OMEGA-3, OMEGA-6, OMEGA-9 FATTY ACIDS: SOURCES, METABOLISM AND SUPPLEMENTS

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Contents

Summary

This chapter is concerned about the importance of essential fatty acids, omega-3, omega-6, and omega-9. The balance of dietary intake of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) is the most important determinant of cell membrane composition under normal conditions. Their balance in human diet ensures homeostasis which is required for normal development of the body for healthy life. It traces the way in which the relative proportions of these acids has changed since prehistoric times in human diet starting from the hunter-gatherer era through agricultural age to the present industrial age. This chapter discusses biosynthesis of

Omega-6 and Omega-3 Fatty Acids and Genetic variants at Fatty Acid Desaturases (FADS) 1 and FADS2, eicosanoid metabolism, specialized pro-resolving mediators (SPMs), biological effects, and metabolic functions of omega-6 and omega-3 fatty acids. It then looks at inflammation and susceptibility to Covid-19 in the light of genetic variation in these. It also presents some aspects of clinical studies of intervention for omega-3/omega-6 essential fatty acid (EFA) balance showing evidence from gene transfer studies. Effects of EFA on obesity, brain function, nutrition of infants of mothers are also discussed. Mediterranean food is discussed for its mono-unsaturated fatty acids (MUFA). A section on olive oil as part of a Mediterranean diet is included with emphasis in terms of its fatty acid composition and polyphenol content contributing to decreasing inflammation and the risk for cardiovascular disease. Olive oil does not compete with the incorporation of omega-3 fatty acids into the cell membrane phospholipids, whereas omega-6 fatty acids do. For that reason the Mediterranean diet with a balanced omega-6/omega-3 ratio is the healthiest diet. General recommendations of a meeting on the subject are referred to a report listed in the bibliography.

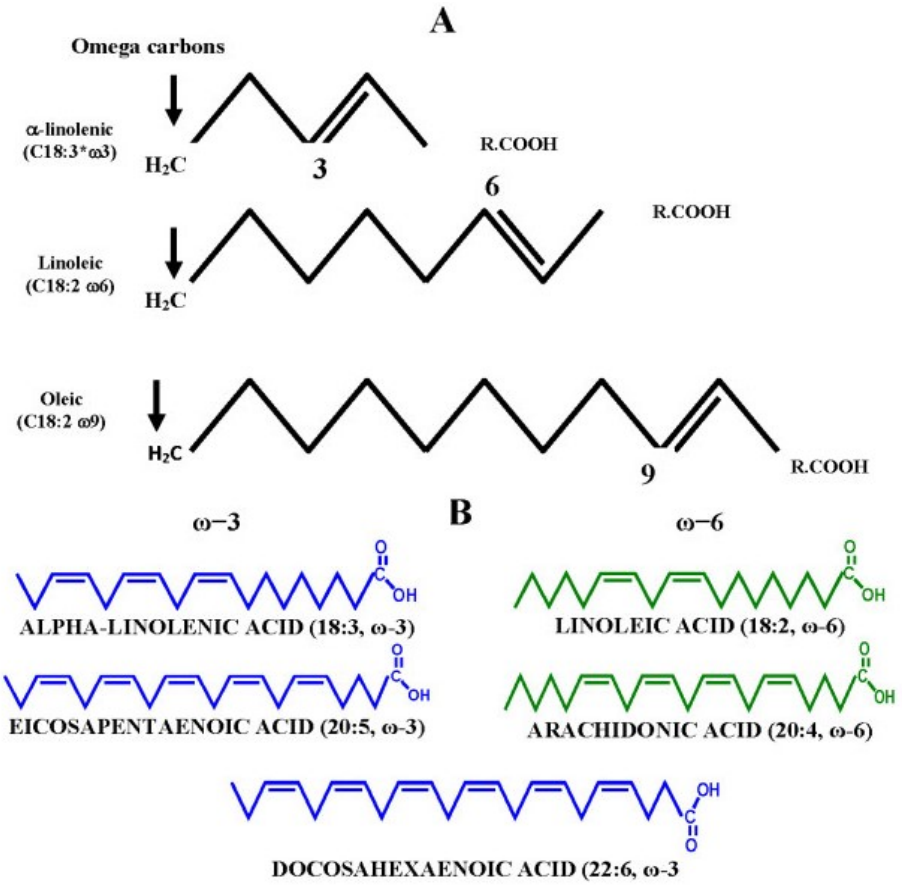
1. Introduction

Studies on the evolutionary aspects of diet indicate that human beings evolved on a diet that was balanced in the omega-6 and omega-3 essential fatty acids (EFAs), whereas current (Western) diets are high in omega-6 fatty acids and deficient in omega-3s. This imbalance leads to a pro-inflammatory state, which is at the base of practically all chronic diseases. The omega-6 and omega-3 EFA are metabolically and functionally distinct, are not inter-convertible, are important components of practically all cell membranes, and have opposing properties. The balance of dietary intake of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) is the most important determinant of cell membrane composition under normal conditions. Their balance is important for homeostasis and normal development throughout life. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are important in neurogenesis and decrease the risk for neurogenerative diseases. It is therefore important to consider omega-3 PUFAs not in isolation but in association with omega-6 PUFA levels. Oleic acid supports the incorporation of omega-3's into the cell membranes, whereas linoleic acid (LA) competes and prevents their incorporation. Research advances using lipidomics indicate that specialized pro-resolving lipid mediators (SPMs) from EPA or DHA control the inflammation that is produced by the high intake of LA, and ARA, and lead to resolution of inflammation, thus lowering the risk of obesity, coronary heart disease (CHD), diabetes, rheumatoid arthritis, depression and some forms of cancer. Because omega-3s are essential for growth and development throughout the life cycle, they should be included in the diets of all humans during pre-pregnancy, gestation, and throughout lactation. DHA is essential for the normal functional development of the retina and brain, particularly for premature infants. By selecting cooking oils low in omega-6 fatty acids, but high in monounsaturated fatty acids (like a Mediterranean diet) eating fish or taking supplements ALA, EPA + DHA, the omega-6/omega-3 fatty acid ratio can be reduced between 1/1 to 4/1 which is consistent with good health.

