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*JB Review*

# Very long-chain fatty acids: elongation, physiology and related disorders

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**Very long-chain fatty acids (VLCFAs) are fatty acids (FAs) with a chain-length of ≥22 carbons. Mammals have a variety of VLCFAs differing in chain-length and the number of double bonds. Each VLCFA exhibits certain functions, for example in skin barrier formation, liver homeostasis, myelin maintenance, spermatogenesis, retinal function and anti-inflammation. These functions are elicited not by free VLCFAs themselves, but through their influences as components of membrane lipids (sphingolipids and glycerophospholipids) or precursors of inflammation-resolving lipid mediators. VLCFAs are synthesized by endoplasmic reticulum membrane-embedded enzymes through a four-step cycle. The most important enzymes determining the tissue distribution of VLCFAs are FA elongases, which catalyze the first, rate-limiting step of the FA elongation cycle. Mammals have seven elongases (ELOVL1–7), each exhibiting a characteristic substrate specificity. Several inherited disorders are caused by mutations in genes involved in VLCFA synthesis or degradation. In this review, I describe the molecular mechanism of FA elongation and the responsible enzymes in mammals and yeast, as well as VLCFA-related disorders in human.**

*Keywords*: fatty acid/lipid/membrane/sphingolipid/very long-chain fatty acid.

*Abbreviations*: ARVD, arrhythmogenic right ventricular dysplasia; AOX, acyl-CoA oxigenase; DBP, D-bifunctional protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ER, endoplasmic reticulum; FA, fatty acid; LCFA, long-chain FA; LOX, lipoxygenase; MUFA, monounsaturated FA; NADPH, nicotinamide adenine dinucleotide phosphate; PC, phosphatidylcholine; PUFA, polyunsaturated FA; SFA, saturated FA; STGD3, Stargardt disease type 3; VLCFA, very long-chain FA; X-ALD, X-link adrenoleukodystrophy.

## Classification of Cellular Fatty Acids

Fatty acids (FAs) are components of most cellular lipids, such as glycerolipids, sphingolipids and cholesterol esters. Major FA species include long-chain FAs (LCFAs) with carbon (C) chain lengths of 12–20, although very long-chain FAs (VLCFAs) with $C \geq 22$ also exist. The definition of VLCFAs differs among researchers and can include FAs with $C \geq 20$, $C \geq 24$ and $C \geq 26$; in this review, I will refer to FAs with $C \geq 22$. Any of these VLCFAs have important functions that cannot be substituted for by LCFAs, such as functions in skin barrier formation, retinal functions, resolution of inflammation, maintenance of myelin, sperm development and maturation and liver homeostasis.

FAs are classified into saturated FAs (SFAs), monounsaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs), depending on the number of double bonds (Fig. 1). PUFAs are further divided into $n-6$ (ω6) and $n-3$ (ω3) series, based on a particular double bond. The nomenclature $n-x$ (ω$x$) denotes a double bond between the $x$ and $x+1$ positions as counted from the carbon on the terminus opposite that of the carboxyl group, whereas $\Delta y$ represents a double bond between the $y$ and $y+1$ positions as counted from the carbon in carboxyl group. Palmitic acid (C16:0) synthesized *de novo* by FA synthase, or dietary FAs can be converted intracellularly to other FA species by elongation, desaturation or β-oxidation (Fig. 1). FAs are elongated by the addition of two carbons to the carboxylic acid side, resulting in an increase in the $\Delta y$ number to $\Delta y+2$. However, the $n-x$ (ω$x$) number remains unchanged by FA elongation. Therefore, representation of certain FA elongation pathways by $n-x$ (ω$x$) is useful.

## Mechanism of FA Elongation

FAs are elongated by endoplasmic reticulum (ER) membrane-embedded enzymes following their conversion to acyl-CoAs. FA elongation occurs by cycling through a four-step process (condensation, reduction, dehydration and reduction) (*1*, *2*). In the first, rate-limiting step, acyl-CoA is condensed with malonyl-CoA to produce 3-ketoacyl-CoA (Fig. 2). This reaction is catalyzed by an FA elongase. Mammals have seven FA elongases (ELOVL1–7) (Table I), and each exhibits a characteristic substrate specificity (discussed later) (*2*, *3*). The FA elongation reactions and responsible enzymes are conserved among eukaryotes, and the yeast *Saccharomyces cerevisiae* possesses three FA elongases (Elo1, Fen1/Elo2 and Sur4/Elo3) (*4*) (Table I). In the second step, 3-ketoacyl-CoA is reduced to 3-hydroxyacyl-CoA by a 3-ketoacyl-CoA reductase (KAR in mammals and Ybr159w in yeast) (*5*, *6*) (Fig. 2 and Table I). Nicotinamide adenine dinucleotide phosphate (NADPH) is used as a reducing agent in this

