in *anteiso*-BCFA than in *iso*-BCFA, and also among BCFAs with more than one methylene group. In turn, in the epidermis of children, medium- and long-chain MUFAs, including 12:1, 14:1 and 18:1 were higher. VLC-MUFAs were observed at higher levels in the adult epidermis. Also, n-6 and n-3 PUFA in adults were at higher levels. ARA was 5-fold higher, DGLA 3.5-fold, and EPA, DPA, docosahexaenoic acid (DHA; 22:6 n-3) and AdA were almost 3 times higher in adults. ALA and LA were observed at very similar levels in the epidermis irrespective of age.

Analysis of lipid fractions in the human epidermis

Neutral lipid (NL) fractions were detected at higher levels in the epidermis of children, but in adults, significantly more neutral glycosphingolipids were observed. Moreover, there is also a trend towards higher levels of FFAs in the epidermis of adults. In children, the ceramide fraction showed a trend towards higher levels, and the SM fraction was at the same level as in adults (Fig. 2a). Summarizing, the SPL fraction, which contained ceramide as a basic component in the epidermis, constituted 19.5% of total lipids in children and 37.0% in adults (Fig. 1 and Fig. 2b).

Table 3. Differences in FA profile in the epidermis depending on age body (% FA content of total lipids).

	Children [mean±SD]	Adults [mean±SD]	*p*–value
Age	**6.33±3.32**	**47.5±8.50**	**< 0.001**
8:0	0.001±0.000	0.001±0.000	–
10:0	0.008±0.004	0.015±0.013	–
12:0	0.58±0.28	0.37±0.14	–
14:0	3.26±0.85	2.93±0.58	–
16:0	21.8±3.22	21.8±1.25	–
18:0	4.90±1.50	6.21±1.42	–
20:0	0.22±0.09	0.269±0.102	–
22:0	0.068±0.033	0.36±0.18	0.001†
24:0	0.13±0.08	1.44±0.71	0.001†
26:0	0.077±0.056	1.28±0.75	0.001†
28:0	0.023±0.020	0.50±0.32	0.001†
30:0	0.008±0.004	0.11±0.09	0.001†
32:0	0.003±0.004	0.011±0.011	–
Total ECFA	**31.1±4.84**	**35.3±3.33**	**–**
9:0	0.001±0.000	0.001±0.000	–
11:0	0.004±0.003	0.004±0.003	–
13:0	0.013±0.005	0.024±0.012	0.044†
15:0	0.32±0.12	0.59±0.14	0.001
17:0	0.22±0.09	0.34±0.08	0.011
19:0	0.022±0.010	0.036±0.012	0.025
21:0	0.010±0.000	0.022±0.010	0.014†
23:0	0.012±0.004	0.12±0.07	0.001†
25:0	0.022±0.016	0.38±0.22	0.001†
27:0	0.007±0.004	0.12±0.09	0.007
29:0	0.004±0.003	0.044±0.031	0.002†
31:0	0.001±0.000	0.006±0.007	–
Total OCFA	**0.63±0.22**	**1.69±0.53**	**< 0.001**
iso 10-M-12:0	0.001±0.000	0.010±0.008	0.013†
iso 12-M-13:0	0.013±0.005	0.023±0.013	–
iso 13-M-14:0	0.035±0.022	0.037±0.021	–
iso 14-M-15:0	0.060±0.029	0.064±0.014	–
iso 15-M-16:0	0.082±0.038	0.056±0.013	–
iso 16-M-17:0	0.003±0.000	0.005±0.003	–
iso 17-M-18:0	0.062±0.020	0.070±0.019	–
iso 18-M-19:0	0.003±0.000	0.003±0.000	–
iso 20-M-21:0	0.003±0.000	0.005±0.003	–
iso 22-M-23:0	0.003±0.000	0.007±0.004	0.024†
iso 24-M-25:0	0.007±0.004	0.018±0.010	0.014
iso 26-M-27:0	0.003±0.000	0.009±0.003	0.003†
***iso* BCFA**	**0.27±0.11**	**0.31±0.08**	**–**
anteiso 11-M-12:0	0.003±0.000	0.009±0.005	0.011†
anteiso 12-M-14:0	0.073±0.043	0.12±0.05	–
anteiso 13-M-15:0	0.003±0.000	0.006±0.004	–
anteiso 14-M-16:0	0.11±0.06	0.072±0.015	–
anteiso 20-M-22:0	0.003±0.000	0.005±0.003	–

anteiso 22-M-24:0	0.004±0.003	0.016±0.007	0.002†
anteiso 24-M-26:0	0.002±0.001	0.011±0.006	0.003†
***anteiso* BCFA**	**0.20±0.10**	**0.24±0.07**	**–**
2.6.10-triM-12:0	0.008±0.004	0.033±0.028	0.004†
4.8.12-triM-13:0	0.001±0.000	0.010±0.009	0.013†
4.8.12-triM-14:0	0.003±0.000	0.021±0.014	< 0.001
Tri-methyl-BCFA	**0.012±0.004**	**0.064±0.050**	**0.001†**
8-M-16:0	0.004±0.003	0.008±0.003	–
10-M-16:0	0.003±0.000	0.021±0.015	0.003†
18-M-24:0	0.003±0.000	0.005±0.003	–
20-M-26:0	0.002±0.001	0.005±0.003	0.017†
Other BCFA	**0.012±0.003**	**0.039±0.018**	**0.003†**
Total BCFA	**0.50±0.21**	**0.65±0.21**	**–**
Total SFA	**32.2±5.02**	**37.6±3.81**	**0.028**
10:1	0.001±0.000	0.003±0.004	–
12:1	0.018±0.010	0.007±0.004	0.017†
9-14:1	0.47±0.17	0.30±0.12	0.037
13-14:1	0.020±0.009	0.16±0.13	0.001†
16:1	6.95±1.73	6.60±1.81	–
18:1	48.1±3.25	39.4±3.99	0.006
19:1	0.017±0.005	0.019±0.007	–
20:1	0.66±0.13	0.57±0.15	–
22:1	0.032±0.012	0.061±0.024	0.024†
24:1	0.023±0.008	0.12±0.06	0.001†
26:1	0.003±0.000	0.006±0.004	–
Total MUFA	**56.2±4.51**	**47.2±5.05**	**0.006†**
COCA	0.001±0.000	0.005±0.005	–
CPOA2H	0.21±0.05	0.21±0.06	–
CFA	**0.21±0.05**	**0.22±0.07**	**–**
16:2 n-6	0.011±0.005	0.015±0.005	–
LA	10.3±1.89	11.6±1.46	–
ARA	0.38±0.12	1.89±0.69	< 0.001
DGLA	0.11±0.02	0.38±0.11	0.001†
20:2 n-6	0.12±0.05	0.15±0.06	–
AdA	0.083±0.020	0.22±0.07	< 0.001
Total PUFA n-6	**11.0±2.02**	**14.2±2.02**	**0.009**
ALA	0.027±0.012	0.034±0.012	–
EPA	0.038±0.013	0.11±0.03	< 0.001
DHA	0.12±0.10	0.32±0.16	0.011†
DPA	0.078±0.019	0.22±0.05	< 0.001
Total PUFA n-3	**0.27±0.11**	**0.69±0.21**	**< 0.001**