The 1985 Conference on The Health Effects of Polyunsaturated Fatty Acids in Seafoods defined the importance of a balanced ω−6 and ω−3 essential fatty acids (EFAs) ratio in

growth and development and in the prevention and management of chronic diseases. The 1980's were a period of expansion in our knowledge about polyunsaturated fatty acids (PUFAs) in general and especially the role of ω−6 and ω−3 EFAs in human metabolism. Today we know that their balance is important in health and disease, and plays an important role in the prevention and treatment of coronary artery disease (CAD), hypertension, arthritis, autoimmune disorders, cancer and other inflammatory diseases, including depression. Research has been carried out in animal models, tissue cultures and humans, including controlled clinical trials. This chapter will focus on the latest research advances in the field, including genetic variants of omega-6 and omega-3 PUFA in the desaturation and elongation of their metabolic pathways, which influence fatty acid levels in the tissues, and their function in health and disease.

A special section on ω−9 oleic acid, a non-essential fatty acid, and the predominant fatty acid in the Mediterranean diet and its relationship to ω−6 and ω−3 fatty acids is included. The importance of a balanced omega-6/omega-3 ratio, and omega-9 fatty acids in our diet and how to obtain them through diet and/or supplementation will be a major part of the chapter.



(A) The first number (before the colon) gives the number of carbon atoms in the molecule and the second gives the number of double bonds. ω−3, ω−6, and ω−9 indicate position of the first double bond in a given fatty acid molecule. (B) Structure of commonly found ω−3 and ω−6 fatty acids.

Figure 1. Schematic and structural formulas for ω−3 (a-linolenic), ω−6 (linoleic), and ω−9 (oleic) fatty acids

Polyunsaturated fatty acids have two or more double bonds. There are two classes of PUFAs, omega-3 (18:3-ω3) and omega-6 fatty acids (18:2-ω6). The distinction between ω−6 and ω−3 fatty acids is based on the location of the first double bond, counting from the methyl end of the fatty acid molecule. Unsaturated fatty acids consist of mono-unsaturates like oleic acid 18:1-ω9 the predominant fatty acid in olive oil, has one double bond (Figure 1).

Omega-3 and omega-6 fatty acids are essential fatty acids, because humans cannot make them and must obtain them from their diet. Omega-3 fatty acids are represented by alpha-linolenic acid (ALA) 18:3ω−3 and omega-6 fatty acids by linoleic acid (LA) 18:3ω−6, which are the parent fatty acids of the two series. Monounsaturated fatty acids are represented by oleic acid 18:1-ω9, which can be synthesized by all mammals including humans.

2. Evolutionary Aspects of Diet: Sources and the Omega-6/Omega-3 Balance

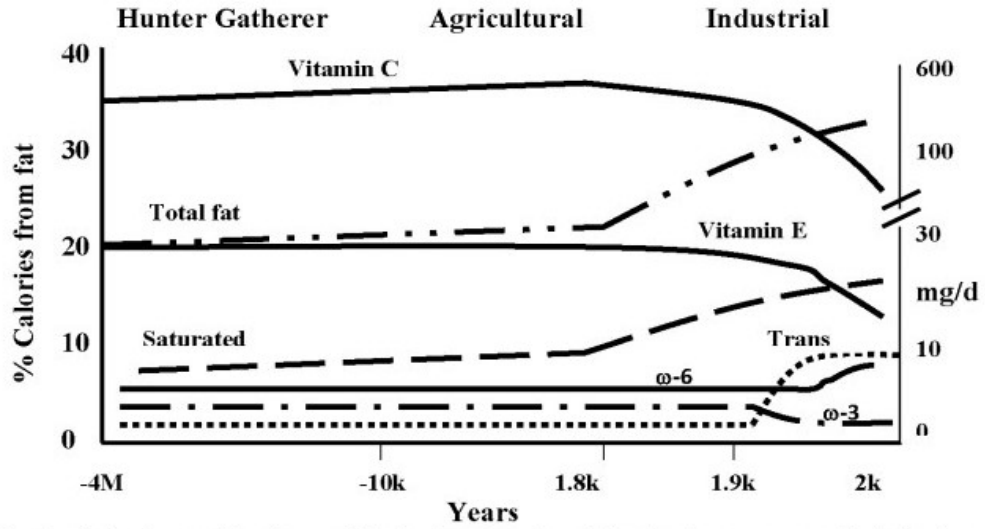
The foods that were commonly available to pre-agricultural humans, lean meat, fish, green leafy vegetables, fruits, nuts, berries and honey, were the foods that shaped modern humans' genetic nutritional requirements. A number of anthropological, nutritional and genetic studies indicate that humans' overall diet, including energy intake and energy expenditure, has changed over the past 10,000 years, with major changes occurring during the past 130 years in the type and amount of fat and in Vitamins C and E intake (Table 1) (Figure 2).

Plant Sources	
LA	4.28
ALA	11.40
Animal Sources	
LA	4.56
ALA	1.21
Total	
LA	8.84
ALA	12.60
Animal Sources	
AA (ω6)	1.81
EPA (ω3)	0.39
DTA (ω6)	0.12
DPA (ω3)	0.42
DHA (ω3)	0.27

Ratios of ω6/ω3	
LA/ALA	0.70
ARA+DTA/EPA+DPA+DHA	1.79
Total ω6/ω3	0.79[b]

LA, linoleic acid; ALA, linolenic acid; ARA, arachidonic acid; EPA, eicosapentaenoic acid; DTA, docosatetranoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

Table 1. Estimated omega-3 and omega-6 fatty acid intake in the late Paleolithic period (g/d)



Hypothetical scheme of fat, fatty acid (ω6, ω3, trans and total) intake (as percentage of calories from fat) and intake of vitamins E and C (mg/d). Data were extrapolated from cross-sectional analyses of contemporary hunter-gatherer populations and from longitudinal observations and their putative changes during the preceding 100 years.

