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Fatty acids from marine fish and their implications in health and diseases

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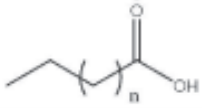
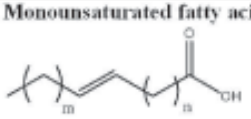
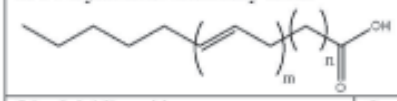
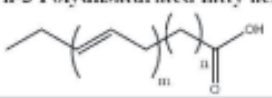
Fatty acids and their classification

Fatty acids are carboxylic acids with long hydrocarbon chains (usually C_{12-22}). Dietary fats are used to build every cell in the body and cell membranes are made of a variety of individual fatty acids. The essential fatty acids from marine fish have protective mechanisms against coronary heart disease, which became apparent in the investigations of the health status of Greenland Eskimos who consumed diets very high in fat from seals, whales, and fish, and yet had a low rate of coronary heart disease events. This paradox was explained by the fact that Eskimos consumed contained large quantities of the very-long-chain and highly polyunsaturated fatty acids with C_{20-22} carbons and 5-6 olefinic bonds, which are abundant in marine fish, and are scarce or absent in land animals and plants. Classification of fatty acids is based on to denote hydrocarbon chain length and number and positions of olefinic bonds. However, the most accepted system of classification is based on the number of olefinic bonds. Saturated fatty acids (SFAs) donot possess olefinic bonds in hydrocarbon chain. Examples of SFAs are lauric acid, myristic acid, palmitic acid, stearic acid, arachidic acid, behenic acid, and lignoceric acid (Table 1). Monounsaturated fatty acids (MUFAs) possess one double bond, the typical examples being myristoleic acid, palmitoleic acid, elaidic acid, oleic acid, erucic acid, and nervonic acid. Fatty acids with e" 2 double bonds are termed as polyunsaturated fatty acids (PUFAs). The tetrahedral bond angles on carbon results in a molecular geometry for saturated fatty acids that is relatively linear. Olefinic bonds in hydrocarbon chain of unsaturated fatty acids results in kinks in their structure results in weak stacking. PUFAs are broadly divided into two major families' viz., ù-3 and ù-6 PUFAs (otherwise termed as n-3 and n-6 PUFAs). However, ù-3 fatty acids are found to be abundantly available in marine sources particularly fish and phytoplanktons. These fatty acids affect many physiological processes including cognitive function, visual acuity, immunosuppressive, and anti-thrombic activities along with having major role on glucose and lipid metabolism. Table 1 illustrates the details regarding the differential changes of fatty acids and their structures including their abbreviated formulae, molecular formulae, and molecular weight.

Biosynthetic route of fatty acids

Fatty acid synthesis is a metabolic process to combine eight C_2 – moieties (-$CH_3C(=O)$ group from $CH_3COSCoA$) to synthesize saturated fatty acid with C_{16}-moiety ($C_{16}H_{32}O_2$) , which thereafter modified to form homologous fatty acid analogues. These modifications include: elongase-catalyzed

