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CHAPTER 7

Fatty acid desaturation and chain elongation in eukaryotes

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1. Introduction

Although the contribution of lipid molecules to the hydrophobic character of membranes was recognized late in the nineteenth century, the nutritional importance of specific lipid molecules was first revealed through the pioneering work of Burr and Burr in 1929. They fed rats a fat-free diet and observed that retarded growth, scaly skin, tail necrosis and eventual death were reversed by feeding specific fats. Linoleic acid was recognized as the active agent and the term 'essential fatty acid' was coined.

During the 1950s, the advent of chromatographic techniques (gas–liquid and thin-layer chromatography) and greater availability of appropriate substrates and precursors labelled with radioisotopes contributed in a major way to current understanding of fatty acid metabolism. In the 1960s, studies focused on in vitro assays of specific enzymatic steps [1]. Δ9 desaturase was measured in yeast, rat liver microsomes and plants. It was determined that polyunsaturated fatty acid (PUFA) formation in animal tissues involved Δ6 and Δ5 desaturation. Chain elongation of long chain fatty acids was found in the endoplasmic reticulum (ER) and mitochondria [2,3]. Relationships between essential fatty acids and prostaglandins (Chapter 13) were elucidated. Subsequent work led to an understanding of primary, alternate and competitive pathways of desaturation and chain elongation and of factors that regulate fatty acid metabolism.

The 1990s and 21st century have seen the advent of molecular and genetic systems that support in-depth analysis of the regulation of fatty acid elongation and desaturation pathways [4–9]. More complete understanding of the roles of particular unsaturated fatty acids in the regulation of cell biology and human health and disease continues to evolve.

1.1. Physical consequences of fatty acyl chain desaturation and elongation

Among the forces holding lipid molecules within membranes, London–Van der Waals interactions have a major impact along the fatty acyl chains. These relatively weak forces are additive, proportional to the number of overlapping methylene groups, and inversely proportional to the distance between them. Thus, for long fatty acyl chains, total London–Van der Waals bonding strength is greater than electrostatic and hydrogen bonding of polar head groups. Accordingly, length of a fatty acyl chain is a crucial

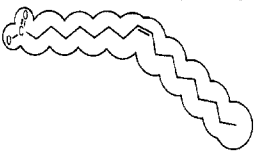
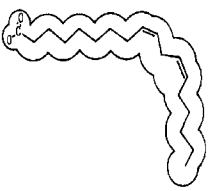
FATTY ACID	ABBREVIATION	MELTING POINT	SPATIAL WIDTH(nm)	POSSIBLE CONFIGURATION
Stearic Acid	18:0	70°	0.25	
Oleic Acid	c-18:1n-9	16°	0.72	
Elaidic Acid	t-18:1n-9	43°	0.31	
Linoleic Acid	c,c-18:2n-6	-5°	1.13	

Fig. 1. Physical characteristics of fatty acids. See Table 1 for nomenclature of the fatty acids. Single bonds have a length of 0.154 nm and an angle of approximately 111°; double bonds have a length of 0.133 nm and an angle of approximately 123°. *c*, *cis*; *t*, *trans*.

parameter in its contribution to membrane structure and stability. Restricted solubility in an aqueous environment is governed by the tendency of acyl chains to remain associated with one another (oil does not dissolve well in water). Similarly, response to temperature is modulated by the extent to which thermal influences dissociate acyl chains. Thus, as acyl chain length increases, solubility decreases and melting point increases. Where more loosely packed membrane structures are advantageous, rigidity of lengthy saturated acyl chains can be countered by double bonds. A double bond of *cis* geometric configuration results in a 30° angle change from the linearity of the saturated chain (Fig. 1). Double bonds also are non-rotating, restrict chain movement and increases polarity through charge concentration.

Double bonds cause bends in fatty acyl chains within biological membranes (potentially, but probably not in a membrane milieu, an acyl chain with six double bonds could assume nearly circular shape) and decrease rigidity. Accordingly, within membranes, acyl chain length and the number and position of double bonds markedly influence fluidity, permeability and stability of biological membranes.

1.2. Fatty acyl chains and biology

Many (but not all) required fatty acids also can be biosynthesized by mammalian cells. Altering fatty acyl composition of membrane lipids influences physical properties and thus changes the function of integral proteins. Release of specific fatty acids from

membrane stores regulates cellular signal transduction and gene transcription with specificity determined by differences in the unsaturation and chain length [10–13].

Fatty acids with several double bonds are known as PUFA. Some PUFA can only be synthesized from dietary fats as animal cells do not have the ability to make double bonds at some positions along the fatty acyl chain. Required dietary fatty acids are known as 'essential fatty acids'. PUFA serve as precursors of biologically active prostaglandins and leukotrienes (Chapter 13) that are required to mount immune and inflammatory responses to infection and pain. In the nucleus, PUFA also control gene transcription through separate classes of receptors called peroxisomal proliferator activated receptors (PPARs) [14] and sterol response elements binding proteins (SREBPs) [15] (also see Chapters 10 and 15). PUFA released from membrane phospholipids by agonist-induced stimulation of phospholipases (Chapter 11) can be involved in signal transduction through modulation of cellular proteins including isoforms of protein kinase C or cyclases, and in translocation of specific enzymes to membranes [12]. Many of these diverse functions are modulated by a specific type of fatty acid. Thus, a large variety of fatty acyl chains are required in the lipids of biological membranes and storage depots. Types obtained through dietary intake and de novo synthesis are insufficient to meet the varied demands of cells, so there is substantial metabolism and rearrangement as development, growth, and aging proceed. Knowledge of how the array of fatty acyl chains is derived and modified, and what regulates metabolism of fatty acyl chains by desaturation and chain elongation are described in sections that follow.

2. *Chain elongation of long chain fatty acids*

De novo synthesis of fatty acids (Chapter 6) produces mainly 16-carbon palmitic acid, with minor amounts of 18-carbon stearic acid (Table 1). Quantitatively, these chain lengths are major components of many membrane lipids; qualitatively, they are related to optimal width of the membrane lipid bilayer. Many major chains are longer than 16 or 18 carbons, constituting more than half of the total acyl chains of many tissues. For example, in myelin surrounding axonal processes of neuronal cells, fatty acyl chains of 18 carbons or greater are more than 60% of the total, and in sphingolipids, 24-carbon acyl chains are prominent. Chain lengths of 28–36 carbons have been reported in phospholipids of retinal photoreceptors.

Many eukaryotic cells have capacity for 2-carbon chain elongation of both endogenously synthesized and dietary fatty acids (Fig. 2). In liver, brain and other tissues, two primary systems — one in the endoplasmic reticulum (ER) and the other in mitochondria — provide chain elongation. The ER system predominates quantitatively. Mitochondrial elongation is distinct from reversal of fatty acid catabolism through β-oxidation (Chapter 5). Peroxisomes also contain an acetyl-CoA dependent elongation system that is enhanced markedly after treatment with specific fatty acids and drug mimics known collectively as peroxisomal proliferators (Chapter 10).

Table 1
Nomenclature and bond positions of major long chain fatty acids

Common name	Systematic name [a]	Abbreviations	Δ Bond positions
Palmitic acid	hexadecanoic acid	16:0	
Palmitoleic acid	9-hexadecenoic acid	16:1 n-7	9
	6-hexadecenoic acid	16:1 n-10	6
Stearic acid	octadecanoic acid	18:0	
Oleic acid	9-octadecenoic acid	18:1 n-9	9
Vaccenic acid	11-octadecenoic acid	18:1 n-7	11
Petroselenic acid	6-octadecenoic acid	18:1 n-12	6
Elaidic acid	*t*-9-octadecenoic acid	*t*-18:1 n-9	9
Linoleic acid	9,12-octadecadienoic acid	18:2 n-6	9,12
Linoelaidic acid	*t,t*-9-12-octadecadienoic acid	*t,t*-18:2 n-6	9,12
α-Linolenic acid	9,12,15-octadecatrienoic acid	18:3 n-3	9,12,15
γ-Linolenic acid	6,9,12-octadecatrienoic acid	18:3 n-6	6,9,12
Stearidonic acid	6,9,12,15-octadecatetraenoic acid	18:4 n-3	6,9,12,15
Arachidic acid	eicosanoic acid	20:0	
Gadoleic acid	9-eicosenoic acid	20:1 n-11	9
Gondoic acid	11-eicosenoic acid	20:1 n-9	11
Dihomo-γ-linolenic acid	8,11,14-eicostrienoic acid	20:3 n-6	8,11,14
Mead acid	5,8,11-eicosatrienoic acid	20:3 n-9	5,8,11
Arachidonic acid	5,8,11,14-eicosatetraenoic acid	20:4 n-6	5,8,11,14
Timnodonic acid	5,8,11,14,17-eicosapentaenoic acid	20:5 n-3	5,8,11,14,17
Behenic acid	docosanoic acid	22:0	
Cetoleic acid	11-docosenoic acid	22:1 n-11	11
Erucic acid	13-docosenoic acid	22:1 n-9	13
Adrenic acid	7,10,13,16-docosatetraenoic acid	22:4 n-6	7,10,13,16
Docosapentaenoic acid	4,7,10,13,16-docosapentaenoic acid	22:5 n-6	4,7,10,13,16
Docosapentaenoic acid	7,10,13,16,19-docosapentaenoic acid	22:5 n-3	7,10,13,16,19
Clupanodonic acid	4,7,10,13,16,19-docosahexaenoic acid	22:6 n-3	4,7,10,13,16,19
Lignoceric acid	tetracosanoic acid	24:0	
Nervonic acid	15-tetracosenoic acid	24:1 n-9	15
Cerotic acid	hexacosanoic acid	26:0	
Ximenic acid	17-hexacosenoic acid	26:1 n-9	17

[a] All double bonds are of *cis* geometric configuration except where *t* indicates a *trans* double bond.