Value is mean ±S.D. Content of FA given as a percentage (%). *p*–value Student's *t*–test, † *p*–value Mann–Whitney Rank Sum Test. AdA, adrenic acid (22:4 n–6); ALA, α–linolenic acid (18:3 n–3); ARA, arachidonic acid (20:4 n–6); BCFA, branched–chain fatty acids; CFA, Cyclopropane fatty acids; COCA, Cyclooctanecarboxylic acid; CPOA2H, Cyclopropaneoctanoic A2–hexyl; DGLA, dihomo– γ –linolenic acid (20:3 n–6); DHA, docosahexaenoic acid (22:6 n–3); DPA, docosapentaenoic acid (22:5 n–3); ECFA, even–chain fatty acids; EPA, eicosapentaenoic acid (20:5 n–3); LA, linoleic acid (18:2 n–6); MUFA, monounsaturated fatty acids, OCFA, odd–chain fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Bold type represents the main groups of fatty acids.

Table 4. Comparison of FA profiles in selected lipid fractions of the epidermis of children and adults.

	Neutral lipids			Free fatty acids			Ceramides			Glycosphingolipids			Sphingomyelin		
FA	Children % FA content [mean±SD]	Adults % FA content [mean±SD]	*p*–value	Children % FA content [mean±SD]	Adults % FA content [mean±SD]	*p*–value	Children % FA content [mean±SD]	Adults % FA content [mean±SD]	*p*–value	Children % FA content [mean±SD]	Adults % FA content [mean±SD]	*p*–value	Children % FA content [mean±SD]	Adults % FA content [mean±SD]	*p*–value
8:0	0.001±0.000	0.001±0.000	–	ND	ND	–	ND	ND	–	ND	ND	–	ND	ND	–
10:0	0.020±0.000	0.043±0.025	–	0.003±0.000	0.012±0.012	–	0.013±0.006	0.008±0.004	–	0.008±0.004	0.008±0.004	–	0.003±0.000	0.005±0.004	–
12:0	0.61±0.20	0.43±0.08	–	0.037±0.021	0.12±0.11	–	0.66±0.39	0.45±0.55	–	0.17±0.06	0.34±0.08	0.039	0.047±0.012	0.12±0.02	0.005
14:0	2.79±0.40	3.63±0.55	–	1.31±0.11	1.71±0.06	0.022	4.30±1.50	3.07±1.94	–	1.84±0.28	1.64±0.12	–	1.95±0.15	1.52±0.08	0.012
16:0	21.18±3.53	23.8±0.39	–	39.5±0.66	34.2±4.24	–	25.8±2.82	16.8±3.03	0.020	36.9±1.98	31.2±1.33	0.014	35.5±1.32	29.8±2.95	0.038
18:0	3.69±1.29	4.09±1.43	–	28.3±1.75	24.3±2.69	–	11.3±0.83	10.6±0.67	–	22.7±1.43	17.5±1.82	0.018	30.1±1.20	27.3±3.42	–
20:0	0.16±0.09	0.11±0.06	–	0.80±0.04	0.85±0.24	–	0.48±0.10	0.96±0.52	–	0.37±0.09	0.52±0.06	–	0.66±0.07	0.55±0.15	–
22:0	0.023±0.015	0.023±0.015	–	1.13±0.18	1.73±0.62	–	0.79±0.14	1.99±1.21	–	0.60±0.14	0.89±0.05	0.025	0.49±0.13	0.57±0.18	–
24:0	0.013±0.006	0.057±0.035	–	3.16±0.63	6.75±1.98	0.052	3.04±0.53	12.0±4.31	0.024	0.67±0.31	1.91±0.51	0.023	0.60±0.04	0.70±0.07	–
26:0	0.001±0.000	0.020±0.010	0.030	2.04±0.89	4.57±0.82	0.049	2.35±0.36	13.0±2.26	–	0.29±0.08	1.07±0.42	0.035	0.23±0.06	0.21±0.06	–
28:0	0.001±0.000	0.007±0.005	–	0.80±0.36	1.53±0.16	–	0.92±0.32	5.13±0.86	0.001	0.14±0.03	0.46±0.21	0.058	0.063±0.068	0.11±0.05	–
30:0	ND	0.001±0.000	–	0.17±0.06	0.30±0.04	–	0.22±0.08	0.65±0.10	0.004	0.023±0.015	0.080±0.020	0.018	0.021±0.016	0.037±0.031	–
32:0	ND	ND	–	0.033±0.015	0.045±0.021	–	0.017±0.006	0.017±0.006	–	0.005±0.004	0.005±0.004	–	0.003±0.000	0.011±0.017	–
Total ECFA	**28.50±4.85**	**32.2±1.43**	**–**	**77.2±2.99**	**76.1±3.23**	**–**	49.9±3.89	64.6±5.85	–	**63.7±2.83**	**55.6±3.07**	**0.028**	**69.7±1.89**	**60.9±6.67**	**–**
9:0	0.001±0.000	0.001±0.000	–	ND	ND	–	ND	ND	–	ND	ND	–	ND	ND	–
11:0	0.001±0.000	0.007±0.005	–	0.001±0.000	0.006±0.006	–	0.007±0.005	0.004±0.005	–	0.004±0.005	0.001±0.000	–	0.001±0.000	0.004±0.005	–
13:0	0.010±0.000	0.030±0.017	–	0.023±0.006	0.045±0.007	0.032	0.057±0.021	0.027±0.015	–	0.027±0.015	0.030±0.010	–	0.023±0.015	0.017±0.006	–
15:0	0.22±0.10	0.61±0.18	0.031	0.77±0.06	1.12±0.42	–	0.63±0.16	0.69±0.09	–	0.71±0.07	1.00±0.31	–	0.99±0.06	0.77±0.15	–
17:0	0.14±0.04	0.25±0.07	–	0.75±0.12	0.78±0.04	–	0.41±0.04	0.49±0.16	–	0.61±0.04	0.79±0.07	0.016	0.66±0.18	0.77±0.16	–
19:0	0.017±0.006	0.013±0.006	–	0.083±0.023	0.11±0.05	–	0.063±0.032	0.12±0.06	–	0.053±0.015	0.083±0.006	0.033	0.070±0.020	0.087±0.012	–
21:0	0.010±0.000	0.010±0.000	–	0.33±0.07	0.39±0.07	–	0.083±0.012	0.22±0.10	–	0.087±0.040	0.077±0.032	–	0.27±0.07	0.19±0.12	–
23:0	0.003±0.000	0.005±0.004	–	0.32±0.14	0.55±0.35	–	0.30±0.19	0.98±0.48	–	0.073±0.012	0.16±0.08	–	0.077±0.029	0.090±0.030	–
25:0	0.001±0.000	0.017±0.006	0.009	0.77±0.17	1.55±0.69	–	0.76±0.22	3.84±0.55	< 0.001	0.11±0.04	0.34±0.12	0.034	0.14±0.03	0.19±0.07	–
27:0	ND	0.007±0.006	–	0.20±0.05	0.35±0.12	–	0.29±0.11	1.33±0.26	0.003	0.027±0.012	0.11±0.05	0.041	0.005±0.004	0.014±0.014	–
29:0	ND	0.001±0.001	–	0.10±0.02	0.11±0.04	–	0.10±0.03	0.39±0.08	0.003	0.013±0.006	0.047±0.012	0.011	0.029±0.044	0.011±0.009	–
31:0	ND	ND	–	0.010±0.000	0.025±0.021	–	0.017±0.012	0.017±0.006	–	0.004±0.005	0.001±0.000	–	0.001±0.000	0.004±0.006	–
Total OCFA	**0.40±0.13**	**0.95±0.25**	**0.028**	**3.35±0.18**	**5.01±1.75**	**–**	2.71±0.74	8.11±1.50	0.005	**1.72±0.05**	**2.64±0.18**	**0.001**	**2.27±0.31**	**2.15±0.33**	**–**