Figure 2. Evolutionary Aspects of Diet

2.1. Large Scale Production of Vegetable Oils High In Omega-6 Fatty Acids

The increased consumption of omega-6 fatty acids in the last 130 years is due to the development of technology at the turn of the19th century that marked the beginning of the modern vegetable oil industry, and to modern agriculture with the emphasis on grain feeds for domestic livestock (grains are rich in omega-6 fatty acids). The invention of the continuous screw press, named Expeller R by V.D. Anderson, and the steam-vacuum deodorization process by D. Wesson made possible the industrial production of cottonseed oil and other vegetable oils for cooking. Solvent extraction of oil seeds came into increased use after First World War, and the large-scale production of vegetable oils became more efficient and more economical. Subsequently, hydrogenation was applied to oils to solidify them. The partial selective hydrogenation of soybean oil reduced the ALA content of the oil, while leaving a high concentration of LA. ALA content was reduced Because ALA in soybean oil caused many organoleptic problems. It is now well known that the hydrogenation process, and particularly the formation of trans fatty acids has led to increases in serum cholesterol concentrations, whereas LA, in its regular state in oil, is associated with a reduced serum cholesterol concentration. The availability of methods for the production of vegetable oils high in omega-6 fatty acids, and their use in lowering serum cholesterol concentrations, despite the fact that omega-6 fatty acids did not lower mortality from CAD, led to an increase in both the fat content of the diet, and the greater increase in vegetable oils rich in omega-6 fatty acids.

2. 2. Agribusiness and Modern Agriculture Decreased the Omega-3 Fatty Acid Content of Foods, while Increased the Omega-6 Fatty Acid Content

Modern agriculture with its emphasis on production has decreased the omega-3 fatty acid content in many foods. Wild animals and birds who feed on wild plants are very lean with a carcass fat content of only 3.9% and contain about five times more PUFAs

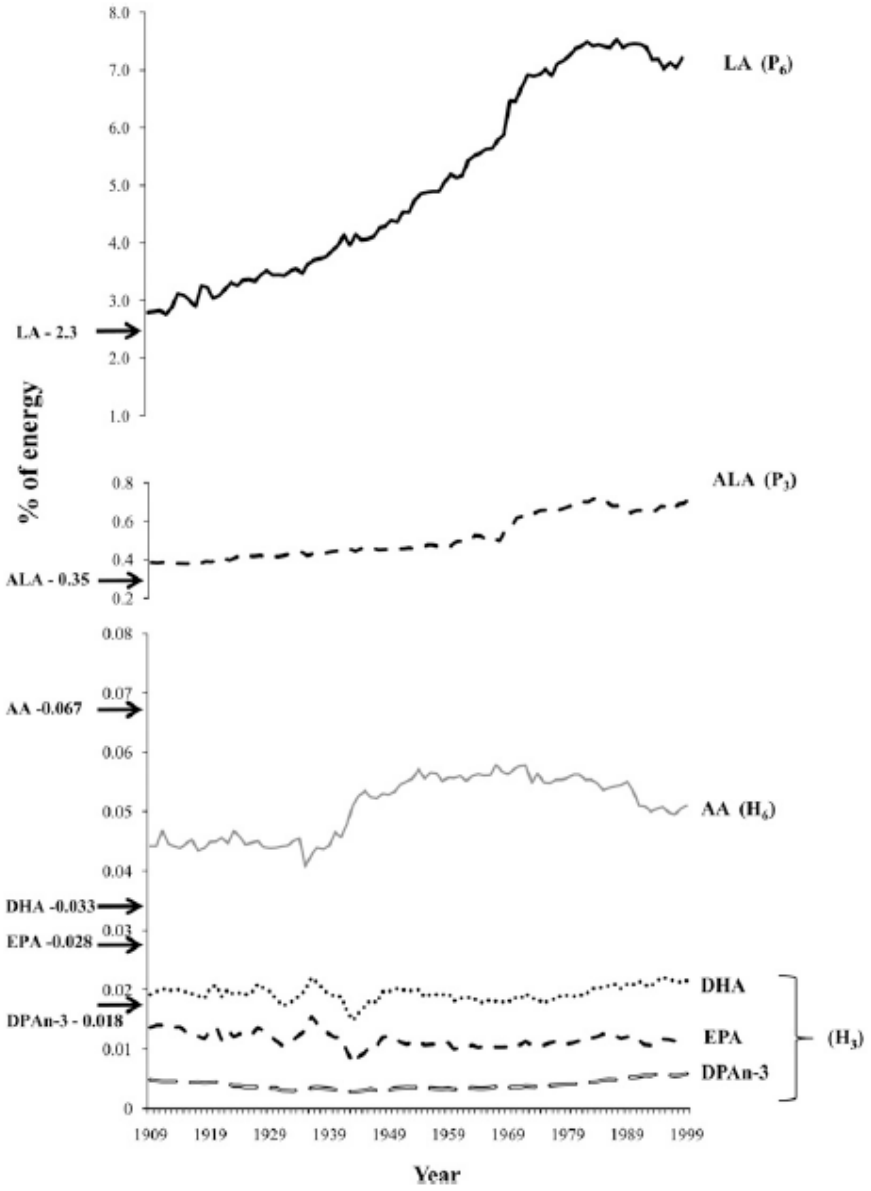
per gram than is found in domestic livestock. Four percent (4%) of the fat of wild animals is EPA. Domestic beef contains very small amounts of ALA in their meat because cattle are fed grains rich in omega-6 fatty acids and poor in omega-3s (ALA). In addition to the changes in the fatty acid composition of animal meats, cultivated green leafy vegetables, eggs from domesticated chickens and even fish from aquaculture contain less omega-3 fatty acids than those in the wild. Table 2 shows the composition of oils and foods and the omega-6/omega-3 ratio (Table 2).

