Progress in Lipid Research 87 (2022) 101180

Contents lists available at ScienceDirect

Progress in Lipid Research

journal homepage: www.elsevier.com/locate/plipres

Review

Very long chain fatty acids

Lucie Kyselová [a], Milada Vítová [b], Tomáš Řezanka [c,*]

[a] Research Institute of Brewing and Malting, Lípová 511, 120 44 Prague, Czech Republic
[b] Institute of Botany, Czech Academy of Sciences, Centre for Phycology, Dukelská 135, 379 01 Třeboň, Czech Republic
[c] Institute of Microbiology, Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague, Czech Republic

A B S T R A C T

Very long chain fatty acids (VLCFAs) are important components of various lipid classes in most organisms, from bacteria to higher plants and mammals, including humans. VLCFAs, or very long chain polyunsaturated fatty acids (VLCPUFAs), can be defined as fatty acids with 23 or more carbon atoms in the molecule. The main emphasis in this review is on the analysis of these acids, including obtaining standards from natural sources or their synthesis. Furthermore, the occurrence and analysis of these compounds in both lower (bacteria, invertebrates) and higher organisms (flowering plants or mammals) are discussed in detail. Attention is paid to their biosynthesis, especially the elongation of very long chain fatty acids protein (ELOVL4). This review deals with papers describing these very interesting compounds, whose chemical, biochemical and biological properties have not been fully explored.

1. Introduction

Fatty acids (FAs) are a ubiquitous component of almost every cell, from the simplest bacteria to higher plants (flowering plants) or mammals. Only microorganisms of the domain (superkingdom) Archaea contain isoprenoid chains in their cell walls, bound by an ether linkage to glycerol. The content of FAs, mostly bound in lipids, whether simple or complex, depends on many factors. Each species and genus is characterized by the composition of their FAs and their quantitative representation also depends on the environment in which the organism lives. Eukaryotic and prokaryotic organisms can differ in their lipid compositions, although this rule does not apply in general. An example is the presence of polyunsaturated fatty acids (PUFAs) in some bacteria [1]. It has long been assumed that bacteria cannot biosynthesize PUFAs because they do not have the appropriate desaturases. However, PUFAs in the bacteria of genus *Shewanella,* and other bacteria, are biosynthesized using a different enzymatic complex, i.e., by polyketide synthase.

Fatty acids usually contain 12–22 carbon atoms (most often 16–20). Palmitic and oleic acids are the most abundant in most organisms. Saturated and unsaturated fatty acids with carbon chains shorter than 12 and longer than 22 carbon atoms are less common. When it comes to very long-chain fatty acids (VLCFAs), the first problem that needs to be addressed is the definition of how long are the VLCFA chains. There are various definitions, the most common being the use of the name VLCFA for acids with 22 or more carbon atoms [2]. With this definition, many problems arise because some FAs with 22 carbons, e.g., docosahexaenoic acid (DHA, 22:6(n-3), all-cis-4,7,10,13,16,19-docosahexaenoic acid) are widespread and are referred to as VCLFAs. Other definitions include FAs with a carbon number of no <23 or 24 carbons [3], or FAs longer than C20 [4]. Unfortunately, VLCFAs are also simply defined as FAs with >18 carbon atoms [5]. The best is to call VLCFAs those acids with a chain length of 23 or more carbon atoms. Sometimes the name ultra-long-chain polyunsaturated fatty acids for 28–36 carbon acids is also used [6]. Based on these definitions and our previous results [7,8], we support the definition of a VLCFAs as comprising 23 or more carbon atoms.

Another problem is the nomenclature of fatty acids. Because FAs were among the most intensively studied compounds in the early days of organic chemistry, they were given trivial names. Some names are listed in Table 1a and 1b. Systematic names are formed by appending the suffix " –oic acid " to the parent hydrocarbon name chain; the carboxylic carbon is taken as C1. According to this system, the FA is denoted by two numbers separated by a colon; the first number is the total number of carbon atoms in the FA and the second is the number of double bond(s). Thus, the designation 24:1 means a C24 acid with one double bond, i.e., tetracosenoic acid. The position of the double bond is given by another number, the position of the double bond being calculated from the COOH (carboxyl group) and at the same time the geometric cis (according to the current nomenclature *Z*) configuration is assumed, which is the most common in natural sources. In the second system (often used in biochemistry), the position(s) of the double bond (single methylene group between the cis or Z double bonds) is calculated from the end, i.e., from the CH_3 group. The position of the terminal double bond can also

* Corresponding author.
E-mail addresses: kyselova@beerresearch.cz (L. Kyselová), milada.vitova@ibot.cas.cz (M. Vítová), rezanka@biomed.cas.cz (T. Řezanka).

https://doi.org/10.1016/j.plipres.2022.101180
Received 12 May 2022; Received in revised form 21 June 2022; Accepted 4 July 2022
Available online 8 July 2022

Table 1a
Examples of some of the more common VLCFAs.

Common name	Systematic name	Short notation
Lignoceric	Tetracosanoic	24:0
Cerotic	Hexacosanoic	26:0
Montanic	Octacosanoic	28:0
Melissic	Triacontanoic	30:0
Lacceroic	Dotriacontanoic	32:0
Ghedoic	Tetratriacontanoic	34:0
Ceroplastic	Pentatriacontanoic	35:0
Nervonic	15Z-Tetracosenoic	15Z-24:1
Ximenic	17Z-Hexacosenoic	17Z-26:1
Lumequeic	21Z-Triacontenoic	21Z-30:1

Table 1b
Examples of some names used for C22 and C24 PUFAs.

Common name	Systematic name	Short notation
Adrenic	7Z,10Z,13Z,16Z-docosa-7,10,13,16-tetraenoic	22:4n-6
Clupanodonic	7Z,10Z,13Z,16Z,19Z-docosa-7,10,13,16,19-pentaenoic	22:5n-3
Ozubondo (Osbond) acid	4Z,7Z,10Z,13Z,16Z-docosa-4,7,10,13,16-pentaenoic	22:5n-6
Cervonic (Docosahexaenoic)	4Z,7Z,10Z,13Z,16Z,19Z-docosa-4,7,10,13,16,19-hexaenoic	22:6n-3
uncommon	9Z,12Z,15Z,18Z,21Z-tetracosa-9,12,15,18,21-pentaenoic	24:5n-3
Nisinic	6Z,9Z,12Z,15Z,18Z,21Z-tetracosa-6,9,12,15,18,21-hexaenoic	24:6n-3

be described as (n), where n is the number of carbon atoms from the last double bond, provided that all other double bonds are interrupted by methylene. Thus, 4,7,10,13,16,19-docosahexaenoic acid is 22:6n-3 acid. Other letters are often used, such as omega (ω) or w. Abbreviations of the "br" type are also used for branched fatty acids (branched FA), often with the determination of the position at which the chain is branched (e.g., br2–24:0, 2-methyl-tricosanoic acid). Special cases that occur relatively frequently are the penultimate (iso, i-) and antepenultimate (anteiso, ai-) isomers (biosynthesized from precursors, i.e., valine, leucine and/or isoleucine). Cyclopropane rings can best be referred to as "c", e.g., c-11-19:0 (lactobacillic acid, 11,12-methylene octadecanoic acid).

2. Occurrence of VLCFAs and VLCPUFAs in nature

Saturated and monounsaturated VLCFAs are present, but mostly as minor components, in almost all organisms. Examples are bacteria [7–9], fungi [7,10], and including yeasts [2,11]. They are also present in photosynthetic organisms such as algae [5,12,13]. In higher plants they are found mostly as wax esters on the surface of leaves, stems, etc. However, there are plant oils where the dominant components are not triacylglycerols, but wax esters. Examples are jojoba oil [14] or ximenia oil [15]. There are relatively large concentration differences in animals such as dinoflagellates (protists) [16–18] or sponges [19,20]. Sponges contain up to tens of percent of total FAs, sometimes called demospongic acids (fatty acids with a delta 5,9 unsaturation system and with a large range of chain-lengths (C16–C32)) [21]. A typical example is 5,9,19–26:3 [21]. Their distribution in vertebrates is diverse (see for example Table 2).

3. Standards

In general, all straight chain saturated fatty acids up to 31:0 are commercially available. Some branched FAs, predominantly iso and/or anteiso FAs, are also commercially available. Of the unsaturated acids, several companies offer nervonic acid (24:1n-9) in their price list, other

Table 2
Tissue occurrence, degree of unsaturation and compound lipids of C28–C36 fatty acids.

Tissue	Degree of unsaturation	Compound lipids	References
Retina	Polyunsaturated	Phosphatidylcholines	[22,23]
Brain	Polyunsaturated	Phosphatidylcholines	[24]
Meibomian gland	Saturated and monounsaturated	Cholesteryl esters	[25,26]
Testis	Polyunsaturated	Triacylglycerols	[27]
		Ceramides	[28]
		Glycosphingolipids	[29]
Epidermis	Saturated and monounsaturated	Acylceramides, glucosylceramides	[30–34]
Vernix caseosa	Saturated and monounsaturated	Ceramides	[35]

acids such as ximenic (26:1n-9) or lumequeic (30:1n-9) FA are no longer commercially available but can be obtained from vegetable oils. However, there are exceptions when some companies offer, for example, 35Z-tetratetracontenoic acid. One very-long chain polyunsaturated fatty acid (VLCPUFA) is also offered, namely 32:6n-3 (14Z,17Z,20Z,23Z,26Z,29Z-dotriacontahexaenoic acid). Companies only offer those FAs that are in demand, and these are FAs up to C22, e.g., docosahexaenoic acid (22:6n-3).

There are two other ways to obtain the standards, and that is either isolation from a natural material or organic synthesis [36,37]. As an example, Mansour [12] used preparative reversed-phase high-performance liquid chromatography RP-HPLC of fatty acid methyl esters (FAMEs) from dinoflagellates of the genus *Scrippsiella* to obtain octacosaoctaenoic acid (28:8n-3). However, obtaining dinoflagellate biomass would probably be more difficult than isolation by RP-HPLC. The same is true for other sources that are not easily obtainable, such as marine sponges. These sponges contain up to tens of percent VLCPUFAs, also often named as demospongic acids (fatty acids with a Δ5,9 unsaturation and chain-lengths of C16–C32) [19]. It is however, necessary to draw attention to a possible source of errors, where, for example, tank settlings derived from cooling sunflower oil at 15 °C consist of a wax in which odd and even VLCFAs up to 30:0 were identified. If the crude oil were analyzed, there is a possibility that the VLCFAs would be considered to be derived from triacylglycerols (TAGs) [38].

4. Enrichment of sample

One way to prove the presence of VLCFAs and/or VLCPUFAs in an organism is to enrich the sample with these acids. A few techniques are available, the first of which is to enrich the sample with the appropriate lipids. In many organisms, VLCPUFAs have been reported to be bound in phosphatidylcholine (PC) [39]. By isolating them after chromatography, mostly by HPLC, the collected fraction can be further analyzed. The second option is the selective isolation of VLCFAs from the total FAs fraction, e.g., separation of FAs by RP-HPLC and collection of the eluent at a certain retention time [13]. Typically, a standard such as 28:0 is used and total eluent with a higher retention time (tR) than the standard is collected. It is thus possible to enrich the sample by up to two orders of magnitude. None of these methods however, can enrich the sample with, for example, an odd VLCFAs. Recent advances in laboratory instrumentation such as liquid chromatography–mass spectrometry (LC-MS) instruments, including high-resolution MS, make it possible to identify odd VLCFAs that are usually in eukaryotic organisms at levels one to two orders of magnitude less than even VLCFAs.

Another option, which can be used also on an industrial scale, is to enrich the VCLPUFAs-containing sample by vacuum distillation. Breivik and Svensen [40] described the use of short path distillation of ethylated herring oil at 113 °C, with a flow rate of 7.4 kg/h and a vacuum of 0.01 mbar. In the distillation residue (as free FAs) after several further

distillations, e.g., 4.1 mL/min and a pressure of 10^{-3}–10^{-4} mbar, the content of 28:8n-3 increased from 0.13% to a total VLCPUFA of 11.17%. In addition, several VLCPUFAs were identified that were below the limit of detection in the original sample, i.e., 28:4n-3, 28:5n-3, 28:5n-3, 30:5n-3, 30:6n-3, etc. Similar results have been obtained with other fish oils, such as sardine and mackerel oils.

Sample enrichment of VLCFAs and/or VLCPUFAs has advantages but also disadvantages. The disadvantage of enrichment is mainly that there may be changes in the representation of individual FAs, so the results of the analysis are rather qualitative, maximally semi-quantitative. On the contrary, the advantages include, in particular, proof of the presence of VLCFAs and/or VLCPUFAs, which in the original sample can reach concentrations up to hundredths of percent of total FAs. Another advantage, if the enrichment is carried out on a preparative scale, e.g., by vacuum distillation, (see above), is to obtain gram and larger amounts of a mixture of VLCFAs and/or VLCPUFAs. This mixture can be used, for example, for biological experiments such as special diets (enrichment of the diet with these FAs).

5. Synthesis

Even VLCPUFAs from C32 to C40 containing six double bonds, e.g., 22Z,25Z,28Z,31Z,34Z,37Z-tetracontahexaenoic acid (40:6n-3), were synthesized by the manganese-based Wurtz type coupling of alkyl bromides with 22-bromo-3Z,6Z,9Z,12Z,15Z,18Z-docosahexaene, which was obtained from docosahexaenoic acid (22:6n-3) [41]. Similarly, 32:6n-3 was synthesized, see Fig. 1 [42]. One source, in which the synthesis of VLCPUFA is described, is the patent of Raman et al. [43]. This patent describes the synthesis of 48 VLCPUFAs from 28:4n-3 to 42:6n-6. In principle, a PUFA is always used as the starter unit(s), e.g., 22:6n-3 and 22:5n-6. Both acids were obtained as a starter unit from commercially available sources. This preparation was an oil obtained by culturing some dinoflagellates, such as *Crypthecodinium cohnii*. Ethyl esters of acids were obtained from the oil (triacylglycerols) after transesterification and fractional distillation, and after further reduction to the corresponding alcohols and other reactions, (see Fig. 2), the corresponding VLCPUFA homologues were obtained. In principle, by selecting a starter unit (PUFA) with the appropriate number of carbon atoms and magnesium bromide, not only even but also odd VLCPUFAs can be obtained.

As an example of the synthesis of VLCFAs and VLCPUFAs, three papers by Rezanka et al. [15,16,44] that describe the synthesis of both saturated VLCFAs (36:0 and 46:0), monoenoic VLCFAs (27Z-34:1 and 25Z-34:1), and VLCPUFAs (36:7n-6 and 36:8n-3) are listed.

6. Analysis

VLCFA analysis is generally not a problem. Two methods can be used to analyze them. First, simple derivatives, such as methyl esters, are analyzed by GC–MS (Table 3). Nitrogen derivatives - pyrrolidides, 4,4-dimethyloxazolines and 3-pyridylcarbinol (formerly picolinyl) esters are used to determine the positions of double bonds, especially in VLCPUFAs. However, analytical techniques for simple derivatives such as methyl esters have not changed much since then. With newly developed stationary phases for GC, there have been two fundamental changes; shortened retention times and increased thermal stability of the stationary phases. The shortening of tR was achieved mainly by reducing column length while maintaining efficiency in the column, and the use of thermostable stationary phases based on polysiloxanes or their derivatives (Dexils, i.e., polycarboranesiloxanes). This is well documented with a few examples.

