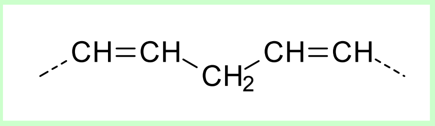
FATTY ACIDS: METHYLENE-INTERRUPTED DOUBLE BONDS

STRUCTURES, OCCURRENCE AND BIOCHEMISTRY

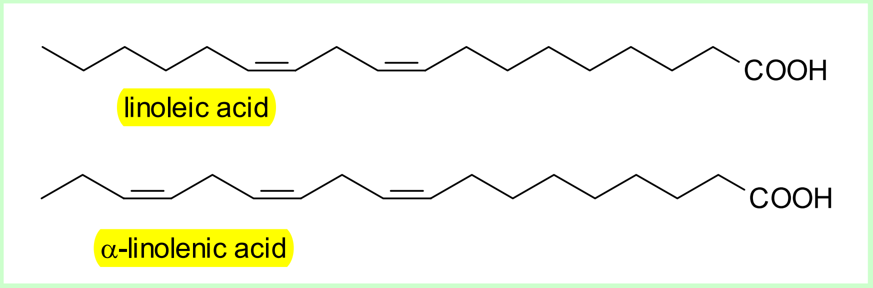
Structure and Nomenclature



$$\cdots CH{=}CH\underset{CH_2}{\smile}CH{=}CH\cdots$$

The lipids of all higher organisms contain appreciable quantities of polyunsaturated fatty acids ('PUFA') with methylene-interrupted double bonds, i.e. with two or more double bonds of the *cis*-configuration separated by a single methylene group. The term 'homo-allylic' is occasionally used to describe this molecular feature.

In higher plants, the number of double bonds in fatty acids only rarely exceeds three, but in algae and animals there can be up to six. Two principal families of polyunsaturated fatty acids occur in nature that are derived biosynthetically from **linoleic** (9-*cis*,12-*cis*-octadecadienoic) and **α-linolenic** (9-*cis*,12-*cis*,15-*cis*-octadecatrienoic) acids.



In the shorthand nomenclature, these are designated 9*c*,12*c*-18:2 and 9*c*,12*c*,15*c*-18:3 respectively. The number before the colon specifies the number of carbon atoms, and that after the colon, the number of double bonds. The position of the terminal double bond can be denoted in the form (*n*-x), where *n* is the chain-length of the fatty acid and x is the number of carbon atoms from the last double bond, assuming that all the other double bonds are methylene-interrupted. Thus linoleate and α-linolenate are 18:2(*n*-6) and 18:3(*n*-3), respectively (18:2ω6 and 18:3ω3 in the older literature).

Both of the parent fatty acids can be synthesised in plants, but not in animal tissues, and they are therefore essential dietary components (see below). Polyunsaturated fatty acids can be found in most lipid classes, but they are especially important as constituents of the phospholipids, where they appear to confer distinctive properties to the membranes, in particular by decreasing their rigidity. The exception is the sphingolipids, where they are rarely detected in other than trace amounts.

The (*n*-6) Family of Polyunsaturated Fatty Acids

Linoleic acid is a ubiquitous component of plant lipids, and of all the seed oils of commercial importance. For example, corn, sunflower and soybean oils usually contain over 50% of linoleate, and safflower oil contains up to 75%. Although all the linoleate in animal tissues must be acquired from the diet, it is usually the most abundant di- or polyenoic fatty acid in mammals (and in most lipid classes) typically at levels of 15 to 25%, although it can amount to as much as 75% of the total fatty acids of heart cardiolipin. It is also a significant component of fish oils, although fatty acids of the (*n*-3) family tend to predominate in this instance.

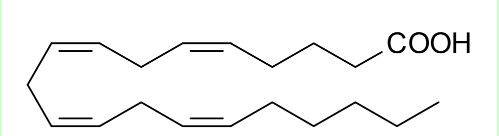
Analogues of linoleic acid with *trans*-double bonds are occasionally found in seed oils. For example 9*c*,12*t*-18:2 is reported from *Dimorphotheca* and *Crepis* species, and 9*t*,12*t*-18:2 is found in *Chilopsis linearis*.