Dietary fat	**Saturated fat**	**Monosaturated fat**	**LA**	**ALA**	**LA/ ALA**	**Cholesterol**
Flaxseed oil	10	20	16	53	0.3	0
Canola oil	6	62	22	10	2.2	0
Walnut oil	12	18	58	12	4.8	0
Safflower oil	10	13	77	Trace	77	0
Sunflower oil	11	20	69	_-	69	0
Corn oil	13	25	61	1	61	0
Olive oil	14	77	8	1	8.0	0
Soybean oil	15	24	54	7	7.7	0
Margarine	17	49	32	2	16	0
Peanut oil	18	49	33	-	33	0
Palm oil	51	39	9	0.3	30	0
Coconut oil	92	7	2	0	2.0	11
Chicken fat	31	47	21	1	21	12
Lard	41	47	11	1	11	14
Beef fat	52	44	3	1	3.0	33
Butterfat	66	30	2	2	1.0	
Grass-fed Beef					1.54	
Grain-fed Beef					5.01	
Chicken Breast, Skinless					16.25	
Chicken Thigh					17.64	
Pork Chop					27.45	
Salmon, Farm Raised					0.80	
Ultra-processed Foods					11:1	
Minimally processed Foods					5:1	
USDA Egg					19.4*	
Greek Egg					1.3*	

*USDA Egg 19.4 is the total omega-6/omega-3 ratio (LA + ARA/ALA, EPA + DHA).
*Greek Egg is 1.3, is the total omega-6/omega-3 ratio (LA + ARA/ALA, EPA + DHA).

Table 2. Composition of Selected Dietary Oils and Foods

An absolute and relative change of omega-6/omega-3 fatty acids in the food supply of Western societies, and now worldwide, have occurred over the last 100 years (Figure 3).



Availability of essential fatty acids from 1909 to 1999. 1909 data are indicated by solid arrows for LA (2.23% of energy), ALA (0.35% of energy), arachidonic acid (AA) (0.67% of energy), docosahexaenoic acid (DHA) (0.033% of energy), eicosapentaenoic acid (EPA) (0.028% of energy), and docosapentaenoic acid (DPAn23) (0.018% of energy).

Figure 3. Essential fatty acids intake in the 20th Century

A balance existed between omega-6 and omega-3 fatty acids for millions of years, during the long evolutionary history of the genus Homo, and genetic changes occurred partly in response to these dietary influences. During evolution, omega-3 fatty acids were found in all foods consumed, meat, wild plants, eggs, fish, nuts and berries. In the traditional diet of Crete, omega-3 fatty acids were found in every meal the people of Crete ate. Today ultra-processed foods account for 54% of all foods eaten. The Hall

study showed that the omega-6/omega-3 ratio is 11/1 in the ultra-processed foods and 5/1 in the minimally processed foods, which may account for gaining of body weight of two pounds in 2 weeks. Rapid dietary changes that have occurred and continue to occur due to food processing – and the increase in imitation foods, such as plant-based fish, meat and chicken based on plant proteins, is a totally new phenomenon in human evolution leading to different omega-6/omega-3 fatty acid ratios worldwide (Table 3).

Population	ω6/ω3
Paleolithic	0.79
Greece prior to 1960	1.00 – 2.00
Current United States	16.74
United Kingdom and northern Europe	15.00
Japan	4.00
India rural	5 – 6.1
India urban	38 – 50

Table 3. ω-6: ω-3 ratios in various populations

2.3. Sources of Omega-6 and Omega-3 Fatty Acids

LA is the predominant EFA in Western diets. It is found in the seeds of most plants, except for coconut and cocoa (Table 4). ALA is found in the seeds of flaxseed (linseed), rapeseed, chia, perilla and in the chloroplast of green leafy vegetables (Table 5). Table 6 presents the omega-3 content and other lipids in fish. Fish is a good source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), whereas meat, dairy, and eggs are high in arachidonic acid (ARA), particularly from grain-fed animals.

Oils High in LA	Amount of LA
Sunflower oil	71%
Safflower oil	78%
Corn oil	59%
Soybean oil	56%
Cottonseed oil	53%

Table 4. Sources of Omega-6 Fatty Acid (Linoleic Acid)

Oils High in ALA	Amount of ALA
Flaxseed oil	55%
Perilla oil	58%
Canola oil	12%
Rapeseed oil	10%
Chia	64%

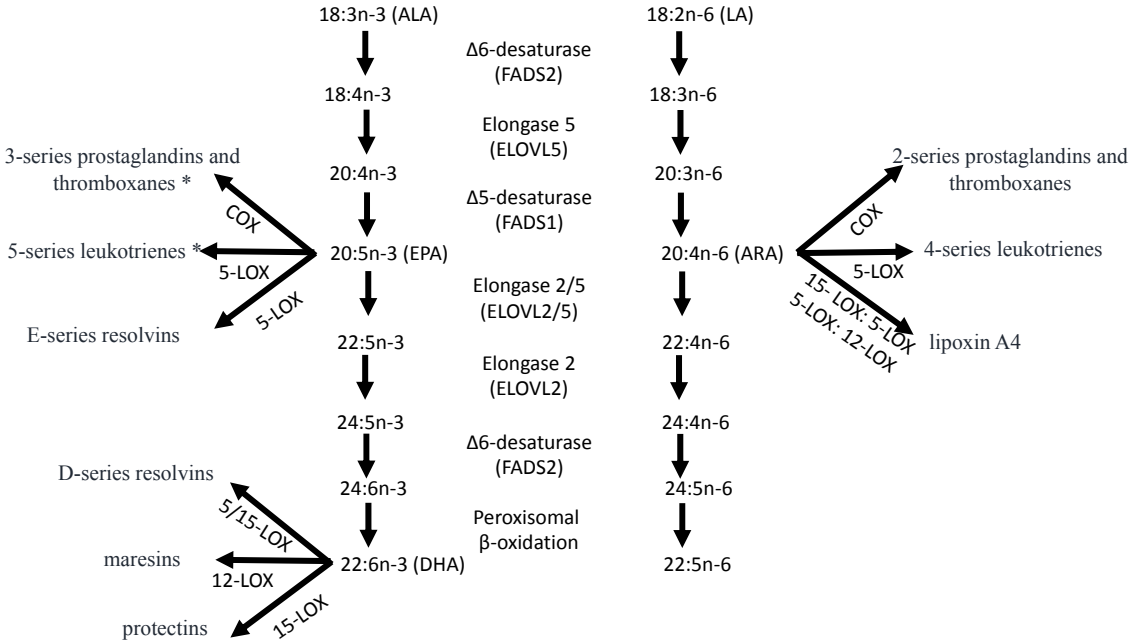
Table 5. Sources of Omega-3 Fatty Acid (Alpha-linolenic acid)