The second method used more often in recent years is the LC-MS method (Table 4). Examples of analysis by both methods were summarized in a review published 20 years ago [58].

Some other examples of analysis either by GC or HPLC, described for individual organisms, are listed below. A comparison of five derivatization methods for VLCFAs from 24:0 to 36:0 has been reported. These VLCFAs are contained in a lipid fraction called D003, which is derived from sugar cane wax. Differences in derivatization methods and in the quantitative determination of derivatives were minimal [72].

The identification of VLCFAs in the form of cholesterol esters by liquid chromatography-mass spectrometry/atmospheric pressure chemical ionization (LC-MS/APCI) has been described [25]. These esters were obtained from the human meibomian gland. Analysis by single ion monitoring at *m/z* 369 showed that even monoenoic FAs from 24:1 to 30:1 was present. Unfortunately, the position of double bonds was not determined.

Method classes gas chromatography–mass spectrometry (GC–MS), LC-MS and shotgun analysis were compared in the identification of VLCFAs and VLCPUFAs in biological samples [49]. The detection limit for VLCFAs by negative electrospray ionization (ESI) and the corresponding selected ion-monitoring ion $[RCOO]^-$ was 10 nmol/L (~11 pg/μL) for TAG and 8.6 nmoles/L (~8 pg/μL) for PC. The use of LC-MS was suitable for VLCPUFAs with up to eight double bonds due to analysis time and the lower thermal stability of PUFAs compared with saturated FAs, but GC–MS was more suitable for the analysis of saturated VLCFAs. The advantages and disadvantages of using GC and HPLC, mostly associated online with a mass spectrometer, are listed above. Based on already published papers [49] it is clear that the GC method is more suitable for saturated and monoenoic FAs, (see also Table 3). HPLC (LC-MS) is more suitable for VLCPUAFs, mainly due to the lower thermal stability of these FAs. Both tables provide examples of analyzes of biological samples, including important conditions (column type, stationary phase, temperature program, etc. for GC, and column type, stationary and mobile phase, etc. for HPLC) for their identification.

6.1. Identification of double bonds

Analysis of the position of double bonds of both monoenoic and polyenoic FAs is often neglected or not performed at all. Unfortunately, the mass spectra of FAMEs, such as the positional isomers of hexacosanoic acid (e.g., methyl 17-hexacosenoate versus methyl 19-hexacosenoate), are almost completely identical [73]. The retention times of the positional isomers of FAMEs in GC (HPLC) analysis are, of course, different, (see Table 5 for retention times of the positional isomers of methyl octadecenoates) [74]. Similar results of the analysis were also published in the paper by Delmonte et al. [75], where positional isomers were separated on the ionic liquid SLB-IL111 column (100 m × 0.25 mm, 0.2 μm thickness). This highly polar phase separates all positional isomers of octedecenoic fatty methyl esters, the only exception being the cis6/cis7 isomers. Similar results were described in a second paper by Delmonte et al. [76].

For this reason, derivatizations are used to analyze the positions of the double bonds of FAs. During this derivatization, DMDS is added to the double bond(s) and the analysis of these adducts can determine the position of the double bond(s) [11,77]. In addition to DMDS, other derivatives were used (e.g., deuteration or Diels-Alder (4-methyl-1,2,4-triazoline-3,5-dione) adducts for conjugated double bonds); their list can be found, for example, at www page [78].

In the case of the use of nitrogen derivatives, (see below), the mass spectra of these compounds are far more interpretable, and the position of the double bond (s) can be decided on the basis of key ions.

6.1.1. Double bond(s) identification based on key ions of FAMEs

The identification of double bonds in the methyl esters of both FAs with a chain length of C16–C20 and VLCFAs has been published (Table 6). A classic case is the identification of two very common FAs, α- and γ-linolenic acids (18:3n-3 and 18:3n-6, respectively). FAMEs of these acids in the mass spectrum obtained by electron ionization (EI) show characteristic ions, often referred to as “omega ions” (ω-ions), at *m/z* 108 and at *m/z* 150 [79,80]. In the case of less common PUFAs (n-9 and/or n-4), these are ions at *m/z* 192 or at *m/z* 122. There are also ions

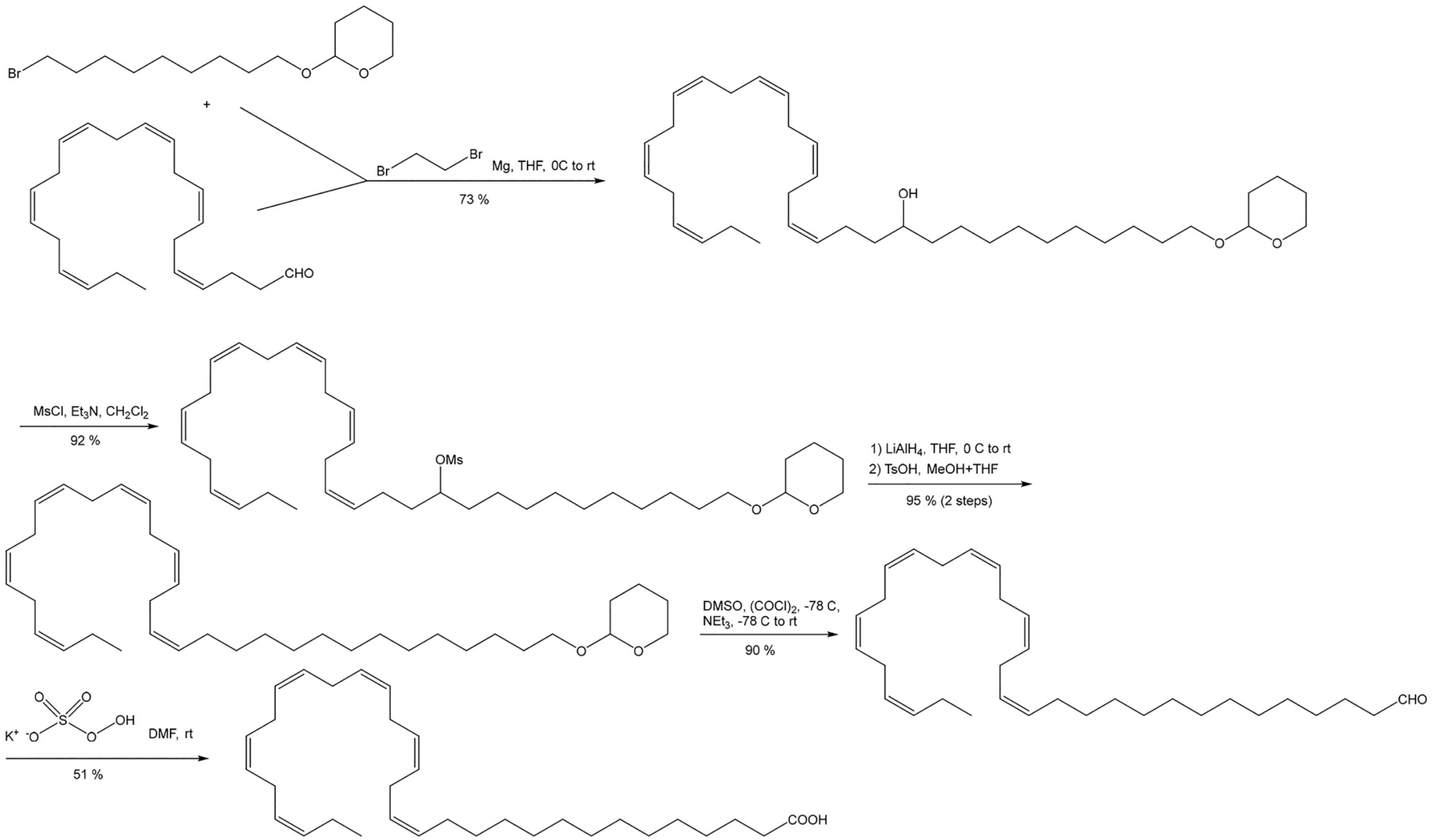

Fig. 1. Synthesis of 32:6n-3 acid by manganese-based Wurtz type coupling of alkyl bromides with 22-bromo-3Z,6Z,9Z,12Z,15Z,18Z-docosahexaene [42].

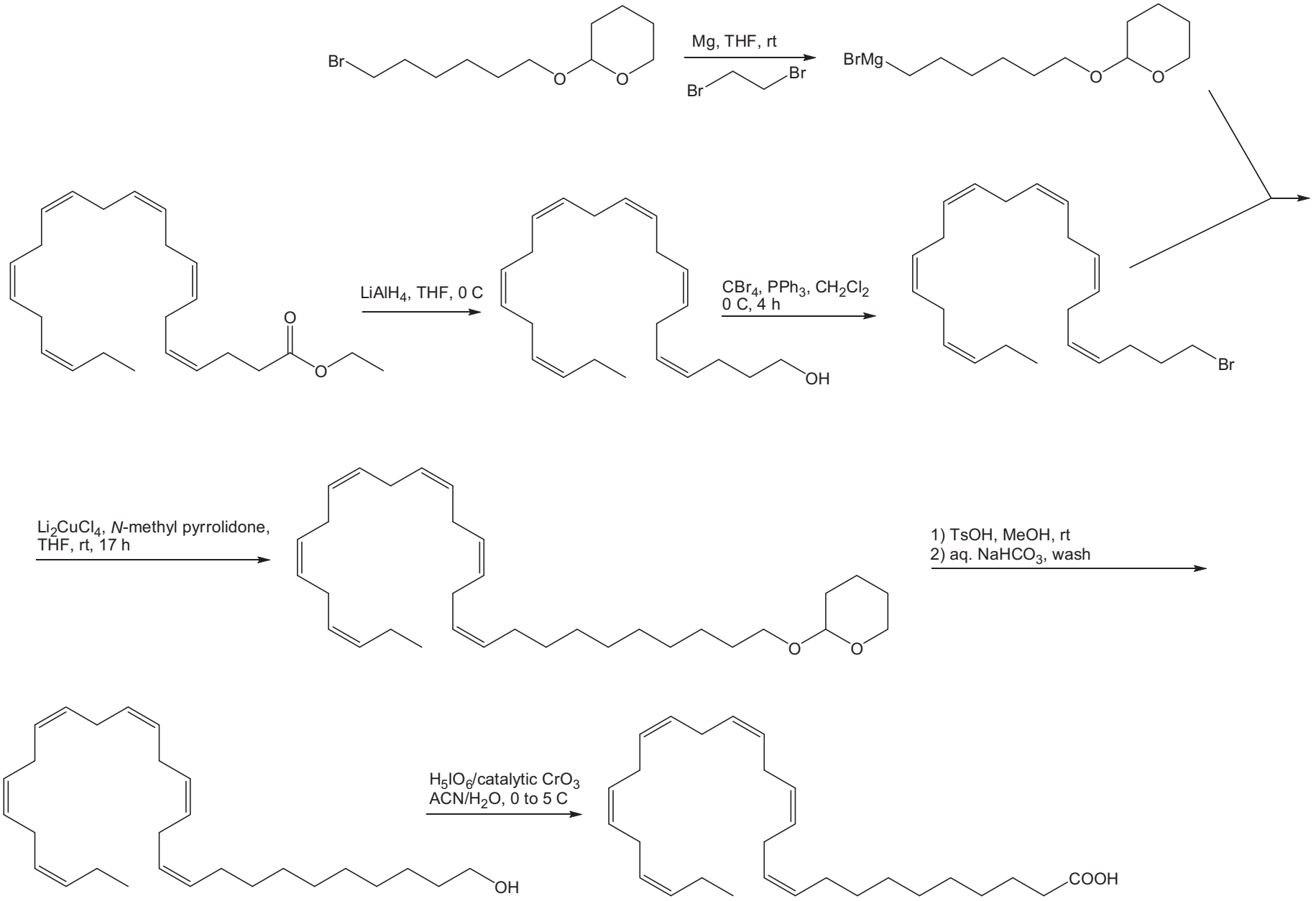

Fig. 2. Synthesis of 28:6n-3 from docosahexaenoic acid and 6-bromohexan-1-ol [43].

Table 3
Survey of literature data on the separation of fatty acids by GC. References not listed in the main article text are given below.

Column	Stat. phase	Temperature program	tR (min)	tR (min)	Detection	Sample	References
Omegawax 250	30 m × 0.25 mm i.d., 0.25 μm	180–270 °C (5 min) at 3 °C /min	22:6 tR = 25		FID	FAMEs from cod liver oil	[45]
Omegawax 250	15 m × 0.1 mm i.d., 0.1 μm	180–270 °C at 40 °C /min	22:6 tR = 2.03		FID	FAMEs from cod liver oil	[45]
Supelcowax 10	60 m × 0.25 mm, 0.25 μm	100 °C (1 min) - 230 °C over 7 min, 230 °C - 280 °C (10 min) over 25 min	22:6 tR = ~ 29.2	28:8 tR = ~ 32.7	GC–MS	crustacea of the order Bathynellacea	[46]
Ultra-1	25 m × 0.25 mm × 0.1 μm	50 °C (5 min) - 320 °C (15 min) at 10 °C /min			GC–MS	cyanobacterial mat of *Calothrix* sp.	[47]
Supelcowax 10	60 m × 0.25 mm, 0.25 μm	100–320 °C at 4 °C /min			GC–MS	dinoflagellates	[17]
SP™-2380	60 m × 0.25 mm, 0.2 μm	150 °C (1 min) - 180 °C at 20 °C/min, 180 °C- 250 °C (1 min) at 2 °C/min	15–24:1 tR = 38.73	21–30:1 tR = 50.29	GC–MS	*Saccharomyces pastorianus*	[11]
SE - 54	15 m × 0.32 mm	70–280 °C at 3 °C /min			FID	meibomian lipids of the mouse	[48]
Supelcowax 10	10 m × 0.10 mm, 0.10 μm	170 °C, at 20 °C/min to 270 °C; (0.5 min)	22:6 tR = 3.05	28:8n-3 tR = 4.90	Positive chemical ionization	algae	[49]
VF-5HT	15 m × 0.32 mm × 0.10 μm	130 °C (1 min); 20 °C/min to 340 °C; 2.5 min	FAME 26:4n6 7.06 min,	FAME 42:6n3 11.51 min	APCI-(Q)TOF MS range *m/z* 50–1000, FWHM 15,000	gene encoding an Elovl4 from gilthead sea bream (*Sparus aurata*) in transgenic yeast (*Saccharomyces cerevisiae*)	[50]
HP-5MS	30 m × 0.25 mm × 0.25 μm	60 °C (1 min); 40 °C /min to 180 °C;10 °C/min to 320 °C (4 min)	FAME 24:6n3 tR = ~13 min	FAME 32:6n3 18.61 min	APCI-(Q)TOF MS range m/z 50–650, FWHM 15,000	fish eyes of European seabass, gilthead seabream, Atlantic salmon, and Senegalese sole	[51]
TRB-WAX	30 m × 0.25 mm × 0.25 μm	50 °C to 220 °C			GC–MS (EI) + SIM mode	recombinant yeast (*S. cerevisiae*); genes from Gilthead seabream and Senegalese sole	[52]
Rxi-5MS	30 m × 0.25 mm × 0.25 μm	60 °C; 5 °C/min to 170 °C; 1 °C /min to 180 °C; 2 °C /min to 240 °C; 4 °C/min to 290 °C; and a hold at 290 °C for 35 min	FAME 24:5n6, tR = 23.27 min	FAME 34:6n3, 58.49 min	EI with full scan and SIM at *m/z* 79, 108, 150	retina of knockout mice with *Elovl4* deleted, fed with 32:6n3 loaded liposomes	[53]
SGE BPX70	60 m × 0·25 mm × 0·25 μm	50 °C for 1.2 min to 170 °C at 4 °C/min, to 200 °C at 0·5 °C/min to 280 °C at 10 °C/min			FID	feeding and experimental diets Atlantic salmon	[54]
DB-23	60 m × 0.32 mm × 0.25 μm	130 °C for 1.0 min, to 170 °C at 6.8 °C/min, to 215 °C at 2.9 °C/min. Held 11.4 min, to 230 °C at 42 °C/min, held for 9.6 min			EI with full scan and SIM at m/z 79.1, 108.1, 150.1	wild, $Nrl^{+/-}$, and $Nrl^{-/-}$ (neural leucine zipper transcription) mice	[55]
Supelcowax 10 and MDN-5S	30 m × 0.25 mm	FAME 160 CC to 260 °C at 2 °C/min, held 25 min			EI 70 eV	abyssal and hadal invertebrates, e.g. 32:5n3 (*Hyalonema* sp.) or 26:7n3 brittle star (*Ophiura irrorata*)	[56]
Rxi-5MS	30 m × 0.25 mm × 0.25 m	60 °C; 10 °C/min to 240 °C; 1 °C/min to 290 °C; and 290 °C for 5 min	24:5n6 tR =23.27 min	34:5n3 tR =59.72 min	liquid chemical ionization and EI modes were used to identify VLC-PUFAs, SIM mode with *m/z* 79, 108, and 150	VLC-PUFAs, C24–C38 from human and mouse retina	[57]