iso 10-M-12:0	0.001±0.000	0.017±0.020	–	0.004±0.005	0.010±0.000	–	0.017±0.006	0.007±0.006	–	0.008±0.004	0.008±0.004	–	0.001±0.000	0.001±0.000	–
iso 12-M-13:0	0.008±0.004	0.033±0.023	–	0.013±0.006	0.025±0.021	–	0.033±0.021	0.020±0.000	–	0.008±0.004	0.013±0.006	–	0.014±0.010	0.008±0.004	–
iso 13-M-14:0	0.023±0.012	0.040±0.010	–	0.047±0.006	0.050±0.000	–	0.16±0.06	0.070±0.010	0.053	0.023±0.006	0.030±0.010	–	0.067±0.015	0.050±0.000	–
iso 14-M-15:0	0.043±0.021	0.070±0.000	–	0.050±0.010	0.10±0.04	–	0.20±0.07	0.083±0.021	0.038	0.040±0.026	0.080±0.036	–	0.10±0.03	0.15±0.04	–
iso 15-M-16:0	0.050±0.017	0.067±0.012	–	0.030±0.000	0.035±0.021	–	0.030±0.010	0.033±0.015	–	0.030±0.017	0.047±0.012	–	0.047±0.012	0.077±0.015	0.053
iso 16-M-17:0	0.001±0.000	0.001±0.000	–	0.010±0.000	0.002±0.001	0.002	0.013±0.006	0.010±0.000	–	0.003±0.000	0.011±0.009	–	0.027±0.029	0.017±0.012	–
iso 17-M-18:0	0.04±0.01	0.067±0.006	0.035	0.13±0.02	0.11±0.03	–	0.18±0.01	0.10±0.01	< 0.001	0.12±0.02	0.14±0.03	–	0.38±0.07	0.24±0.13	–
iso 18-M-19:0	0.003±0.000	0.003±0.000	–	0.007±0.006	0.025±0.007	0.049	0.011±0.009	0.008±0.004	–	0.005±0.004	0.008±0.004	–	0.023±0.015	0.037±0.015	–
iso 20-M-21:0	0.001±0.000	0.001±0.000	–	0.053±0.023	0.080±0.014	–	0.027±0.015	0.010±0.000	–	0.008±0.004	0.010±0.000	–	0.027±0.006	0.027±0.006	–
iso 22-M-23:0	0.001±0.000	0.007±0.005	–	0.013±0.006	0.020±0.014	–	0.030±0.010	0.023±0.015	–	0.010±0.000	0.020±0.010	–	0.027±0.021	0.023±0.015	–
iso 24-M-25:0	0.000±0.000	0.001±0.000	–	0.010±0.010	0.030±0.014	–	0.011±0.009	0.043±0.023	–	0.005±0.004	0.013±0.006	–	0.003±0.000	0.012±0.016	–
iso 26-M-27:0	0.000±0.000	0.001±0.001	–	0.030±0.010	0.035±0.007	–	0.027±0.021	0.010±0.000	–	0.010±0.000	0.011±0.009	–	0.041±0.069	0.002±0.001	–
***iso* BCFA**	**0.17±0.06**	**0.31±0.05**	**0.050**	**0.40±0.08**	**0.52±0.04**	**–**	0.75±0.03	0.41±0.04	< 0.001	**0.27±0.07**	**0.39±0.08**	**–**	**0.75±0.14**	**0.65±0.08**	**–**
anteiso 11-M-12:0	0.001±0.000	0.010±0.010	–	0.004±0.005	0.007±0.005	–	0.013±0.006	0.008±0.004	–	0.005±0.004	0.010±0.000	–	0.003±0.000	0.003±0.000	–
anteiso 12-M-14:0	0.043±0.021	0.12±0.06	–	0.090±0.020	0.16±0.04	–	0.13±0.05	0.073±0.040	–	0.047±0.015	0.060±0.026	–	0.11±0.00	0.103±0.012	–
anteiso 13-M-15:0	0.003±0.000	0.008±0.004	–	0.017±0.006	0.020±0.000	–	0.020±0.000	0.021±0.019	–	0.011±0.009	0.014±0.014	–	0.012±0.016	0.011±0.009	–