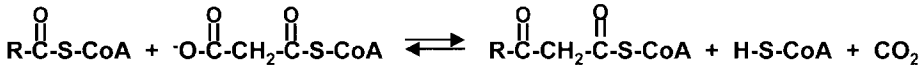
2.1. Endoplasmic reticulum elongation system

Fatty acyl chain elongation associated with the ER is highly active. Fatty acids must be activated to CoA derivatives; the CoA moiety provides energy for attachment of the donor group to a growing fatty acyl chain. Several fatty acyl-CoA synthetases with specificities for chain length and degree of unsaturation have been purified and their cDNAs cloned. Describing their precise subcellular locations is a needed step in determining links to the synthesis of particular lipids.

Distinctive to the ER elongation system, the 2-carbon elongation unit is donated from 3-carbon malonyl-CoA; interestingly, malonyl-CoA itself is not synthesized in the ER. Microsomal chain elongation is active with both saturated and unsaturated fatty acids, with γ-linolenate, 18:3 n-6, being the most effective substrate. In a manner analogous

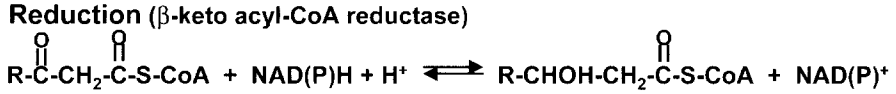
1. Condensation

a. Microsomes

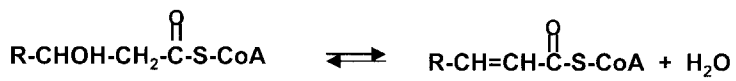


b. Mitochondria

2. Reduction (β-keto acyl-CoA reductase)



3. Dehydration (β-hydroxy acyl-CoA dehydrase)



4. Reduction (2–trans enoyl-CoA reductase)

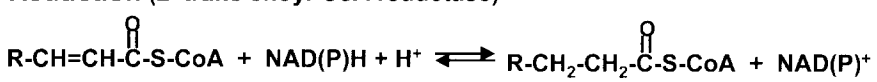


Fig. 2. Reactions in 2-carbon chain elongation of long chain fatty acids.

to de novo synthesis of 16-carbon acyl chains, a four-component reaction occurs in the 2-carbon elongation process of long chain fatty acyl-CoA (Fig. 2). Condensation of fatty acyl-CoA and malonyl-CoA to β-ketoacyl-CoA is the initial step. It is rate-limiting, determines fatty acyl specificity, and results in addition of the 2-carbon moiety. The second reaction is catalyzed by a reductase that utilizes NADPH (in preference to NADH) to form β-hydroxy acyl-CoA. It is not known if the ability to use both electron donors is due to two separate reductases. Flow of reducing equivalents from NADPH or NADH to β-keto reductase appears to involve cytochrome *b*5 and cytochrome *P*-450 reductase. The third reaction in chain elongation involves dehydration to enoyl-CoA, and the final reaction is a second reductase step catalyzed by 2-*trans*-enoyl-CoA reductase that requires NADPH. With possible exception of the dehydrase, active sites of all components of the ER elongation system have a cytosolic orientation. Partial purification of components of the elongation system suggest that the enzymes are discrete entities (in contrast to a single polypeptide for de novo fatty acid synthase of animal tissues); acyl-CoA derivatives can be isolated at each intermediate step. Despite such evidence, some investigations suggest covalent linkage of acyl-CoA to a multi-functional complex of the enzymes after condensation.

Regulation of the ER chain elongation system is not well understood. The rate-limiting condensation enzyme, but not the reductases or dehydrase, can be influenced by diet. Fasting depresses elongation and refeeding a carbohydrate diet increases overall chain elongation, whereas refeeding a high protein diet does not. Dietary effects seem

similar for saturated and PUFA substrates. In rat liver, elongation activity peaks at two weeks after birth and declines before another increase after weaning; old adult rats have lower elongation activities than young adult rats. Elongation of 16:0 by rat brain microsomes declines with age but elongation of PUFA increases and remains high for several months. Little is known about developmental changes of elongation activities in human tissues. Inhibition of elongation by induction of a diabetic state and reversal by insulin is greater for 16:0 than for 18:3 n-3 suggesting divergent regulation of elongation of acyl chain classes. Xenobiotics, including plasticizers or hypolipidemic agents such as fibrates, markedly alter chain elongation, primarily at the condensation step. Studies with brain indicate different elongation enzymes reacting with dissimilar acyl chain lengths. In Quaking mice (a genetic mutant with defective myelination), chain elongation of 20:0 to 22:0 and 24:0 was reduced by 70%, whereas elongation of 16:0 and 18:0 was unaltered relative to control mice. Thus, more than one elongation system with specificity based on length and unsaturation of acyl chains appears to be operative in the ER.

2.2. Mitochondrial elongation system

Although less active than the microsomal system, mitochondrial chain elongation has been extensively investigated, particularly in liver and brain. The 2-carbon condensing donor in mitochondria is acetyl-CoA (Fig. 2; reaction 1b). Generally, monounsaturated fatty acyl-CoA is more active than saturated-CoA and both support higher activity than PUFA, particularly in brain. Maximal mitochondrial elongation in liver, brain, kidney, and adipose tissue seems to require both NADPH and NADH, whereas heart, aorta and muscle require only NADH.

During the 1970s, mechanisms of mitochondrial chain elongation were elucidated [3]. Although β-oxidation (Chapter 5) and mitochondrial chain elongation have the same organelle location, reversal of β-oxidation is not feasible; the FAD-dependent acyl-CoA dehydrogenase of β-oxidation is substituted by a more thermodynamically favorable enzyme, enoyl-CoA reductase (Fig. 2; reaction 4), to produce overall negative free energy for the sequence. Enoyl-CoA reductase from liver mitochondria is distinct from the ER reductase, based on pH optima and specificities for saturated and unsaturated acyl-CoAs. Kinetic studies suggest that enoyl-CoA reductase is rate-limiting in mitochondrial elongation.

2.3. Functions of elongation systems

Chain elongation in the ER appears to be the most important source of acyl chains greater than 16 carbons for membrane phospholipids during growth and maturation, when long chain acids may not be supplied adequately in the diet. 18- to 24-carbon saturates and monoenes and 20- and 22-carbon polyunsaturates are required for neural growth and myelination, regardless of dietary fluctuations.

The function of the mitochondrial elongation system is less clear but it may participate in biogenesis of mitochondrial membranes or in transfer of reducing equivalent between carbohydrates and lipids. However, in view of the relatively low activity toward

16- and 18-carbon acyl chains, a primary role in the formation of long acyl chains for membrane synthesis is questionable.

Specific roles for elongation in peroxisomes have not been defined but this organelle may produce the very long chain saturated and polyenoic fatty acids of 24–36 carbons. Import of very long chain fatty acids into peroxisomes is defective in patients with the inherited disease, X-linked adrenoleukodystrophy, and this results in increased cellular and serum levels of very long chain fatty acids. The gene whose mutation leads to the degenerative disease apparently codes for a transporter required for importing very long chain fatty acids into peroxisomes for subsequent activation by a peroxisomal very long chain fatty acyl-CoA synthase. The fate of long chain acyl-CoAs formed in peroxisomes is not yet understood.

Studies using the yeast *Saccharomyces cerevisiae* resulted in the isolation of three genes required for elongation of fatty acyl-CoAs — *ELO1*, *ELO2*, and *ELO3* [7]. *ELO1* encoded protein prefers to elongate 14-carbon fatty acyl-CoAs, the *ELO2* encoded protein elongates 20- and 22-carbon chains, and the *ELO3* gene product elongates a broad range of fatty acyl CoA-chains to 26-carbon products. Inactivation of the *ELO2* or *ELO3* gene results in defective sphingolipid synthesis. In both yeast and mammals, sphingolipids store fatty acids greater than 24 carbons indicating that *ELO2* and *ELO3* elongation enzymes are coupled directly to synthesis of sphingolipids. Inactivation of *ELO2* or *ELO3* genes gives yeast cells the ability to survive in the absence of v-SNARES required for targeting of membrane vesicles from the Golgi to the plasma membrane. This suggests a role for long chain fatty acids or sphingolipids, mediated by *ELO2* and *ELO3* encoded proteins, in regulated fusion of biological membranes.