called "alpha ions" (α-ions), which in the above two FAMEs, have a value of 192 Da (for 18:3n-6) and 236 Da (for 18:3n-3). For FAMEs with four or more double bonds, α + 27 Da ions can also be used. If the FAME methylene is not interrupted, then great care must be taken in the interpretation of the mass spectrum and it is better to use other derivatives (see below). In the case of the methyl ester of VLCPUFAs, the abundance ratio of the above ions was also used to determine whether it was n-3 or n-6 FAME (see below).

In a paper of Liu et al. [81], the identification of VLCPUFA methyl esters was performed in EI using a ratio of ions at *m/z* 108 and at *m/z* 151. Thus, over twenty FAs up to 34:6n-3 were identified. Fatty acids from C24 to C34 have always been from tetraenes to hexaenes and n-3 or n-6 (e.g., 32:4n-6, 32:5n-6, 32:5n-3, and 32:6n-3). In their next paper, Liu et al. [57] developed a sensitive GC–MS method for VLCPUFA analysis in 4 mm punches of human retina or a single pair of mouse retinas. VLCPUFAs were also identified in blubber of ringed reals (*Phoca hispida* sp.) caught from Lakes Saimaa and Ladoga, the Baltic Sea, and Spitsbergen. Again, based on the intensity of the ion at *m/z* 150 versus the ion at *m/z* 108, the structure of either n-6 or n-3 was determined [84]. A complete series of homologues was identified, up to 28:7n-3.

In the paper by Garlito et al. [51], the ratio of $C_9H_{13}^+$ (121.1012 Da) and $C_{12}H_{19}^+$ (163.1480 Da) ion abundances was used to determine the position of double bonds in FAME 32:6n-3. Mass spectra were in APCI mode and were measured in high-energy ionization. Two years later, the same team of authors [50] states in their paper: "Unfortunately, the fragmentation observed in high-energy ionization was found to be

Table 4
Survey of literature data on the separation of fatty acids by HPLC. References not listed in the main article text are given below.

Column	Stat. phase	Mobil. phase	tR (min)	tR (min)	Detection	Sample	References
C18	75 × 4.6, 3 μm	gradient water to acetonitrile	22:6 tR = 14.5	28:8 tR = 21.3	MS2	sea anemone+ dinoflagellate	[59]
UPLC C18	150 mm × 2.1 mm, 1.7 μm	water to acetonitrile-2-propanol + aqueous ammonium acetate	22:6 tR = 6.5	28:1 tR = 49.2	positive ESI-MS, MS2	human plasma, porcine brain	[60]
C18	50 mm × 2.0 mm i.d., 3 μm	linear gradient water with 0.1% ammonium acetate and methanol with 0.1% ammonium acetate	22:6 tR = 4.76	28:1 tR = 6.91	LC-FTMS	human meibum	[61]
C18	250 mm × 4.6 mm i.d., 5 μm	isocratic acetonitrile-water (97.5:2.5, *v*/v)	22:6 tR = ~ 6	28:8 tR = ~ 11	evaporative light-scattering detector	dinoflagellate	[12]
preparative LC C18	300 mm × 22 mm, 10 μm	gradient acetonitrile-$CHCl_3$	22:6 tR =15	28:8 tR = 18	evaporative light-scattering detector	dinoflagellate	[12]
C8	2.1 × 50 mm, 1.7 μm	water-acetonitrile +1 mM ammonium acetate	22:6 tR = 4.5	28:1 tR = 9.7	negative ESI-MS	fibroblasts and plasma with peroxisomal diseases	[62]
Supelcosil LC-C18	250 × 2.1 mm, 3 μm	acetonitrile + dichloromethane + propionitrile	22:6 tR = ~ 20	28:7 tR = 22.8	LC-MS with APCI	crustacea of the order Bathynellacea	[63]
C8	150 mm × 4.6 mm, 5 μm	methanol + water	22:0 tR = ~39	24:0 tR = ~47	HPLC-FD	ancient pottery	[64]
HIRPB-250 AM	250 mm × 2.1 mm, 5 μm	acetonitrile + dichloromethane + propionitrile	30:1 tR = 31.5	34:1 tR =37.2	LC-MS with APCI	*Chlorella kessleri*	[13]
C8	150 mm × 3.9 mm, 5 μm	methanol + water	22:0 tR = ~8	26:0 tR = ~15	HPLC-FD	VLCFA spiked in plasma	[65]
C18	150 mm × 4.6 mm, 5 μm	methanol or methanol+2-propanol	22:0 tR = ~10	28:0 tR = ~21	LC-MS with API	standards	[66]
Zorbax ODS and C8	5–6 μm, 250 mm × 4.6 mm and 150 mm × 4.6 mm or 250 mm × 4.6 mm	acetonitrile + aqueous phosphoric acid	22:6 tR = ~ 22	18:0 tR = ~80	UV at 205 nm	bovine brain lipids	[67]
C18	100 × 8 mm, 10 μm	p-dioxane in acetonitrile	26:0 tR = ~26	26:1 tR = ~23	radioactivity flow detector	*Mycobacterium tuberculosis* H37Ra	[68]
HIRPB-250 AM	250 mm × 2.1 mm, 5 μm	acetonitrile + dichloromethane + propionitrile	36:0 tR = ~40	49:0 tR = ~60	LC–MS/APCI	sugar cane wax	[44]
HIRPB-250 AM	250 mm × 2.1 mm, 5 μm	acetonitrile + dichloromethane + propionitrile	22:0 tR = ~ 26	28:1 tR = ~ 31	LC–MS/APCI	*Ximenia* oil	[15]
HIRPB-250 AM	250 mm × 2.1 mm, 5 μm	acetonitrile + dichloromethane + propionitrile	22:6 tR = ~ 22	22:8 tR = ~ 26	LC-MS with APCI	*Amphidinium carterae*	[16]
HIRPB-250 AM	250 mm × 2.1 mm, 5 μm	acetonitrile + dichloromethane + propionitrile	23:6 tR = ~ 17	29:6 tR = ~ 37	LC-MS with APCI	*Amphidinium carterae*	[18]
C18	50 mm × 2.1 mm, 1.7 μm	acetonitrile with pentadecafluorooctanoic acid	20:0 tR = 2.0	20:6 tR = 3.3	LC-MS/MS	VLCFA in plasma	[69]
C 8	10 cm × 2.1 mm, 2.7 μm	gradient acetonitrile - 2-propanol 100:0, linear from 0 min to 6 min to 10:90	30:1n-9 tR = 4.38	28:8n-3 tR = 1.81	LC-MS/MS (APCI)	algae	[49]
BEH C8	100 mm × 2.1 mm × 1.7 μm	ternary gradient 5 mM NH_4HCO_3/water, acetonitrile, isopropanol			selected reaction monitoring $[M + H]^+ \rightarrow 184$	developing and adult mouse retina, PC up to 60:12	[70]
ACQUITY UPLC CSH C18	100 mm × 2.1 mm × 1.7 μm	acetonitrile/water (3:2, v/v) containing 5 mM ammonium formate and acetonitrile/2-propanol (1:9, v/v) containing 5 mM ammonium formate, gradient			ESI^+, multiple reaction monitoring (e.g. 264.3 Da for ceramides)	ceramides, cholesteryl esters, and wax esters containing of mice meibomian glands iso- and anteiso-VLCFAs	[71]
CAPCELL PAK C18	50 mm × 2.1 mm × 3.0 μm	acetonitrile/methanol/water (2:2:1, by vol.) with 0.1% formic acid and 0.028% ammonia (A), isopropanol with 0.1% formic acid and 0.028% ammonia (B), gradient			positive ion mode parallel reaction monitoring	colorectal tissue specimen of the patients 26:0–18:1–18:1, 26:0–18:0–18:2-TAGs, etc.	[3]

insufficient for identification purposes".

6.1.2. Double bond(s) identification using DMOX derivatives

The mass spectra obtained by EI provide a limited amount of information regarding the position of double bonds, or rather, identification by both alpha and omega ions is significant only when position verification is otherwise available. For these reasons, other derivatives of both VLCFAs and VLCPUFAs are used. The most common are nitrogen derivatives, especially 3-pyridylcarbinol, 4,4-dimethyloxazoline (DMOX) and pyrrolidine derivatives.

Eyeballs from calf and from nine human donors were analyzed for VLCPUFAs content by GC–MS of DMOX [82]. DMOX mass spectra of two VLCPUFAs (32:6n-3 and 32:4n-6) yielded intense molecular ions at *m/z* 521 and at *m/z* 525, respectively, which are consistent with a low molecular weight PUFA. Mass intervals of 12 Da were identified instead of the regular 14 Da between two adjacent homologous fragments containing n-1 and n carbon atoms, confirming the presence of a double bond. As with the shorter DMOX derivatives of PUFAs, a gap of 40 Da was found between the two fragments containing n-2 and n + 1 carbon atoms located adjacent to the double bond.

Fukuda and Ando [85] described the analysis of all-cis-5,8,11,14,17,20,23-hexacosaheptaenoic acid (26:7n-3) in roughscale sole *Clidoderma asperrimum* flesh lipids. It was enriched by 26:7n-3 by reversed phase thin layer chromatography (RP-TLC) and silver ion thin-layer chromatography (Ag-TLC) to a final 83% in the fraction containing VLCPUFAs. The structure of this VLCPUFA was demonstrated by the mass spectrum of the DMOX derivative. Again, characteristic gaps were found, clearly proving the structure of the acid.

Two VLCPUFAs were isolated from seven marine dinoflagellates, namely 4,7,10,13,16,19,22-octacosaheptaenoic acid (28:7n-6) and

Table 5
Equivalent chain-lengths (ECLs) of the methyl ester of isomeric C18 monoenoic fatty acids on a Carbowax 20M™ phase [74].

Fatty acid	ECL
3–18:1	18.44
4–18:1	18.19
5–18:1	18.09
6–18:1	18.18
7–18:1	18.14
8–18:1	18.14
9–18:1	18.16
10–18:1	18.19
11–18:1	18.23
12–18:1	18.30
13–18:1	18.37
14–18:1	18.46
15–18:1	18.56
16–18:1	18.84
17–18:1	18.54

4,7,10,13,16,19,22,25-octacosaoctaenoic acid (28:8n-3) [86]. Both DMOX derivatives 28:7n-6 and 28:8n-3 from *Prorocentrum micans* (i.e., dinoflagellate) gave corresponding electron impact (EI) mass spectra, i.e., with the gaps described above.

The occurrence of VLCPUFAs has been reported in lipids from *Acanthamoeba castellanii*, which is a genus of amoebae that are commonly recovered from soil, fresh water, etc. Using MS of DMOX derivatives, the structure of an unusual non-methylene interrupted VLCPUFA, 4,21,24–30:3, was demonstrated, in addition to other VLCFAs (e.g., 5,21–28:2, 21,24–30:2, etc.) [77].

Tanaka et al. [87] analyzed monoenoic and branched (iso and/or anteiso) VLCFAs in the meibomian gland and dry eye. Both n-7 and n-9 FA were identified, e.g., 26:1n-7, 28:1n-7, 30:1n-9, 30:1n-7, as well as branched FAs (i-24:0, i-26:0, i-28:0, ai-25:0, ai-27:0, ai-29:0). The structure of these acids was determined on the basis of DMOX derivatives for monoenoic acids, using mass spectra of 3-pyridylcarbinol esters for branched FAs.

The structure of four hexacosapolyenoic acids from deep brittle stars (e.g., *Ophiopholis aculeata*) caught near the Kuril Islands was confirmed by mass spectra of DMOX derivatives of these VLCPUFAs, i.e., 26:6n-6, 26:7n-3, 26:6n-3 and 26:5n-3 [88].

6.1.3. Identification of double bond(s) using pyridylcarbinol esters

VLCPUFAs from *Amphidinium carterae* (dinoflagellate) have been identified by LC-MS/APCI as 3-pyridylcarbinol esters up to 36:7n-3, 36:7n-6 and 36:8n-3 [16]. For complete confirmation of the structures, two VLCPUFAs were also synthesized (36:7n-6 and 36:8n-3). Odd VLCPUFAs from *A. carterae* were analyzed by RP-HPLC/MS-APCI [18]. Analysis with 3-pyridylcarbinol esters showed the longest to be 33:6n-3. Mass spectra of 29:6n-3 and 29:5n-6 of 3-pyridylcarbinol esters are shown in this paper.

One of the longest and most unsaturated VLCPUFA has been isolated from *Bathynella natans* [89], a crustacean that lives interstitially in groundwater, e.g., in caves. The unpublished mass spectrum of pyridylcarbinol 40:9n-6 acid is shown (Fig. 3).

6.1.4. Identification of double bond(s) as DMDS adducts

The structure of all-cis-5,8,11,14,17,20,23-hexacosaheptaenoic acid (26:7n-3) in *Clidoderma asperrimum* was also confirmed by the mass spectra of dimethyl disulfide (DMDS) adducts of 26:1 six isomers formed by hydrazine hydrogenation of the 26:7n-3 acid [85]. The monoenoic structures of VLCFA, e.g., 21–28:1, were determined using the mass

Table 6
Identification of double bonds.