The remaining members of the (*n*-6) family of fatty acids are synthesised from linoleate in animal and plant tissues by a sequence of elongation and desaturation reactions as described below. Shorter-chain components may also be produced by *alpha* or *beta*-oxidation. Most can function as essential fatty acids.

γ-Linolenic acid ('GLA' or 6-*cis*,9-*cis*,12-*cis*-octadecatrienoic acid or 18:3(*n*-6)) is usually a minor component of animal tissues in quantitative terms (< 1%), as it is rapidly converted to higher metabolites. It is found in a few seed oils, and those of evening primrose, borage and blackcurrant have some commercial importance. Evening primrose oil contains about 10% GLA, and is widely used both as a nutraceutical and a medical product.

8-*cis*,11-*cis*,14-*cis*-Eicosatrienoic acid (dihomo-γ-linolenic acid or 20:3(*n*-6)) is the immediate precursor of arachidonic acid, and of a family of eicosanoids (PG_1 prostaglandins). However, it does not accumulate to a significant extent in animal tissue lipids, and is typically about 1-2% of the phospholipid fatty acids.

Arachidonic acid (5-*cis*,8-*cis*,11-*cis*,14-*cis*-eicosatetraenoic acid or 20:4(*n*-6)) is the most important metabolite of linoleic acid in animal tissues, both in quantitative and biological terms. It is often the most abundant polyunsaturated component of the phospholipids, and can comprise as much as 40% of the fatty acids of phosphatidylinositol. As such, it has an obvious role in regulating the physical properties of membranes, but the free acid also is involved in the mechanism by which apoptosis is regulated.



arachidonic acid

Several families of **eicosanoids** are derived from arachidonate, including prostaglandins (PG_2 series), thromboxanes, leukotrienes, and lipoxins, with phosphatidylinositol being the primary source. These have an enormous range of essential biological functions that are discussed in elsewhere in these web pages. In addition, 2-arachidonoylglycerol and anandamide (*N*-arachidonoylethanolamine) have important biological properties as endocannabinoids, although they are minor lipids in quantitative terms. While arachidonate is found in all fish oils, polyunsaturated fatty acids of the (*n*-3) families tend to be present in much larger amounts. Arachidonic acid is frequently found as a constituent of mosses, liverworts and ferns, but there appears to be only one definitive report of its occurrence in a higher plant (*Agathis robusta*). The fungus *Mortierella alpina* is a commercial source or arachidonate via a fermentation process.

4,7,10,13,16-Docosapentaenoic acid (22:5(*n*-6)) is usually a relatively minor component of animal lipids, but it is the main C_{22} polyunsaturated fatty acid in the phospholipids of testes. It can amount to 70% of the lysobisphosphatidic acid, for example. In this instance, C_{22} fatty acids of the (*n*-3) family are present at relatively low levels, in contrast to most other reproductive tissues.

Other fatty acids of the (*n*-6) family that are found in animal tissues include 20:2(*n*-6), 22:3(*n*-6) and 22:4(*n*-6). The last of these, 7,10,13,16-docosatetraenoic or adrenic acid, is a significant component of the phospholipids of the adrenal glands and of testes. Tetra- and pentaenoic fatty acids of the (*n*-6) family from C_{24} to C_{28} have been found in testes, and even longer homologues occur in retina. Very-long-chain fatty acids were first reported from human brain in patients with the rare inherited disorder, Zellweger's syndrome, but it was subsequently demonstrated that such fatty acids with up to 38 carbon atoms and with from 3 to 6 methylene-interrupted double bonds are present at low levels the brain of normal young humans, with 34:4(*n*-6) and 34:5(*n*-6) tending to predominate. The function of these is not known.

Until recently, the most highly unsaturated fatty acid of the (*n*-6) family was believed to be 28:7(*n*-6) (4,7,10,13,16,19,22-octacosaheptaenoate), which has been found in the lipids of marine dinoflagellates and herring muscle. Now, 4,7,10,13,16,19,22,25,28-tetratriacontanonaenoic acid (34:9(n-6)) has been identified in the freshwater crustacean species *Bathynella natans*.