anteiso 14-M-16:0	0.080±0.035	0.14±0.01	0.055	0.067±0.006	0.080±0.028	–	0.090±0.017	0.057±0.006	0.034	0.037±0.006	0.037±0.021	–	0.070±0.026	0.12±0.01	0.038
anteiso 20-M-22:0	0.001±0.000	0.001±0.000	–	0.043±0.012	0.040±0.014	–	0.040±0.010	0.027±0.012	–	0.010±0.000	0.008±0.004	–	0.030±0.020	0.013±0.006	–
anteiso 22-M-24:0	ND	0.007±0.005	–	0.050±0.035	0.065±0.049	–	0.067±0.025	0.050±0.036	–	0.010±0.000	0.013±0.006	–	0.033±0.025	0.030±0.026	–
anteiso 24-M-26:0	ND	0.004±0.006	–	0.013±0.006	0.030±0.014	–	0.013±0.006	0.020±0.017	–	0.008±0.004	0.010±0.000	–	0.003±0.000	0.003±0.000	–
***anteiso* BCFA**	**0.13±0.06**	**0.29±0.07**	**0.037**	**0.28±0.07**	**0.40±0.11**	**–**	0.37±0.09	0.26±0.10	–	**0.13±0.01**	**0.15±0.05**	**–**	**0.26±0.07**	**0.28±0.04**	**–**
2.6.10-triM-12:0	0.003±0.000	0.047±0.038	–	0.033±0.006	0.035±0.007	–	0.080±0.017	0.047±0.012	0.050	0.017±0.006	0.027±0.012	–	0.070±0.030	0.023±0.006	0.057
4.8.12-triM-13:0	0.003±0.000	0.011±0.009	–	0.017±0.012	0.025±0.007	–	0.030±0.000	0.017±0.012	–	0.010±0.000	0.017±0.012	–	0.009±0.010	0.008±0.004	–
4.8.12-triM-14:0	0.003±0.000	0.037±0.025	–	0.057±0.021	0.040±0.000	–	0.043±0.021	0.043±0.015	–	0.027±0.006	0.040±0.052	–	0.10±0.02	0.070±0.035	–
Tri-methyl-BCFA	**0.009±0.000**	**0.094±0.070**	**–**	**0.11±0.04**	**0.10±0.01**	**–**	0.15±0.02	0.11±0.04	–	**0.053±0.006**	**0.083±0.075**	**–**	**0.18±0.05**	**0.10±0.04**	**–**
8-M-16:0	0.003±0.000	0.014±0.010	–	0.023±0.006	0.025±0.007	–	0.030±0.000	0.020±0.010	–	0.013±0.006	0.014±0.010	–	0.060±0.044	0.027±0.015	–
10-M-16:0	0.003±0.000	0.033±0.025	–	0.037±0.006	0.050±0.014	–	0.047±0.015	0.047±0.015	–	0.017±0.006	0.040±0.052	–	0.077±0.006	0.037±0.012	0.006
18-M-24:0	ND	0.001±0.000	–	0.017±0.006	0.020±0.000	–	0.033±0.006	0.027±0.029	–	0.003±0.000	0.014±0.014	–	0.005±0.004	0.018±0.014	–
20-M-26:0	ND	0.001±0.001	–	0.007±0.006	0.010±0.000	–	0.008±0.004	0.013±0.006	–	0.003±0.000	0.008±0.004	–	0.001±0.000	0.001±0.000	–
Other BCFA	**0.006±0.000**	**0.049±0.034**	**–**	**0.083±0.021**	**0.11±0.01**	**–**	0.12±0.01	0.11±0.04	–	**0.036±0.010**	**0.076±0.067**	**–**	**0.14±0.04**	**0.082±0.010**	**–**
Total BCFA	**0.32±0.12**	**0.74±0.22**	**0.044**	**0.87±0.20**	**1.12±0.17**	**–**	1.39±0.09	0.88±0.21	0.019	**0.49±0.07**	**0.71±0.27**	**–**	**1.33±0.25**	**1.12±0.09**	**–**