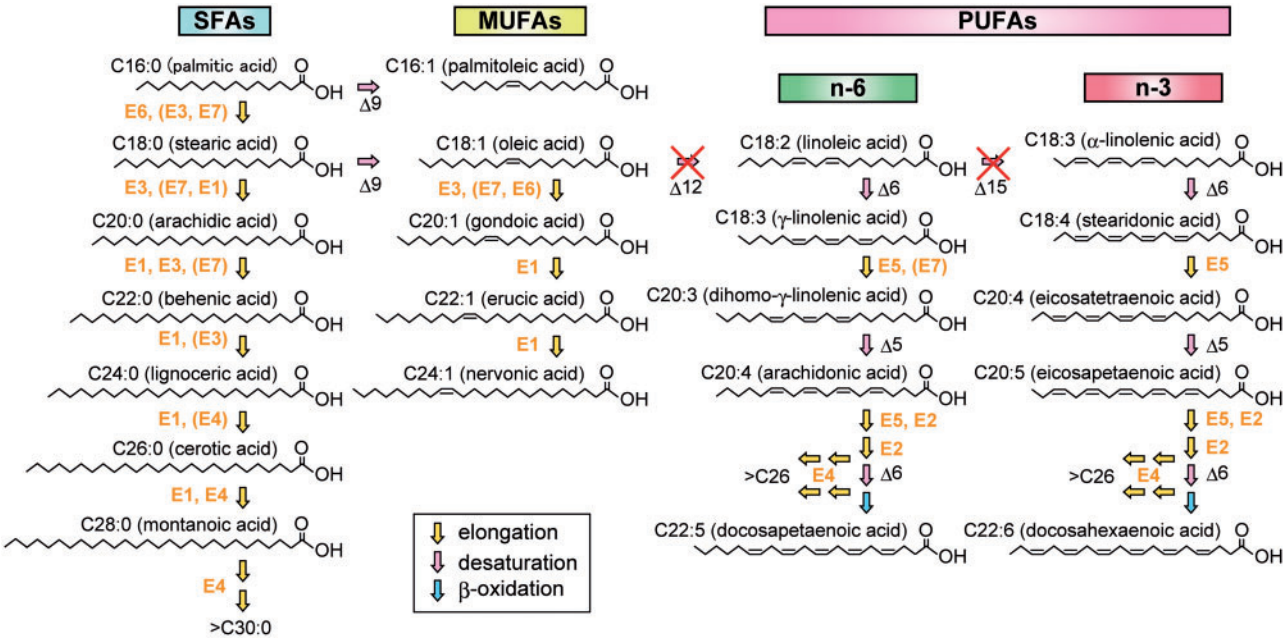


**Fig. 1 FA elongation pathways in human.** Human FA elongation pathways and the ELOVLs involved in each pathway are illustrated. Neither Δ12 nor Δ15 desaturase exists in humans, so $n-6$ and $n-3$ FAs are not synthesized *de novo*. E*x* and Δ*x* represent ELOVL*x* and Δ*x* desaturase, respectively, where *x* denotes the enzyme number. Parentheses indicate ELOVLs having weak activities in the indicated reactions.