The (*n*-3) Family of Polyunsaturated Fatty Acids

α-Linolenic acid (9-*cis*,12-*cis*,15-*cis*-octadecatrienoic acid or 18:3(*n*-3)) is a major component of the leaves and especially of the photosynthetic apparatus of algae and higher plants, where most of it is synthesised. It can amount to 65% of the total fatty acids of linseed oil, where its relatively susceptibility to oxidation has practical commercial value in paints and related products. In contrast, soybean and rapeseed oils have up to 7% of linolenate, and this reduces the value of these oils for cooking purposes. α-Linolenic acid is the biosynthetic precursor of jasmonates in plants, which appear to have functions that parallel those of the eicosanoids in animals.

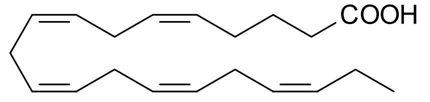
In animal tissue lipids, α-linolenic acid tends to be a minor component (<1%), the exception being grazing non-ruminants such as the horse or goose, where it can amount to 10% of the adipose tissue lipids.

As with linoleate, the remaining members of the (*n*-3) family of fatty acids are synthesised from α-linolenate in animal and plant tissues by a sequence of elongation and desaturation reactions as described below, while shorter-chain components may also be produced by *alpha* or *beta*-oxidation. They are essential fatty acids.

11,14,17-Eicosatrienoic acid (20:3(*n*-3)) can usually be detected in the phospholipids of animal tissue but rarely at above 1% of the total. Somewhat higher concentrations may be found in fish oils.

Stearidonic acid (6,9,12,15-octadecatetraenoic or 18:4(*n*-3)) is occasionally found in plants as a minor component, and it occurs in algae and fish oils.

3,6,9,12,15-Octadecapentaenoic acid or 18:5(*n*-3) is a significant component of the lipids of dinoflagellates, and it can enter the marine food chain from this source.

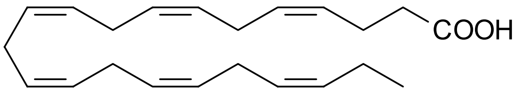


eicosapentaenoic acid (20:5(*n*-3))

8,11,14,17-Eicosatetraenoic acid (20:4(*n*-3)) is found in most fish oils and as a minor component of animal phospholipids. It is frequently encountered in algae and mosses, but rarely in higher plants. **5,8,11,14,17-Eicosapentaenoic acid** ('EPA' or 20:5(*n*-3)) is one of the most important fatty acids of the (*n*-3) family. It occurs widely in algae and in fish oils, which are major commercial sources, but there are few definitive reports of its occurrence in higher plants. It is an important constituent of the phospholipids in animal tissues, especially in brain, and it is the precursor of the PG_3 series of **prostaglandins** and **resolvins**, which have anti-inflammatory effects (see the appropriate web page). There is currently great interest in the role of this acid in treating neurological disorders such as schizophrenia.

7,10,13,16,19-Docosapentaenoic acid (22:5(*n*-3)) is an important constituent of fish oils, and it is usually present in animal phospholipids at a level of 2-5%.

4,7,10,13,16,19-Docosahexaenoic acid ('DHA' or 22:6(*n*-3)) is usually the end point of α-linolenic acid metabolism in animal tissues. It is a major component of fish oils, especially from tuna eyeballs, and of animal phospholipids, those of brain synapses and retina containing particularly high proportions. Indeed, there is evidence that increased levels of this fatty acid are correlated with improved cognitive and behavioural function in the development of the human infant. Dietary supplements may also benefit the elderly. While DHA is found in high concentrations in many species of algae, especially those of marine origin, it is not present in higher plants.



docosahexaenoic acid

DHA is not a substrate for the prostaglandin synthase/cyclooxygenase enzymes, and indeed it inhibits them. However, via the action of lipoxygenases, it is the precursor of the docosanoids, termed '**resolvins**' or 'protectins', which are analogous to the eicosanoids and have potent anti-inflammatory and immuno-regulatory actions.

The concentration of DHA in tissues has been correlated with a number of human disease states, and it is essential to many neurological functions. Particular attention has been given to its role in the retina where it is a major structural component of the photoreceptor outer segment membranes. For example, it binds strongly to specific sites on rhodopsin, the primary light receptor in the eye, modifying its stability and activity. It affects signalling mechanisms involved in photo-transduction, enhancing activation of membrane-bound retinal proteins, and it may be involved in rhodopsin regeneration. In some cases, sight defects have been ameliorated with DHA supplementation. It is intimately involved with **phosphatidylserine** metabolism in neuronal tissue.