Table 1. Nomenclature of fatty acids

			Abbreviated nomenclature		Molecular formulae	Molecular weight
Saturated fatty acids 	**m**	**n**	**With respect to -COOH group**	**With respect to n (or ω)-group**		
Butyric acid	0	1	4:0	4:0	$C_4H_8O_2$	88.11
Caproic acid	0	3	6:0	6:0	$C_6H_{12}O_2$	116.16
Caprylic acid	0	5	8:0	8:0	$C_8H_{16}O_2$	144.21
Capric acid	0	7	10:0	10:0	$C_{10}H_{20}O_2$	172.26
Undecanoic acid	0	8	11:0	11:0	$C_{11}H_{22}O_2$	186.29
Lauric acid	0	9	12:0	12:0	$C_{12}H_{24}O_2$	200.32
Tridecanoic acid	0	10	13:0	13:0	$C_{13}H_{26}O_2$	214.34
Myristic acid	0	11	14:0	14:0	$C_{14}H_{28}O_2$	228.37
Pentadecanoic acid	0	12	15:0	15:0	$C_{15}H_{30}O_2$	242.4
Palmitic acid	0	13	16:0	16:0	$C_{16}H_{32}O_2$	256.42
Heptadecanoic acid	0	14	17:0	17:0	$C_{17}H_{34}O_2$	270.45
Stearic acid	0	15	18:0	18:0	$C_{18}H_{36}O_2$	284.48
Arachidic acid	0	17	20:0	20:0	$C_{20}H_{40}O_2$	312.53
Heneicosanoic acid	0	18	21:0	21:0	$C_{21}H_{42}O_2$	326.53
Behenic acid	0	19	22:0	22:0	$C_{22}H_{44}O_2$	340.58
Tricosanoic acid	0	20	23:0	23:0	$C_{23}H_{46}O_2$	354.61
Lignoceric acid	0	21	24:0	24:0	$C_{24}H_{48}O_2$	368.64
Monounsaturated fatty acids 						
Myristoleic acid	2	7	$14:1\Delta^{9}$	14:1n9	$C_{14}H_{26}O_2$	226.36
Cis-10-Pentadecenoic acid	2	8	$15:1\Delta^{10}$	15:1n10	$C_{15}H_{28}O_2$	240.36
Palmitoleic acid	4	7	$16:1\Delta^{9}$	16:1n9	$C_{16}H_{30}O_2$	254.41
Cis-10-Heptadecenoic acid	5	8	$17:1\Delta^{10}$	17:1n10	$C_{17}H_{32}O_2$	268.43
Elaidic acid	7	7	$18:1\Delta^{9}$	18:1n9 trans	$C_{18}H_{34}O_2$	282.46
Oleic acid	7	7	$18:1\Delta^{9}$	18:1n9 cis	$C_{18}H_{34}O_2$	282.46
Cis-11-eicosenoic acid	7	9	$20:1\Delta^{11}$	20:1n11	$C_{20}H_{38}O_2$	310.51
Erucic acid	7	11	$14:1\Delta^{13}$	22:1n13	$C_{22}H_{42}O_2$	338.57
Nervonic acid	7	13	$14:1\Delta^{15}$	24:1n15	$C_{24}H_{46}O_2$	366.62
n-6 Polyunsaturated fatty acids 						
Linolelaidic acid	2	6	$18:2\Delta^{9,12}$	18:2n6 trans	$C_{18}H_{32}O_2$	280.45
Linoleic acid	2	6	$18:2\Delta^{9,12}$	18:2n6 cis	$C_{18}H_{32}O_2$	280.45
γ-Linolenic acid	3	3	$18:2\Delta^{6,9,12}$	18:3n6	$C_{18}H_{30}O_2$	278.43
Cis-11,14-Eicosadienoic acid	2	8	$20:2\Delta^{11,14}$	20:2n6	$C_{20}H_{36}O_2$	308.5
Cis-8,11,14-Eicosatrienoic acid	3	5	$20:2\Delta^{8,11,14}$	20:3n6	$C_{20}H_{34}O_2$	306.48
Arachidonic acid	4	2	$20:2\Delta^{5,8,11,14}$	20:4n6	$C_{20}H_{32}O_2$	304.47
Cis-13,16-Docosadienoic acid	2	10	$22:2\Delta^{13,16}$	22:2n6	$C_{22}H_{40}O_2$	336.55
n-3 Polyunsaturated fatty acids 						
Linolenic acid	3	6	$18:2\Delta^{9,12,15}$	18:3n3	$C_{18}H_{30}O_2$	278.43
Cis-11,14,17-Eicosatrienoic acid	3	8	$20:2\Delta^{11,14,17}$	20:3n3	$C_{20}H_{34}O_2$	306.48
Cis-5,8,11,14,17-Eicosapentaenoic acid	5	2	$20:2\Delta^{5,8,11,14,17}$	20:5n3	$C_{20}H_{30}O_2$	302.45
Cis-4,7,10,13,16,19-Docosahexaenoic acid	6	1	$22:2\Delta^{4,7,10,13,16,19}$	22:6n3	$C_{22}H_{32}O_2$	328.49