3. Formation of monounsaturated fatty acids by oxidative desaturation

The spectrum of fatty acyl chains needed to meet requirements of lipid storage, membrane synthesis and maintenance, and lipid regulation of cellular processes cannot be provided by diet or de novo fatty acid synthesis and chain elongation alone. Unsaturated fatty acids also must be synthesized in cells, supplemented by essential fatty acids in the diet.

3.1. Nomenclature to describe double bonds

Before discussing desaturation enzymes, abbreviations to describe the number and position of double bonds in acyl chains (Table 1) will be outlined using linoleic acid as an example.

(1) To indicate that linoleic acid is an 18-carbon fatty acid with two double bonds, the shorthand 18:2 is used. The number before the colon denotes the number of carbon atoms and the number following refers to the number of double bonds.

(2) To assign the position of an individual double bond or specificity of an enzyme inserting it, the delta (Δ) nomenclature is used. This describes a bond position relative to the carboxyl (number one) carbon of the acyl chain. For linoleic acid, the double bonds are in the $\Delta 9$ and $\Delta 12$ positions, between carbons 9–10 and

12–13, and are introduced into the 18-carbon chain by Δ9 and Δ12 desaturase enzymes.

(3) To designate an individual fatty acid within a 'family' of structurally related acids, the n- nomenclature is used. Here, the position of the first double bond from the methyl end is described. Thus, 18:2 n-6 indicates that the double bond closest to the methyl end is 6 carbons from the methyl group and in the Δ12 position. This convention is particularly useful in designating groups of fatty acids derived from the same parent compound as metabolic reactions do not occur on the methyl side of an existing double bond.Among other conventions, the ω6 designation is still widely used to describe the position of a double bond from the methyl end (ω-carbon). It is similar to the n- nomenclature. Linoleic acid is an ω6 fatty acid.

(4) To indicate the geometric configuration of a double bond, the designation is preceded by a *c-* for *cis* or *t-* for *trans*. Thus, *c,c*-18:2 n-6 distinguishes linoleic acid from a *trans*-containing isomer, such as a conjugated linoleic acid (CLA; e.g., 9c,11t-18:2). Generally, double bond configuration is *cis*.

3.2. Characteristics of monounsaturated fatty acid-forming desaturation enzymes

Monounsaturated fatty acids are formed in mammalian systems by direct oxidative desaturation (removal of two hydrogens resulting in the introduction of a double bond) of a preformed long chain saturated fatty acid. The oxygenase type of enzyme is associated with the ER of liver, mammary gland, brain, testes and adipose tissue. The Δ9 desaturase is the predominant, if not exclusive, desaturation enzyme for saturated acids in these tissues and is rate-limiting in the formation of 18:1 n-9. Δ9 desaturase acts on fatty acyl-CoA. For most tissues, 14- to 18-carbon saturated fatty acyl chains are good substrates, with stearoyl-CoA being most active. Reduced pyridine nucleotide is required and generally NADH is more active than NADPH. The Δ9 desaturase has an absolute requirement for molecular oxygen which acts as an electron acceptor for two pairs of hydrogens — one from NADH and the other from the fatty acyl-CoA.

The Δ9 desaturation system consists of three major protein components: (1) NADH-cytochrome *b*5 reductase, (2) cytochrome *b*5, and (3) a desaturase domain that is cyanide-sensitive and rate-limiting in double bond formation (Fig. 3). In some systems, such as those in insects, cytochrome *P*-450 may substitute for cytochrome *b*5. Integral association of Δ9 desaturase with the ER has retarded characterization of the complex. Loss of activity during solubilization was largely overcome through use of controlled ratios of detergents to protein and combinations of mild extraction solvents. The purified desaturase component has one atom of non-heme iron per molecule as the prosthetic group and 62% non-polar amino acid residues. Thus, the desaturase is largely within the microsomal membrane, with the active centre exposed to the cytosol. Interaction with specific reagents suggests that arginyl residues play a role at the binding site for the negatively charged CoA moiety of the substrate and tyrosine residues are involved in chelation of the iron prosthetic group. Isolation, characterization and site-directed mutagenesis of the cDNA coding for mammalian Δ9 desaturase has confirmed the essential catalytic role of histidine residues.

NADH-cytochrome *b*5 reductase and cytochrome *b*5 are more readily solubilized

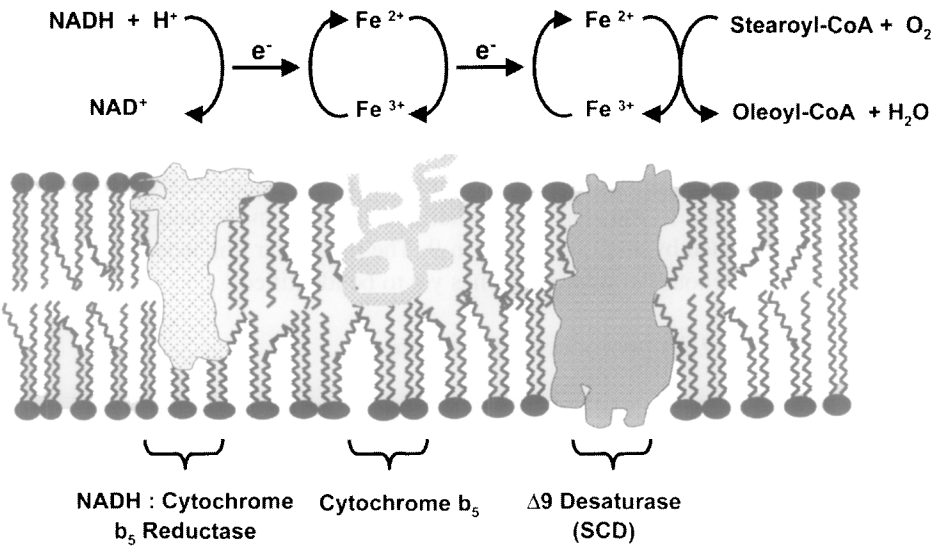


Fig. 3. Representation of the Δ9 (stearoyl-CoA) desaturase (SCD) complex, including electron transport proteins.

than the desaturase. Cytochrome *b*5 has a hydrophilic region of 85 residues (including the NH2-terminal) and the protein terminates in a hydrophobic COOH-terminal tail of 40 amino acids that attaches the protein to the membrane. It is uncertain whether one or two cytochrome *b*5 molecules per complex are required to transfer two electrons from NADH to oxygen.

Stereochemical studies have shown that the *D*-hydrogens at positions 9 and 10 are removed concertedly to give a *cis* double bond without involving an oxygen-containing intermediate. Attempts to demonstrate a hydroxyacyl intermediate have been negative and hydroxyacyl-CoAs are not readily desaturated. In insect systems oxygen free radical is involved.

Δ9 desaturase is translated on soluble cytoplasmic polysomes with post-translational binding of iron and insertion into the ER. Plasmid expression vectors have been used to synthesize desaturase peptides that can be reconstituted with cytochrome *b*5 and cytochome b5 reductase to give Δ9 desaturase activity. Dietary induction of Δ9 desaturase facilitated isolation of poly(A+) RNA and preparation of cDNA for stearoyl-CoA desaturase (SCD). mRNA increased 40- to 60-fold following refeeding of fasted animals and during induced differentiation of preadipocytes. Two genes (*SCD-1* and *SCD-2*) encoding isoforms of Δ9 desaturase with 87% homology were identified. Whereas liver exclusively expresses SCD-1, brain, spleen, heart and lymphocytes express only SCD-2; both SCD-1 and SCD-2 are expressed in adipose tissue, lung and kidney.

A structural gene for Δ9 desaturase from yeast (*OLE1*) has been isolated and characterized [16]. The *OLE1* gene encodes the only fatty acid desaturase in yeast and *OLE1* protein is found in the ER. The predicted protein amino acid sequence has a

high degree of similarity to mammalian SCD. Indeed, expression of rat SCD in yeast can substitute for yeast *OLE1*. If the *OLE1* gene is genetically inactivated, yeast are no longer viable, although life can be rescued in medium containing 14:1, 16:1, 18:1, or 18:2 fatty acids. A temperature-sensitive allele of the *OLE1* gene results in intact protein at 25°C but inactive protein at 37°C; at the latter temperature, there is an increase in saturated, and decrease in unsaturated, fatty acids. At the non-permissive temperature, dividing cells are unable to transfer mitochondria into newly made daughter cells, but the defect can be rescued by addition of 18:1 n-9. The molecular link between fatty acid desaturation and mitochondrial inheritance has yet to be identified.

3.3. Modification of Δ9 desaturase activities in vitro

Cyanide completely inhibits Δ9 desaturation in rat liver by acting on the terminal desaturase component (cyanide-sensitive factor), apparently related to accessibility to the non-heme iron. Some Δ9 desaturases (e.g., in insects and yeast) are not inhibited by cyanide.