DHA is believed to have specific effects on gene transcription that regulate a number of proteins involved in fatty acid synthesis and desaturation, for example. It has been demonstrated to have beneficial effects upon inflammatory disorders of the intestine and in reducing the risk of colon cancer, which may be mediated through associations with specific signalling proteins in membranes.

As a phospholipid constituent, it has profound effects on the properties of membranes, modulating their structure and function. In such an environment, DHA is believed to be more compact than more saturated chains with an average length of 8.2Å at 41°C compared to 14.2Å for oleic chains. This is the result of adoption of a conformation with pronounced twists of the chain, which reduce the distance between the ends. The methyl group with its extra bulk is located in the interior region. In mixed-chain phospholipids, a further consequence is a marked increase in the conformational disorder of the saturated chain. There appears to be an incompatibility between the rigid structure of cholesterol and the highly flexible chains of DHA, promoting the lateral segregation of membranes into PUFA-rich/cholesterol-poor and PUFA-poor/cholesterol-rich regions. The latter may ultimately become the membrane microdomains known as **rafts**.

PUFA-rich/cholesterol-poor membrane microdomains are technically less easy to study than rafts, but they may also contain distinctive proteins and have important biological functions. It has been proposed that changes in the conformation of signalling proteins when they move between these very different domains may have the potential to modulate cell function in a manner that may explain some of the health benefits of dietary consumption of DHA.

Other fatty acids of the (*n*-3) family that are found in nature include 22:3(*n*-3) from animal tissues and 16:3(*n*-3), which is a common constituent of leaf lipids (see our web pages on **mono-and digalactosyldiacylglycerols**). 16:4(*n*-3), 16:4(*n*-3), 21:5(*n*-3), 24:5(*n*-3) and 24:6(*n*-3) are occasionally present in marine organisms, including fish. Heptaenoic fatty acids of the (*n*-3) family (38:7(*n*–3) and 40:7(*n*–3)) have been reported from brains of patients with a defined genetic defect, but the most highly unsaturated fatty acid of the n-3 family yet found is 4,7,10,13,16,19,22,25-octacosaoctaenoate (28:8(*n*-3)) from marine dinoflagellates.

The (*n*-9) Family of Polyunsaturated Fatty Acids

Oleate can be chain elongated and desaturated in animal tissues with **5,8,11-eicosatrienoic acid** (20:3(n-9) or 'Mead's acid') as the most important product. This only accumulates in tissues when the animals are suffering from essential fatty acid deficiency (see below). Other fatty acids of this family that may also be found at low levels include 18:2(*n*-9), 20:2(*n*-9) and 22:3(*n*-9).

Other Families of Polyunsaturated Fatty Acids

9,12-Hexadecadienoic acid (16:2(*n*-4)) is found in marine microorganisms and is presumably the biosynthetic precursor of other fatty acids with an **(*n*-4)** terminal structure, i.e. 18:2(*n*-4), 20:2(*n*-4), 16:3(*n*-4) and 18:3(*n*-4). Fatty acids of an **(*n*-1)** family, also found in marine organisms, are believed to be derived biosynthetically by further desaturation (Δ15) of 6,9,12-hexadecatrienoic acid (16:3*n*-4)). The main naturally occurring fatty acids of this type are 16:4(*n*-1) and 18:4(*n*-1), but 18:5(*n*-1) has also been detected. Trace amounts of polyunsaturated fatty acids of an **(*n*-7)** family are occasionally encountered in tissues and are presumably metabolites of 9-16:1.

Biosynthesis of Linoleic and Linolenic Acids

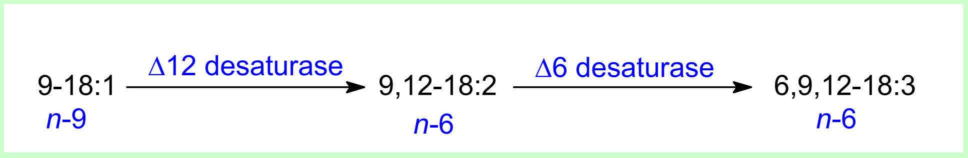
Linoleic and α-linolenic acids are synthesised in plant tissues from oleic acid by the introduction of double bonds between the existing double bond and the terminal methyl group by the sequential action of Δ12 and Δ15 desaturases.