chain elongation to synthesize fatty acids with longer hydrocarbon chain, e.g., stearic acid ($C_{18}H_{36}O_2$), arachidic acid ($C_{20}H_{40}O_2$), and so on. These SFAs, on desaturation yield unsaturated fatty acid analogues. In general, fatty acid synthesis takes place in cytoplasm of liver, adipose, central nervous system, and lactating mammary gland tissues of human. Glycolytic breakdown of glucose y**ields acetyl CoA through** pyruvate ($CH_3COCOOH$) by aerobic glycolysis that is starting material for fatty acid synthesis. Acetyl CoA serves as substrate to synthesize citrate that transported out of mitochondria to cytosol and generates acetyl CoA. The overall reaction of anabolism of fatty acids to form unsaturated faty acids is as follows:

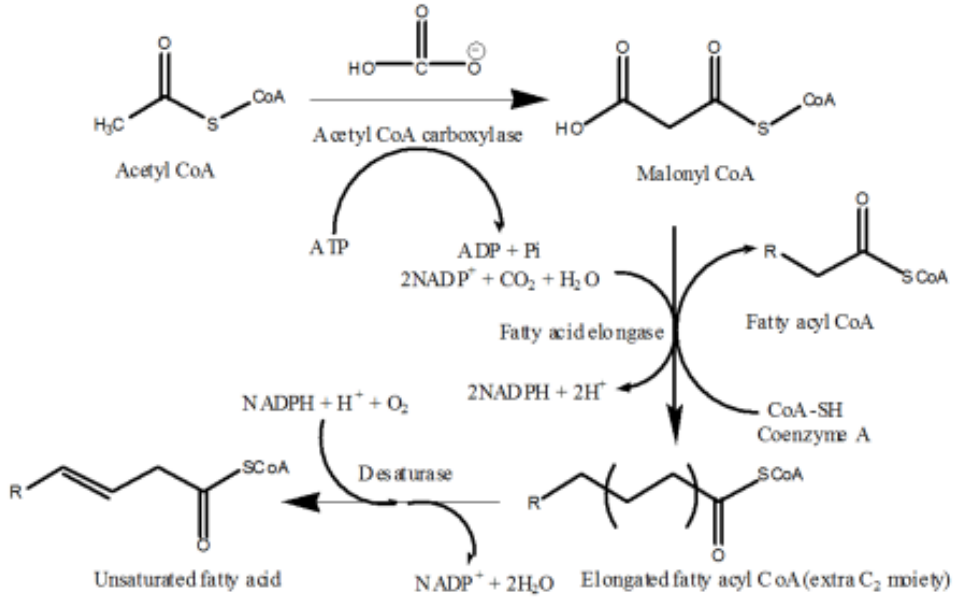


Figure 1. Synthesis of unsaturated fatty acids from acetyl CoA

Fatty acids are stored in adipocytes as triacylglycerol that must be hydrolyzed to release free fatty acids.

Polyunsaturated fatty acids and their importance in health and disease

To prevent cancer

Among dietary factors postulated to influence cancer development are long chain polyunsaturated ù-3 fatty acids, found in fish. Earlier studies revealed inverse relation between marine fatty acid consumption and mortality rates of prostate (Hebert et al., 1998) and breast cancer (Hebert et al., 1996). The mechanisms proposed how the intake of marine fatty acids might lower the risk of cancer is the inhibition of eicosanoid biosynthesis from AA, a ù-6 fatty acid. Prostaglandins converted from AA by the cyclooxygenase-2 enzyme, notably PGE_2, have been linked to carcinogenesis viz., mammary tumor development, proliferation of breast and prostate cancer (Erickson, 1986). Tumor cells typically produce large amounts of AA-derived PGE2, which may impede immune system function, possibly through their role in the generation of suppressor T cells (Erickson, 1986). Marine fatty acids were reported to inhibit cyclooxygenase-2 and the oxidative metabolism of AA to PGE2. EPA and DHA also inhibit lipoxygenases which metabolize AA to HETEs and leukotrienes. 12-HETE

has been linked to the suppression of apoptosis, stimulation of angiogenesis, stimulation of tumor cell adhesion, and expression of the invasive phenotype. It is apparent that both EPA and DHA can inhibit the biological activity of eicosanoids and androgens (Liang et al., 1992), which are known to have a stimulating effect on cell growth and uncontrolled cell proliferation (Ghosh & Myers, 1997). It is well established that in animal models and in human cancer cell lines, EPA and DHA were found to suppress cell growth. However, because intakes of fish and marine fatty acids are highly correlated, it is difficult to disentangle the effect of fatty acids from the effect of fish *per se*.