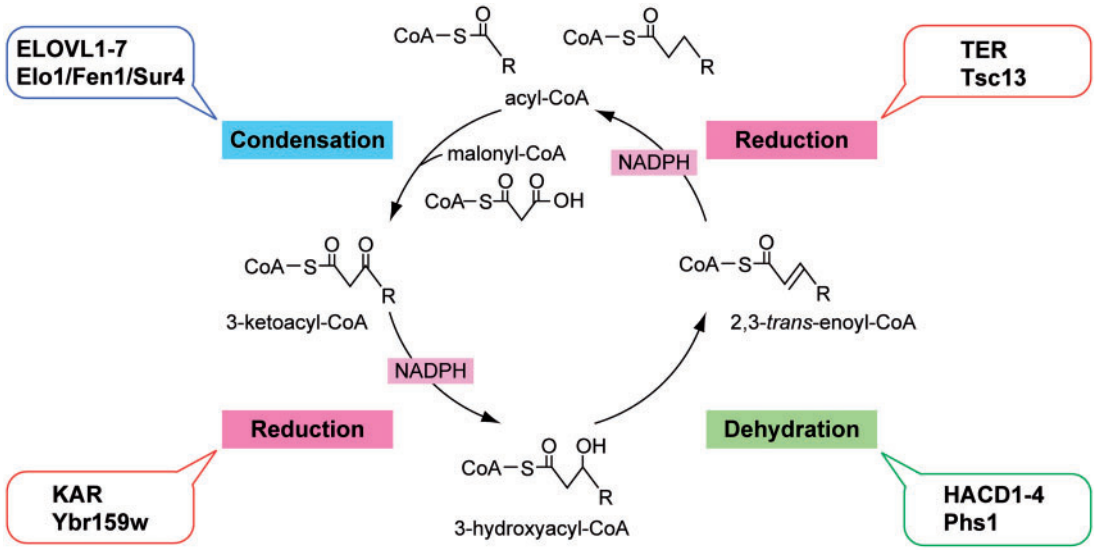


**Fig. 2 FA elongation cycle.** The FA elongation cycle and the mammalian and yeast enzymes involved in each step are illustrated. Acyl-CoA is elongated to have two more carbon units in each elongation cycle, which consists of four steps (condensation, reduction, dehydration and reduction).

reaction. 3-Hydroxyacyl-CoA is then dehydrated by 3-hydroxyacyl-CoA dehydratase, generating 2,3-*trans*-enoyl-CoA. Mammals have four isozymes (HACD1–4) as their 3-hydroxyacyl-CoA dehydratases, whereas the yeast has only one (Phs1) (*7*, *8*) (Table I). Finally, 3-hydroxyacyl-CoA is reduced to an acyl-CoA having two more carbon chain units than the original acyl-CoA (Fig. 2). A 2,3-*trans*-enoyl-CoA reductase (TER in mammals and Tsc13 in yeast) catalyzes this reaction using NADPH as a reductant (*6*, *9*) (Table I). The enzymes responsible for the FA elongation cycle can interact with each other and form elongase complex(es) (*7*, *8*, *10*, *11*).

The FA elongases ELOVL1-7 exhibit characteristic substrate specificities towards acyl-CoAs (*2*, *3*) (Fig. 1). ELOVL1, 3, 4, 6 and 7 are involved in the elongation of SFAs and MUFAs. ELOVL6 is responsible for the elongation of C16:0-CoA or shorter, saturated acyl-CoAs. ELOVL3 and ELOVL7 elongate both saturated and unsaturated C16–C22 acyl-CoAs with the highest activities towards C18-CoAs (*3*, *12*). ELOVL1 elongates saturated C18:0–C26:0 and monounsaturated C20:1$n-9$ and C22:1$n-9$ acyl-CoAs (*3*). In mammalian cells, relatively high amounts of sphingolipids having C24:0 or C24:1$n-9$ FAs exist; ELOVL1 is the major FA elongase responsible for

**Table I. Enzymes involved in FA elongation, their human and yeast genes and related disorders.**

| Enzyme | Human gene | Yeast gene | Disorder |
|---|---|---|---|
| FA elongase | *ELOVL1–7* | *ELO1*, *FEN1*, *SUR4* | STGD3 and ichthyosis (human *ELOVL4*) |
| 3-Ketoacyl-CoA reductase | *KAR* | *Ybr159w*, *AYR1* | |
| 3-Hydroxyacyl-CoA dehydratase | *HACD1–4* | *PHS1* | Centronuclear myopathy (dog *HACD1*) |
| 2,3-*Trans*-enoyl-CoA reductase | *TER* | *TSC13* | Non-syndromic mental retardation (human *TER*) |

the production of C24 sphingolipids (*3*). ELOVL4 is responsible for extremely long SFAs, such as >C26 FAs found in skin, as well as >C26 PUFAs found in brain, retina and spermatozoa (*13*). In contrast to these five enzymes active towards SFAs and MUFAs, ELOVL2 and ELOVL5 are strictly PUFA-specific elongases. ELOVL2 prefers longer acyl-CoAs than ELOVL5. ELOVL2 and ELOVL5 elongate C22-CoAs and C18-CoAs, respectively, and both can elongate intermediate C20-CoAs (*3*).

Each ELOVL exhibits a characteristic tissue distribution pattern (*3*) and differences in FA composition among tissues can be explained, at least partly, by the differential expression of ELOVLs. For example, *ELOVL1*, *5* and *6* mRNAs are widely expressed among tissues, consistent with ubiquitously observed products or derivatives thereof (ELOVL1, C24 sphingolipids; ELOVL5, arachidonic acid (C20:4$n-6$); ELOVL6, stearic acid (C18:0)). On the other hand, the expression of ELOVL4 is restricted to tissues where extremely long FAs exist. *ELOVL4* mRNA is expressed highest in retina, followed by skin, brain and testis (*14*, *15*).