Cyclopropenoid fatty acids found in stercula and cotton seeds are potent inhibitors of Δ9 desaturase. Sterculoyl- and malvaloyl-CoA (18- and 16-carbon derivatives with cyclopropene rings in the Δ9 position) specifically inhibit Δ9 desaturation. Hens fed meal containing cyclopropene fatty acids have decreased 18:1/18:0 ratios in their egg yolks. Cyclopropene acids have been used in vitro to differentially alter Δ9 and Δ6 desaturase activities, distinguishing relative contributions of these two enzymes in perinatal brain development.

3.4. Age-related, dietary and hormonal regulation of Δ9 desaturase

Extreme response to dietary alterations is a remarkable feature of Δ9 desaturase. Levels of *SCD-1* and *SCD-2* encoded Δ9 desaturase are highly regulated at the levels of transcription and of transcript and protein stability [6,8,9,15]. When rats are not fed for 12–72 h, liver Δ9 desaturase (SCD-1) activity declines to less than 5% of control values (Fig. 4). After refeeding, Δ9 desaturase activity increases dramatically to more than 2-fold above normal. The restoration has been termed 'super-induction' as levels of enzyme activity can be more than 100-fold above the fasted state, particularly when the rats are refed a fat-free diet enriched in carbohydrate or protein. Protein synthesis inhibitors and immunological techniques have been used to show that synthesis of the desaturase component is altered quantitatively. Liver SCD-1 mRNA is dramatically altered by dietary changes; for example, a nearly 100-fold increase occurs in rat pups nursed by mothers on an essential fatty acid deficient diet compared to controls, and a 45-fold increase occurs in liver upon refeeding fasted mice with a fat-free, high-carbohydrate diet. Responses of liver enzyme to dietary intake probably explain the 'circadian changes' in Δ9 desaturase, where liver activities can fluctuate 4-fold over a 24-h period; highest activity (around midnight) corresponds to maximal food intake in the nocturnal rat.

In contrast to the liver enzyme, brain Δ9 desaturase (SCD-2) is little altered by dietary restrictions. This ensures continuing activity during crucial stages of brain

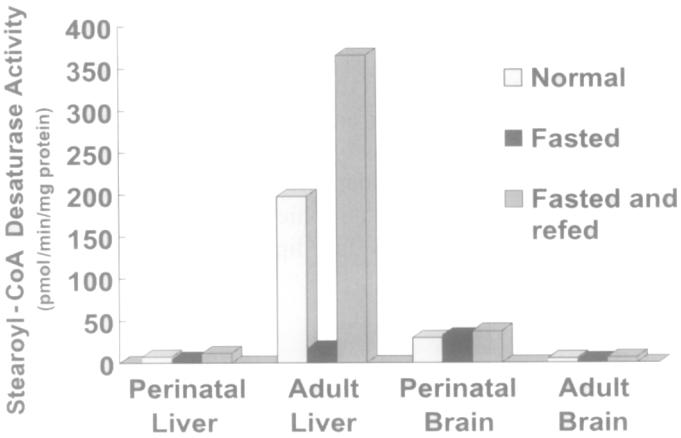


Fig. 4. Effects of fasting for 48 h and subsequent refeeding with a normal diet for 24 h on in vitro Δ9 desaturase activities of brain and liver from 10-day-old and adult rats. Adapted from Cook and Spence [29].

development. Brain Δ9 desaturase activity is greatest during the perinatal and suckling period in rats and is generally higher than in liver. However, when rats are weaned, brain Δ9 desaturase activity slowly declines. In contrast to the extreme changes in liver SCD-1 noted above, brain SCD-2 mRNA increases only 2-fold in neonates that are suckling mothers on an essential fatty acid deficient diet.

Transcription of *SCD-1* and *SCD-2* is regulated through specific elements in the promoter of each gene [8]. Although there are differences in the promoter region of *SCD-1* and *SCD-2* that lead to tissue-specific expression, shared regions appear to be responsible for decreased transcription in response to cholesterol (or conversely, increased transcription in response to sterol depletion). The regulation of SCD-1 and SCD-2 in response to cholesterol is through the binding of the transcription factor SREBP-1a and nuclear factor-Y (that heterodimerize) to three conserved regions within the *SCD-1* and *SCD-2* promoters. Addition of PUFA, particularly 18:2 n-6 or 20:4 n-6 [17], also decreases the level of SCD-1 and SCD-2 mRNA; whether this is due to decreased transcription or increased mRNA degradation, or both, is still unclear. SCD proteins also turn over rapidly in cells, possibly to ensure that transcriptional responses quickly alter SCD protein levels. The N-terminal 33-amino acids of SCD are required for turnover. The protease system responsible for degradation of SCD protein is not known.

Hormonal regulation of Δ9 desaturase is complex and not understood fully. Rats with genetic diabetes, or made diabetic by destruction of pancreatic β-cells, have depressed Δ9 desaturase activity in liver, mammary gland and adipose tissue. Insulin restores activity in vivo but is without effect in an in vitro assay. Insulin appears essential for basal transcription of the *SCD-1* gene and markedly induces transcription through a process requiring synthesis of a protein regulator. Significant changes in cytochrome *b*5 and the reductase are not elicited by insulin. Other hormones and effectors, such as glucagon and cyclic AMP, do not alter Δ9 desaturase activity, whereas epinephrine and thyroxine do.

Studies of SCD mutations in mice support an integration of monoene formation with neutral lipid and lipoprotein metabolism as well as unpredicted effects on the skin and eyes [17–19]. Mice containing a natural mutation that inactivates SCD-1 display a dramatic decrease in synthesis of liver triacylglycerols and cholesterol esters, leading to lower levels of these lipids in very low and low density lipoproteins. Specific genetic inactivation of the mouse SCD-1 gene produces similar effects on triacylglycerols. In addition, these mice develop skin abnormalities and atrophied sebaceous glands (that secret oil into the skin) and meibomian glands (that produce lipid secretions for the eye), resulting in an inability of these mice to blink.

4. Formation of polyunsaturated fatty acids

4.1. Characteristics in animal systems

All eukaryotic organisms contain polyenoic fatty acyl chains in their membrane lipids, and most mammalian tissues modify acyl chain composition by introducing more than one double bond.

(1) The first double bond introduced into a saturated acyl chain is generally in the Δ9 position so that substrates for further desaturation contain either a Δ9 double bond or one derived from the Δ9 position by chain elongation. Relatively large amounts of 16:1 n-10 and 18:1 n-10 in neonatal rat brain are exceptions but the qualitative significance during brain development is unclear.

(2) Like Δ9 desaturation that inserts the first double bond, further desaturation is an oxidative process requiring molecular oxygen, reduced pyridine nucleotide and electron transfer involving a cytochrome and related reductase.

(3) Animal systems cannot introduce double bonds beyond the Δ9 position. Thus, second and subsequent double bonds are always inserted between an existing bond and the carboxyl end of the acyl chain, never on the methyl side (Fig. 5). Plants, on the other hand, introduce second and third double bonds between the existing double bond and the terminal methyl group (Chapter 4). Diatoms, *Euglena*, insects, snails and slugs can desaturate on either side of an existing bond; at least 15 insect species form 18:2 n-6 by de novo synthesis and some produce 20:4 n-6. Consequently, double bonds are found at the Δ9, Δ6, Δ5 and Δ4 positions as a result of desaturation in animals, at the Δ9, Δ12 and Δ15 positions in plants, and at the Δ5, Δ6, Δ9, Δ12 and Δ15 positions in insects and other invertebrates. Well-established evidence confirms distinct Δ9, Δ6 and Δ5 desaturases in many animal tissues. In contrast, the longstanding assumption that a Δ4 desaturase exists in the classical pathways of PUFA metabolism (Fig. 6) has not been confirmed by direct enzyme characterization. The abundance of long chain polyenoic acids containing Δ4 double bonds in tissues such as brain, retina and testes, and their formation in vivo and in vitro from more saturated precursors, indicate that mammalian tissues can produce PUFA with a Δ4 double bond. A new dimension to the classical pathway, erroneously thought to include direct Δ4 desaturation, is now recognized. In rat liver, fish and human monocytes (and probably other