To combat atherosclerosis and cardiovascular diseases

Eating ù-3 fatty acids abundantly available in marine fish were reported to protect human beings from heart failure (*European Heart Journal*. doi:10.1093/eurheartj/ehp111). Researchers in the USA and Sweden followed 39,367 Swedish men, aged between 45-79, from 1998 to 2004. They recorded details of the men's diet and tracked the men's outcome through Swedish inpatient and cause-of-death registers. PUFAs in the diet have long been considered essential to the growth and proper nutrition of humans and other vertebrates. It was reported that atherosclerosis and thrombosis represent essential fatty acid deficiencies, but rather that the polyunsaturated fat may affect these pathological processes through other mechanisms. There is evidence from epidemiology that marine n-3 PUFA is associated with a reduced risk of coronary heart disease. This was originally found in Greenland Eskimos with an extremely high intake of n-3 PUFA (10–14 g/day) and later also reported in several other populations (Schmidt et al., 2005; Kris-Etherton et al., 2002) including Western populations with an average intake of marine n-3 PUFA below 0.2–0.4 g/day. Recently, a meta-analysis was published on fish consumption and CHD mortality from 13 cohort studies including a total of 222,364 individuals with an average of 11.8 years of follow-up (He et al., 2004). Fish consumption was inversely related with fatal CHD and sudden cardiac death (He et al., 2004).

Estimation of fatty acids in laboratory

Broadly fatty acid estimation is divided under the broad categories, viz., (1) lipid extraction and acid-catalyzed transesterification of fatty acid to methyl esters (FAMEs) and *N*-acyl pyrrolidides; and (3) gas-liquid chromatography and gas chromatography-mass spectrometry (GC/MS) analysis of FAMEs. Below are illustrated the details under each head.

Lipid extraction

Lipid from the crude sardine oil was extracted by using $CHCl_3$-CH_3OH-H_2O (Bligh, & Dyer, 1959). In brief, about 10 g tissue together with chloroform methanol mixture (2:1) ratio is homogenized, and $CHCl_3$-CH_3OH mixture (15 times) was added and mixed (to 1/3rd of the total volume). The resulting solution was filtered, and the filtrate was collected. The process was repeated two more times with rest of the $CHCl_3$-CH_3OH mixture. To the filtrate, add distilled water (20% of the total volume of the filtrate) and leave overnight. The water-soluble residue diffuses away from the solvent and occupies the top position in the separating funnel. Solvent containing lipid (bottom layer) is collected by filtering through anhydrous Na_2SO_4. Evaporate to dryness and make up the volume using $CHCl_3$. On extraction with $CHCl_3$-CH_3OH, lipid (bottom layer) is separated from the sample and is collected by filtering through anhydrous Na_2SO_4. After saponification of the dried extract PUFA is determined using gas chromatograph as illustrated below.

Extraction and derivatization of fatty acids to fatty acid methyl esters (FAME) and *N*-acyl pyrrolidides

The lipid extract thus obtained was saponified with 0.5 N KOH in CH_3OH. After removal of the nonsaponifiable material with *n*-hexane and acidification with 1 N HCl, the saponifiable materials were extracted with petroleum etherdiethyl ether (1:1 v/v) and transesterified to furnish fatty acid methyl esters (FAME) by reaction (30 min under reflux) with a methylating mixture (14% BF_3/ CH_3OH, 5 mL) in a boiling water bath under an inert atmosphere of N_2 (Metcalf, Schimtz, & Pleka, 1966). The FAME thus obtained was cooled to ambient temperature, and distilled water (20 mL) was added. The solution was extracted with *n*-hexane (10 mL X 6), and the upper *n*-hexane layer was removed and concentrated under an inert atmosphere of N_2. The resulting FAME concentrate was reconstituted in petroleum ether, flushed with N_2 in glass vials, and stored in deep freeze (-20^{o}C) until required for GC/GC-MS analyses. Analysis was performed in triplicate.