## Saturated VLCFAs and Sphingolipids

Most saturated and monounsaturated VLCFAs in mammals are found as constituents of sphingolipids. Widely conserved in eukaryotes, sphingolipids are abundant in the outer leaflet of the plasma membrane. Ceramide, the backbone of sphingolipids, is composed of two amide-linked hydrophobic chains, a long-chain base and an FA (*16*) (Fig. 3). In mammals, the polar head group of each sphingolipid is phosphocholine (sphingomyelin) or a sugar (glycosphingolipids), and hundreds of glycosphingolipids exist, differing in species, number and linkage position of sugars.

In mammals, the major FAs in sphingolipids are C16:0 FA (palmitic acid) and C24 FAs (C24:0 lignoceric acid and C24:1 nervonic acid) (Fig. 3), although in some tissues, such as brain and skeletal muscle, the content of C18:0 stearic acid in sphingolipids is unusually high (*16*). Generally, the ratio of C24 sphingolipids to total sphingolipids varies among tissues. For example in mice, C24 sphingomyelin reflects ~25% of the total sphingomyelin in brain, ~15% in testis, ~15% in skeletal muscle, ~45% in kidney and ~60% in liver (*17*).

Ceramide synthesis is catalyzed by ceramide synthases, of which mammals have six (CerS1–6). Each ceramide synthase exhibits a characteristic substrate specificity towards an acyl-CoA (CerS1, C18; CerS2, C22 and C24; CerS3, ≥C26; CerS4, C20; CerS5, C16; and CerS6, C16) (*18*). The difference in the tissue-specific expression pattern of each ceramide synthase may partly account for the tissue distribution patterns of sphingolipids with certain acyl-chains. For example, the abundance of C24 sphingomyelin in liver and kidney correlates with high expression of *CerS2* mRNA (*17*). Similarly, the expression levels of *CerS1* are high in brain and skeletal muscle, tissues in which the CerS1 products, C18 sphingolipids, are abundant (*17*), whereas the ceramide synthase CerS3 is expressed at high levels in skin and testis, where sphingolipids with ≥C26 FAs are found (*17*, *19*).

C24 sphingolipids exhibit unique physical properties that differ completely from those of C16 sphingolipids or glycerophospholipids. These properties include unique abilities to interdigitate with the opposing leaflet of a lipid bilayer and to cluster with other lipids to form lipid microdomains (*20*). Moreover, the long FA portion of C24 sphingolipids can alter the thickness of the plasma membrane locally. Such C24 sphingolipid-containing lipid microdomains are important as signalling platforms for certain proteins. For example, C24 lactosylceramides (glycosphingolipids containing glucose and galactose) are important for the activation of the Src family kinase Lyn and for cell signalling in neutrophils (*20*). In HeLa cells, a shift in sphingolipid composition from C24 to C16 by knockdown of either *ELOVL1* or *CerS2* causes a change in cell membrane properties and increases cellular susceptibility to apoptosis (*21*). Recent studies using *CerS2*-deficient mice having grossly reduced C24 sphingolipid levels revealed a requirement for C24 sphingolipids in liver homeostasis and myelin maintenance (*22*, *23*). Myelin normally contains high levels of galactocylceramides and sulfatides with C24 and C22 VLCFAs, but in the *CerS2*-deficient mice, both are reduced (*22*).

In the stratum corneum of the epidermis, the extracellular spaces of corneocytes are filled with a hydrophobic lipid mixture organized into multi-lamellar membranous structures known as lipid lamellae, which play a pivotal function in skin barrier formation (*18*, *24*). Ceramide is a major lipid component of lipid lamellae, and the ceramide species in epidermis is quite unique. Unusually long FAs, even >C30, are found in epidermis ceramides (*25*). Moreover, some ceramides are ω-hydroxylated at the FA portion and esterified with linoleic acid (C18:2$n-6$) (Fig. 3) or with envelope proteins (*24*). The importance of ceramides with extremely long FA and ω-acyl-ceramides in skin barrier function was revealed by studies in *Elovl4* mutant mice (*26*, *27*). *Elovl4* mutant mice exhibit a neonatal lethal phenotype due to a skin barrier defect. In these mice,