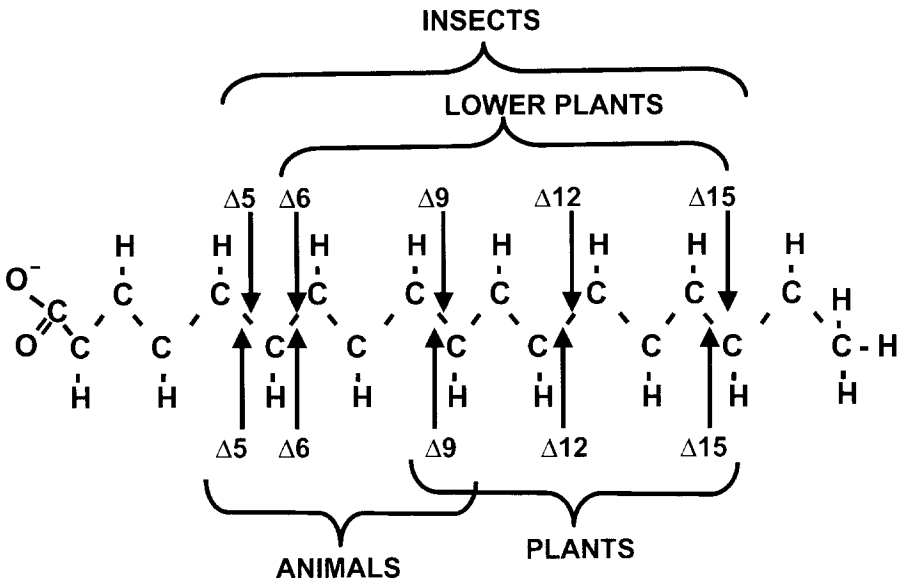


Fig. 5. Positions of fatty acyl chain desaturation by enzymes of animals, plants, insects and lower plants.

human tissues), formation of 22:6 n-3 from 22:5 n-3 or of 22:5 n-6 from 22:4 n-6 involves direct chain elongation of these respective precursors and then Δ6 desaturation followed by a 2-carbon chain shortening, the latter by β-oxidation in peroxisomes. This alternative to direct Δ4 desaturation has become known as the Sprecher pathway [20]. Comparative studies with tissue explants, primary cultures and neoplastic cells suggest that introduction of the Δ4 bond into 22:6 n-3 is characteristic of differentiated tissue and may be restricted in undifferentiated cells.

(4) In most organisms, and certainly in higher animals, methylene interruption between double bonds is usually maintained with conjugated double bonds being rare.

(5) All bonds introduced by oxidative desaturation in animals are in the *cis* geometric configuration.

4.2. Essential fatty acids — a contribution of plant systems

Requirements for PUFA cannot be met by de novo metabolic processes in mammalian tissues. Animals are absolutely dependent on plants (or insects) for providing double bonds in the Δ12 and Δ15 positions of the two major precursors of the n-6 and n-3 fatty acids, linoleic and linolenic acids. In animal tissues these acyl chains are converted to fatty acids containing 3–6 double bonds. Even very long chain PUFA of 28–36 carbons apparently do not have more than 6 double bonds.

4.3. Families of fatty acids and their metabolism

Relationships among fatty acids in the metabolic pathways can be evaluated by considering groups or families of fatty acids based on the parent unsaturated acid in the

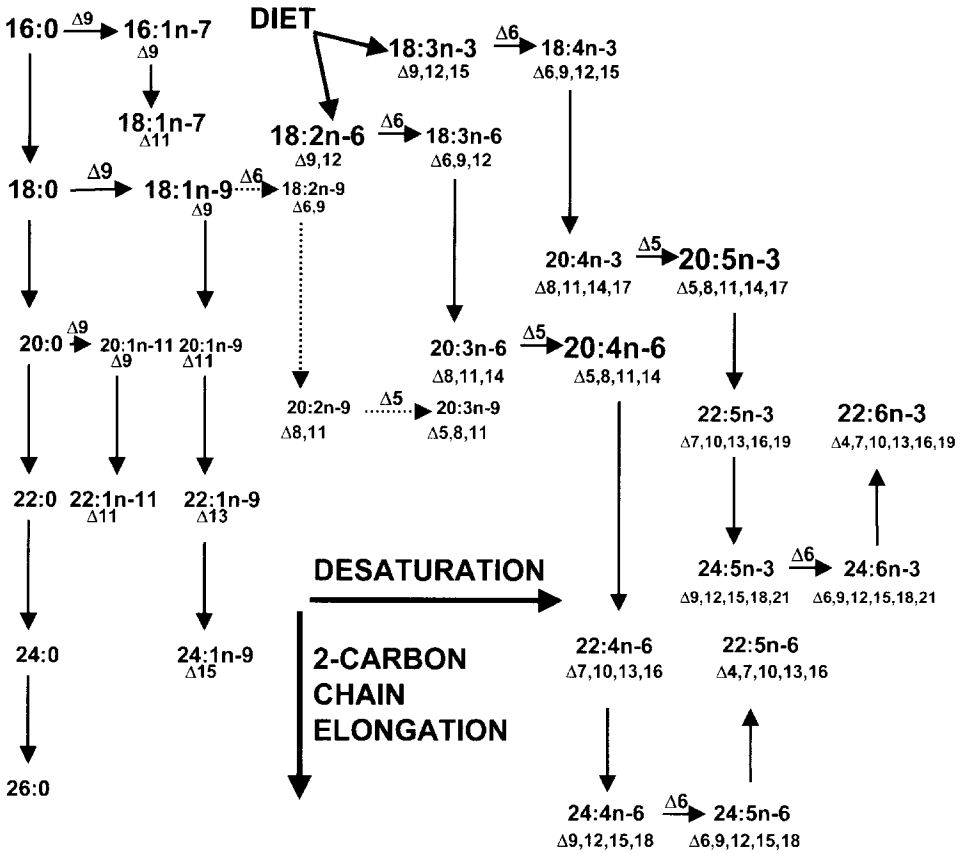


Fig. 6. Major pathways of fatty acid desaturation and chain elongation in animal tissues. Note the alternating sequence of desaturation in the horizontal direction and chain elongation in the vertical direction in the formation of polyunsaturated fatty acids from dietary essential fatty acids.

sequence. The predominant fatty acid families (Fig. 6) are:

n-6 acids derived from 18:2 n-6
n-3 acids derived from 18:3 n-3
n-9 acids derived from 18:1 n-9, and
n-7 acids derived from 16:1 n-7.

4.3.1. The n-6 family

Arachidonate, 20:4 n-6, is an abundant polyenoic acyl chain found in most animal tissues. 20:4 n-6 can be derived from 18:2 n-6 by the alternating sequence of Δ6 desaturation, chain elongation of the 18:3 n-6 intermediate, and Δ5 desaturation of 20:3 n-6 (Fig. 6). Δ6 desaturation is considered rate-limiting in most situations. 20:4 n-6 is a component of phospholipids contributing to the structural integrity of membranes and is the primary precursor of important biological signalling molecules (e.g., prostaglandins and leukotrienes) with a variety of biological activities (Chapter 13). Frequently, 20:4

n-6 is referred to as an essential fatty acid as there is an absolute requirement for it; however, 18:2 n-6 can be converted to adequate 20:4 n-6 by most mammalian cell types. Neutrophils are a major exception, requiring 20:4 (-6) for leukotriene production but being unable to synthesize it from 18:2 n-6.

In liver and most other tissues of animals in a normal, balanced state, only 18:2 n-6 and 20:4 n-6 accumulate in relatively large quantities; much lower levels of the intermediates, 18:3 n-6 and 20:3 n-6, are detected. Such observations support a rate-limiting role for Δ6 desaturase in the sequence. When Δ6 desaturase activity is limited, this can be by-passed by providing 18:3 n-6 from enriched sources (such as evening primrose oil) for subsequent elongation and Δ5 desaturation to 20:4 n-6.

The genes and cDNAs coding for mammalian Δ6 and Δ5 desaturase enzymes have been isolated and characterized [4,5]. In humans, both desaturases are on chromosome 11. Mouse and human Δ6 desaturases are comprised of 444 amino acids with 87% homology between species. A surprising feature in analysis of the amino acid sequences is that both Δ6 and Δ5 desaturases have a cytochrome *b*5 domain at the N-terminus of the polypeptide chain with a C-terminal catalytic domain very similar to that found in Δ9 desaturase, including the positioning of specific catalytic histidine residues. Yeast Δ9 desaturase also has an N-terminus cytochrome *b*5 domain; genetic inactivation of the yeast cytochrome *b*5 gene still allows for functional Δ9 desaturation, while deletion of the *OLE1* encoded Δ9 desaturase cytochrome *b*5 domain results in inactive Δ9 desaturase. Thus, cellular cytochrome *b*5 can not substitute for the covalently attached N-terminal cytochrome *b*5 domain. By analogy, the N-terminal cytochrome *b*5 domain also is likely functional and essential for mammalian Δ6 and Δ5 desaturases.

mRNA transcript levels for Δ6 and Δ5 desaturases, determined for various mammalian tissues, correlate reasonably with detectable enzyme activities. Highest levels of transcript and activity for both Δ6 and Δ5 desaturases are found in liver, followed by lower amounts in heart, brain, placenta and lung. Liver Δ6 and Δ5 desaturase mRNA levels and enzyme activities are affected by fasting and refeeding with the level of PUFA in the diet being highly influential; high PUFA diets result in decreased liver Δ6 and Δ5 desaturase mRNA and enzyme activity levels and diets lacking PUFA result in high Δ6 and Δ5 desaturase mRNA and enzyme activities. Enhancement of desaturase activity observed upon refeeding after fasting can be suppressed by glucagon or dibutyryl cAMP for Δ6 desaturase activity but not for Δ5 desaturase. In rat liver, Δ6 desaturase activity is low during fetal and neonatal development, increases dramatically around weaning and remains high throughout adulthood. Brain Δ6 desaturase activity is relatively high in the fetus and neonates and markedly declines by weaning, remaining low throughout adulthood. Δ5 desaturase follows similar trends. Liver microsomes from human neonates have Δ6 and Δ5 desaturases at lower levels than for adult humans or rodents.