		Fatty acids						
Fish	**Total fat**	**Total saturated**	**Total mono-unsaturated**	**Total poly-unsaturated**	**18:3**	**20:5**	**22:6**	**Cholesterol**
			g/100g					mg/100g
Anchovy, European	4.8	1.3	1.2	1.6	-	0.5	0.9	-
Bass, striped	2.3	0.5	0.7	0.8	Tr	0.2	0.6	80
Blue fish	6.5	1.4	2.9	1.6	-	0.4	0.8	59
Carp	5.6	1.1	2.3	1.4	0.3	0.2	0.1	67
Catfish, brown Bullhead	2.7	0.6	1.0	0.8	0.1	0.2	0.2	75
Catfish, channel	4.3	1.0	1.6	1.0	Tr	0.1	0.2	58
Cod, Atlantic	0.7	0.1	0.1	0.3	Tr	0.1	0.2	43
Croaker, Atlantic	3.2	1.1	1.2	0.5	Tr	0.1	0.1	61
Flounder, unspecified	1.0	0.2	0.3	0.3	Tr	0.1	0.1	46
Grouper, red	0.8	0.2	0.1	0.2	-	Tr	0.2	-
Haddock	0.7	0.1	0.1	0.2	Tr	0.1	0.1	63
Halibut, Greenland	13.8	2.4	8.4	1.4	Tr	0.5	0.4	46
Halibut, Pacific	2.3	0.3	0.8	0.7	0.1	0.1	0.3	32
Herring, Pacific	13.9	3.3	6.9	2.4	0.1	1.0	0.7	77
Herring, round	4.4	1.3	0.8	1.5	0.1	0.4	0.8	28
Mackerel, king	13.0	2.5	5.9	3.2	-	1.0	1.2	53
Mullet, striped	3.7	1.2	1.1	1.1	0.1	0.3	0.2	49
Ocean perch	1.6	0.3	0.5	0.5	Tr	0.1	0.1	42
Plaice, European	1.5	0.3	0.5	0.4	Tr	0.1	0.1	70
Pollock	1.0	0.1	0.1	0.5	-	0.1	0.4	71
Pompano, Florida	9.5	3.5	2.6	1.1	-	0.2	0.4	50
Salmon, Chinook	10.4	2.5	4.5	2.1	0.1	0.8	0.6	-
Salmon, pink	3.4	0.6	0.9	1.4	Tr	0.4	0.6	-
Snapper, red	1.2	0.2	0.2	0.4	Tr	Tr	0.2	-
Sole, European	1.2	0.3	0.4	0.2	Tr	Tr	0.1	50
Swordfish	2.1	0.6	0.8	0.2	-	0.1	0.1	39
Trout, rainbow	3.4	0.6	1.0	1.2	0.1	0.1	0.4	57
Tuna, albacore	4.9	1.2	1.2	1.8	0.2	0.3	1.0	54
Tuna, unspecified	2.5	0.9	0.6	0.5	-	0.1	0.4	-

Table 6. Content of ω−3 fatty acids and other fat components in selected fish

3. Biosynthesis of Omega-6 and Omega-3 Fatty Acids and Genetic variants at FADS1 and FADS2

Humans and animals except for carnivores, such as lions and possibly cats, can convert LA to AA and ALA to EPA and DHA. This conversion was shown by using deuterated ALA.



Alpha-linolenic acid (18:3n-3; ALA) is desaturated and elongated to eicosapentaenoic acid (20:5n-3; EPA) and docosahexaenoic acid (22:6n-3; DHA) while the n-6 polyunsaturated fatty acid linoleic acid (18:2n-6) produces arachidonic acid (20:4n- 6; ARA). Importantly not only do ALA and LA compete for the same enzymes, but recent work demonstrates that genetic differences in the FADS genes regulate activity and tissue PUFA levels. ARA is the precursor to the prostaglandins, thromboxanes and leukotrienes, and lipoxins, while EPA produces prostaglandins, thromboxanes and leukotrienes with diminished activity relative to those from ARA. EPA and DHA are also precursors to the specialized proresolving mediators (SPMs), E-series resolvins from EPA, D-series resolvins, maresins and protectins from DHA and DPA.

Figure 4. Biosynthesis of Omega-6 and Omega-3 Fatty Acids

FADS1 and FADS2 located at chromosome 12.2–13.1) and elongase genes (ELOVL2 at chromosome 6p24.2 and ELOVL5 located at chromosome 6p12.1) (Figure 4) mediate the endogenous biosynthesis of ARA, EPA, and DHA. There is competition between omega-3 and omega-6 fatty acids for the desaturation enzymes FADS 1 and FADS2. However, both FADS1 and FADS2 prefer the omega-3 to omega-6 fatty acid pathway. There is some evidence that FADS2 decreases with age, premature infants, hypertensive individuals and some diabetics are limited in their ability to make EPA and DHA from ALA. These findings are important and need to be considered when making dietary recommendations.

ALA is the parent fatty acid of the omega-3 family of EFA and is the principal ω−3 PUFA in the Western diet. Typical consumption of ALA in US, Europe, and Australia ranges between 0.6 -1.7 g/day in men and 0.5 -1.4 g/day in women. The ratio of

LA/ALA varies in countries but it is higher in the US, Australia, UK and the Netherlands about 16-20/1 than in Greece, where the ratio that was balanced 1/1 ω−6/ ω−3 until 1960 it is now about 6/1, ω−6/ ω−3 due to increased consumption of omega-6 rich sunflower and corn oil, because they are cheaper than olive oil.