Total SFA	**29.22±4.70**	**33.9±1.68**	**–**	**81.4±3.25**	**82.2±1.31**	**–**	54.0±3.29	73.6±7.29	0.013	**65.9±2.76**	**58.9±2.70**	**0.035**	**73.3±1.34**	**64.1±6.72**	**–**
10:1	0.001±0.000	0.001±0.000	–	ND	ND	–	ND	ND	–	ND	ND	–	ND	ND	–
12:1	0.027±0.021	0.007±0.005	–	ND	0.005±0.007	–	ND	ND	–	ND	ND	–	ND	ND	–
9-14:1	0.51±0.23	0.44±0.25	–	0.033±0.006	0.020±0.014	–	0.25±0.02	0.047±0.023	< 0.001	0.010±0.000	0.010±0.000	–	0.030±0.017	0.023±0.006	–
13-14:1	0.023±0.015	0.27±0.19	–	0.040±0.010	0.060±0.014	–	0.033±0.006	0.073±0.029	–	0.027±0.006	0.037±0.029	–	0.10±0.05	0.040±0.030	–
16:1	7.61±2.61	9.04±3.10	–	2.20±0.30	2.62±0.91	–	6.23±0.89	4.01±1.10	0.053	2.28±0.24	2.88±0.91	–	2.96±0.32	2.10±0.38	0.041
18:1	50.57±2.14	44.2±1.34	0.012	12.3±2.55	10.2±0.36	–	30.9±1.71	13.5±4.33	0.003	16.7±1.86	17.0±1.31	–	14.5±0.55	14.0±1.78	–
19:1	0.020±0.000	0.023±0.006	–	0.023±0.012	0.015±0.007	–	0.080±0.010	0.033±0.006	0.002	0.017±0.006	0.017±0.006	–	0.060±0.035	0.043±0.023	–
20:1	0.74±0.18	0.61±0.23	–	0.087±0.023	0.15±0.06	–	0.15±0.04	0.093±0.031	–	0.15±0.04	0.18±0.04	–	0.39±0.11	0.25±0.09	–
22:1	0.027±0.012	0.020±0.010	–	0.083±0.021	0.47±0.29	–	0.50±0.22	0.50±0.28	–	0.060±0.000	0.037±0.006	0.002	0.25±0.03	0.16±0.06	–
24:1	0.010±0.000	0.010±0.000	–	0.023±0.006	0.065±0.035	–	0.15±0.04	0.30±0.08	0.037	0.22±0.05	0.58±0.22	0.051	0.15±0.03	0.21±0.03	–
26:1	ND	0.001±0.000	–	0.017±0.012	0.035±0.021	–	0.027±0.015	0.020±0.010	–	0.011±0.009	0.014±0.010	–	0.003±0.000	0.005±0.004	–
Total MUFA	**59.5±4.66**	**54.6±3.2**	**–**	**14.8±2.85**	**13.6±1.08**	**–**	38.3±2.30	18.6±5.76	0.005	**19.4±2.13**	**20.7±1.86**	**–**	**18.4±0.84**	**16.8±2.23**	**–**
COCA	0.001±0.000	0.004±0.005	–	0.004±0.005	0.007±0.005	–	0.013±0.006	0.008±0.004	–	0.005±0.004	0.005±0.004	–	0.001±0.000	0.001±0.000	–
CPOA2H	0.21±0.06	0.32±0.12	–	0.077±0.021	0.16±0.10	–	0.18±0.03	0.11±0.07	–	0.083±0.021	0.11±0.03	–	0.13±0.05	0.17±0.03	–
CFA	**0.21±0.06**	**0.32±0.13**	**–**	**0.081±0.026**	**0.17±0.11**	**–**	**0.19±0.04**	**0.12±0.07**	–	**0.09±0.025**	**0.12±0.03**	**–**	**0.13±0.05**	**0.17±0.03**	**–**
16:2 n-6	0.010±0.000	0.010±0.000	–	0.013±0.006	0.015±0.007	–	0.080±0.017	0.047±0.006	0.034	0.013±0.006	0.027±0.006	0.047	0.023±0.006	0.017±0.006	–
LA	10.32±1.54	9.61±1.30	–	2.82±0.54	3.19±0.62	–	6.22±1.21	5.90±1.23	–	9.66±0.90	11.94±0.75	0.028	3.73±0.93	5.67±1.35	–
ARA	0.23±0.06	0.59±0.13	0.012	0.17±0.01	0.18±0.06	–	0.73±0.15	1.24±0.49	–	3.79±0.67	6.20±0.88	0.02	3.34±0.94	9.60±2.49	–
DGLA	0.090±0.010	0.22±0.19	–	0.037±0.012	0.030±0.000	–	0.077±0.040	0.12±0.02	–	0.46±0.15	0.77±0.09	0.037	0.13±0.04	0.34±0.22	–
20:2 n-6	0.13±0.01	0.17±0.10	–	0.020±0.010	0.020±0.000	–	0.030±0.020	0.030±0.010	–	0.077±0.025	0.11±0.05	–	0.050±0.010	0.063±0.029	–
AdA	0.060±0.020	0.19±0.13	–	0.020±0.010	0.007±0.005	–	0.040±0.010	0.063±0.015	–	0.13±0.03	0.25±0.02	0.005	0.30±0.14	0.89±0.35	0.054
Total PUFA n-6	**10.84±1.49**	**10.8±1.84**	**–**	**3.08±0.52**	**3.44±0.57**	**–**	7.18±1.23	7.40±1.59	–	**14.1±1.08**	**19.3±0.81**	**0.003**	**7.58±1.97**	**16.6±4.24**	**0.029**
ALA	0.033±0.012	0.033±0.006	–	0.037±0.006	0.040±0.014	–	0.057±0.012	0.033±0.015	–	0.027±0.006	0.027±0.015	–	0.027±0.012	0.037±0.015	–
EPA	0.033±0.015	0.10±0.01	0.005	0.50±0.25	0.49±0.43	–	0.080±0.040	0.12±0.04	–	0.11±0.03	0.27±0.10	0.049	0.11±0.06	0.32±0.17	–
DHA	0.073±0.031	0.12±0.02	–	0.057±0.055	0.035±0.007	–	0.13±0.04	0.10±0.06	–	0.19±0.07	0.35±0.07	0.052	0.36±0.04	1.30±0.54	0.039
DPA	0.067±0.031	0.18±0.07	0.053	0.027±0.021	0.007±0.005	–	0.077±0.015	0.043±0.015	0.056	0.10±0.01	0.24±0.07	0.026	0.14±0.02	0.63±0.22	0.019
Total PUFA n-3	**0.21±0.04**	**0.43±0.08**	**0.012**	**0.62±0.20**	**0.57±0.44**	**–**	0.35±0.07	0.29±0.03	–	**0.43±0.10**	**0.89±0.22**	**0.028**	**0.64±0.08**	**2.29±0.89**	**–**