$$\underset{n\text{-}9}{\text{9-18:1}} \xrightarrow{\Delta 12\text{ desaturase}} \underset{n\text{-}6}{\text{9,12-18:2}} \xrightarrow{\Delta 15\text{ desaturase}} \underset{n\text{-}3}{\text{9,12,15-18:3}}$$

The main substrate for the Δ12 desaturase is 1-acyl,2-oleoyl-phosphatidylcholine in the endoplasmic reticulum of the cell (although other lipids may also be substrates in chloroplasts). The newly formed linoleate is then transferred by a variety of mechanisms to other lipids. Phosphatidylcholine can also be the substrate for further desaturation, but in leaf tissue in a number of plant species it appears that most α-linolenate is formed by desaturation of linoleic acid linked to monogalactosyldiacylglycerols. Those plants that produce significant amounts of 16:3(*n*-3) add further complications to the problem, and it is evident that much remains to be learned of the overall process.

In fact, two distinct desaturases have been characterized that can insert the Δ12 double bond, i.e. a plastidial enzyme (FAD6), which uses the terminal methyl group as a reference point and is an ω6 desaturase as it introduces the double bond six carbons from the terminal carbon, and secondly an extra-plastidial oleate Δ12 desaturase (FAD2) that is selective for C-12,13 oxidation independently of chain length. The latter is related closely to an enzyme in the seeds of castor oil (*Ricinus communis*) that converts oleate to (*R*)-12-hydroxystearate. Indeed, whether the product is a hydroxyl group or a double bond may depend on the nature of only four amino acid residues. Less is know of the desaturase (FAD3) that converts linoleate to α-linolenate, but it is argued that it should be considered as an ω3 rather than as a Δ15 enzyme. It also has much in common with hydroxylase enzymes.

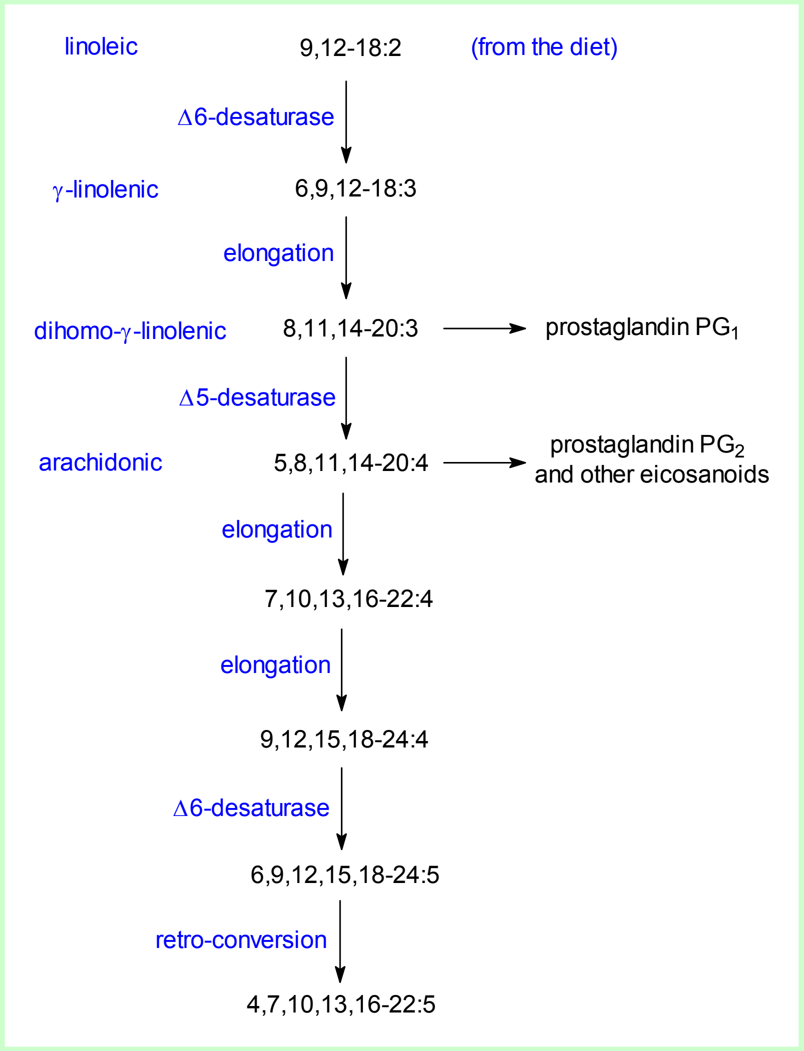
Infrequently in plants, a double bond is inserted between an existing double bond and the carboxyl group as in the biosynthesis of γ-linolenic acid in evening primrose and borage seed oils.