LA and ALA and their long-chain derivatives are important components of animal and plant cell membranes. In mammals and birds, the omega-3 fatty acids are distributed selectively among lipid classes. ALA is found in triglycerides cholesterol esters and in very small amounts in phospholipids. EPA is found in cholesterol esters, triglycerides and phospholipids. DHA is found mostly in phospholipids. In mammals, including humans, the cerebral cortex retina and testis and sperm are particularly rich in DHA. DHA is one of the most abundant components of the brain's structural lipids. DHA, like EPA, can be derived only from direct ingestion or in small amounts by synthesis from dietary EPA and ALA.

The concentration of ALA in phospholipids, plasma, red blood cells (RBCs) and tissues is typically less than 0.5% of total fatty acids and the dietary intake of EPA, DPA and DHA are about 25 to 15 times lower than those of ALA, which is about 1.4 g/d and the LA is 14.4 g/day (UK data). The effect of ALA deficiency on neurological function supports the role of ALA as a precursor to longer-chain ω−3 PUFA, which are critical in the function of the Central Nervous System. ALA is absorbed across the GI tract and its secretion into the blood stream is efficient and similar to LA and oleic acid.

Since both ω−6 and ω−3 PUFA are metabolized by the same desaturation/elongation pathway, there is competition between these two families of EFA. The initial conversion of ALA to 18:4 ω−3 by the action of D6-desaturase is the rate limiting reaction of the pathway. Despite the fact that the affinity of D6-desaturase for ALA is greater than for LA, because of the high levels of LA in Western diets, LA is found in higher concentrations than ALA in cellular pools, which results in greater conversion of LA to higher levels of ω−6 PUFAs, especially ARA. Increasing the amount of ALA leads to increased levels of EPA in both plasma and RBCs. Because of competition in their metabolism between LA and ALA, the LA content of the diet influences the conversion of ALA to longer-chain derivatives. At a given intake of ALA the production of EPA is greater when LA intake is decreased. Human studies show that it is possible to increase the biosynthesis from ALA to EPA, and DPA, by reducing LA or increasing ALA intake, but LA levels need to be reduced to < 2.5% of energy before DHA levels can be increased. Since LA levels in Western Diets account between 6-8% of energy, such levels reduce the production of DHA. In today's Western diets with very high intakes of LA than even before in the history of humans, it is necessary to decrease the intake of vegetable oils high in ω−6 fatty acids in order to improve the status of ω−3 long-chain PUFA, EPA, DPA and DHA. The conversion of ALA to longer-chain ω−3 PUFA is dependent on the cooking oils of the food supply. Because Western diets are high in LA and low in ALA and fish intake, the population is in a state of "Omega-3 deficiency." Studies have shown that in women of reproductive age – about 28 years of age, the conversion of ALA to EPA and DHA was substantially greater indicating a gender-related difference in the activity, possibly due to the action of estrogens. There is additional evidence that strongly supports the suggestion that sex hormones regulate the activity of the desaturation/elongation pathway in humans; i.e. DHA levels were higher

in women taking oral contraceptives. A possible biological role for greater synthesis of DHA in women may be the need in meeting the demands of the fetus and newborn for DHA.

In addition to its role in the production of EPA, DPA and DHA, ALA could have by itself, a beneficial role against cardiovascular disease (CVD) and some cancers. A recent systematic review and meta-analysis of prospective cohort studies examined the dietary intake and biomarkers of alpha-linolenic acid and risk of all cause, cardiovascular and cancer mortality. The findings show that dietary ALA intake is associated with a reduced risk of mortality from all causes, CVD, and CHD, and a slightly higher risk of cancer mortality, whereas higher blood levels of ALA are associated with a reduced risk of all cause and CHD mortality only. Each 1 g/day increase in ALA intake was associated with a 5% lower risk of CVD mortality and each 1 SD increment in blood levels of ALA was associated with an 8% lower risk of CHD mortality.

In our studies of the diet of Crete the people of Crete had a ratio of omega-6/omega-3 of 1-2/1, whereas Western diets had a ratio of 20-16/1. A few years ago, studies on the Mediterranean diet did not include omega-3s. But our studies showed that their diet was balanced in omega-6/omega-3 PUFAs, more people began to pay attention to the omega-6/omega-3 ratio and the current definition of the Mediterranean diet includes the need for a balanced omega-6/omega-3 ratio. Additional studies compared the fatty acids of the population of Crete to the Zutphen population in the Seven Countries Study. The data showed that the Greeks had 33% more ALA ($p < 0.001$) and 20% lower LA ($P<0.001$) in their plasma, but in their report, there weren't any data regarding EPA and DHA levels. A positive association between spontaneous adherence to Mediterranean diet as assessed by a dietary score, and plasma EPA and DHA has been observed in both the ATTICA and the Three-City studies, which independently evaluated the dietary habits of a Greek and French cohort in relation to the plasma fatty acid contents. In the French cohort individuals with higher Mediterranean diet adherence score showed DHA and total omega-3 PUFA levels 10% ($p < 0.004$) higher than individuals in the lower score category. Similarly, consumption of a Mediterranean-type diet by healthy subjects for 4 weeks, resulted in a 47% increase in DHA plasma content ($p < 0.0001$) compared with an ordinary Swedish diet. Although the above studies lack the absolute quantification of plasma omega-3 PUFA content, they demonstrate that a Mediterranean-type diet ensures higher omega-3 PUFAs bioavailability than the common Western diets, and hence at least part of the protective effects exerted by Mediterranean diets may be attributed to its vegetable, animal and marine content (meat and fish) of omega-3 PUFAs ALA, EPA, DHA, the low content of LA and ARA, and the high content of oleic acid in olive oil. Thus, both plant- and marine- derived omega-3 PUFAs may be considered as valuable mediators of protection by traditional Mediterranean diets along with oleic acid.