Gas-liquid chromatography and gas chromatography-mass spectrometry (GC/MS) analysis of fatty acid derivatives

Quantitative and qualitative analyses of FAME obtained by transesterification were performed on gas chromatograph using a flame ionization detector (FID). FAMEs were identified by comparison of retention times with the known standards. In another process, FAMEs were derivatized to *N*-acyl pyrrolidides by condensation of fatty acid methyl ester with a mixture of pyrrolidine (1 mL) and acetic acid (0.1 mL) at 100 ?C under reflux (2 h) for GC-MS analyses (Andersson, 1978). The GC-MS analyses need to be performed by GC interfaced with mass spectrometer for confirmation of fatty acid identification.

Mass Spectroscopic Analyses of FAME Derivatives

The following are the mass spectrometric data of FAME derivatives.

Methyl Palmitate. EI-MS *m/z* (relative intensity, %): 270 (M+, 61.11), 239 (15.74), 227 (31.48), 213 (7.41), 199 (14.81), 185 (12.96), 171 (12.96), 157 (7.41), 143 (31.48), 129 (11.11), 87 (74.07), 74 (*100*), 55 (18.52).

Methyl Oleate. EI-MS *m/z* (relative intensity, %): 296 (M+, 20.00), 111 (76.67), 264 (33.33), 222 (26.67), 180 (18.33), 166 (23.33), 152 (23.33), 123 (23.33), 110 (38.33), 97 (75.00), 83 (70.00), 74 (66.67), 69 (78.33), 55 (*100*).

Methyl Linoleate. EI-MS *m/z* (relative intensity, %): 294 (M+, 52.46), 263 (24.59), 220 (8.20), 178 (13.11), 164 (19.67), 150 (21.31), 136 (18.03), 123 (18.85), 109 (37.70), 95 (70.49), 81 (*100*), 67 (91.80), 55 (50.82).

Methyl Linolenate. EI-MS *m/z* (relative intensity, %): 292 (M+, 16.67), 261 (5.00), 236 (6.67), 173 (6.67), 163 (6.67), 149 (20.00), 135 (20.00), 121 (25.00), 108 (56.67), 95 (58.33), 79 (*100*), 67 (56.67), 55 (35.00).

Methyl Arachidonate. EI-MS *m/z* (relative intensity, %): 318 (M+, 1.82), 290 (1.82), 264 (1.82), 175 (5.45), 150 (7.27), 133 (7.27), 105 (30.91), 91 (70.91), 79 (*100*), 67 (80.00), 55 (49.09).

Methyl Eicosapentaenoate. EI-MS *m/z* (relative intensity, %): 315 (M+, 1.67), 175 (6.67), 161 (8.33), 145 (11.67), 131 (18.33), 119 (31.67), 108 (31.67), 91 (70.00), 79 (*100*), 67 (68.33), 55 (48.33).

Methyl Docosahexaenoate. EI-MS *m/z* (relative intensity, %): 342 (M+, 0.60), 145 (4.20), 131 (6.60), 119 (10.80), 108 (11.40), 91 (28.20), 79 (*100*), 67 (20.40). (Chakraborty et al, 2010).

Mass Spectroscopic Analyses of *N*-Acyl Pyrrolidide Derivatives

The following are the mass spectrometric data of *N*-acyl pyrrolidide derivatives.

1-(Pyrrolidin-1-yl)hexadecan-1-one/Palmitoylpyrrolidine. EI-MS *m*/*z* (relative intensity, %): 309 (M+, 16.00), 294 (2.00), 168 (8.00), 140 (10.00), 126 (16.00), 113 (*100*), 98 (8.00), 70 (12.00), 55 (14.00).

1-(Pyrrolidin-1-yl)octadec-9-en-1-one. EI-MS *m*/*z* (relative intensity, %): 335 (M+, 27.56), 250 (8.62), 236 (10.34), 208 (6.90), 196 (5.17), 182 (12.07), 126 (53.45), 113 (*100*), 98 (18.97), 85 (8.62), 72 (20.69), 55 (27.59).