In this instance, the double in position 6 is inserted after those in positions 9 and 12.

Biosynthesis of the (*n*-6) Family of Polyunsaturated Fatty Acids

In animal tissues, additional double bonds can only be inserted between an existing double bond and the carboxyl group. The linoleic acid, which is the primary precursor molecule for the (*n*-6) family of fatty acids, must come from the diet. Biosynthesis of polyunsaturated fatty acids requires a sequence of chain elongation and desaturation steps, as illustrated below, and the various enzymes require the acyl-Coenzyme A esters as substrates not intact lipids (unlike plants). The liver is the main organ involved in the process.

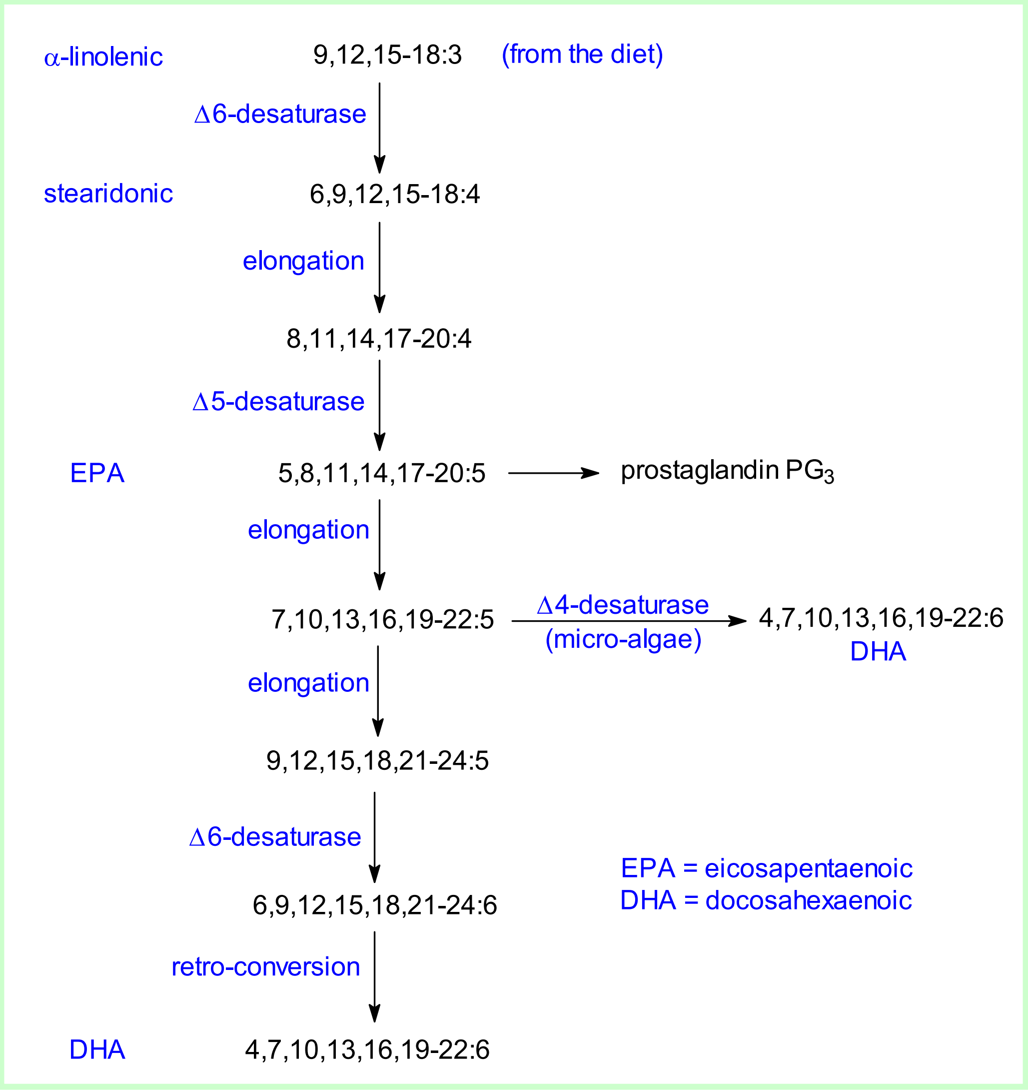


The first step is believed to be rate limiting and involves desaturation with the introduction of a double bond in position 6 to form γ-linolenic acid. Chain elongation by a two-carbon unit gives 20:3(*n*-6), which is converted to arachidonic acid by a Δ5 desaturase. This is the main end-product of the process. However, two further chain-elongation steps yield 24:4(*n*-6), which can be further desaturated by a Δ6 desaturase to 24:5(*n*-6). All the enzymes to this stage are located in the endoplasmic reticulum of the cell, but the last fatty acid (24:5(*n*-6) must be transferred to the peroxisomes for retro-conversion (β-oxidation) to 22:5(*n*-6).

The marine parasitic protozoon *Perkinus marinus* (and at least three other unrelated unicellular organisms) synthesises arachidonic acid by an alternative pathway in which elongation of linoleic to 11,14-eicosadienoic acid is followed by sequential desaturation by Δ8 and Δ5 desaturases.

Biosynthesis of the (*n*-3) Family of Polyunsaturated Fatty Acids

Again, the α-linoleic acid, which is the primary precursor molecule for the (*n*-3) family of fatty acids in animal tissues, must come from the diet. The main pathway to the formation of docosahexaenoic acid (22:6(*n*-3)) requires a sequence of chain elongation and desaturation steps *(*Δ5 *and* Δ6 desaturases), as illustrated below, with acyl-Coenzyme A esters as substrates. Thus, α-linoleic acid is sequentially elongated and desaturated, with double bonds being inserted between existing double bonds and the carboxyl group, as far as 24:6(*n*-3).