4. Eicosanoid Metabolism, Specialized Pro-Resolving Mediators (SPMS), Biological Effects, And Metabolic Functions of Omega-6 and Omega-3 Fatty Acids

When humans ingest fish or fish oil, the EPA and DHA from the diet, partially replace the omega-6 fatty acids, especially ARA, in the membrane of probably all cells, but

especially in the membranes of platelets, erythrocytes, neutrophils, monocytes, and liver cells.

4.1. Eicosanoids

As discussed above ARA and EPA are the parent compounds for eicosanoid metabolism (Figure 4).

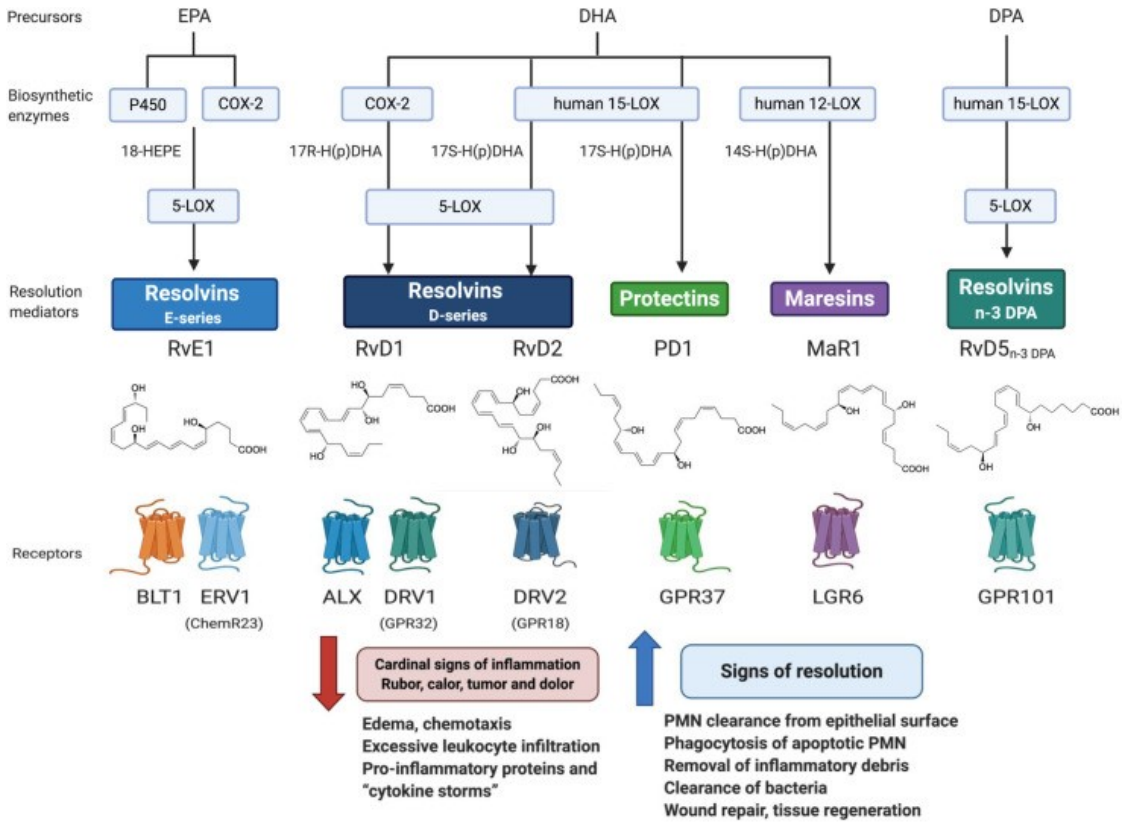
Due to increased amounts of omega-6 fatty acids in Western diets, the eicosanoid metabolic products from ARA – specifically prostaglandins, thromboxanes, leukotrienes, hydroxy fatty acids and lipoxins, are formed in larger quantities than those formed from omega-3 fatty acids, specifically EPA, DPA and DHA. The eicosanoids from ARA are biologically active in very small quantities and if they are formed in large amounts, they contribute to the formation of thrombus and atheromas, to allergic and inflammatory disorders, particularly in susceptible people, and to proliferation of cells. Thus, a diet rich in omega-6 fatty acids, such as the current Western diet, shifts the physiological state to one that is pro-thrombotic and pro-aggregatory with increases in blood viscosity, vasospasm and vasoconstriction, and decreases in bleeding time. The anti-thrombotic aspects and the effects of different doses of fish oil on the prolongation of bleeding time have been investigated. A dose of 1.8 g/d EPA did not result in any prolongation in bleeding time, but at 4 g/d, the bleeding time increased and the platelet count decreased without any adverse effects. In human studies, there has never been a case of clinical bleeding, even in patients undergoing angioplasty while they were on fish oil supplements. The effects of EPA and DHA from eating fish or taking fish oil supplements on eicosanoid metabolism of ARA are summarized in the following:

- Decreased production of prostaglandin E_2 (PGE_2) metabolites
- A decrease in thromboxane A_2, a potent platelet aggregator and vasoconstrictor
- A decrease in leukotriene B_4 formation, an inducer of inflammation, and a powerful inducer of leukocyte chemotaxis and adherence
- An increase in thromboxane A_3, a weak platelet aggregator and weak vasoconstrictor
- An increase in prostacyclin PGI_3, leading to an overall increase in total prostacyclin by increasing PGI_3 without a decrease in PGI_2, both PGI_2 and PGI_3 are active vasodilators and inhibitors of platelet aggregation
- An increase in leukotriene B_5, a weak inducer of inflammation and a weak chemotactic agent

4.2. Specialized Pro-resolving Mediators: Controlling Infections, Inflammation and its Resolution

As discussed earlier ARA produces eicosanoids that are pro-inflammatory and EPA produces eicosanoids that are less inflammatory. However, ARA produces also lipoxin A_4 that is anti-inflammatory, and it participates in the control of inflammation and its resolution. The acute inflammatory response is protective and aimed toward neutralizing invading microbes. When uncontrolled, excessive inflammation contributes to many widely occurring diseases in all organs of the body e.g., neurodegenerative diseases, depression, cardiovascular diseases, diabetes, aging, and cancer. The acute

inflammatory response is classically divided into two phases, initiation and resolution. In the initiation phase of the acute response ARA is mobilized to enzymatically produced prostaglandins and leukotrienes by leukocytes, platelets and surrounding damaged tissues. Lipoxin A_4 from ARA and resolvins from EPA, protectins and maresins from DHA and resolvins from DPA lead to resolution of inflammation.