Relationships between PUFA metabolism and insulin are complex: PUFA formation is impaired in diabetes, insulin increases the activities of Δ6 and Δ5 desaturases, essential fatty acid deficiency prevents induced diabetes and supplementation with fish-oil (containing n-3 fatty acids) worsens hyperglycemia in diabetic patients. Epinephrine suppresses Δ6 and Δ5 desaturases through β-receptors. Triiodothyronine inhibits both Δ6 and Δ5 desaturases. Generally, Δ6 and Δ5 desaturases respond similarly to

glucocorticoids, other steroids and adrenocorticotropic hormone. Potential roles of protein kinases as mediators of hormone action in control of PUFA biosynthesis remain to be defined. Vitamin E supplementation increases brain Δ6 desaturase and vitamin B6 deficiency markedly decreases liver Δ6 desaturation. Some cultured cell lines have lost Δ6 desaturase but retain Δ5 desaturase activity. The seed extract sesamin inhibits Δ5, but not Δ6, desaturase and the hypocholesterolemic drug simvastatin specifically increases Δ5 desaturation. These differences in activity are likely mediated through alterations in mRNA levels, although whether this is due to changes in the level of transcription or mRNA stability has yet to be determined.

In some tissues, 22:4 n-6 and 22:5 n-6 are quantitatively significant. Although numerous earlier reports concluded that there is Δ4 desaturation, the Sprecher pathway appears to be responsible primarily for production of 22:5 n-6 [20]. Exogenous 22:4 n-6 also is a substrate for 'retroconversion'. This process of partial degradation involves loss of either a 2-carbon fragment or a double bond and 2 or 4 carbons by β-oxidation. In general, retroconversion utilizes fatty acids of 20 carbons or greater. Since only double bonds in the Δ4 position are lost, this process could provide fatty acids with the first double bond in the Δ5 position. The quantitative significance of this process is not established. Deficiency of retroconversion of 22:6 n-3 to 20:5 n-3 in fibroblasts from a Zellweger patient with defective assembly of peroxisomes supports a role for peroxisomal β-oxidation (Chapter 5) in retroconversion.

A physical relationship of desaturases with chain elongation enzymes in ER membranes remains to be demonstrated. Indirect evidence supports a sequence of reactions (including esterification of products into phospholipids) that proceeds in a concerted manner without release of free fatty acyl intermediates [21].

4.3.2. The n-3 family

The most abundant n-3 acyl chains are 20:5 n-3 and 22:6 n-3. These fatty acids are esterified to phospholipids in cerebral cortex, retina, testes, muscle and liver. In retinal rod outer segments, the fatty acids in phospholipids are 40–60% 22:6 n-3. Many animal tissues convert dietary 18:3 n-3 to 20:5 n-3 and 22:6 n-3 by desaturation and elongation combined with peroxisomal chain shortening (Fig. 6). Whether the Δ6 desaturase in the Sprecher pathway converting 22:5 n-3 to 22:6 n-3 (or 22:4 n-6 to 22:5 n-6) is the same as the Δ6 desaturase acting directly on 18:3 n-3 (or 18:2 n-6) is unresolved with evidence for and against a common enzyme. Alternative sequences for initial steps of 18:3 n-3 (and 18:2 n-6) metabolism involving chain elongation followed by sequential Δ5 and Δ8 desaturation have been detected but their significance is unclear.

Potential benefits of increased consumption of fish and fish oil products abundant in 20:5 n-3 and 22:6 n-3 have been investigated extensively in animals and humans [22]. Enzymes in phytoplankton consumed by fish, or in fish themselves, produce these fatty acids from 18:3 n-3. Consumption of n-3 fatty acids leads to altered acyl chain composition of phospholipids in plasma, platelets, neutrophils and red cells of mammals, with further enrichment of n-3 fatty acids in peripheral body tissues taking longer. Generally, increases in n-3 content are at the expense of n-6 acids. Some populations, such as Greenland Inuit, routinely consume high levels of n-3 fatty acids and have a lower incidence of ischemic heart disease and longer bleeding times

related to a reduction in platelet aggregation. Clinical trials support positive effects of n-3 fatty acids in decreasing platelet aggregation, lowering blood pressure, reducing circulating triacylglycerols and producing modest beneficial changes in cholesterol and lipoproteins.

Some studies indicate that n-3 PUFA reduce severity of arthritis or impaired kidney functions involving abnormal immune and inflammatory responses. Other areas of potential n-3 fatty acid involvement include enhanced insulin sensitivity in the diabetic state, countering n-6 fatty acids in chemically induced, transplanted or metastatic tumors, and altered visual acuity and response to learning tests. Each fatty acid family has a role in overall nutritional balance believed largely to be manifested at the level of eicosanoid production (Chapter 13). The high concentration of n-3 fatty acids in some body tissues with specialized functions (e.g., brain and retina) and tenacious retention of n-3 acids during dietary deprivation suggest important structural and physical roles.

Suggestions that 0.2–0.3% of energy as n-3 fatty acids is adequate for adults and 0.5% during pregnancy, lactation and infancy must be matched with the need for n-3 to n-6 ratios of 1:4 to 1:8 to promote normal growth and development. Reported deficiencies in humans on long-term intravenous or gastric tube feeding have been corrected by supplementation with n-3 fatty acids. Interest in potential positive benefits of concentrated fish oils in the diet should be balanced by attention to potential negative or toxological side effects.

Competition among fatty acids of the n-3 and n-6 families for desaturation and chain elongation enzymes is extensively documented from a variety of in vivo and in vitro experiments. 18:3 n-3 is a better substrate for $\Delta 6$ desaturase than is 18:2 n-6; accordingly, abundance of 18:3 n-3 can effectively decrease formation of 20:4 n-6. In humans, conversion of 18:3 n-3 to 20:5 n-3 and 22:6 n-3 is much greater than conversion of 18:2 n-6 to 20:4 n-6.

4.3.3. The n-9 family

The prominent acyl chain in this family is 18:1 n-9. Generally, competition from 18:2 n-6 and 18:3 n-3 for $\Delta 6$ desaturase prevents formation and accumulation of more unsaturated n-9 acids. However, in animals on a diet deficient in essential fatty acids, competition by 18:2 n-6 is removed and 18:1 n-9 is utilized as a substrate for the rate-limiting $\Delta 6$ desaturase (Fig. 6). Further chain elongation of 18:2 n-9 to 20:2 n-9 and $\Delta 5$ desaturation results in accumulation of 20:3 n-9. While 20:3 n-9 may partially substitute for some physical functions within membranes, it is not a precursor of prostaglandins and cannot alleviate the signs of essential fatty acid deficiency.

Since deficiency of essential fatty acids markedly reduces 20:4 n-6 while increasing 20:3 n-9, a ratio of triene to tetraene (20:3 n-9/20:4 n-6) of less than 0.2 in tissues and serum usually indicates essential fatty acid deficiency. However, use of this ratio has limitations as inhibition of $\Delta 6$ desaturase will reduce formation of both 20:3 n-9 and 20:4 n-6 and result in a deficiency state without altering the ratio. Total amount of n-6 acids may be a better reflection of essential fatty acid deficiency.

4.3.4. The n-7 family

The primary n-7 acid in membranes and circulating lipids is 16:1 n-7. As most analyses do not distinguish specific 18:1 isomers, the contribution of 18:1 n-7 to the 18:1 fraction is seldom appreciated even though in developing brain 18:1 n-7 comprises 25% of total 18-carbon monoene. Potentially, 20:4 n-7, with only a single carbon shift of the double bonds compared to 20:4 n-6, could be formed from 16:1 n-7; however, high levels of PUFA derived from 16:1 n-7 are not detected even on a fat-free diet, although increased levels of 16:1 n-7 frequently accompany deficiency of essential fatty acids.

5. *Unsaturated fatty acids with trans double bonds*

5.1. General properties

Trans-unsaturated fatty acids, the geometric isomers of naturally occurring *cis*-acids, are not produced by mammalian enzymes but are formed by microorganisms in the gastrointestinal tract of ruminant mammals and chemically during commercial partial hydrogenation of fats and oils [23,24]. *Trans*-acids have been inaccurately labelled as unnatural, foreign or non-physiological. From diets containing beef fat, milk fat, margarines and partially hydrogenated vegetable oils, *trans*-acids are ingested, incorporated and modified in animal tissues.