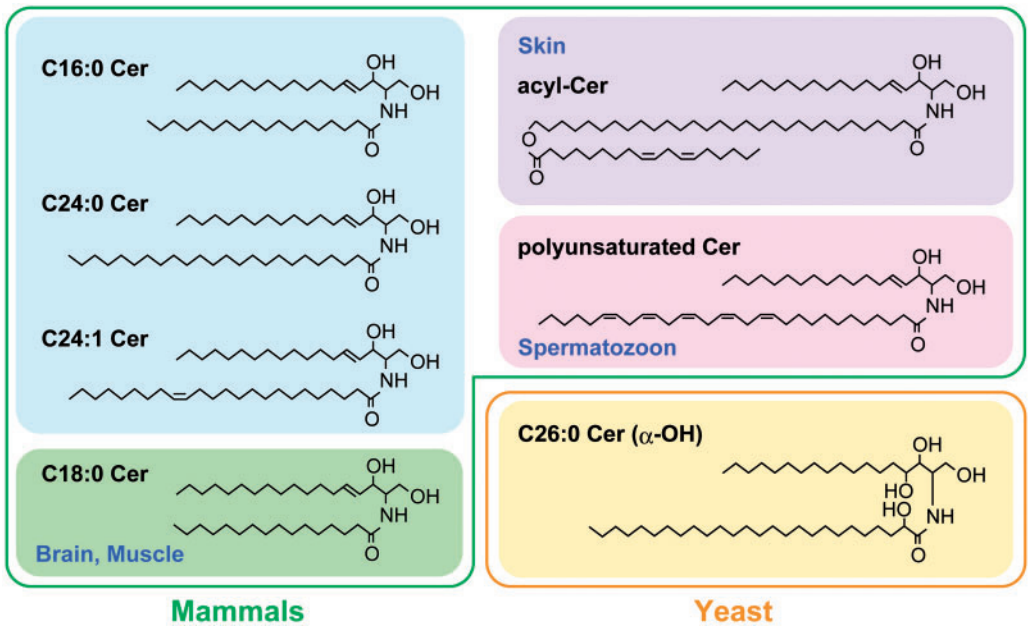


**Fig. 3 Ceramide species in mammals and yeast.** Structures of ceramides found in mammals and yeast are illustrated. C16 ceramide and C24 ceramide with C24:0 or C24:1 FA exist ubiquitously in mammalian tissues, although the ratio of C16 ceramide to C24 ceramide varies among tissues. In contrast, C18:0 ceramide levels are unusually high in brain and skeletal muscle. Acyl-ceramide is specific to skin, and polyunsaturated ceramide exists only in the testis germ cells and spermatozoa. The FA of yeast ceramide is C26:0 with or without α-hydroxylation. Cer, ceramide.

the levels of ceramides/glucosylceramides with ≥C28 are greatly reduced, and acyl-ceramides are absent. Among the Cer synthases, CerS3 is responsible for the production of ceramides with extremely long chain FAs (≥C26) (*28*). It is not surprising, then, that *CerS3* knockout mice exhibit a skin barrier defect similar to that in *Elovl4* mutant mice and die shortly after birth (*28*).

## Very Long-Chain PUFAs

In humans, palmitic acid (C16:0) and stearic acid (C18:0) can be converted to palmitoleic acid (C16:1$n-7$) and oleic acid (C18:1$n-9$), respectively, by Δ9 desaturase (stearoyl-CoA desaturase). However, neither Δ12 desaturase, which produces linoleic acid (C18:2$n-6$) from oleic acid, nor Δ15 desaturase, which converts linoleic acid to α-linolenic acid (C18:3$n-3$), exists in humans (Fig. 1), so linoleic acid and α-linolenic acid must be consumed through food. Dietary linoleic acid and α-linolenic acid can be converted to other n − 6 and n − 3 FAs, respectively (Fig. 1). For example, α-linolenic acid is metabolized to eicosapentaenoic acid (EPA; C20:4$n-3$) via desaturation by Δ6 desaturase and subsequent elongation. EPA is further converted to the well-known very long-chain PUFA (VLC-PUFA) docosahexaenoic acid (DHA; C22:6$n-3$) by two successive elongation cycles, desaturation by Δ6 desaturase and β-oxidation in a peroxisome (Fig. 1). DHA is abundant in glycerolipids found in the grey matter of the brain and is important in the functional development of the brain (*29*). High levels of DHA also exist in glycerolipids found in the retinal photoreceptor outer segment membranes, where it may function in regulating phototransduction (*30*).