Column	Length × i.d. × film thickness	Gradient	Range m/z	FWHM	IONIZATION	Určeni polohy C=C	Source	tR 28:5n3 (min)	tR 28:5n6 (min)	tR 28:6n3 (min)	References
fused silica HP-5MS	30 m × 0.25 mm × 0.25 μm	60 °C (1 min), 40 °C/min to 180 °C, 10 °C/min to 320 °C (4 min).	50–650	15,000	APCI or EI cone voltage was set to 20 V	ratio of m/z 121 and m/z 163	fish eye samples	~13	–	~14.5	[51]
Rxi-5MS	30 m × 0.25 mm × 0.25 μm	60 °C; 10 °C/min to 240 °C; 1 °C/min to 290 °C; and 290 °C for 5 min	50–650	?	liquid chemical ionization (LCI) and EI	EI ratio of 108 and 150 m/z	human eyes	34.42	~33.2	33.61	[81]
CP-Sil 88	100 m × 0.25 mm i.d. × 0.2 μm	60 °C (5 min), 15 °C/min to 165 (1 min) 2 °C/min to 225C, held at 225 °C for 17 min	–	–	FID	–	bovine and human retina	~27.5 DMOX			[82]
SGE BPX70	35 m × 0.22 mm × 0.25 um	90 °C (10 min), 3 °C/min to 290 °C, 290 °C (12.3 min)			EI + SIM	m/z ratios 79.1, 108.1, 150.1 in SIM	rat neonatal cardiomyocytes	7.2[a]	7.2[a]		[83]
INNOWAX	?	100 °C, to 240 °C (15 °C/min), 10 min			EI 70 eV + SIM		fibroblasts and peroxisomal diseases	7.2[a]		6.8[a]	[62]
VF-5HT	15 m × 0.32 mm × 0.10 μm	130 °C (1 min), 20 °C/min to 340 °C (2.5 min)	50–1000	15,000	APCI		gilthead sea bream (brain, eye, and gonads)	7.72		7.65	[50]
Supelcowax 10	10 m × 0.10 mm × 0.10 um	170 °C, 20C/min to 270 °C, (0.5 min)			chemical ionization (CI)		algae	4.56		4.58	[49]

[a] position C=C was not determined.

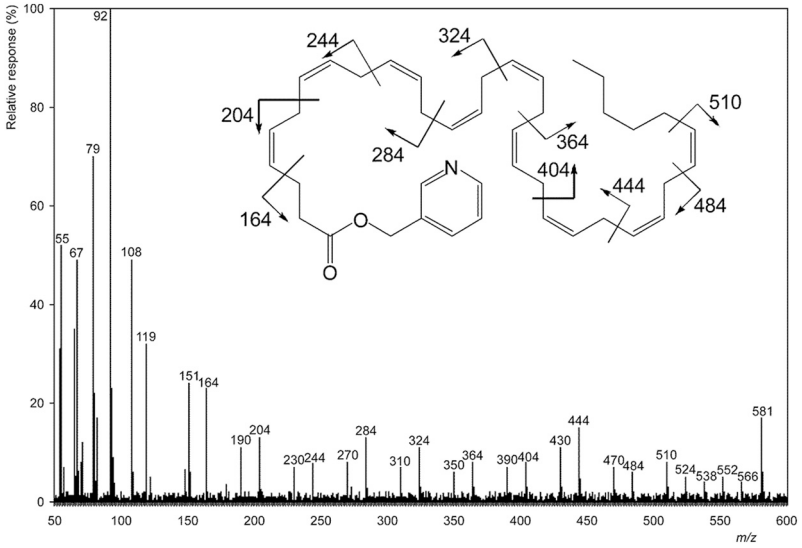

Fig. 3. The unpublished mass spectrum of pyridylcarbinol 40:9n-6 acid from crustacean *Bathynella natans* [89].

spectra of DMDS adducts [77].

In the phospholipids isolated from brewer's yeast, monoenoic VLCFAs up to 30:1 were identified by GC–MS [11]. The structure of both n-7 and n-9 was demonstrated by DMDS adducts and also by 3-pyridylcarbinol esters.

7. Occurrence of VLCFA and or VLCPUFA in mammals (humans) and diseases

The presence of VLCPUFA in specialized mammalian organs or tissues was discovered in the second half of the 1980s. A brief summary of known occurrences of VLCFA-containing lipids is given in Table 2. Examples of lipid structures are shown in Fig. 4. Sharp et al. [90] published the structure and lipid distribution of VLCPUFAs in the brain of peroxisome-deficient patients, i.e., having Zellweger syndrome. Using ^{1}H NMR and fast atom bombardment mass spectrometry (FAB MS), he proved that the main phospholipid containing VLCPUFAs was phosphatidylcholine. In the same year, Aveldano and Sprecher [22] published EI-MS of 32:4n-6, 32:5n-3, and 32:6n-3. Poulos et al. [91] separated from PC using TLC, a minor phospholipid (designated as 'X') that migrated between PC and phosphatidylethanolamine (PE) zones and contained VLCPUFAs. In another mammalian biological sample, spermatozoa, VLCPUFAs (e.g., 30:4n-6, 30:6n-3, 32:6n-3 and 34:6n-3) of sphingomyelins have been identified [92]. This fact was later confirmed, see e.g. the paper of Furland et al. [28], where they analyzed the head of mammalian spermatozoa and demonstrated the presence of VLCPUFAs as major acids of sphingomyelins and ceramides. Further, Furland et al. [27] identified VLCPUFAs in murine testicular triacylglycerols and cholesterol esters. With the advantage of constantly evolving analytical methods, odd VLCPUFAs were also identified, e.g., 29:5n-6, 31:5n-6 and 33:5n-6, albeit in quantities below one ppm of total FAs.

By comparing the materials and methods used, Aveldano [93] and Berdeaux et al. [82] clearly show what progress has been made in lipid analysis over 20 years. While Aveldano used a series of separation and other methods, i.e., TLC on silica gel, phospholipase C hydrolysis, derivatization of diacylglycerols, separation by Ag-TLC and RP-HPLC, Berdeaux et al. used LC-ESI-MS normal-phase chromatography in positive and/or negative ESI for separation of lipid classes from human and bovine retina, followed by analysis of these lipid classes using tandem MS. Several dozen molecular species of the following lipid classes were identified: PE, phosphatidylinositol (PI), PS, PC, lysophosphatidylcholine (LPC) and sphingomyelin (SM) including plasmalogens. The VLCPUFAs were always in the *sn*-1 position while PUFAs at the *sn*-2 position were exclusively 22:6n-3, e.g., 30:6/22:6-PC, 32:6/22:6-PC, 34:6/22:6-PC and 36:6/22:6-PC.

A method for detecting a number of VLCPUFAs that occur in peroxisomal diseases, such as Zellweger syndrome and X-linked adrenoleukodystrophy, has been described [62]. The use of ultra-performance liquid chromatography-mass spectrometry with negative ESI allowed the detection of VLCPUFAs up to 44:13, including 44:10, 44:11, and 44:12. The fatty acid 44:13n-3 is probably the longest and most unsaturated VLCPUFA so far analyzed in fibroblasts and plasma of patients with peroxisomal diseases [62] (Fig. 5).

Dozens of molecular species of PC, as well as PE, PS, TAG or cholesteryl esters, in fibroblasts from patients with Zellweger syndrome and X-linked adrenoleukodystrophy were identified by LC-MS/tandem ESI [94]. Based on tandem MS, it was also possible to determine acyls in *sn*-1 or *sn*-2 positions. It has been reported that *sn*-2 acyls are more readily cleaved in tandem MS to form the 1-acyl-2-lyso forms than acyls at the *sn*-1 position [95]. Based on this analysis, molecular species 30:2/20:4-PC, 26:5/20:4-PC, 26:5/18:2-PC, etc. were identified.

Two VLCFAs, 26:0 and 26:1, were identified in human breast milk by liquid extraction surface analysis coupled with high-resolution mass spectrometry [96]. This paper is another demonstration that VLCFAs can be detected using a specialized technique, even in such a commonly studied lipid source. Although in this paper [97], the authors did not analyze VLCPUFAs in milk, we still mention it due to the fact that modern analytical methods make it possible to determine trace amounts of FAs. Fifty-one TAGs containing mainly EPA, DPA and DHA were identified by LC-MS.

Natarajan et al. [98] pointed out the unreliability of the analysis of VLCFA in carnitines. Carnitines, with 24:0 and 26:0 acids, were used as markers for newborn screening of X-linked adrenoleukodystrophy and were analyzed by flow injection analysis-tandem mass spectrometry.

Quantification of three saturated FAs, i.e., 22:0, 24:0, and 26:0 were performed by enrichment of trimethyaminoethyl-VLCFA using multi-walled carbon nanotubes [99]. Analysis of these derivatives using surface-assisted laser desorption/ionization-time-of-flight mass spectrometry showed that the limit of detection was 0.5–1 μg/mL. Such a sensitive analysis was used to detect peroxisomal disorders.

Molecular species of VLCPUFAs in sphingomyelins with 28–34 carbon FAs were detected in sperm samples, most of which were C28 and C30 VLCPUFAs [100]. Statistical analyzes showed that lower VLCPUFA-SM levels were positively correlated with lower total motile count and lower total sperm count, while total VLCPUFAs and 22:6n3 content did

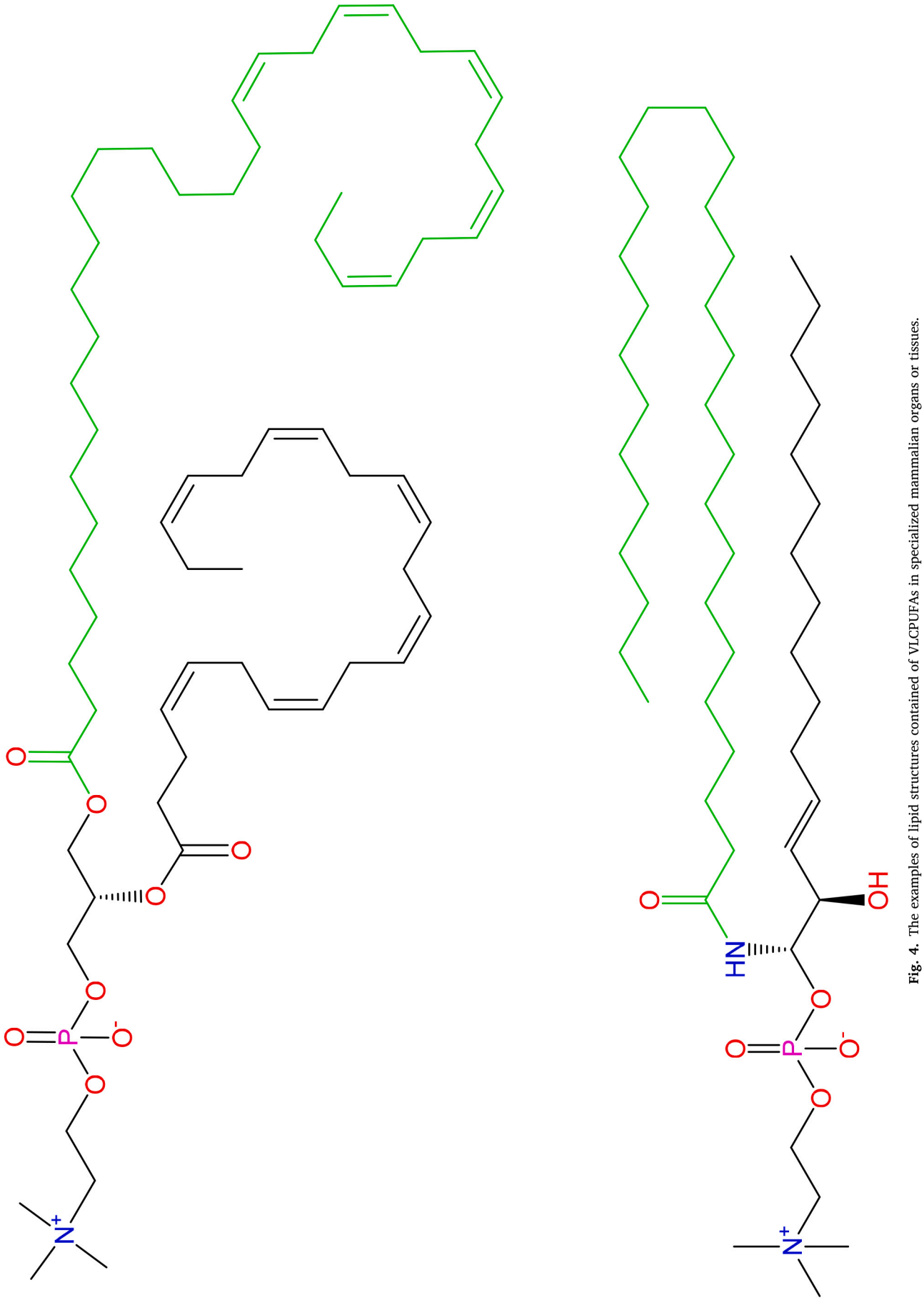

Fig. 4. The examples of lipid structures contained of VLCPUFAs in specialized mammalian organs or tissues.

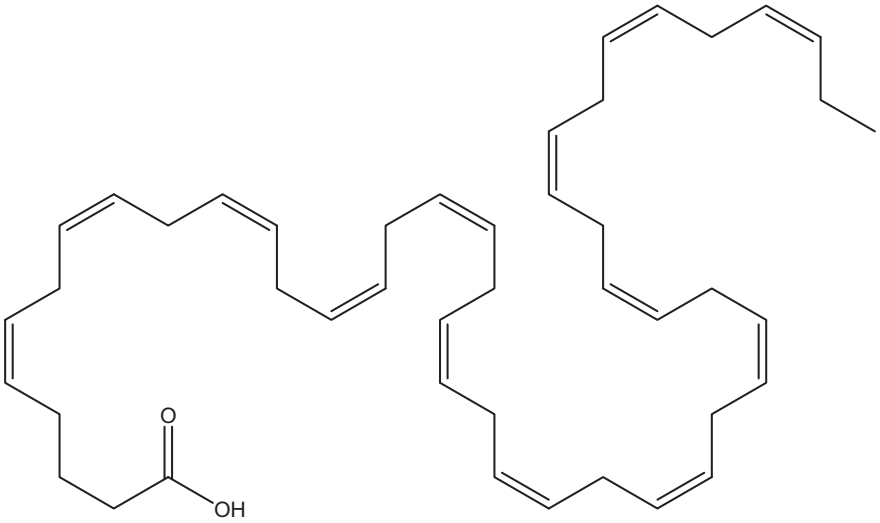

Fig. 5. The structure of VLCPUFA 44:13n-3, i.e., (5Z,8Z,11Z,14Z,17Z,20Z,23Z,26Z,29Z,32Z,35Z,38Z,41Z)-tetratetraconta-5,8,11,14,17,20,23,26,29,32,35,38,41-tridecaenoic acid. Probably the longest and most unsaturated VLCPUFA so far analyzed in fibroblasts and plasma of patients with peroxisomal diseases [62].

not correlate. This study revealed a positive correlation between VLCPUFA levels with sperm count and total sperm motility, and suggests that sperm quality and quantity may depend on the presence of VLCPUFAs. The lack of correlation between VLCPUFA levels and 22:6n3, on the other hand, suggest that low levels of VLCPUFAs are not due to inadequate PUFA precursors.

An attempt to treat X-linked adrenoleukodystrophy, a disease characterized by increased VLCFA content, e.g., 26:0, has been described [101]. Irbesartan is a medication used to treat high blood pressure, heart failure, and diabetic kidney disease. When the drug was administered at a concentration between 2 μM and 10 μM, both C26-LPC (lysophosphatidylcholine) and 26:0 and 28:0 levels in total fibroblast lipids were reduced.