The final steps of what has been termed the 'Sprecher' pathway involve retro-conversion, i.e. removal of the first two carbon atoms by a process of β-oxidation, and take place in the peroxisomes of the cell (as in the case of the (*n*-6) family of fatty acids).

All the various intermediates may be found in tissues, especially those of fish, but eicosapentaenoic (20:5(*n*-3)), docosapentaenoic (22:5(*n*-3)) and docosahexaenoic (22:6(*n*-3)) acids tend to be by far the most abundant. In human tissues, the rates of conversion of α-linoleic acid to longer-chain metabolites is very low, suggesting that a high proportion of the latter must come from the diet (meat, eggs and fish) in normal circumstances.

Δ4, Δ5 and Δ8 Desaturases have been found in certain micro-algae of marine origin (e.g. *Pavlova salina*), suggesting that a more direct route may exist in these organisms, i.e. via desaturation of 22:5(*n*-3).

With acetyl-CoA as the primary precursor, the synthesis of 22:6(*n*-3) by the route described above involves approximately 30 distinct enzymes and 70 reactions. However, an entirely different and much simpler pathway catalysed by a distinct polyketide synthase has been found in marine microbes. The conventional view of polyketides is of secondary metabolites consisting of multiple building blocks of ketide groups ($–CH_2–CO–$), which are synthesised by a polyketide synthase. This is an enzyme system similar to the fatty acid synthase in bacteria in that it uses acyl carrier protein as a covalent attachment for chain synthesis and proceeds in iterative cycles. However, the double bonds are introduced during the process of fatty acid synthesis in contrast to the elongation-desaturation pathway. Much remains to be learned of this process in relation to DHA synthesis, but it is believed that as the chain elongates the ketones groups are reduced to hydroxyls, and this is followed by dehydration reactions to introduce the double bonds. Thus, aerobic desaturation is not required for introducing double bonds into the existing acyl chain, and it is sometimes termed an 'anaerobic' pathway, although it can occur under aerobic conditions.

In contrast to higher plants and mammals, the nematode *Caenorhabditis elegans* possesses all of the enzymes required for the synthesis of 20:4(*n*-6) and 20:5(*n*-3) fatty acids *de novo*, feats that can also be accomplished by the fungus, *Mortierella alpina*, and some mosses and red algae.