DHA also functions as a precursor of the recently identified lipid mediators active in resolving the inflammatory response, such as D-series resolvins (resolvin D1–D4), protectin D1 and maresin 1 (*31*). Conversion of DHA to 17*S*-hydroperoxy-DHA by 15-lipoxygenase (LOX), and subsequent conversion of 17*S*-hydroperoxy-DHA by 5-LOX or 15-LOX produces D-series resolvins or protectin D1, respectively (*31*). On the other hand, conversion of DHA to 14*S*-hydroperoxy-DHA by 12-LOX, which is present in macrophages and platelets, and subsequent enzymatic epoxidation and hydrolysis produces maresin 1 (*32*). Resolvin D exerts anti-inflammatory actions by reducing neutrophil migration and infiltration (*31*, *33*). Protectin, named for its tissue-protective effects on the brain, the immune system and retina, displays potent anti-inflammatory activity and activates the resolution of inflammation, in addition to its cell-protective activity (*31*). Maresin 1 reduces neutrophil migration and stimulates phagocytosis by macrophages (*32*).

VLC-PUFAs with >C24 uniquely exist in mammalian brain, retina, testis and spermatozoa (*13*). In brain, phosphatidylcholine (PC) contains $n-3$ or $n-6$ series VLC-PUFAs having up to C38 and four to six double bonds at the $sn-1$ position (*13*, *34*). In the brains of peroxisome-deficient Zellweger's syndrome patients, increased levels of $n-6$ PUFAs having up to C40 with 5 and 6 double bonds have been observed (*34*), suggesting a role for VLC-PUFAs in brain physiology.

In human retina, PC can have FAs with up to C36 and three to six double bonds at the $sn-1$ position and DHA at the $sn-2$ position (*13*, *30*, *35*). Stargardt disease type 3 (STGD3) is a juvenile-onset

macular dystrophy characterized by gradual vision loss, lipofuscin accumulation and window defects in the macula (*13*, *36*). STGD3 is caused by dominant mutations in the *ELOVL4* gene (discussed later); ELOVL4 is responsible for the elongation of >C26 FAs. *Stgd3*-knockin mice that carry the human pathogenic 5-bp deletion in the mouse *Elovl4* gene (an animal model of STGD3) are defective in the production of PC having C32-C36 VLC-PUFAs (*37*). These findings suggest that these extremely long VLC-PUFAs, in addition to DHA, are important for the maintenance of normal retinal function.

Although the FA moieties of sphingolipids are usually saturated or monounsaturated, there are unique sphingolipids present in testis germ cells and spermatozoa that contain VLC-PUFAs with C26–32 and 4–6 double bonds at $n-6$ and $n-3$ (*38*) (Fig. 3). These VLC-PUFA-containing sphingolipids include sphingomyelin and a class of fucosylated gangliosides (glycosphingolipids containing sialic acid). In testis, spermatogenesis proceeds as spermatogonia differentiate into primary spermatocytes and undergo meiosis, generating spermatids that maturate into spermatozoa. Sphingolipids containing VLC-PUFA first appear at the pachytene spermatocyte stage, one of the prophase substages of the first meiotic division (*39*). VLC-PUFA-containing sphingomyelin are abundant in the head of spermatozoa. During capacitation (the destabilization of the sperm head membrane that is the penultimate step in the maturation of mammalian spermatozoa and is required to render them competent for oocyte fertilization), VLC-PUFA sphingomyelin is converted to VLC-PUFA ceramide (*40*). From these results, VLC-PUFA-containing sphingolipids are thought to function in spermatogenesis and capacitation.

## Disorders Associated with Mutations in VLCFA-Related Genes

Several inherited diseases are related to VLCFAs. As described earlier, STGD3 is one such disorder caused by mutations in the *ELOVL4* gene (Table I). To date, three distinct *ELOVL4* mutations have been reported to cause STGD3, all resulting in a frameshift that leads to the production of C-terminally truncated proteins (*13*, *36*). The loss of the C-terminal region of the protein abolishes its enzymatic activity and results in its mislocalization to aggresomes or the Golgi, because this region contains a C-terminal ER retention signal (*13*, *36*). Moreover, the mutant protein forms oligomers with wild type ELOVL4 and with components of the elongase complex, causing mislocalization and/or inactivation of the interacting proteins (*10*, *36*). Such oligomerization is assumed to be the basis for the autosomal, dominant mode of inheritance of the STGD3. Although no patients with a homozygous *STGD3* mutation have been reported so far, individuals carrying recessive *ELOVL4* mutations different from the *STGD3* mutations have recently been found (*41*). Two patients with these mutations display a neurocutaneous disorder of ichthyosis, seizures, mental retardation and spasticity (*41*). Considering information obtained in studies of the *Elovl4* mutant mice, VLC-ceramides and acyl-ceramides in the epidermis of the homozygous *ELOVL4* patients may be affected.