Analysis of patients suffering from X-linked adrenoleukodystrophy revealed that their fibroblasts contained sphingolipids (cerebrosides, lactosylceramides, gangliosides, etc.) having VLCFAs with 0 to 2 double bonds and with 24 and 26 carbon atoms (24:0 to 26:2) [102]. LC-MS/tandem ESI with multiple reaction monitoring (MRM) mode was used for analysis.

Below are listed some reviews in which the reader can find more detailed information about VLCFAs in healthy and sick people. Lemaitre and King published a review on very long-chain saturated fatty acids in diabetes and cardiovascular disease [103], as well as a paper describing the transport of very long-chain acyl-CoA into peroxisomes [104]. Furthermore, a review of VLCFA, their elongation, physiology and related disorders has been published [105].

A review by three authors describing the metabolism and function of VLCPUFAs (> C24) in mammals has been published [106], addressing the distribution and abundance of VLCPUFAs in retinal, testicular, spermatozoa and cerebral tissues, their biosynthesis, physiological role, and diseases associated with their presence and absence.

8. Occurrence in lower organisms

Although >10 years have passed since our last review [7], very few new sources of VLCFAs or VLCPUFAs have been published. The main direction of research focused on global developments in the analysis of fatty acids, particularly in intact lipids. Another area in which great progress has been made is the elucidation of biosynthesis using genetic methods. Nevertheless, some examples of isolation and identification of both VLCFAs and VLCPUFAs from significant sources are described briefly below. One of the main sources of commercially produced VLCPUFAs are dinoflagellates. This group of microorganisms, taxonomically classified among algae as well as protists, is characterized by the production of PUFAs, predominantly docosahexaenoic acid. Based on published results [107], it can be assumed that two biosynthetic pathways are responsible for the high content of DHA and other VLCPUFAs. PUFAs are synthesized both by the "classical" biosynthetic route (stearic-oleic-linoleic acids) and by a polyketide synthase complex (PKS pathway), as confirmed using $^{13}CO_2$.

The lipidomic profile of three species of dinoflagellates (*Amphidinium carterae*, *Cystodinium* sp., and *Peridinium aciculiferum*) containing VLCPUFAs was investigated using high-resolution mass spectrometry with positive and negative ESI [17]. Individual lipid classes were separated by hydrophilic interaction liquid chromatography (HILIC), and the major lipid classes were identified by neutral loss scan $[M + H\text{-}28{:}8]^+$ and by precursor ion scan, i.e., ion $[C_{27}H_{39}COO]^-$. Of the dozens of lipid classes contained in total lipids, only two classes of neutral lipids, (major TAGs and minor diacylglycerols), and two classes of phospholipids (phosphatidic acid (PA) and/or PC) contained VLCPUFAs. Further chromatography, RP-HPLC, showed that the molecular species of TAGs were *sn*-28:7/28:8/28:8, *sn*-26:7/28:7/28:8, or *sn*-26:7/28:8/28:8. Also in PA and PC, *sn*-28:8/16:0-PA and *sn*-28:8/16:0-PC and the corresponding lyso-forms, 28:8- lysophosphatidylcholine (LPC) and 28:8-lysophosphatidic acid (LPA), were identified.

Symbiotic dinoflagellates living in hydrocorals (*Millepora dichotoma* and *M. platyphylla*) contain molecular species 18:0/28:8 and 18:0/28:7 of diacylglyceryl-3-*O*-carboxyhydroxymethylcholines [108].

VLCFAs up 30:2 were identified in four strains of the green alga *Botryococcus braunii* using HILIC and subsequent RP-HPLC with positive and negative high resolution ESI tandem MS identification [39]. Based on tandem MS, VLCFAs were found to be bound at the *sn*-1 position of PC and molecular species of TAGs contained one or two acyls of a VLCFAs attached to the primary alcohol group (*sn*-1 or *sn*-3 position).

Rezanka et al. [109] describes the separation and identification of TAGs containing VLCFAs and/or VLCPUFAs. Analysis by RP-HPLC/APCI tandem MS and chiral liquid chromatography, also with APCI identification, were used to separate and identify the molecular species of these TAGs. Known sources of both VLCFAs and VLCPUFAs, i.e., ximenia oil (*Ximenia americana*), green alga (*Botryococcus braunii*), brewer's yeast (*Saccharomyces pastorianus*) and a dinoflagellate (*Amphidinium carterae*), were analyzed as biological samples. The analysis found that very long chain acyls were preferentially bound in the *sn*-1 position of TAGs. Dinoflagellate analysis was performed several times [16–18] and VLCPUFAs up to 36:7n-6, but also odd VLCPUFAs up to 33:6n-3, were identified. Because dinoflagellates form a substantial proportion of

phytoplankton, both in the seas and in freshwater waters, it is hypothesized that some VLCPUFAs, e.g., 28:8n-3 [12] identified in fish oil [40], are derived from food (plankton).

One interesting fact is the presence of VLCPUFAs in bacteria. It is known that some bacteria, such as the genus *Shewanella*, but also many other bacteria (*Aureispira*, *Colwellia*, *Moritella*, etc., see Rezanka et al. [110], biosynthesize PUFA not in the classical way, i.e., by a combination of elongases and aerobic desaturases, but by a polyketide synthase complex. Synthesis proceeds under anaerobic conditions, through several intermediates (e.g., 3,6,9,12,15-cis C18-ACP (acyl-carrier-protein), 5,8,11,14,17-cis-C20-ACP) to DHA. VLCPUFAs have not yet been found in these organisms, although it is questionable whether this is due to poor analysis or whether the organisms concerned have higher levels of PUFA than DHA.

The Gram-negative bacterium *Oligotropha carboxidovorans* can utilize carbon monoxide as a sole source of carbon. Two VLCFAs (26:0(25-OH) and 28:0(27-OH)) were located in lipid A [111].

In the bacterium *Bradyrhizobium*, a Gram-negative soil bacterium that fixes nitrogen, an unusual straight and branched (ω-1)-hydroxylated VLCFA has been identified in lipopolysaccharides [112]. Methyls at position n-10 or n-11 and hydroxyls at position n-1 were identified by GC–MS derivatives (4,4-dimethyloxazolines and trimethylsilyl). This clarified the partial structure of bradyrhizobial lipid A.

The typical organisms where VLCPUFAs are found are sponges. Demospongic acids are commonly found in these organisms, i.e., fatty acids with unusual position of double bonds (Δ5,9-double bonds).

Invertebrates such as cephalopod mollusks were also analyzed, confirming that both sponges and other invertebrates contained VLCPUFAs, and it is hypothesized again that the presence of VLCPUFAs in these animals is from phytoplankton [113]. Some dinoflagellates even live in symbiosis with sea corals.

A relatively complicated study was performed on the cold-water coral-associated sponge *Hymedesmia* (*Stylopus*) *coriacea* [20]. Sponges contain symbiotic organisms, probably nitrifying and/or sulfur-oxidizing bacteria, capable of fixing $^{13}CO_2$ in the dark. Subsequent analysis of phospholipid-derived fatty acids confirmed the presence of ^{13}C in VLCFAs, e.g., 26:2, 26:3, or 28:3. The authors assume the transfer of precursors/building blocks (e.g., 9–16:1 and 11–18:1) for the synthesis of VLCFAs from sponge-associated bacteria to the sponge.

The sponges (genera *Haliclona*, *Aplysina* and *Dysidea*) from three localities, one of which contained brackish water, were examined for the presence of natural abundance of ^{13}C FAs [114]. Typical demospongic acids have been identified (e.g., 5,9,17–26:3, 5,9,21–28:3 or 5,9,23–30:3). Stable carbon isotope ratios ($\delta^{13}C$) in demospongic acids showed very limited seasonal variation in all sponges. Caribbean sponge *Asteropus niger* was analyzed and new demospongic acids, which are α-methoxylated ((2R, 5Z, 9Z)-2-methoxy-25-methyl-5,9-hexacosadienoic and (2R, 5Z, 9Z)-2-methoxy-24-methyl-5,9-hexacosadienoic acids) have been identified as potent inhibitors of topoisomerases IB [115]. In deep-sea sponges (class Demospongiae) two types of FAs were identified [116]. These were mainly standard bacterial FAs, such as mid-chain branched FAs (8- and 9-Me-16:0 and 10- and 11-Me-C18:0) and, obviously, demospongic acids with a chain length of 24–28 carbon atoms and with predominantly the typical Δ5,9-, Δ9,19-, and yet undescribed Δ11,21- position of double bonds. The ^{13}C isotopic abundance was also investigated and its values ranged around −21.8 ‰ at 26:2, while the expected precursor (16:0) was found to be −21.6 ‰. Based on values for $\Delta^{13}C$, the authors proposed biosynthetic pathways of (microbial) precursors to VLCFAs. Fresh-water sponge *Baikalospongia intermedia* from Lake Baikal was analyzed and tens of fatty acids were identified, both monoenoic up to 27:1 and polyenoic up to 27:2 [117]. The position of double bonds has not been determined.

One paper clarifies the conformation of ultra-long-chain fatty acids (ULCFAs) where the unsaturated chain contains C32 VLCPUFA at the *sn*-1 position in three types of phospholipid bilayers [118]. This phenomenon was studied by molecular dynamics simulation. It was found that the ultra-long chain (32:6n-3) flips between the two leaflets and twists in the opposite direction (towards the polar head).

9. Plants and herbicides

Various stressors, such as salt, drought, pathogen defense, temperature, etc., effect the levels of VLCFAs in plants [119]. The reviewers concluded that the VLCFAs content increased in response to the various stresses listed above. Stress factors therefore, stimulate the biosynthesis and accumulation of VLCFAs, or even the biosynthesis of new VLCFAS, which are not normally found in plant tissues.

One of the interesting aspects of VLCFAs is the effect of herbicides on their biosynthesis and thus on the complete cessation of cell division, growth and tissue deformation, leading to plant death [120]. Chloroacetamides and thiocarbamates have been and continue to be used worldwide to control weeds (grasses) in corn, rice, soybeans and wheat crops. These herbicides are applied as a pre-emergence herbicide (herbicide used before crop germination). Chloroacetamides act as cell division/inhibitors of VLCFAS synthesis, while thiocarbamates are inhibitors of lipid synthesis. Both groups are more active on monocotyledonous than on dicotyledonous plants.

The effect of two herbicides, metazachlor and mefluidide, on the inhibition of 3-ketoacyl-CoA synthase, which catalyzes the condensation of a C2 unit from malonyl-CoA to long acyl-CoA, and thus the extension of FA in *Arabidopsis thaliana* and *Nicotiana benthamiana,* has been investigated in pollen tube and root growth [121].

The levels of phytoceramides (PhytoCer), phytoceramides with hydroxylated fatty acyls (PhytoCer-OHFA), and phyto-glucosylceramides (Phyto-GluCer) were examined using ultrahigh performance liquid chromatography-tandem MS (UHPLC-MS/MS) for a prominent industrial crop - cotton, and glycosyl-inositol-phospho-ceramides (GIPC) [122]. Changes in the representation of individual classes of sphingolipids in cotton fiber cells after days post-anthesis were found. Anthesis is the period during which a flower is fully open and functional. The composition and content of sphingolipids at different stages of growth and development of fibrous cells, as well as changes in molecular species of sphingolipids, were analyzed. The majority of sphingolipids involved in fiber elongation and secondary cell wall synthesis are PhytoCer, PhytoCer-OHFA, phyto-GluCer and GIPC. For specific molecular species, tri-hydroxylated, unsaturated long-chain bases (LCBs) and hydroxylated saturated VLCFAs (up to C26) were identified in increased amounts. Based on these results, the authors state that sphingolipids play an important role in the elongation of cellulose fibers.

A review describing the production of nervonic acid (24:1n-9, cis-15-tetracosenoic acid) in plants has been published [123]. The review presents the possibilities of biosynthesis and sources of nervonic acid, as well as the use of genetic engineering to increase its production.

A recent review describing the synthesis of C20-–38 FAs in plant tissues was published [124]. This review describes the biosynthesis of VLCFAs, including the expression disorder of genes involved in VLCFA synthesis. This leads to several phenotypic consequences, from growth retardation to embryo death.

10. Elongation of very long chain fatty acids protein 4 (ELOVL4)

The protein ELOVL4 that catalyzes the elongation of FAs is required for the biosynthesis of VLCFAs and is a member of the ELOVL family of FA elongases that are jointly responsible for catalyzing the formation of fatty acids (Fig. 6). ELOVL4 is the only member of the family that catalyzes the production of VLCFA and/or VLCPUFA.

A large number of articles deal with the levels of VLCFAs and VLCPUFAs in the diagnosis and pathophysiology of human diseases. Well known and widespread examples are various inherited diseases such as ichthyosis, macular degeneration, myopathy, mental retardation and demyelination, which are caused by mutations in genes encoding VLCFA metabolizing enzymes [4]. Elongation of FAs by the enzyme

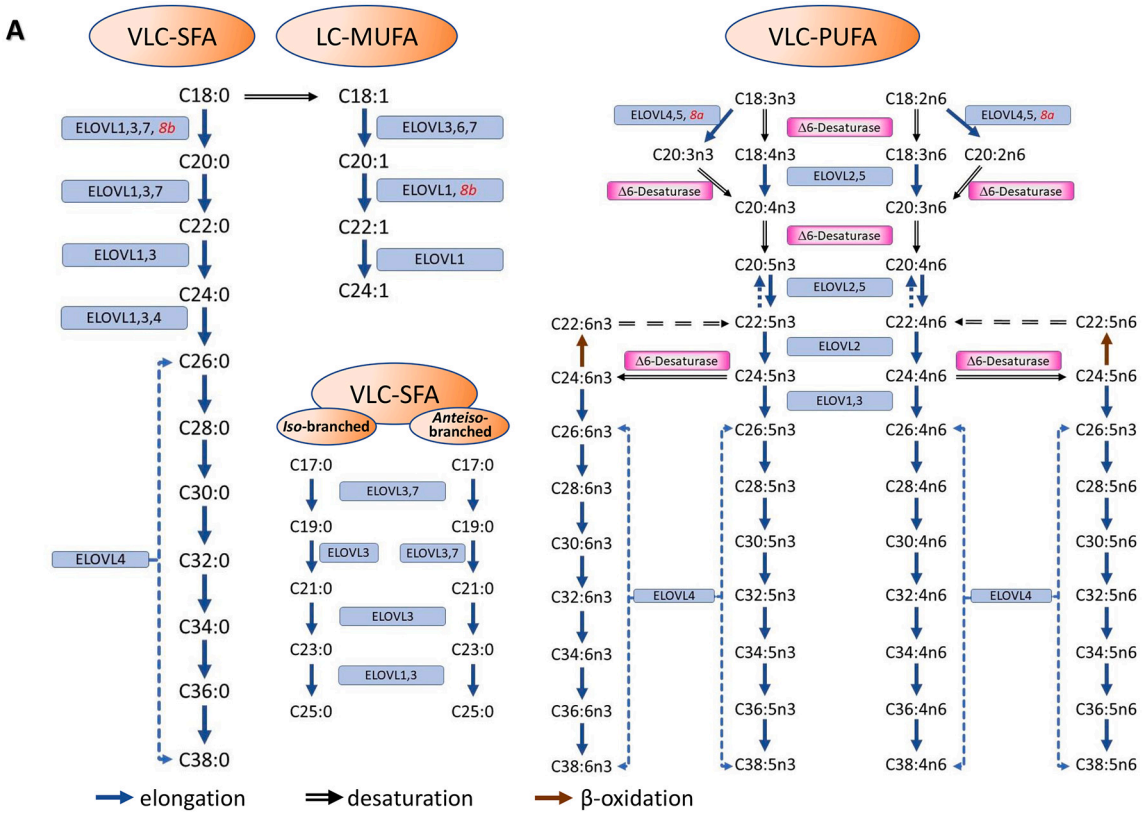

Fig. 6. Biosynthesis of different type of VLCFAs and VLCPUFAs.