Value is mean ±S.D. Content of FA given as a percentage (%). *p*–value Student's *t*–test, † *p*–value Mann–Whitney Rank Sum Test. AdA, adrenic acid (22:4 n–6); ALA, α–linolenic acid (18:3 n–3); ARA, arachidonic acid (20:4 n–6); BCFA, branched–chain fatty acids; CFA, Cyclopropane fatty acids; COCA, Cyclooctanecarboxylic acid; CPOA2H, Cyclopropaneoctanoic A2–hexyl; DGLA, dihomo– γ –linolenic acid (20:3 n–6); DHA, docosahexaenoic acid (22:6 n–3); DPA, docosapentaenoic acid (22:5 n–3); ECFA, even–chain fatty acids; EPA, eicosapentaenoic acid (20:5 n–3); LA, linoleic acid (18:2 n–6); MUFA, monounsaturated fatty acids, OCFA, odd–chain fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids. Bold type represents the main groups of fatty acids.

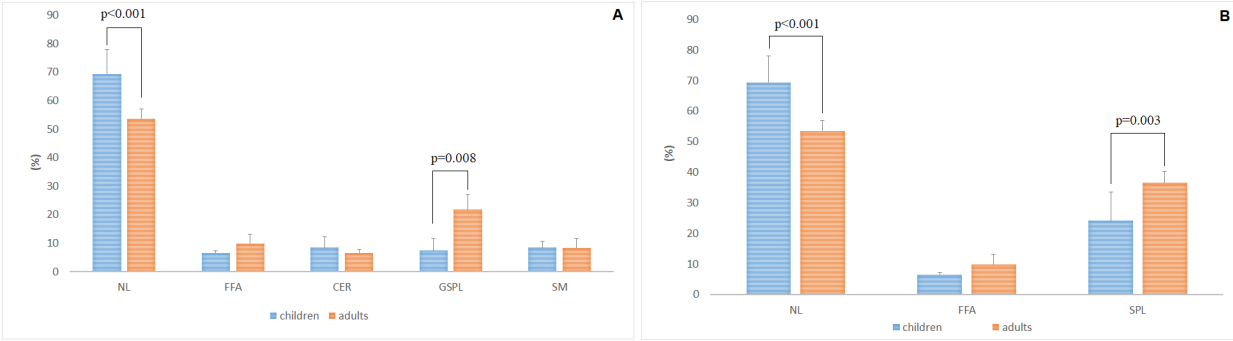

Figure 2. Comparison levels of selected lipid fractions in the epidermis of children and adults.
(**A**) All fractions identified in total lipids; (**B**) CER, GSPL and SM presented as one fraction – SPL. Values are mean ± S.D. *p*-value Student's *t*-test. CER, ceramides; FFA, free fatty acids; GSPL, neutral glycosphingolipids; NL, neutral lipids; SM, sphingomyelin; SPL, sphingolipids.

Fatty acid profiles in lipid fractions

In the epidermis of adults, we observed small but significant differences between the FA profiles in various lipid fractions, whereas in children these differences were more pronounced (Table 4). In adults, a higher content of VLCFAs was found in CER, although the content long-chain FAs (14:0–18:0) was higher in FFA. Also, in this fraction, the total SFA content was the highest compared with other lipid groups. In NL, MUFAs were significantly higher. The level of LA, the main component of ceramides, was higher in GSPL, similar to the levels of DGLA and 20:2n-6. ARA and almost all n-3 PUFA were found to be at levels several times higher in SM fractions, and the levels of VLCFAs 20:0-24:0 were higher in the FFA in the epidermis of children. Only 25:0-30:0 showed a tendency to dominate in CER. Several BCFA representatives were at higher levels in the CER fraction. Similarly, the highest total content of *iso*- and *anteiso*-BCFA was detected in this fraction, and this relationship was not recorded in the epidermis of adults. However, the content of MUFAs was also higher in the NL fraction in children than in adults, and similar to the epidermis of adults, VLC-MUFAs were at higher levels in fractions with ceramides as the main component. The level of LA in the epidermis of children was higher in the NL fraction compared with adults, more n-6 PUFA representatives were observed in GSPL, and n-3 PUFA representatives were at higher levels in the SM and FFA fractions.

Very small differences between adults and children were observed in the NL and FFA fractions (Table 4). We detected significantly higher levels of 25:0, 26:0 and 15:0 in the epidermis of adults. The level of *iso*-BCFA was found to be almost two times higher and *anteiso*-BCFA more than 2-fold in the epidermis of adults compared to children. Similarly, among PUFAs, only the levels of ARA, EPA and DPA were higher, at almost threefold. However, the total content of n-6 PUFAs in the epidermis of children and adults was the same, except for 18:1, which was higher in the epidermis of children. Although ceramide is the main component of CER, GSPL and SM fractions, FA profiles depend greatly on the age of the examined person. The CER and GSPL fractions showed the same relationship for both even- and odd-chain VLCFAs, being several times higher in the epidermis of adults, while among the very long-chain MUFAs, only 24:1 acid was elevated statistically significantly in adults, both in the CER and GSPL fractions. Interestingly, all the *iso*- and *anteiso*-BCFAs and BCFAs with three CH_3 groups were at higher levels in the epidermis of children, which was in contrast to the result of the FA profile analysis in total lipids. In turn, BCFAs in GSPL showed a tendency towards higher levels in the epidermis of adults, but no statistically significant differences were noted. Higher amounts of long-chain MUFAs, including 16:1, 18:1 and 19:1, were also observed in children in the ceramide fraction, and the total content of MUFAs was twice as high in the epidermis of children as in adults. Levels of n-3 and n-6 PUFAs in the CER fraction were similar in children and adults. However, almost all representatives of n-3 and n-6 PUFA, as well as the total content of n-3 and n-6 PUFA, were statistically at higher levels in the epidermis of adults in the GSPL fraction. In summary, in the CER and GSPL fractions, only the content of VLCFAs was similar. In the SM fraction, we observed higher levels of of medium and long-chain FAs, including 12:0, 14:0, 16:0 and 16:1, in the epidermis of children. In turn, higher levels of total n-6 PUFA (2-fold), and almost three times higher levels of DHA and DPA were detected in the epidermis of adults. In SM fraction, the smallest differences were observed.

DISCUSSION

FA are a crucial component of epidermis, across which they compose different types and subtypes of complex lipids. The detailed biochemical composition of the epidermis and its interactions with external and internal factors still remain largely unknown, with the exception of the stratum corneum, which is extensively investigated (Knox & O'Boyle, 2021). The novel analytical methods emerged as well (Dapic *et al.*, 2018; Sjövall *et al.*, 2018). However, according to our best knowledge, none of the studies published thus far presents such a wide profile of FAs including the different lipid fractions in the full-thickness human epidermis. Thus, the results obtained by us broaden the knowledge about lipid composition of the female and childish skin and give further insight into its complexity and plasticity. First of all, we show the changes in lipid profiles in various body sites. In the epidermis of the limb, we observed significantly lower amounts of LA and ARA compared to the abdomen and breast, respectively. These FAs are precursors of pro-inflammatory eicosanoids. Much greater amounts of VLCFAs in the epidermis of the breast and abdomen than in the epidermis of the arm could result from the fact that the epidermis of the limb, which is more ex-