Mutations in the *ABCA12* gene, which encodes a member of the ATP-binding cassette transporters, also cause ichthyosis (*42*). The ABCA12 protein is expressed in granular layer keratinocytes and is involved in the transport of glucosylceramides into the inner side of lamellar granules. Most ceramides, including acyl-ceramides, in the lipid lamella of stratum corneum are derived from glucosylceramides (*24*). Glucosylceramides in lamellar granules are released in the stratum corneum and hydrolyzed to ceramides.

The *TER* (*TECR*) gene encodes the 2,3-*trans*-enoyl-CoA reductase, which catalyzes the fourth step of the FA elongation cycle (*6*). A mutation in the *TER* gene that substitutes Pro182 to Leu (P182L) in the gene product causes autosomal recessive non-syndromic mental retardation (*43*) (Table I). To date, no other 2,3-*trans*-enoyl-CoA reductase gene involved in VLCFA generation has been reported in mammals. VLCFA production is essential for organogenesis and embryonic survival, as revealed by the studies in knockout mice for the *KAR* (*HSD17B12*) gene (*44*), which encodes the 3-ketoacyl-CoA reductase responsible for the second step of the FA elongation cycle. Therefore, it is reasonable to consider that the TER(P182L) mutant protein may exhibit residual but reduced enzyme activities.

The *HACD1–4* genes encode 3-hydroxyacyl-CoA dehydratases and function in the third step of the FA elongation cycle (*8*). *HACD1* (*PTPLA*) mRNA is highly tissue specific, and its expression is restricted to heart and skeletal muscle (*45*). An insertion mutation in the Labrador Retriever canine *HACD1* gene causes centronuclear myopathy (Table I), which is characterized by skeletal muscle atrophy accompanied by a type II fibre deficiency (*46*). Although involvement of the *HACD1* mutation in the pathogenesis of human myopathy has not yet been reported, a point mutation in the human *HACD1* gene has been implicated, but not established, in arrhythmogenic right ventricular dysplasia (ARVD) (*45*). ARVD, a major cause of sudden death in the young and in athletes, is an inherited heart disease characterized by a gradual loss of right ventricular myocardium and replacement by adipose and fibrous tissue (*47*). A point mutation (K64Q) was found in all affected individuals in a large ARVD family ($n=10$), yet the mutation was also detected in normal control subjects (3 alleles per 100 chromosomes) (*45*). Biochemical analyses demonstrated that the HACD1 (K64Q) mutant protein exhibited normal enzyme activity (*11*). Nevertheless, although biochemical studies did not support any direct involvement of the *HACD1* mutation in ARVD, it is still possible that long-term accumulation of undetectable defects of the mutant protein affects heart function.

Defects in VLCFA degradation/β-oxidation also have causative effects in pathogenic disorders. VLCFAs are β-oxidized to LCFAs in peroxisomes after being converted to VLC acyl-CoAs. Transport

of VLC acyl-CoAs to peroxisomes involves the ABC transporter ABCD1 (*48*). Mutations in the *ABCD1* gene result in the accumulation of C24 and C26 VLCFAs in plasma, brain, adrenal gland and other tissues, and cause X-link adrenoleukodystrophy (X-ALD) (*48*), including the two major clinical phenotypes of X-ALD, adrenomyeloneuropathy and the more severe cerebral adrenoleukodystrophy. β-oxidation of VLCFAs involves four reactions in each cycle: dehydrogenation, hydration, dehydrogenation and thiolytic cleavage (*49*). Peroxisomal straight-chain acyl-CoA oxigenase (AOX) catalyzes the first dehydrogenation reaction. Both the second and third reactions are catalyzed by the bifunctional enzyme D-bifunctional protein (DBP), and 3-ketoacyl-CoA thiolase is responsible for the fourth reaction. Deficiencies in AOX cause hypotonia, delayed motor development, sensory deafness and retinopathy. Similarly, DBP deficiency leads to hypotonia, craniofacial dysmorphia, neonatal seizures, hepatomegaly and developmental delay (*49*). In conclusion, then, the homeostasis of VLCFAs is important generally for maintaining normal tissue and cellular functions.

## VLCFAs in Yeast

VLCFAs in the yeast *S. cerevisiae* consist almost entirely of C26:0 FA with or without α-hydroxylation (*50*). C26:0 FA mainly exists in sphingolipids but also in small amounts in glycosylphosphatidylinositol anchors (*4*). Although FA moieties of mammalian sphingolipids are either long-chain (C16 or C18) or VLC (mainly C24) (*16*), those of yeast sphingolipids are exclusively C26 VLCFA (Fig. 3) (*50*).