ELOVL4 is the best-known example of an enzyme responsible for the extension of C16 and C18 on VLCFAs.

The crucial role of ELOVL4 in VLCPUFAs synthesis and retinal function has been described [82]. A mutation in elongase (ELOVL4) is responsible for an autosomal dominant form of Stargardt's disease, and a type of juvenile macular degeneration. VLCPUFAs were identified in PCs and PEs using photoreceptor-specific knockout mice and using LC-MS. Several molecular species have been identified, e.g., 32:6/22:6-PC, 34:6/22:6-PC, 36:6/22:6-PC, and their respective lyso-PCs. VLCPUFAs were always identified in position *sn*-1, while in position *sn*-2 is 22:6.

In humans, see Table 5 schematically below, VLCPFAs and/or VLCPUFAs have so far been identified in several tissues (organs). Several conclusions can be drawn from these results. In humans in particular, VLCPUFAs are present only in a minority, unlike DHA. Furthermore, VLCPUFAs are biosynthesized only in tissues that contain the enzyme ELOVL4 and are hardly synthesized by other enzymes [125]. The effect of diet (fish oil with VLCPUFA n-3) on availability and content in rat, fish and mouse tissues was also investigated [54]. When fed a diet containing VLCPUFAs in the form of a concentrate, these acids were bioavailable and stored in both phospholipids and TAGs in all tissues studied (skin, eye, brain, testis, liver and heart), and their distribution appeared to be tissue dependent, but not species specific.

The ELOVL4-like elongase from Atlantic salmon (*Salmo salar*) has been transformed into *Saccharomyces cerevisiae* [126]. Heterologous expression in yeast has shown that salmon ELOVL4 effectively extended saturated FAs up to 36:0, with 24:0 and 26:0 appearing to be preferred substrates. Furthermore, this enzyme in yeast efficiently transformed C20 and C22 PUFAs (e.g., 20:5n-3, 20:4n-6, 22:5n-3, 22:4n-6, and 22:6n-3) into VLCPUFA up to C36 (36:4n-6, 36:5n-3, 36:6n-3).

The change in salinity of the water in which the fish *Pampus argenteus* lived led to a change in the content of both PUFAs and VLCPUFAs [127]. VLCPUFAs up to 36:5n-3, were identified in liver and gill samples by GC–MS analysis. The role of two enzymes, i.e., ELOVL4a and ELOVL4b, in the biosynthesis of VLCPUFAs from lower MW precursors, e.g., 18:4n-3, 20:5n-3, 22:5n-3, etc. was also examined.

Further analysis of iso and anteiso-FA meibomian glands, especially cholesteryl and wax esters, was performed [71]. FA elongases such as ELOVL1, ELOVL3 and ELOVL7 extended branched chain saturated acyl-CoA. ELOVL3 was highly active against iso-17:0 and anteiso-17:0 acyl-CoA and extended them to iso-23:0 and anteiso-25:0 acyl-CoA. ELOVL1 elongated both iso- and anteiso-23:0 acyl-CoA to 25:0 acyl-CoA. Dozens of molecular species of ceramides (up to d18:1/32:0), cholesteryl esters up to 29:0, and wax esters (to 18:0/32:0) were identified by LC-tandem MS.

Other ELOVL fatty acid elongases have been described, e.g., ELOVL8 (a, b), which elongate zebrafish (*Danio rerio*) polyunsaturated fatty acids 18:2n-6 and 18:3n-3 to 20:2n-6 and 20:3n-3. In this case, however, it is not VLCFA (VLCPUFA) [128]. Another two reviews were published, the first of which describes retinal very long-chain PUFAs and new insights from studies on ELOVL4 protein [129], the second review deals with novel insights of ELOVL4 and very long chain fatty acid-containing lipids [130].

A review describing knowledge about the biosynthesis of polyunsaturated fatty acids, including desaturases and elongases in aquatic invertebrates (sponges, cnidarians, rotifers, mollusks, annelids, arthropods, etc.) has been published [131].

Further review describing PUFAs in chordates summarizes both the occurrence and biosynthesis of these FAs [132]. Attention is paid to the enzyme ELOVL4, which is an enzyme necessary for the biosynthesis of VLCFAs [133]. ELOVL4 is responsible for biosynthesis of both saturated VLCFAs (e.g., 26:0, 28:0 and 30:0), which are components of sphingolipids and ceramides, as well as the biosynthesis of VLCPUFAs, which are contained, for example, in the PC retina, brain or testes.

There are also known compounds called elovanoids [134], which are very-long chain (C32 and C34) oxylipins produced by the action of

elongases, especially ELOVL4 - hence the name, on DHA in brain and retina. These are converted to protectin analogues under conditions of oxidative stress, i.e., to 20,27-dihydroxy-32:6n-3 (ELV-N32) and 22,29-dihydroxy-34:6n-3 (ELV-N34). They are low-abundance, high-potency, protective mediators. In contrast to DHA per se, which is located in position *sn*-2 of phospholipids in these tissues, precursors of the elovanoids are located in position *sn*-1. This implies that the stimulus for release of the precursors and formation of novel mediators must be somewhat different from that of protectins.

11. Conclusion

Further development of knowledge and new scientific findings in the field of VLCFAs and VLCPUFAs can be expected mainly in two basic areas. The first is new methods of analysis. In the last decade, research in this area has focused on classes of lipids that these acids contain, rather than on the determination of acids in the form of synthetic derivatives, typically methyl esters, DMOX or pyridylcarbinol esters. This is mainly related to the development of instrumentation technologies, where LC-MS devices are widely available. Another area where a significant increase in knowledge, and thus in published papers, can be expected is biosynthesis, or rather the connection with genetic methods and comprehension of all functions of e.g., ELOVL proteins, especially ELOVL4. Although VLCFAs (VLCPUFAs) are minor in total fatty acids, the same cannot be said of their functions in organisms. These acids play a significant role in the field of human metabolic disorders (Zellweger syndrome, adrenoleukodystrophy, etc.) or in specialized organs (retina, spermatozoa, Meibomian gland, etc.). Another possibility is to prove the presence of VLCPUFAs in bacteria, where they can be biosynthesized by polyketide synthase. This review should also provide motivation to unify the rules of what can be considered VLCFAs (VLCPUFAs).

Acknowledgements

This study was supported by Czech Science Foundation (GACR) project 21-07247S, Institutional Research Concept (Institute of Microbiology, Prague, Czech Republic), grant number RVO61388971 and the Ministry of Agriculture of the Czech Republic within the institutional support No. MZE-RO1918. We acknowledge prof. J. D. Brooker for critical reading and language editing of the text.

References

[1] Nichols DS, Nichols PD, McMeekin TA. Anaerobic production of polyunsaturated fatty acids by *Shewanella putrefaciens* strain ACAM 342. FEMS Microbiol Lett 1992;98:117–22. https://doi.org/10.1111/j.1574-6968.1992.tb05499.x.

[2] Erdbrügger P, Fröhlich F. The role of very long chain fatty acids in yeast physiology and human diseases. Biol Chem 2021;402:25–38. https://doi.org/10.1515/hsz-2020-0234.

[3] Hama K, Fujiwara Y, Hayama T, Ozawa T, Nozawa K, Matsuda K, et al. Very long-chain fatty acids are accumulated in triacylglycerol and nonesterified forms in colorectal cancer tissues. Sci Rep 2021;11:6163. https://doi.org/10.1038/s41598-021-85603-w.

[4] Sassa T, Kihara A. Metabolism of very long-chain fatty acids: genes and pathophysiology. Biomol Ther (Seoul) 2014;22:83–92. https://doi.org/10.4062/biomolther.2014.017.

[5] Batsale M, Bahammou D, Fouillen L, Mongrand S, Joubès J, Domergue F. Biosynthesis and functions of very-long-chain fatty acids in the responses of plants to abiotic and biotic stresses. Cells 2021;10:1284. https://doi.org/10.3390/cells10061284.

[6] Ritchie SA, Ahiahonu PW, Jayasinghe D, Heath D, Liu J, Lu Y, et al. Reduced levels of hydroxylated, polyunsaturated ultra long-chain fatty acids in the serum of colorectal cancer patients: implications for early screening and detection. BMC Med 2010;8:13. https://doi.org/10.1186/1741-7015-8-13.

[7] Rezanka T, Sigler K. Odd-numbered very-long-chain fatty acids from the microbial, animal and plant kingdoms. Prog Lipid Res 2009;48:206–38. https://doi.org/10.1016/j.plipres.2009.03.003.

[8] Rezanka T. Very-long-chain fatty acids from the animal and plant kingdoms. Prog Lipid Res 1989;28:147–87. https://doi.org/10.1016/0163-7827(89)90011-8.

[9] Kinji U. Occurrence of saturated and mono-unsaturated fatty acids with unusually-long-chains (C20-C30) in *Lactobacillus heterohiochii*, an alcoholophilic bacterium. BBA - Lipids Lipid Metab 1974;348:86–93. https://doi.org/10.1016/0005-2760(74)90095-2.

[10] Fisher DJ, Holloway PJ, Richmond DV. Fatty acid and hydrocarbon constituents of the surface and wall lipids of some fungal spores. Microbiology 1972;72:71–8. https://doi.org/10.1099/00221287-72-1-71.

[11] Rezanka T, Kolouchova I, Gharwalova L, Palyzova A, Sigler K. Identification and characterization of phospholipids with very long chain fatty acids in brewer's yeast. Lipids 2017;52:1007–17. https://doi.org/10.1007/s11745-017-4294-6.

[12] Mansour M. Reversed-phase high-performance liquid chromatography purification of methyl esters of C16–C28 polyunsaturated fatty acids in microalgae, including octacosaoctaenoic acid [28: 8(n-3)]. J Chromatogr A 2005;1097:54–8. https://doi.org/10.1016/j.chroma.2005.08.011.

[13] Rezanka T. Identification of very long chain fatty acids by atmospheric pressure chemical ionization liquid chromatography-mass spectroscopy from green alga *Chlorella kessleri*. J Sep Sci 2002;25:1332–6. https://doi.org/10.1002/jssc.200290004.

[14] Sánchez M, Avhad MR, Marchetti JM, Martínez M, Aracil J. Jojoba oil: a state of the art review and future prospects. Energ Conver Manage 2016;129:293–304. https://doi.org/10.1016/j.enconman.2016.10.038.

[15] Rezanka T, Sigler K. Identification of very long chain unsaturated fatty acids from *Ximenia* oil by atmospheric pressure chemical ionization liquid chromatography-mass spectroscopy. Phytochemistry 2007;68:925–34. https://doi.org/10.1016/j.phytochem.2006.11.034.

[16] Rezanka T, Nedbalova L, Sigler K. Identification of very-long-chain polyunsaturated fatty acids from *Amphidinium carterae* by atmospheric pressure chemical ionization liquid chromatography-mass spectroscopy. Phytochemistry 2008;69:2391–9. https://doi.org/10.1016/j.phytochem.2008.06.011.

[17] Rezanka T, Lukavsky J, Nedbalova L, Sigler K. Lipidomic profile in three species of dinoflagellates (*Amphidinium carterae*, *Cystodinium* sp., and *Peridinium aciculiferum*) containing very long chain polyunsaturated fatty acids. Phytochemistry 2017;139:88–97. https://doi.org/10.1016/j.phytochem.2017.04.912.

[18] Rezanka T, Nedbalova L, Sigler K. Odd-numbered very-long-chain polyunsaturated fatty acids from the dinoflagellate *Amphidinium carterae* identified by atmospheric pressure chemical ionization liquid chromatography-mass spectrometry. Phytochemistry 2008;69:2849–55. https://doi.org/10.1016/j.phytochem.2008.08.021.

[19] Dembitsky VM, Rezanka T, Srebnik M. Lipid compounds of freshwater sponges: family Spongillidae, class Demospongiae. Chem Phys Lipids 2003;123:117–55. https://doi.org/10.1016/S0009-3084(03)00020-3.

[20] van Duyl F, Lengger S, Schouten S, Lundalv T, van Oevelen D, Muller C. Dark CO2 fixation into phospholipid-derived fatty acids by the cold-water coral associated sponge *Hymedesmia (Stylopus) coriacea* (Tisler reef, NE Skagerrak). Mar Biol Res 2020;16:1–17. https://doi.org/10.1080/17451000.2019.1704019.

[21] Kornprobst J-M, Barnathan G. Demospongic acids revisited. Mar Drugs 2010;8:2569–77. https://doi.org/10.3390/md8102569.

[22] Aveldano M, Sprecher H. Very long-chain (C-24 to C-36) polyenoic fatty-acids of the n-3 and n-6 series in dipolyunsaturated phosphatidylcholines from bovine retina. J Biol Chem 1987;262:1180–6.

[23] McMahon A, Jackson SN, Woods AS, Kedzierski W. A Stargardt disease-3 mutation in the mouse Elovl4 gene causes retinal deficiency of C32–C36 acyl phosphatidylcholines. FEBS Lett 2007;581:5459–63. https://doi.org/10.1016/j.febslet.2007.10.050.

[24] Robinson BS, Johnson DW, Poulos A. Unique molecular species of phosphatidylcholine containing very-long-chain (C24-C38) polyenoic fatty acids in rat brain. Biochem J 1990;265:763–7. https://doi.org/10.1042/bj2650763.

[25] Butovich IA. Cholesteryl esters as a depot for very long chain fatty acids in human meibum*. J Lipid Res 2009;50:501–13. https://doi.org/10.1194/jlr.M800426-JLR200.

[26] Butovich IA, Uchiyama E, McCulley JP. Lipids of human meibum: mass-spectrometric analysis and structural elucidation1. J Lipid Res 2007;48:2220–35. https://doi.org/10.1194/jlr.M700237-JLR200.

[27] Furland NE, Maldonado EN, Aveldaño MI. Very long chain PUFA in murine testicular triglycerides and cholesterol esters. Lipids 2003;38:73–80. https://doi.org/10.1007/s11745-003-1033-9.

[28] Furland NE, Zanetti SR, Oresti GM, Maldonado EN, Aveldaño MI. Ceramides and sphingomyelins with high proportions of very long-chain polyunsaturated fatty acids in mammalian germ cells*. J Biol Chem 2007;282:18141–50. https://doi.org/10.1074/jbc.M700708200.

[29] Sandhoff R, Geyer R, Jennemann R, Paret C, Kiss E, Yamashita T, et al. Novel class of glycosphingolipids involved in male fertility*. J Biol Chem 2005;280:27310–8. https://doi.org/10.1074/jbc.M502775200.