Early studies suggested selective exclusion of *trans*-acids from metabolic processes and incorporation into membrane lipids, particularly in the central nervous system. Collectively, further studies have indicated little selectivity for absorption, esterification or β-oxidation compared to *cis*-isomers. Discrimination against specific positional isomers of *trans*-acids (e.g., Δ13 *trans*) may occur. In general, *trans*-monoenoic acids are recognized as a distinct class with properties intermediate between saturated and *cis*-monounsaturated acids, particularly in specificity for esterification to phospholipids. Accumulation of *trans*-acids in tissues generally is proportional to dietary levels. Lack of preferential accumulation over the long term suggests that *cis* and *trans* isomers turn over similarly. Nonetheless, potential negative influences of *trans*-unsaturated fatty acids in biological systems continue to be controversial.

Specific interactions of *trans*-acids with desaturation and chain elongation enzymes of animal tissues have been reported. Positional isomers of *t*-18:1 (except for the Δ9 isomer) are substrates for Δ9 desaturase of rat liver microsomes, resulting in *cis,trans*-dienoic isomers that, in some cases, are desaturated again by Δ6 desaturase to unusual polyunsaturated structures.

5.2. Trans-polyenoic fatty acids

Isomers of *trans,trans*-dienoic fatty acids, including *t,t*-18:2 n-6, are substrates for Δ6 desaturation in liver and brain, albeit at a lower rate than for *c,c*-18:2 n-6. Dienoic *trans*-isomers of 18:2 clearly lack properties of essential fatty acids and interfere with normal conversion of 18:2 n-6 to 20:4 n-6, primarily at the Δ6 desaturase. Diets containing *trans*-acids intensify signs of mild essential fatty acid deficiency; conversion

of available 18:2 n-6 to 20:4 n-6 is reduced as is 18:1 n-9 to 20:3 n-9. Although complex interactions are possible, *trans*-dienes are minor components (usually less than 1%) of hydrogenated vegetable oils used in human food products.

Many investigators conclude that *trans*-acids are undesirable dietary components. The actual influence of increased *trans* fatty acid intake during early development, on genetic disorders or on cardiovascular disease remains controversial and unresolved. It is reasonable to attempt to reduce their human consumption but continued investigations of *trans*-acids as a distinct and significant class of fatty acids are necessary.

5.3. Fatty acids with conjugated double bonds

While research interests in *trans* unsaturated fatty acids have oscillated somewhat over the last few decades, much recent attention has been directed to a specific class of dietary conjugated fatty acids, some with *trans* bonds, known as conjugated linoleic acid isomers (CLA; e.g., 9c,11t; 9t,11t; 10t,12c; 10t,12t; 10c,12c isoforms — compared to 9c,12c-linoleic acid) [24,25]. Intriguing, but poorly understood, health benefits have been implicated for CLAs including suppression of cancer cell growth, weight gain, diabetes and atherosclerosis. CLA isoforms can be metabolized to isoforms of 20:4 n-6 and some interfere with $\Delta 9$ desaturation or 20:4 n-6 and prostaglandin production. However, information on their relative metabolism, interference with normal *cis* isomers such as 18:1 n-9 and 18:2 n-6 or alterations by deficiency and disease states is limited.

6. Abnormal patterns of distribution and metabolism of long chain saturated and unsaturated fatty acids

Despite the diversity of enzymes involved in unsaturated fatty acyl chain formation, documented clinical defects specific to unsaturated acyl chain metabolism are few. Most alterations of acyl chain patterns reflect dietary deficiency states or impaired activities but not absence of specific fatty acid metabolizing enzymes. This indicates that desaturation and elongation enzymes are essential in supporting life-sustaining cellular processes. However, defects or deficiencies resulting in abnormal patterns of unsaturated fatty acid distribution have been documented.

6.1. Deficiency of essential fatty acids and related nutrients

In experimental animals, severe effects are observed in the absence of dietary essential fatty acids. These include a dramatic decrease in weight, dermatosis and increased permeability to water, enlarged kidneys and reduced adrenal and thyroid glands, cholesterol accumulation, impaired reproduction, and ultimate death. Substantial changes in fatty acid composition of tissue and circulating lipids occur. Brain is exceptionally resistant to loss of essential fatty acids, but modification of acyl patterns can be achieved if a deficient diet is started at an early age and continued long enough. The four n-6 acids in the sequence from 18:2 n-6 to 20:4 n-6 individually have similar potency in reversing

effects of deficiency, whereas the capacity of 18:3 n-3 is much lower. Collective assessment of nutritional studies indicates that 2–4% 18:2 n-6 and 0.2–0.5% 18:3 n-3 are adequate, but such requirements depend on the demands of specific tissues and the stage of growth, development and metabolism.

Inadequate supply of essential fatty acids resulting in deficiency signs described for rats is rare in humans. Normal diets contain enough 18:2 n-6 and 18:3 n-3, or their metabolic products, to meet tissue demands. Adipose stores provide a protective buffer against temporary limited intake. However, severe deficiency states have been observed in humans (especially premature infants with restricted adipose stores) on prolonged intravenous feeding or artificial milk formulations without adequate lipid supplements. Marked alterations of serum fatty acid patterns characterized by depletion of n-6 acids and a major increase in the 20:3 n-9 to 20:4 n-6 ratio are accompanied by severe skin rash, loss of hair and irritability. These signs are reversed rapidly by supplementation with lipid emulsions containing 18:2 n-6.

Functions for 18:2 n-6, in addition to a role as precursor of 20:4 n-6, seem likely. Some fatty acids that cannot serve as prostaglandin precursors prevent signs of essential fatty acid deficiency. Cats apparently require both 18:2 n-6 and 20:4 n-6 in their diets but may have relatively low, rather than absence of, $\Delta 6$ desaturase activity.

Gross signs of zinc deficiency are similar to those observed in essential fatty acid deficiency. Possible relationships between zinc, PUFA and eicosanoids have been proposed, but direct connections at the metabolic level have not been shown.

In several human diseases, abnormal patterns of PUFA, attributable to insufficient dietary 18:2 n-6 or to abnormal metabolism, have been described [26]. Some, including acrodermatitis enteropathica, cystic fibrosis, Crohn disease, peripheral neuropathy, and congenital liver disease have diminished capabilities for desaturation or chain elongation of PUFA or conversion to eicosanoids. Alcoholism, cirrhosis, Reye's syndrome, and chronic malnutrition are accompanied by significantly abnormal patterns of essential fatty acids in serum phospholipids. As high intake of n-6 may be proinflammatory, a countering interaction of n-6 PUFA could protect in situations such as rheumatoid arthritis, autoimmune disease (e.g., AIDS) or malignant tumor progression. Balance between PUFA families may be particularly important during stress of recovery from surgery or burn injury.

6.2. Relationships to plasma cholesterol

Considerable evidence supports a correlation between high intake of dietary saturated fats, relative to PUFA, and the occurrence of atherosclerosis and coronary disease. Risk of coronary disease is proportional to serum cholesterol levels and total serum cholesterol can be decreased following dietary intake of lipids enriched in PUFA [22]. Factors such as platelet aggregation, blood pressure and vascular obstruction may be influenced through some of the oxygenated derivatives of PUFA.

7. Regulation through sensors and receptors

7.1. Membrane sensing factors and response elements

Cells closely regulate membrane content of unsaturated fatty acids, although precisely how the level is sensed and a response is initiated is not understood. Genetic studies in yeast resulted in discovery of an unsaturated fatty acids sensing system. A transcription factor encoded by the yeast *STP23* gene is required for efficient transcription of the Δ9 desaturase gene, *OLE1*. Addition of exogenous unsaturated fatty acids results in decreased *OLE1* transcription and synthesis of unsaturated fatty acids. The regulatory *SPT23* protein contains a C-terminal, membrane-spanning domain that anchors the protein to the ER. Upon addition of exogenous unsaturated (but not saturated) fatty acids, *SPT23* protein is ubiquinated, resulting in targeted cleavage of the C-terminus. The N-terminal *SPT23* transcription factor moves to the nucleus and shuts off transcription of the *OLE1* gene [27,28]. Thus, this ER-bound protein is a precursor of a nuclear transcription factor and is similar to SREBP that is derived from an ER precursor protein (Chapter 15).

Based on similarities of the yeast system to mammalian SREBP processing in response to altered sterol levels (Chapter 15), a system for sensing of unsaturated fatty acids levels in mammalian cells also may exist. Both SCD1 and SCD2 are regulated by sterols through SREBP. In transgenic mice, truncated forms of SREBP give a 3- to 4-fold increase in both SCD1 and 2. Both SREBP and PUFA regulation of SCDs through promoter reporter regions are dependent on sterol- and adjacent-response elements.

7.2. Peroxisomal proliferator-activated receptors (PPARs)

Fatty acids and their oxygenated derivatives are among the known activators of members of the PPAR family of nuclear receptors [14]. PPARγ is a transcription factor and critical modulator of fat cell differentiation and function, thus providing a direct link between fatty acid concentrations and regulation of gene transcription in adipocytes. Drugs that act as agonists or antagonists of PPARγ can be used to alter lipid metabolism, insulin resistance and diabetes and to influence related diseases such as obesity, atherosclerosis and hypertension. Although paradoxes remain in the complex interactions mediated by PPARs, studies with gene knockouts or specifically designed modulator molecules are shedding new light on PPARs in relation to uptake, storage and oxidation of fatty acids.