1-(Pyrrolidin-1-yl)octadeca-9,12-dien-1-one. EI-MS *m*/*z* (relative intensity, %): 333 (M+, 77.97), 290 (10.17), 236 (15.25), 222 (20.34), 182 (16.95), 168 (15.25), 140 (22.03), 126 (44.07), 113 (*100*), 98 (25.42), 70 (42.37), 55 (49.15).

1-(Pyrrolidin-1-yl)octadeca-9,12,15-trien-1-one. EI-MS *m*/*z* (relative intensity, %): 331 (M+, 44.00), 182 (22.00), 168 (24.00), 140 (26.00), 126 (60.00), 113 (*100*), 98 (30.00), 72 (64.00), 55 (42.00).

1-(Pyrrolidin-1-yl)icosa-5,8,11,14-tetraen-1-one. EI-MS *m*/*z* (relative intensity, %): 357 (M+, 18.97), 232 (10.34), 180 (10.34), 126 (13.79), 113 (*100*), 85 (17.24), 70 (22.41), 55 (27.59).

1-(Pyrrolidin-1-yl)icosa-5,8,11,14,17-pentaen-1-one. EI-MS *m*/*z* (relative intensity, %): 355 (M+, 3.85), 286 (7.69), 232 (7.69), 126 (13.46), 113 (*100*), 85 (17.31), 72 (26.92), 55 (21.15).

1-(Pyrrolidin-1-yl)octadeca-9,12-dien-1-one. EI-MS *m*/*z* (relative intensity, %): 381 (M+, 3.91), 312 (7.05), 272 (7.29), 232 (16.22), 218 (15.76), 192 (8.24), 166 (23.67), 153 (22.85), 113 (*100*), 98 (46.62), 72 (21.98) (Chakraborty et al, 2010).

Conclusions

Research on exploring sources long-chain PUFAs, viz., DHA, EPA, and AA for use in nutrition have received considerable attention. These PUFAs, which are usually low in abundance in human, are regarded as essential and must be supplied in diet. The importance of PUFAs in human nutrition has been extensively investigated during the past 20 years. DHA is one of the important PUFAs, which maintains structural and functional integrity in larval cell membranes in addition to the neural development and function, while AA and EPA are involved in, respectively, the production and modulation of eicosanoids. Docosahexaenoic acid (22:6ù-3), which is a vital component of the phospholipids of cellular membranes, especially in the brain and retina, is necessary for their proper functioning. The ù-3 fatty acids favorably affect atherosclerosis, coronary heart disease, inflammatory disease, and perhaps even behavioral disorders. Membrane fluidity is essential for proper functioning of these tissues. In the retina, where ù-3 fatty acids are especially important, deficiency can result in decreased vision and abnormal electroretinogram results. The ù-3 fatty acids are essential fatty acids, necessary from conception through pregnancy and infancy and, undoubtedly, throughout life. AA has been an essential function of producing eicosanoids, making it an essential fatty acid because prostaglandins (PGF2R) are produced from 20:4n6, and has roles in reproduction. AA is the basis for cyclo-oxygenase (COX) action to produce PGF2R. AA, being a major component of phosphoinositol, was reported to have a vital role in the transduction signal mechanism. An imbalance in ù-3/ù-6 ratio can accentuate ù-3 fatty acid deficiency state, as shown by earlier studies. The ratio may have increased in industrialized societies because of increased consumption of vegetable oils

rich in ù-6 fatty acids, ie, linoleic acid (18:2n26), and reduced consumption of foods rich in ù-3 fatty acids. Another important feature of ù-3 fatty acids is their role in the prevention and modulation of certain diseases that are common (Importance of n23 fatty acids in health and disease (*W. E. Connor Am J Clin Nutr* 2000;71(suppl):171S–5S). Below is appended a partial list of diseases that may be prevented or ameliorated with ù-3 fatty acids:

- Coronary heart disease and stroke
- Cancers of the breast, colon, and prostate
- Retinal and brain development);
- Immunostimulant
- Hypertension

The first two functions are extremely important and are related directly or indirectly with other diseases as listed earlier.

Suggested Reading

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