[30] McMahon A, Butovich IA, Mata NL, Klein M, Ritter 3rd R, Richardson J, et al. Retinal pathology and skin barrier defect in mice carrying a Stargardt disease-3 mutation in elongase of very long chain fatty acids-4. Mol Vis 2007;13:258–72.

[31] Cameron DJ, Tong Z, Yang Z, Kaminoh J, Kamiyah S, Chen H, et al. Essential role of Elovl4 in very long chain fatty acid synthesis, skin permeability barrier function, and neonatal survival. Int J Biol Sci 2007;3:111–9. https://doi.org/10.7150/ijbs.3.111.

[32] Li W, Sandhoff R, Kono M, Zerfas P, Hoffmann V, Ding BC-H, et al. Depletion of ceramides with very long chain fatty acids causes defective skin permeability barrier function, and neonatal lethality in ELOVL4 deficient mice. Int J Biol Sci 2007;3:120–8. https://doi.org/10.7150/ijbs.3.120.

[33] Vasireddy V, Uchida Y, Salem Jr N, Kim SY, Mandal MNA, Reddy GB, et al. Loss of functional ELOVL4 depletes very long-chain fatty acids (≥C28) and the unique ω-O-acylceramides in skin leading to neonatal death. Hum Mol Genet 2007;16:471–82. https://doi.org/10.1093/hmg/ddl480.

[34] Sandhoff R. Very long chain sphingolipids: tissue expression, function and synthesis. FEBS Lett 2010;584:1907–13. https://doi.org/10.1016/j.febslet.2009.12.032.
[35] Rissmann R, Groenink HWW, Weerheim AM, Hoath SB, Ponec M, Bouwstra JA. New insights into ultrastructure, lipid composition and organization of vernix caseosa. J Invest Dermatol 2006;126:1823–33. https://doi.org/10.1038/sj.jid.5700305.
[36] Maharvi GM, Edwards AO, Fauq AH. Chemical synthesis of deuterium-labeled and unlabeled very long chain polyunsaturated fatty acids. Tetrahedron Lett 2010;51:6426–8. https://doi.org/10.1016/j.tetlet.2010.09.139.
[37] Itoh T, Tomiyasu A, Yamamoto K. Efficient synthesis of the very-long-chain n-3 fatty acids, tetracosahexaenoic acid (C24:6n-3) and tricosahexaenoic acid (C23:6n-3). Lipids 2011;46:455–61. https://doi.org/10.1007/s11745-011-3541-5.
[38] Popov A, Stefanov K. Untersuchungen über die Zusammensetzung der Wachsbodensätze und des Sonnenblumenölwachses. Fette, Seifen, Anstrichmittel 1968;70:234–8. https://doi.org/10.1002/lipi.19680700407.
[39] Rezanka T, Lukavsky J, Vitova M, Nedbalova L, Sigler K. Lipidomic analysis of *Botryococcus* (Trebouxiophyceae, Chlorophyta) - identification of lipid classes containing very long chain fatty acids by offline two-dimensional LC-tandem MS. Phytochemistry 2018;148:29–38. https://doi.org/10.1016/j.phytochem.2018.01.011.
[40] Breivik H, Svensen H. Very long chain polyunsaturated fatty acids from natural oils. US-10501704-B2. 2019.
[41] Hamberg M. A facile and efficient method for the synthesis of labeled and unlabeled very long chain polyunsaturated fatty acids. J Am Oil Chem Soc 2021;98:489–94. https://doi.org/10.1002/aocs.12484.
[42] Wade A, Rallabandi R, Lucas S, Oberg C, Gorusupudi A, Bernstein PS, et al. The synthesis of the very long chain polyunsaturated fatty acid (VLC-PUFA) 32:6 n-3. Org Biol Chem 2021;19:5563–6. https://doi.org/10.1039/d1ob00491c.
[43] Raman K, Tarwade V, Salem Jr N. Synthesis and use of omega-3 and omega-6 very long chain polyunsaturated fatty acids (VLC-PUFA). WO 2011/053892 Al. 2011.
[44] Rezanka T, Sigler K. Identification of very long chain fatty acids from sugar cane wax by atmospheric pressure chemical ionization liquid chromatography–mass spectroscopy. Phytochemistry 2006;67:916–23. https://doi.org/10.1016/j.phytochem.2006.02.014.
[45] Mondello L, Tranchida PQ, Dugo P, Dugo G. Rapid, micro-scale preparation and very fast gas chromatographic separation of cod liver oil fatty acid methyl esters. J Pharm Biomed Anal 2006;41:1566–70. https://doi.org/10.1016/j.jpba.2006.01.027.
[46] Rezanka T, Dembitsky V. Very long chain polyunsaturated fatty acids in crustacea of the order Bathynellacea. Biochem Syst Ecol 1999;27:551–8. https://doi.org/10.1016/S0305-1978(98)00127-6.
[47] Rezanka T, Nedbalova L, Elster J, Cajthaml T, Sigler K. Very-long-chain *iso* and *anteiso* branched fatty acids in *N*-acylphosphatidylethanolamines from a natural cyanobacterial mat of *Calothrix* sp. Phytochemistry 2009;70:655–63. https://doi.org/10.1016/j.phytochem.2009.02.003.
[48] Harvey DJ, Tiffany JM. Identification of meibomian gland lipids by gas chromatography—mass spectrometry: application to the meibomian lipids of the mouse. J Chromatogr A 1984;301:173–87. https://doi.org/10.1016/S0021-9673(01)89187-1.
[49] Rezanka T, Vítová M, Lukavský J, Nedbalová L, Kolouchová I. Rapid screening of very long-chain fatty acids from microorganisms. J Chromatogr A 2019;1605:460365. https://doi.org/10.1016/j.chroma.2019.460365.
[50] Serrano R, Navarro JC, Portolés T, Sales C, Beltrán J, Monroig Ó, et al. Identification of new, very long-chain polyunsaturated fatty acids in fish by gas chromatography coupled to quadrupole/time-of-flight mass spectrometry with atmospheric pressure chemical ionization. Anal Bioanal Chem 2021;413:1039–46. https://doi.org/10.1007/s00216-020-03062-0.
[51] Garlito B, Portolés T, Niessen WMA, Navarro JC, Hontoria F, Monroig Ó, et al. Identification of very long-chain (>C24) fatty acid methyl esters using gas chromatography coupled to quadrupole/time-of-flight mass spectrometry with atmospheric pressure chemical ionization source. Anal Chim Acta 2019;1051:103–9. https://doi.org/10.1016/j.aca.2018.11.001.
[52] Morais S, Torres M, Hontoria F, Monroig Ó, Varó I, Agulleiro MJ, et al. Molecular and functional characterization of Elovl4 genes in *Sparus aurata* and *Solea senegalensis* pointing to a critical role in very long-chain (>C24) fatty acid synthesis during early neural development of fish. Int J Mol Sci 2020;21. https://doi.org/10.3390/ijms21103514.
[53] Gorusupudi A, Rallabandi R, Li B, Arunkumar R, Blount JD, Rognon GT, et al. Retinal bioavailability and functional effects of a synthetic very-long-chain polyunsaturated fatty acid in mice. Proc Natl Acad Sci U S A 2021;118:e2017739118. https://doi.org/10.1073/pnas.2017739118.
[54] Torrissen M, Svensen H, Stoknes I, Nilsson A, Østbye T-K, Berge GM, et al. Deposition and metabolism of dietary n-3 very-long-chain PUFA in different organs of rat, mouse and Atlantic salmon. Br J Nutr 2022;127:35–54. https://doi.org/10.1017/S0007114521000817.
[55] Agbaga M-P, Merriman DK, Brush RS, Lydic TA, Conley SM, Naash MI, et al. Differential composition of DHA and very-long-chain PUFAs in rod and cone photoreceptors. J Lipid Res 2018;59:1586–96. https://doi.org/10.1194/jlr.M082495.
[56] Kharlamenko VI, Odintsova NA. Unusual methylene-interrupted polyunsaturated fatty acids of abyssal and hadal invertebrates. Prog Oceanogr 2019;178:102132. https://doi.org/10.1016/j.pocean.2019.102132.
[57] Liu A, Terry R, Lin Y, Nelson K, Bernstein PS. Comprehensive and sensitive quantification of long-chain and very long-chain polyunsaturated fatty acids in small samples of human and mouse retina. J Chromatogr A 2013;1307:191–200. https://doi.org/10.1016/j.chroma.2013.07.103.
[58] Rezanka T, Votruba J. Chromatography of long chain alcohols (polyprenols) from animal and plant sources. J Chromatogr A 2001;936:95–110. https://doi.org/10.1016/S0021-9673(01)01152-9.
[59] Thomas MC, Dunn SR, Altvater J, Dove SG, Nette GW. Rapid identification of long-chain polyunsaturated fatty acids in a marine extract by HPLC-MS using data-dependent acquisition. Anal Chem 2012;84:5976–83. https://doi.org/10.1021/ac3006523.
[60] Ovcacikova M, Lisa M, Cifkova E, Holcapek M. Retention behavior of lipids in reversed-phase ultrahigh-performance liquid chromatography-electrospray ionization mass spectrometry. J Chromatogr A 2016;1450:76–85. https://doi.org/10.1016/j.chroma.2016.04.082.
[61] Mori N, Fukano Y, Arita R, Shirakawa R, Kawazu K, Nakamura M, et al. Rapid identification of fatty acids and (O-acyl)-omega-hydroxy fatty acids in human meibum by liquid chromatography/high-resolution mass spectrometry. J Chromatogr A 2014;1347:129–36. https://doi.org/10.1016/j.chroma.2014.04.082.
[62] Takashima S, Toyoshi K, Itoh T, Kajiwara N, Honda A, Ohba A, et al. Detection of unusual very-long-chain fatty acid and ether lipid derivatives in the fibroblasts and plasma of patients with peroxisomal diseases using liquid chromatography-mass spectrometry. Mol Genet Metab 2017;120:255–68. https://doi.org/10.1016/j.ymgme.2016.12.013.
[63] Rezanka T. Analysis of very long chain polyunsaturated fatty acids using high-performance liquid chromatography - atmospheric pressure chemical ionization mass spectrometry. Biochem Syst Ecol 2000;28:847–56. https://doi.org/10.1016/S0305-1978(99)00125-8.
[64] Rezanka T, Podojil M. Gradient separation of fatty-acids (C14–C30) by reversed-phase high-performance liquid-chromatography. J Chromatogr 1985;346:453–5.
[65] Nomura A, Yamada J, Yarita T, Sudo Y, Kudo S, Nishizawa Y. Determination of free fatty-acids by supercritical-fluid chromatography on an ods silica-gel column. Anal Sci 1995;11:385–8. https://doi.org/10.2116/analsci.11.385.
[66] Kusaka T, Ikeda M, Nakano H, Namajiri Y. Liquid-chromatography mass-spectrometry of fatty-acids as their anilides. J Biochem 1988;104:495–7. https://doi.org/10.1093/oxfordjournals.jbchem.a122497.
[67] Aveldano M, VanRollins M, Horrocks L. Separation and quantitation of free fatty-acids and fatty-acid methyl-esters by reverse phase high-pressure liquid-chromatography. J Lipid Res 1983;24:83–93.
[68] Qureshi N, Sathyamoorthy N, Takayama K. Biosynthesis of C30 to C56 fatty acids by an extract of *Mycobacterium tuberculosis* H37Ra. J Bacteriol 1984;157:46–52. https://doi.org/10.1128/jb.157.1.46-52.1984.
[69] Al-Dirbashi OY, Santa T, Rashed MS, Al-Hassnan Z, Shimozawa N, Chedrawi A, et al. Rapid UPLC-MS/MS method for routine analysis of plasma pristanic, phytanic, and very long chain fatty acid markers of peroxisomal disorders. J Lipid Res 2008;49:1855–62. https://doi.org/10.1194/jlr.D800019-JLR200.
[70] Hamano F, Kuribayashi H, Iwagawa T, Tsuhako A, Nagata K, Sagara H, et al. Mapping membrane lipids in the developing and adult mouse retina under physiological and pathological conditions using mass spectrometry. J Biol Chem 2021;296:100303. https://doi.org/10.1016/j.jbc.2021.100303.
[71] Tanno H, Sassa T, Sawai M, Kihara A. Production of branched-chain very-long-chain fatty acids by fatty acid elongases and their tissue distribution in mammals. Biochim Biophys Acta Mol Cell Biol Lipids 2021;1866:158842. https://doi.org/10.1016/j.bbalip.2020.158842.
[72] Antolín EM, Delange DM, Canavaciolo VG. Evaluation of five methods for derivatization and GC determination of a mixture of very long chain fatty acids (C24:0–C36:0). J Pharm Biomed Anal 2008;46:194–9. https://doi.org/10.1016/j.jpba.2007.09.015.
[73] Christie WW. The LipidWeb. Methyl Esters of Fatty Acids, Archive of Mass Spectra [online], https://www.lipidmaps.org/resources/lipidweb/lipidweb_html/ms/methesters/me-arch/index.htm#monoene.
[74] Christie WW. Equivalent chain-lengths of methyl ester derivatives of fatty acids on gas chromatography A reappraisal. J Chromatogr A 1988;447:305–14. https://doi.org/10.1016/0021-9673(88)90040-4.
[75] Delmonte P, Fardin Kia A-R, Kramer JKG, Mossoba MM, Sidisky L, Rader JI. Separation characteristics of fatty acid methyl esters using SLB-IL111, a new ionic liquid coated capillary gas chromatographic column. J Chromatogr A 2011;1218:545–54. https://doi.org/10.1016/j.chroma.2010.11.072.
[76] Delmonte P, Fardin-Kia AR, Kramer JKG, Mossoba MM, Sidisky L, Tyburczy C, et al. Evaluation of highly polar ionic liquid gas chromatographic column for the determination of the fatty acids in milk fat. J Chromatogr A 2012;1233:137–46. https://doi.org/10.1016/j.chroma.2012.02.012.
[77] Palusinska-Szysz M, Turska-Szewczuk A, Karas M, Russa R, Drozanski WJ. Occurrence of new polyenoic very long chain acyl residues in lipids from *Acanthamoeba castellanii*. Acta Protozool 2009;48:63–72.
[78] Christie WW. The LipidWeb. Mass Spectrometry of methyl esters, derivatization of double bonds in fatty acids for structural analysis [online], https://www.lipidmaps.org/resources/lipidweb/lipidweb_html/ms/methesters/me-dbderivs/index.htm.
[79] Fellenberg AJ, Johnson DW, Poulos A, Sharp P. Simple mass spectrometric differentiation of the n-3, n-6 and n-9 series of methylene interrupted polyenoic acids. Biomed Environ Mass Spectrom 1987;14:127–9. https://doi.org/10.1002/bms.1200140306.
[80] Brauner A, Budzikiewicz H, Boland W. Studies in chemical ionization mass spectrometry: V†—localization of homoconjugated triene and tetraene units in aliphatic compounds. Org Mass Spectrom 1982;17:161–4. https://doi.org/10.1002/oms.1210170403.

[81] Liu A, Chang J, Lin Y, Shen Z, Bernstein PS. Long-chain and very long-chain polyunsaturated fatty acids in ocular aging and age-related macular degeneration. J Lipid Res 2010;51:3217–29. https://doi.org/10.1194/jlr.M007518.