8. Future directions

Progress in understanding desaturation and chain elongation of fatty acyl chains and the influence of fatty acids on metabolic functions has been exciting over the last half century. At the same time, expansion of knowledge of mechanisms, regulation and functions of these processes, particularly the extent to which information applies to humans, is needed. Appropriate levels and balance of n-6 and n-3 fatty acids and the modulating role of n-3 acids, especially relating to fetal and neonatal development, to

stresses such as surgery, accidental injury or incompetent immunity, and to inherited or acquired diseases require continuing study. Surprisingly, potential deleterious effects of *trans* unsaturated fatty acids still has not been resolved fully. Are monenoic fatty acids a logical substitution if other dietary fatty acids are reduced? In establishing nutritional standards for classes of fatty acids, both individual and species variability and the complex interplay of a variety of physiological functions must be considered.

Application of cloning, molecular probes and genetic technologies to desaturation and chain elongation enzymes has substantially progressed recently but the full power of molecular probes and techniques is yet to be realized in this realm. Specific molecular probes for Δ6 desaturase will be beneficial for determining whether the enzyme(s) acting on 18:2 n-6, 18:3 n-3, 24:4 n-6 and 24:5 n-3 are identical. Modulation of Δ5 desaturase relative to preceding desaturation and elongation steps could be enlightening. Studies based on mutant cell lines or transgenic and gene disrupted mice, deficient in or overexpressing one or more components of the desaturation–chain elongation sequences, should provide exciting insights into regulation. To what extent are the components coordinately regulated and is the sequence of activities physically associated to provide channelled conversion to the major end products? Is the location of desaturation and elongation confined to the ER or might there be significant activities within peroxisomes or nuclear and plasma membranes?

Intracellular signalling roles for PUFA, in addition to being precursors of eicosanoids, are recognized but require more definition particularly in relation to PPAR (or other orphan receptors) classes and functions. How do PUFA functions relate to diseases and the type and amount of dietary fatty acids we ingest? Details of alternatives to classical pathways of n-3 and n-6 fatty acid metabolism must be pursued.

Abbreviations

ER	endoplasmic reticulum
PPAR	peroxisomal proliferator activated receptor
PUFA	polyunsaturated fatty acid
SCD	stearoyl-CoA desaturase
SREBP	sterol response element binding protein

References

1. Mead, J.F. (1981) The essential fatty acids: past, present and future. Prog. Lipid Res. 20, 1–6.
2. Cinti, D.L., Cook, L., Nagi, M.N. and Suneja, S.K. (1992) The fatty acid chain elongation system of mammalian endoplasmic reticulum. Prog. Lipid Res. 31, 1–51.
3. Seubert, W. and Podack, E.R. (1973) Mechanisms and physiological roles of fatty acid chain elongation in microsomes and mitochondria. Mol. Cell. Biochem. 1, 29–40.
4. Cho, H.P., Nakamura, M. and Clarke, S.D. (1999) Cloning, expression, and fatty acid regulation of the human Delta-5 desaturase. J. Biol. Chem. 274, 37335–37339.
5. Cho, H.P., Nakamura, M.T. and Clarke, S.D. (1999) Cloning, expression, and nutritional regulation of the mammalian Delta-6 desaturase. J. Biol. Chem. 274, 471–477.

6. Los, D.A. and Murata, N. (1998) Structure and expression of fatty acid desaturases. Biochim. Biophys. Acta Lipids Lipid Metab. 1394, 3–15.
7. Oh, C.S., Toke, D.A., Mandala, S. and Martin, C.E. (1997) *ELO2* and *ELO3*, homologues of the *Saccharomyces cerevisiae ELO1* gene, function in fatty acid elongation and are required for sphingolipid formation. J. Biol. Chem. 272, 17376–17384.
8. Tabor, D.E., Kim, J.B., Spiegelman, B.N. and Edwards, P.A. (1999) Identification of conserved *cis*-elements and transcription factors required for sterol-regulated transcription of stearoyl-CoA desaturase 1 and 2. J. Biol. Chem. 274, 20603–20610.
9. Tocher, D.R., Leaver, M.J. and Hodgson, P.A. (1998) Recent advances in the biochemistry and molecular biology of fatty acyl desaturases. Prog. Lipid Res. 37, 73–117.
10. Clarke, S.D. and Jump, D.B. (1994) Dietary polyunsaturated fatty acid regulation of gene transcription. Annu. Rev. Nutr. 14, 83–98.
11. Duplus, E., Glorian, M. and Forest, C. (2000) Fatty acid regulation of gene transcription. J. Biol. Chem. 275, 30749–30752.
12. Graber, R., Sumida, C. and Nunez, E.A. (1994) Fatty acids and cell signal transduction. J. Lipid Mediat. 9, 91–116.
13. Stubbs, C.D. and Smith, A.D. (1990) Essential fatty acids in membrane: physical properties and function. Biochem. Soc. Trans. 18, 779–781.
14. Willson, T.M., Lambert, M.H. and Kliewer, S.A. (2001) Peroxisome proliferator-activated receptor γ and metabolic disease. Annu. Rev. Biochem. 70, 341–367.
15. Hannah, V.C., Ou, J.F., Luong, A., Goldstein, J.L. and Brown, M.S. (2001) Unsaturated fatty acids down-regulate SREBP isoforms 1a and 1c by two mechanisms in HEK-293 cells. J. Biol. Chem. 276, 4365–4372.
16. Gonzalez, C.I. and Martin, C.E. (1996) Fatty acid-responsive control of mRNA stability — unsaturated fatty acid-induced degradation of the *Saccharomyces OLE1* transcript. J. Biol. Chem. 271, 25801–25809.
17. Miyazaki, M., Kim, Y.C., Gray-Keller, M.P., Attie, A.D. and Ntambi, J.M. (2000) The biosynthesis of hepatic cholesterol esters and triglycerides is impaired in mice with a disruption of the gene for stearoyl-CoA desaturase 1. J. Biol. Chem. 275, 30132–30138.
18. Miyazaki, M., Man, W.C. and Ntambi, J.M. (2001) Targeted disruption of stearoyl-CoA desaturase1 gene in mice causes atrophy of sebaceous and meibomian glands and depletion of wax esters in the eyelid. J. Nutr. 131, 2260–2268.
19. Miyazaki, M., Kim, Y.C. and Ntambi, J.M. (2001) A lipogenic diet in mice with a disruption of the stearoyl-CoA desaturase 1 gene reveals a stringent requirement of endogenous monounsaturated fatty acids for triglyceride synthesis. J. Lipid Res. 42, 1018–1024.
20. Sprecher, H. (2000) Metabolism of highly unsaturated n-3 and n-6 fatty acids. Biochim. Biophys. Acta Mol. Cell Biol. Lipids 1486, 219–231.
21. Cook, H.W., Clarke, J.T.R. and Spence, M.W. (1983) Concerted stimulation and inhibition of desaturation, chain elongation, and esterification of essential fatty acids by cultured neuroblastoma cells. J. Biol. Chem. 258, 7586–7591.
22. Fernandes, G. and Venkatraman, J.T. (1993) Role of omega-3 fatty acids in health and disease. Nutr. Res. 13, S19–S45.
23. Carlson, S.E., Clandinin, M.T., Cook, H.W., Emken, E.A. and Filer Jr., L.J. (1997) *Trans* fatty acids: infant and fetal development. Am. J. Clin. Nutr. 66, 717S–736S.
24. Ip, C. (1997) Review of the effects of *trans* fatty acids, oleic acid, n-3 polyunsaturated fatty acids, and conjugated linoleic acid on mammary carcinogenesis in animals. Am. J. Clin. Nutr. 66, 1523S–1529S.
25. West, D.B., Delany, J.P., Camet, P.M., Blohm, F., Truett, A.A. and Scimeca, J. (1998) Effects of conjugated linoleic acid on body fat and energy metabolism in the mouse. Am. J. Physiol. Regul. Integr. Comp. Physiol. 275, R667–R672.
26. Holman, R.T. and Johnson, S. (1981) Changes in essential fatty acid profiles of serum phospholipids in human disease. Prog. Lipid Res. 20, 67–73.
27. Hoppe, T., Matuschewski, K., Rape, M., Schlenker, S., Ulrich, H.D. and Jentsch, S. (2000) Activation of a membrane-bound transcription factor by regulated ubiquitin/proteasome-dependent processing. Cell 102, 577–586.

28. Zhang, S.R., Skalsky, Y. and Garfinkel, D.J. (1999) *MGA2* or *SPT23* is required for transcription of the Delta9 fatty acid desaturase gene, *OLE1*, and nuclear membrane integrity in *Saccharomyces cerevisiae*. Genetics 151, 473–483.
29. Cook, H.W. and Spence, M.W. (1973) J. Biol. Chem. 248, 1793–1796.