Sphingolipid synthesis is essential for yeast growth. However, a suppressor mutation in the 1-acylglycerol-3-phosphate acyltransferase *SLC1* gene (*SLC1-1* mutation) allows cell growth of the mutant yeast, even though it cannot synthesize sphingolipids (*51*). In this yeast, a phosphatidylinositol with an unusual C26:0 FA at the $sn-2$ position is produced, which partly substitutes for sphingolipids in function. This observation suggests that the VLCFA portion of sphingolipids has important functions in and of itself.

VLCFA synthesis is also essential for yeast growth. Of the three yeast FA elongases, Fen1 and Sur4 are involved in VLCFA synthesis, and Elo1 is involved in the elongation of C14 FA to C16 FA (*4*, *52*). In a Δ*sur4* mutant C26 VLCFAs are absent, instead C24 and especially C22 VLCFAs are accumulated (*53*). In Δ*fen1* cells, C26 VLCFAs still exist but are largely reduced (*53*). Although both the Δ*sur4* and Δ*fen1* single deletion mutants can grow, a double deletion of the *FEN1* and *SUR4* genes is lethal (*54*). Similarly, cells deficient in the *YBR159w* gene, which encodes the yeast 3-ketoacyl-CoA reductase, still grow, albeit slowly, and they still exhibit weak 3-ketoacyl-CoA reductase activity (*5*). This residual activity has been attributed to the Ayr1 protein, and Δ*ybr159w* Δ*ayr1* cells are inviable (*5*). The *PHS1* and *TSC13* genes encode the yeast 3-hydroxyacyl-CoA dehydratase and 2,3-*trans*-enoyl-CoA reductase, respectively, and both are essential for viability (*7*, *9*).

In mutants of genes involved in VLCFA synthesis, sphingolipid formation is affected so that long-chain bases, long-chain base 1-phosphates and ceramides are increased, whereas complex sphingolipids are decreased (*5*, *7*, *9*, *53*, *55*). Although the physiological functions of VLCFAs still remain largely unknown, several lines of observation suggest that they are involved in protein transport. In a *phs1* mutant, the vacuolar alkaline phosphatase is abnormally processed, and the mature Gas1 protein is reduced (*56*). Mutation in the *FEN1* or *SUR4* gene suppresses the temperature-sensitive growth, and secretion-defective phenotypes of the *snc* mutant, which lacks vesicular membrane proteins known as v-SNAREs (*57*). We recently identified the *VPS21* gene as a multicopy suppressor of temperature-sensitive growth of VLCFA-limited yeast cells. Vps21 is a Rab GTPase involved in vesicular transport to the vacuole. Furthermore, from studies using double yeast mutants bearing both a Δ*sur4* mutation and one of several mutations affecting vacuolar protein sorting (*vps*), including Δ*vps21*, we speculate that VLCFAs are involved in vesicular transport at the late endosome/multivesicular body (our unpublished results). In addition to the proposed functions of VLCFAs in vesicular transport, involvement of VLCFAs has also been suggested in the maintenance of a functional nuclear envelope and in the biogenesis of microautophagic vesicles (*58*, *59*). The chain length of VLCFAs allows them to span both leaflets of the lipid bilayer, which would facilitate generation and stabilization of highly curved membranes. During vesicle budding and fusion, local generation of highly curved membranes is essential, for example at the neck of budding vesicles and at the contact sites of lipid mixing during vesicle fusion. Highly curved membranes also exist around the nuclear pore complex. Thus, it would seem likely that VLCFAs are involved in vesicular trafficking, maintenance of a functional nuclear envelope and microautophagy by stabilizing highly curved membranes.

## Conclusion

The unusually long chain-length in VLCFAs confers unique functions differing from those of LCFAs. The highly hydrophobic nature of the ceramides with extremely long FAs that are found in the epidermis imparts a resistance on the lipid lamella towards exogenous compounds and water loss. Moreover, the VLCFA portions of sphingolipids can interdigitate into the opposite leaflet of the lipid bilayer, an activity that may function in lipid microdomain formation and in stabilizing highly curved membranes. VLC-PUFAs in glycerolipids may also affect membrane functions by altering fluidity, lipid phase properties, permeability and clustering. Although in this review I describe only those disorders affected by mutations in VLCFA-related genes, VLCFA levels are also altered in other many diseases. Furthermore, it is expected that greater and more prominent importance will be ascribed to VLCFAs in physiological and pathological

processes, as future studies uncover more of their varied and unique functions.

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### Conflict of interest

None declared.

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