[82] Berdeaux O, Juaneda P, Martine L, Cabaret S, Bretillon L, Acar N. Identification and quantification of phosphatidylcholines containing very-long-chain polyunsaturated fatty acid in bovine and human retina using liquid chromatography/tandem mass spectrometry. J Chromatogr A 2010;1217:7738–48. https://doi.org/10.1016/j.chroma.2010.10.039.

[83] Agbaga M, Brush R, Mandal M, Henry K, Elliott M, Anderson R. Role of Stargardt-3 macular dystrophy protein (ELOVL4) in the biosynthesis of very long chain fatty acids. Proc Natl Acad Sci U S A 2008;105:12843–8. https://doi.org/10.1073/pnas.0802607105.

[84] Käkelä R, Ackman RG, Hyvärinen H. Very long chain polyunsaturated fatty acids in the blubber of ringed seals (*Phoca hispida* sp.) from Lake Saimaa, Lake Ladoga, the Baltic Sea, and Spitsbergen. Lipids 1995;30:725–31. https://doi.org/10.1007/BF02537799.

[85] Fukuda Y, Ando Y. Occurrence of all-cis-5,8,11,14,17,20,23-hexacosaheptaenoic acid (26:7n-3) in roughscale sole *Clidoderma asperrimum* flesh lipids. Fish Sci 2011;77:875–82. https://doi.org/10.1007/s12562-011-0387-y.

[86] Mansour MP, Volkman JK, Holdsworth DG, Jackson AE, Blackburn SI. Very-long-chain (C28) highly unsaturated fatty acids in marine dinoflagellates. Phytochemistry 1999;50:541–8. https://doi.org/10.1016/S0031-9422(98)00564-0.

[87] Tanaka H, Harauma A, Takimoto M, Moriguchi T. Association between very long chain fatty acids in the meibomian gland and dry eye resulting from n-3 fatty acid deficiency. Prostaglandins Leukot Essent Fatty Acids 2015;97:1–6. https://doi.org/10.1016/j.plefa.2015.02.004.

[88] Svetashev V, Kharlamenko V. Occurrence of hexacosapolyenoic acids 26:7(n-3), 26:6(n-3), 26:6(n-6) and 26:5(n-3) in deep-sea brittle stars from near the Kuril Islands. Lipids 2015;50:691–6. https://doi.org/10.1007/s11745-015-4037-5.

[89] Rezanka T, Dembitsky VM. Tetratriacontanonaenoic acid, first natural acid with nine double bonds isolated from a crustacean *Bathynella natans*. ChemInform 2004;35. https://doi.org/10.1002/chin.200435220.

[90] Sharp P, Poulos A, Fellenberg A, Johnson D. Structure and lipid distribution of polyenoic very-long-chain fatty-acids in the brain of peroxisome-deficient patients (Zellweger syndrome). Biochem J 1987;248:61–7. https://doi.org/10.1042/bj2480061.

[91] Poulos A, Sharp P, Johnson D, Easton C. The occurrence of polyenoic very long chain fatty acids with greater than 32 carbon atoms in molecular species of phosphatidylcholine in normal and peroxisome-deficient (Zellweger's syndrome) brain. Biochem J 1988;253:645–50. https://doi.org/10.1042/bj2530645.

[92] Poulos A, Johnson D, Beckman K, White I, Easton C. Occurrence of unusual molecular-species of sphingomyelin containing 28-34-carbon polyenoic fatty-acids in ram spermatozoa. Biochem J 1987;248:961–4. https://doi.org/10.1042/bj2480961.

[93] Aveldano M. A novel group of very long-chain polyenoic fatty-acids in dipolyunsaturated phosphatidylcholines from vertebrate retina. J Biol Chem 1987;262:1172–9.

[94] Hama K, Nagai T, Nishizawa C, Ikeda K, Morita M, Satoh N, et al. Molecular species of phospholipids with very long chain fatty acids in skin fibroblasts of Zellweger syndrome. Lipids 2013;48:1253–67. https://doi.org/10.1007/s11745-013-3848-5.

[95] Nakanishi H, Iida Y, Shimizu T, Taguchi R. Separation and quantification of sn-1 and sn-2 fatty acid positional isomers in phosphatidylcholine by RPLC-ESIMS/MS. J Biochem 2010;147:245–56. https://doi.org/10.1093/jb/mvp171.

[96] Koulman A, Furse S, Baumert M, Goldberg G, Bluck L. Rapid profiling of triglycerides in human breast milk using liquid extraction surface analysis Fourier transform mass spectrometry reveals new very long chain fatty acids and differences within individuals. Rapid Commun Mass Spectrom 2019;33:1267–76. https://doi.org/10.1002/rcm.8465.

[97] Liu Z, Moate P, Ezerniks V, Cocks BG, Rochfort S. Identification and quantification of triacylglycerols containing n-3 long-chain polyunsaturated fatty acids in bovine milk. J Dairy Sci 2015;98:8473–85. https://doi.org/10.3168/jds.2015-9889.

[98] Natarajan A, Christopher R, Palakuzhiyil SV, Chandra SR. Utility of measuring very long-chain fatty-acyl carnitines in dried blood spots for newborn screening of X-linked Adrenoleukodystrophy. Mol Genet Metab Rep 2021;26:100720. https://doi.org/10.1016/j.ymgmr.2021.100720.

[99] Hsu W-Y, Lin W-D, Hwu W-L, Lai C-C, Tsai F-J. Screening assay of very long chain fatty acids in human plasma with multiwalled carbon nanotube-based surface-assisted laser desorption/ionization mass spectrometry. Anal Chem 2010;82:6814–20. https://doi.org/10.1021/ac100772j.

[100] Craig LB, Brush RS, Sullivan MT, Zavy MT, Agbaga M-P, Anderson RE. Decreased very long chain polyunsaturated fatty acids in sperm correlates with sperm quantity and quality. J Assist Reprod Genet 2019;36:1379–85. https://doi.org/10.1007/s10815-019-01464-3.

[101] Moser AB, Liu Y, Shi X, Schrifl U, Hiebler S, Fatemi A, et al. Drug discovery for X-linked adrenoleukodystrophy: an unbiased screen for compounds that lower very long-chain fatty acids. J Cell Biochem 2021;122:1337–49. https://doi.org/10.1002/jcb.30014.

[102] Fujiwara Y, Hama K, Shimozawa N, Yokoyama K. Glycosphingolipids with very long-chain fatty acids accumulate in fibroblasts from adrenoleukodystrophy patients. Int J Mol Sci 2021;22. https://doi.org/10.3390/ijms22168645.

[103] Lemaitre RN, King IB. Very long-chain saturated fatty acids and diabetes and cardiovascular disease. Curr Opin Lipidol 2022;33:76–82.

[104] Kawaguchi K, Mukai E, Watanabe S, Yamashita A, Morita M, So T, et al. Acyl-CoA thioesterase activity of peroxisomal ABC protein ABCD1 is required for the transport of very long-chain acyl-CoA into peroxisomes. Sci Rep 2021;11:2192. https://doi.org/10.1038/s41598-021-81949-3.

[105] Kihara A. Very long-chain fatty acids: elongation, physiology and related disorders. J Biochem 2012;152:387–95. https://doi.org/10.1093/jb/mvs105.

[106] Murray M, Walchuk C, Suh M. Metabolism and function of very-long-chain polyunsaturated fatty acids (>C24) in mammals. LipidlibraryAocsOrg 2021. https://lipidlibrary.aocs.org/human-nutrition/metabolism-and-function-of-very-long-chain-polyunsaturated-fatty-acids-(c24)-in-mammals.

[107] Remize M, Planchon F, Loh AN, Le Grand F, Lambert C, Bideau A, et al. Identification of polyunsaturated fatty acids synthesis pathways in the toxic Dinophyte *Alexandrium minutum* using C-13-labelling. Biomolecules 2020;10:1428. https://doi.org/10.3390/biom10101428.

[108] Imbs AB, Ermolenko EV, Grigorchuk VP, Dang LTP. A lipidomic approach to the study of biodiversity of symbiotic dinoflagellates in *Millepora* hydrocorals from Vietnam coral reefs. Russ J Mar Biol 2021;47:312–7. https://doi.org/10.1134/S1063074021040064.

[109] Rezanka T, Kolouchová I, Nedbalová L, Sigler K. Enantiomeric separation of triacylglycerols containing very long chain fatty acids. J Chromatogr A 2018;1557:9–19. https://doi.org/10.1016/j.chroma.2018.04.064.

[110] Rezanka T, Kolouchová I, Mat'átková O. Chapter 4 - alternative sources of omega-3 polyunsaturated fatty acids. In: Atta-ur-Rahman, editor. Studies in natural products chemistry. vol. 67. Elsevier; 2020. p. 123–59. https://doi.org/10.1016/B978-0-12-819483-6.00004-7.

[111] Choma A, Zamłyńska K, Mazur A, Pastuszka A, Kaczyński Z, Komaniecka I. Lipid A from *Oligotropha carboxidovorans* lipopolysaccharide that contains two galacturonic acid residues in the backbone and malic acid a tertiary acyl substituent. Int J Mol Sci 2020;21:7991. https://doi.org/10.3390/ijms21217991.

[112] Choma A, Komaniecka I. Straight and branched (ω-1)-hydroxylated very long chain fatty acids are components of *Bradyrhizobium* lipid A. Acta Biochim Pol 2011;58:51–8.

[113] Monroig O, Tocher DR, Navarro JC. Biosynthesis of polyunsaturated fatty acids in marine invertebrates: recent advances in molecular mechanisms. Mar Drugs 2013;11:3998–4018. https://doi.org/10.3390/md11103998.

[114] Koopmans M, van Rijswijk P, Boschker HTS, Marco H, Martens D, Wijffels RH. Seasonal variation of fatty acids and stable carbon isotopes in sponges as indicators for nutrition: biomarkers in sponges identified. Marine Biotechnol 2015;17:43–54. https://doi.org/10.1007/s10126-014-9594-8.

[115] Carballeira NM, Montano N, Amador LA, Rodríguez AD, Golovko MY, Golovko SA, et al. Novel very long-chain α-methoxylated Δ5,9 fatty acids from the sponge *Asteropus niger* are effective inhibitors of topoisomerases IB. Lipids 2016;51:245–56. https://doi.org/10.1007/s11745-015-4114-9.

[116] de Kluijver A, Nierop K, Morganti T, Bart M, Slaby B, Hanz U, et al. Bacterial precursors and unsaturated long-chain fatty acids are biomarkers of North-Atlantic deep-sea demosponges. PLoS One 2021;16. https://doi.org/10.1371/journal.pone.0241095.

[117] Radnaeva LD, Bazarsadueva SV, Taraskin VV, Tulokhonov AK. First data on lipids and microorganisms of deepwater endemic sponge *Baikalospongia intermedia* and sediments from hydrothermal discharge area of the Frolikha Bay (North Baikal, Siberia). J Great Lakes Res 2020;46:67–74. https://doi.org/10.1016/j.jglr.2019.09.021.

[118] Kawaguchi K, Nakagawa KM, Nakagawa S, Shindou H, Nagao H, Noguchi H. Conformation of ultra-long-chain fatty acid in lipid bilayer: molecular dynamics study. J Chem Phys 2020;153:165101. https://doi.org/10.1063/5.0026030.

[119] Zhukov AV, Shumskaya M. Very-long-chain fatty acids (VLCFAs) in plant response to stress. Funct Plant Biol 2020;47:695–703.

[120] Busi R. Resistance to herbicides inhibiting the biosynthesis of very-long-chain fatty acids. Pest Manag Sci 2014;70:1378–84. https://doi.org/10.1002/ps.3746.

[121] Kim J, Lee SB, Suh MC. Arabidopsis 3-ketoacyl-CoA synthase 4 is essential for root and pollen tube growth. J Plant Biol 2021;64:155–65. https://doi.org/10.1007/s12374-020-09288-w.

[122] Chen Q, Xu F, Wang L, Suo X, Wang Q, Meng Q, et al. Sphingolipid profile during cotton fiber growth revealed that a phytoceramide containing hydroxylated and saturated VLCFA is important for fiber cell elongation. Biomolecules 2021;11:1352. https://doi.org/10.3390/biom11091352.

[123] Liu F, Wang P, Xiong X, Zeng X, Zhang X, Wu G. A review of nervonic acid production in plants: prospects for the genetic engineering of high nervonic acid cultivars plants. Front Plant Sci 2021;12:626625. https://doi.org/10.3389/fpls.2021.626625.

[124] Zhukov A, Popov V. Synthesis of C20–38 fatty acids in plant tissues. Int J Mol Sci 2022;23. https://doi.org/10.3390/ijms23094731.

[125] Jakobsson A, Westerberg R, Jacobsson A. Fatty acid elongases in mammals: their regulation and roles in metabolism. Prog Lipid Res 2006;45:237–49. https://doi.org/10.1016/j.plipres.2006.01.004.

[126] Carmona-Antoñanzas G, Monroig Ó, Dick JR, Davie A, Tocher DR. Biosynthesis of very long-chain fatty acids (C>24) in Atlantic salmon: cloning, functional characterisation, and tissue distribution of an Elovl4 elongase. Comp Biochem Physiol B Biochem Mol Biol 2011;159:122–9. https://doi.org/10.1016/j.cbpb.2011.02.007.

[127] Luo J, Monroig Ó, Liao K, Ribes-Navarro A, Navarro JC, Zhu T, et al. Biosynthesis of LC-PUFAs and VLC-PUFAs in *Pampus argenteus*: characterization of Elovl4 elongases and regulation under acute salinity. J Agric Food Chem 2021;69:932–44. https://doi.org/10.1021/acs.jafc.0c06277.

[128] Sun S, Wang Y, Goh P-T, Lopes-Marques M, Castro LFC, Monroig Ó, et al. Evolution and functional characteristics of the novel elovl8 that play pivotal roles

in fatty acid biosynthesis. Genes 2021;12:1287. https://doi.org/10.3390/genes12081287.
[129] Agbaga M-P, Mandal MNA, Anderson RE. Retinal very long-chain PUFAs: new insights from studies on ELOVL4 protein. J Lipid Res 2010;51:1624–42. https://doi.org/10.1194/jlr.R005025.
[130] Yeboah GK, Lobanova ES, Brush RS, Agbaga M-P. Very long chain fatty acid-containing lipids: a decade of novel insights from the study of ELOVL4. J Lipid Res 2021;62:100030. https://doi.org/10.1016/j.jlr.2021.100030.
[131] Monroig Ó, Kabeya N. Desaturases and elongases involved in polyunsaturated fatty acid biosynthesis in aquatic invertebrates: a comprehensive review. Fish Sci 2018;84:911–28. https://doi.org/10.1007/s12562-018-1254-x.
[132] Castro LFC, Tocher DR, Monroig O. Long-chain polyunsaturated fatty acid biosynthesis in chordates: insights into the evolution of Fads and Elovl gene repertoire. Prog Lipid Res 2016;62:25–40. https://doi.org/10.1016/j.plipres.2016.01.001.
[133] McMahon A, Kedzierski W. Polyunsaturated very-long-chain C28-C36 fatty acids and retinal physiology. Br J Ophthalmol 2010;94:1127–32. https://doi.org/10.1136/bjo.2008.149286.
[134] Bazan NG. Overview of how N32 and N34 elovanoids sustain sight by protecting retinal pigment epithelial cells and photoreceptors. J Lipid Res 2021;62:100058. https://doi.org/10.1194/jlr.TR120001137.