posed to various factors and mechanical injuries, is desquamated more often than the abdominal and breast epidermis, and the synthesis of VLCFAs is not efficient enough. This could also happen with the epidermis of the abdomen – more often exposed to external factors than the sensitive epidermis of the breast. Indeed, it was shown that the epidermis of the arm is two times thicker than the epidermis of the face (Boireau-Adamezyk *et al.*, 2014). Authors compared the lipid content of the epidermis of three different body sites, including an exposed (external) arm site, a protected (internal) arm site and the face, and found the highest lipid content in the face, lower in the protected arm site, and the lowest on the epidermis of the exposed arm site. Hence, differences in the composition of FAs between the epidermis of the abdomen, breast and arm can depend on exposure to external factors, UV radiation, mechanical injuries, microbes, etc. (Boireau-Adamezyk *et al.*, 2014; Elias, 2005). However, such a conclusion cannot be unambiguously driven from the present study. Furthermore, we present the age-dependent content of the major lipid fractions. During the differentiation process, FAs released from phospholipids, the major components of cell membranes, are built into ceramides and glycosphingolipids in the form of FFAs (Castiel-Higounenc *et al.*, 2004). Finally, in the lipids of the extracellular matrix of the SC, only CERs, CHOL and FFAs can be distinguished (Sahle *et al.*, 2015). In our studies, we observed the highest amounts of NL (including CHOL) compared to other lipid fractions in the epidermis, regardless of age: NL: 74.0%, SPL: 19.5%, FFA: 6.5% and NL: 55.0%, SPL: 37.0%, FFA: 8.0% in children and adults, respectively. Similar amounts of NL were observed in other studies (Reinertson *et al.*, 1958; Lampe *et al.*, 1983), where the major component of NL is the CHOL fraction, including 7-dehydrocholesterol, which is a precursor of vitamin D. There could be several reasons for the high level of this fraction. Keratinocytes produce vitamin D with the assistance of UV light (Yousef & Sharma, 2018). Children have a much more active lifestyle than adults and are more frequently exposed to skin breakage. Disturbances of the barrier function of the epidermis result in a marked and rapid increase in epidermal CHOL and FA synthesis (Pappas, 2009), whereas the increased synthesis of CER in a skin lesion is significantly delayed compared to FFAs and CHOL (Pappas, 2009). Other authors also indicated the epidermis as a very important and active place for the synthesis of CHOL, due to high activity and high levels of protein and HMG-CoA reductase mRNA (Feingold, 2009). The CHOL content decreases with age (Harding *et al.*, 1996), which is consistent with the results of our research, and also some drugs, such as statins, decrease epidermal CHOL production (Jia & Mustoe, 2017). The second dominating fraction in adults is GSPL, which is one of the major components of lamellar bodies (Yousef & Sharma, 2018). Additionally, since corneocytes are not rapidly cleared in adults, the process of CER formation from GSPL in the stratum spinosum may be slowed down. Also, CER can be transformed into glucosylceramide in the stratum granulosum (Das & Olmsted, 2016).

FAs are synthesized *de novo* by keratinocytes or come from external sources (Khnykin *et al.*, 2011; Rabionet *et al.*, 2014). The dermal synthesis and elongation of FAs lead to the production of greater amounts of VLCFAs, which have a greater affinity for incorporation into SPL than shorter FAs. SPL containing VLCFAs and free CHOL forms a tight type of membrane barrier (Ansari *et al.*, 1970). The level of VLCFAs in adults is significantly higher in the total lipids and in each lipid fraction individually. Also, we detected an almost two times higher SPL level in adults compared to children (37.0% vs 19.5%, respectively). The packing of lipids impacts the general properties of the epidermal barrier and is dependent on the relative quantities of lipids, as well as the chain length of FAs (Assi *et al.*, 2020). In our analyses that included full-thickness epidermis we found that C16, C18, C24 and C26 FA are abundant in the fraction of FFA and ceramides irrespective of age. The concentration of shortened FA was highest, especially among children's ceramides. Other studies have shown that the most abundant fatty acid chains in the ceramides of SC are C24-26 (Sjövall *et al.*, 2018; Kawana *et al.*, 2020). Moreover, Školová *et al.*, proved that the length of ceramide acyl chain is adversely correlated with epidermal permeability (Školová *et al.*, 2013). Smeden *et al.* shown (in SC analysis) that also the free fatty acid chain length correlates with lipid organization and skin barrier function in atopic eczema (van Smeden *et al.*, 2014) and Netherton syndrome patients. Also, Dapic *et al.*, have shown that patients with Atopic Dermatitis have reduced levels of very long chain FFAs (Dapic *et al.*, 2018). In these disorders there was an increased concentration of shortened FA chains (mainly C16 and C18) (Van Smeden *et al.*, 2014). Importantly, it has long been known that there are also other factors influencing barrier function e.g. FFA saturation level or CE headgroup substitutions. Recently published data show that in the model membrane, the increase of short-chain FFAs fraction led to a reduction of barrier capabilities, while CER subclass composition was less pronounced (Uche *et al.*, 2019). Furthermore, studies by Beddoes *et al.*, proved that extracellular lipid matrix composition is rather solid and barrier function is resistant to a certain threshold of change (Beddoes *et al.*, 2021). This observation, along with the variety of epidermal lipid composition, gives a further idea of how highly complex and dynamic the structure of the epidermis in fact is. The amount of ceramides depends on the age, location of the skin site, season and ultraviolet irradiation (Akutsu *et al.*, 2009; Li *et al.*, 2020; Boireau-Adamezyk *et al.*, 2014; Harding *et al.*, 1996). Other authors have found that children (about 6 years) have more CERs than adults in the epidermis (Li *et al.*, 2020), and we also observed a similar trend. In children, the process of epidermal keratosis occurs much faster and the corneocytes formed in the SC layer become smaller and smaller (Akutsu *et al.*, 2009), so the lipid content in the external matrix is also lower. Smaller corneocytes indicate a more frequent replacement of the callous epidermis in children than in adults. Also, smaller corneocytes are on the part of the body that is more exposed to damaging external factors, such as UV radiation (Akutsu *et al.*, 2009). In adults, corneocytes are much larger and the desquamation process takes place more slowly (Boireau-Adamezyk *et al.*, 2014; Akutsu *et al.*, 2009), therefore the accumulation of VLCFA and ceramide fractions may occur. At the same time, some authors indicate a lower activity of CER synthase in adults (Boireau-Adamezyk *et al.*, 2014). This may explain the faster process of keratinization in children and the accumulation of VLCFAs in adults. VCLFAs predominate in CER and glucosylceramides and only trace levels are observed in SM in the epidermis (Uchida, 2011), which was also observed in our study. The high accumulation of VCLFAs in CER is responsible for the stability of the structure of lamellar bodies (Jennemann & Gröne, 2013).