Essential Fatty Acids

As discussed briefly above, linoleic and linolenic acids cannot be synthesised in animal tissues and must be obtained from the diet, i.e. ultimately from plants. There is an absolute requirement for these '**essential fatty acids**' for growth, reproduction and good health. Young animals deprived of these fatty acids in the diet rapidly display the effects, including diminished growth, liver and kidney damage, and dermatitis; these eventually result in death. A key biochemical parameter is the 'triene-tetraene' ratio, i.e. the ratio of 20:3(*n*-9) to 20:4(*n*-6) fatty acids in plasma; levels greater than 0.4 reflect essential fatty acid deficiency. It takes longer for the effects to become apparent in older animals, which may have substantial stores of essential fatty acids in their body fats, but symptoms will appear eventually. The effects of essential fatty acid deficiency have been seen in human infants, on adults on parenteral nutrition or with certain genetic disorders. The absolute requirements are dependent on a number of factors, including species and sex, but are usually considered to be 1-2% for linoleate, and somewhat less for linolenate. In contrast, the requirement for α-linolenate in fish is higher than for linoleate. For some years it was believed that cats lacked a *Delta*-6 desaturase and had an absolute requirement for arachidonic acid especially in their diet, i.e. they were obligate carnivores, but this now appears not to be the case.

Linoleate and linolenate may in fact be less important than their longer-chain metabolites in animal biology. The functions of arachidonic, eicosapentaenoic and docosahexaenoic acids that make them essential are only partly understood. They are signalling molecules and are involved in the regulation of gene expression. They are precursors of **eicosanoids**, including prostaglandins

(PG_1, PG_2 and PG_3 series), thromboxanes, leukotrienes, and lipoxins, which have a variety of important biological properties. In addition, these polyunsaturated fatty acids confer distinctive attributes on the complex lipids that may be required for their function in membranes.

Although the actual requirement for polyunsaturated fatty acids is relatively low, general nutritional advice for the human diet until relatively recently was that they should comprise a substantial part of the daily intake. Now it is recognized that the propensity of such fatty acids for oxidation can lead to potentially harmful levels of hydroperoxides in tissues. Higher relative proportions of monoenes are now recommended. Detailed discussion of this topic is not possible here.

Recommended Reading

- Brash, A.R. Arachidonic acid as a bioactive molecule. *J. Clin Invest.*, **107**, 1339-1345 (2001).
- Cunnane,S.C. Problems with essential fatty acids: time for a new paradigm? *Prog. Lipid Res.*, **42**, 544-568 (2003).
- Gunstone, F.D., Harwood, J.L. and Padley, F.B. (Editors). *The Lipid Handbook (2nd Edition*). Chapman & Hall, London) (1994).
- Gurr, M.I., Harwood, J.L. and Frayn, K. *Lipid Biochemistry (5th Edition)*. (Blackwells, London) (2002).
- Holman, R.T. (Editor). *Progress in Lipid Research*, Volumes 9 (1971) and 25 (1986). - Several chapters.
- Nakamura, M.T. and Nara, T.Y. Essential fatty acid synthesis and its regulation in mammals. *Prostaglandins, Leukotrienes Essential Fatty Acids*, **68**, 145-150 (2003).
- Poulos, A. Very long chain fatty acids in higher animals - a review. *Lipids*, **30**, 1-14 (1995).
- Qiu, X. Biosynthesis of docosahexaenoic acid (DHA, 22:6-4,7,10,13,16,19): two distinct pathways. *Prostaglandins, Leukotrienes Essential Fatty Acids*, **68**, 181-186 (2003).
- SanGiovann, J.P. and Chew, E.Y. The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. *Prog. Retinal Eye Res.*, **24**, 87-138 (2005).
- Vance, D.E. and Vance, J. (Editors). *Biochemistry of Lipids, Lipoproteins and Membranes (4th Edition)*. (Elsevier, Amsterdam) (2002) – Several chapters.
- Wallis, J.G., Watts, J.L. and Browse, J. Polyunsaturated fatty acid synthesis: what will they think of next? *Trends Biochem. Sci.*, **27**, 467-473 (2002).
- Wassall, S.R. and Stillwell, W. Docosahexaenoic acid domains: the ultimate non-raft membrane domain. *Chem. Phys. Lipids*, **153**, 57-63 (2008).

Scottish Crop Research Institute (and MRS Lipid Analysis Unit), Invergowrie,
Dundee (DD2 5DA), Scotland

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