In the cornification process, short-chain FAs are replaced by highly saturated long- and very long-chain

FAs. Most of them have 20 or more C atoms, and FAs with 22–24C atoms predominate (Khnykin *et al.*, 2011; Wohlrab *et al.*, 2018). LA and ALA, the essential FAs, are to some extent substrates for very long-chain PUFA synthesis. The reason for higher levels of all PUFAs in adults may be due to their anti- and proinflammatory properties (Pakiet *et al.*, 2020). High levels of LA both in the epidermis of children and adults are the result of their dominant presence among PUFAs in the ceramides, being necessary to maintain the proper structure of the epidermis (Khnykin *et al.*, 2011). Also, high content of ARA was observed in our study. ARA is transported from the circulation into keratinocytes (Khnykin *et al.*, 2011), which in contrast to other cells prefer the transport of LA and ARA over non-essential FAs, including oleic acid (Khnykin *et al.*, 2011). An intensive conversion of ARA into eicosanoids was observed in the epidermis, and metabolites of ARA in the skin are associated with inflammation, growth regulation and cell differentiation (Chapkin *et al.*, 1986). LA and ARA are also substrates for oxylipin production, a process regulated by pH (Elias, 2005; Sahle *et al.*, 2015). FFAs cause acidic pH on the surface of the SC, regulating desquamation, the antimicrobial barrier, permeability and inflammation (Khnykin *et al.*, 2011). In our research, the content of n-3 PUFA in the total amount of lipids did not exceed 1%, as in most of the lipid fractions, both in the epidermis of children and adults. However, the total level of n-3 PUFA was higher in the epidermis of adults. One of the explanations may be the fact that n-3 PUFA representatives, e.g. EPA and DHA, are precursors of anti-inflammatory metabolites (Ziboh *et al.*, 2000), which may be more profound in adults as the skin is more exposed to dangerous external factors with age.

VLCFAs are synthesized in the epidermis (Nobusawa *et al.*, 2013) and the elongation of fatty acids in the epidermis is extremely important. Animals with a deficient elongation of VLCFAs, like fatty acid elongase 4 (ELOVL4) deficient mice, show a significantly compromised permeability barrier of the skin and die shortly after birth (Feingold, 2009). An increase in the chain length causes a reduction in membrane fluidity, whereas the degree of saturation increases the membrane order, activating the stability of membrane microdomains (Kihara, 2016). In our study, C24:0 was observed at the highest levels among VLCFAs in all sphingolipid fractions. Kihara showed that SPLs with C24:0 are essential for the activation of the kinase Lyn from the Src family, involved in the membrane microdomain function. Moreover, too high a proportion of long-chain FAs compared to VLCFAs in SPL increases the susceptibility to apoptosis (Kihara, 2016). Furthermore, the human skin is the main site of BCFA synthesis, playing the role in increasing cell membrane fluidity and excreting lipids. Many FAs are present in the skin exclusively, examples being very long-chain hydroxylated FAs and BCFAs, and sometimes odd-chain FAs (OCFAs). They can be products of the catabolism of essential branched-chain amino acids, they can originate from diet or be products of the resident skin microflora (Pappas, 2009). In the epidermis of adults, we detected VLCFA, OCFA, BCFA and PUFA levels several times higher in comparison to the epidermis of children. During the analysis of particular fractions of lipids, we observed very small differences in the content of NL and FFA between the epidermis of children and that of adults. More changes were detected in CER and GSPL fractions, which proves that they are very dynamic and FA profiles in these fractions change with age. We observed much fewer differences when comparing different fractions of lipids in the epidermis of adults than that of children. It seems that the skin of adults is a significantly stabilized formation, while in children the cells proliferate rapidly, the epidermis peels off faster and the lipid metabolism is increased (Boireau-Adamezyk *et al.*, 2014; Plewig, 1970; Akutsu *et al.*, 2009).

Last but not least, it should be noted that the main limitation of our study is the fact that the BMI of our female adult group is placed within the range of 25 to 33, which could potentially affect our results. Nevertheless, biopsies were taken from unaffected skin without any sign of barrier distortion. It must be noted that there are few studies only on skin lipid levels in obese people. In 2017 Horie *et al.*, compared levels of cholesterol and fatty acid as well as epidermal structure in the skin samples of an obesity group (BMI from 25 to 35) and a control group (BMI<25). Their results showed decreased levels of cholesterol and fatty acid in the skin of adults with BMI>22 and increased in the group of low-weight patients (BMI<22). Of note, the authors extracted lipids from samples consisting of the dermis and epidermis (Horie *et al.*, 2018). However, microscopic evaluation showed a thickening of the epidermis in the group of obese females. The authors also verified the expression levels of two inflammation markers TNF-α and IL-6 which were not found to be correlated with BMI (Horie *et al.*, 2018). In another study on obese (BMI 35–50) and non-obese (BMI 18–27) postmenopausal women between the ages of 40 and 70 years, no differences in relation to the thickness of the epidermis between those groups were found, despite existing differences in gene expression profile (Walker *et al.*, 2020). Furthermore, another study by Matsumoto *et al.*, revealed that weight reduction leads to a decrease in epidermal thickness by about 50% of analysed obese males (Matsumoto *et al.*, 2018). It is also worth mentioning that in some previously published studies, the BMI of analyzed patients is not given. For example, in the study of Kendall *et al.*, skin for organ culture models was obtained from four healthy female donors (33–47 years) who, similarly to females analyzed by us, were undergoing elective abdominoplasty surgery (Kendall *et al.*, 2017). In the study of Sjövall and others (Sjövall *et al.*, 2018) where the distribution of skin lipids was investigated, normal abdominal skin from anonymous healthy female donors was obtained during plastic surgery procedures. In this case, it is not precise what kind of plastic surgery was performed and what was the BMI of the females. Considering the limitation of published data referring to the influence of obesity on epidermal structure, function, and, most importantly composition, we are not able to state if, and to what extent, the BMI affected the levels of fatty acids in otherwise clinically healthy epidermal samples. In conclusion, we present unique data comprising a profile of 74 FAs in the normal epidermis of adults and children, both in the total lipids and in five fractions, which gives further insight into skin biochemistry.

Both the proportion of lipid fractions and the profile of FAs in the epidermis differ between children and adults. In particular, the FA profiles in CER and GSPL fractions vary, showing that they are very dynamic and change with age.

The comparison of the total FA profile revealed differences depending on the body site in the adult epidermis. The results of our study suggest that age and body site determine the content of lipid fractions and the profile of FAs in the epidermis.

Declatarions

Conflict of interest. The authors declare no conflict of interest. The results have not been presented elsewhere.

Authorship. A.M.: conceptualization, investigation of funding acquisition, methodology, supervision, writing - original draft preparation. A.P.: investigation, methodology. O.S., K.W., K.O., C.K., N.K., B.H.N.: resources. K.W-T.: conceptualization, funding acquisition, methodology, resources, supervision, draft editing.

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