The SPMs depicted are biosynthesized from EPA, n-3 DPA and DHA by human leukocytes. The E series resolvins are produced from EPA and D-series from DHA. Both the protectin family and maresin family are biosynthesized from DHA. The complete stereochemistry and pro-resolving actions of each SPM are established and confirmed by total organic synthesis and commercially available for research. These include SPMs biosynthesized from n-3 DPA. Each of the SPMs reduce and counter-regulate the Cardinal signs of inflammation and stimulate as agonists the major signs of resolution by definition, thus serving as immunoresolvents.

Figure 5. Pro-Resolving Mediators Network: Biosynthesis, Receptors and Functions

Cardinal signs of inflammation consist of rubor, calor, tumor and dolor lead to edema, chemo-taxis, excessive leukocyte infiltration, pro-inflammatory proteins and "cytokine storm." Specialized pro-resolving mediators (SPMs) (resolvins, protectins, maresins) from omega-3 fatty acids-EPA, DPA and DHA are potent anti-inflammatory mediators that lead to resolution of inflammation by increasing polymorphonuclear cell (PMN) clearance from epithelial surfaces, increasing phagocytosis of apoptotic, PMN cells, and removal of inflammatory debris, clearance of bacteria, wound repair, and tissue regeneration. Each of the SPMs biosynthesized from omega-3 fatty acids reduce and counter regulate the cardinal signs of inflammation and stimulate as agonists the major signs of resolution, thus serving as immune resolvents enabling the tissue to return to

function and homeostasis. This precise stereochemistry of the SPMs arises from the chemistry inherited from their omega-3 PUFA precursors present in our diets. The SPMs counter regulate cytokine storms, as well as pro-inflammatory lipid mediators via NF_kB and inflammasome down regulation, and unlike anti-inflammatory agents that eventually become immunosuppressive, SPMs control both the killing and clearance of microbes.

Many experimental studies have provided evidence that incorporation of alternative fatty acids into tissues may modify inflammatory and immune reactions, and that omega-3 fatty acids in particular are potent therapeutic agents for inflammatory diseases. Supplementing the diet with omega-3 fatty acids (3.2g EPA and 2.2g DHA) in normal subjects increased the EPA content in neutrophils and monocytes more than sevenfold without changing the quantities of AA and DHA. The anti-inflammatory effects of fish oils are partly mediated by inhibiting the 5-lipoxygenase pathway in neutrophils and monocytes and inhibiting leukotriene B4 (LTB4) production. Omega-3 fatty acids influence interleukin metabolism by decreasing IL-lb and IL-6. A balance between omega-6and omega-3 fatty acids is a more physiological state in terms of gene expression, eicosanoid metabolism and cytokine production.

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Bibliography

Alvheim AR, Torstensen BE, Lin YH, Lillefosse HH, Lock EJ, Madsen L, Frøyland L, Hibbeln JR, Malde MK. (2014) Dietary linoleic acid elevates the endocannabinoids 2-AG and anandamide and promotes weight gain in mice fed a low-fat diet. *Lipids*, 49, 59–69. [An excellent experimental study showing that LA promotes weight gain in mice by increasing the levels of its endocannabinoid metabolites.]

Ambring A, Johansson M, Axelsen M, Gan L, Strandvik B, Friberg P. (2006) Mediterranean inspired diet lowers the ratio of serum phospholipid n-6 to n-3 fatty acids, the number of leukocytes and platelets, and vascular endothelial growth factor in healthy subjects. *Am J Clin Nutr*;83:575–81. [A clinical intervention study in healthy Swedish subjects who on a Mediterranean inspired diet, lowered their omega-6/omega-3 serum phospholipids indicating that the Mediterranean diet can be adopted by other populations with beneficial results.]

Ameur, A., Enroth, S., Johansson, A., Zaboli, G., Igl, W., Johansson, A.C., Rivas, M.A.,Daly, M.J., Schmitz, G., Hicks, A.A., Meitinger, T., Feuk, L., van Dujin, C., Oostra, B.,Pramstaller, P.P., Rudan, I., Wright, A.F., Wilson, J.F., Campbell, H., Gyllensten, U., (2012). Genetic adaptation of fatty-acid metabolism: a human-specific haplotype increasing the biosynthesis of long-chain omega-3 and omega-6 fatty acids. *Am. J. Hum. Genet*. 90 (5), 809–820.[An excellent study on the genetic variants of FADS1 and FADS2 in various populations worldwide, and the discovery of 2 different haplotypes (A+D) that influence LA + ALA metabolism due to more efficient haplotype D.]

Andrikopoulos NK, Tzamtzis VA, Giannopoulos GA, Kalantzopoulos GK, Demopoulos CA. (1989) Deterioration of some vegetable oils. I. During heating or frying of several foods. *Rev Fr Corps*

Gras.;36:127-129. [A very useful study explaining the temperature of various oils including olive oil in preserving their quality during frying.]

Artemis P. Simopoulos (2022) The Healthiest Diet for You:Scientific Aspects, MDPI: Basel, Switzerland, ISBN 978-3-0365-2735-2 (Hbk), ISBN 978-3-0365-2734-5 (PDF), doi:10.3390/books978-3-0365-2734-5 [The book discusses major advances that have taken place in the last 40 years in the role of nutrition in development and in the prevention and management of chronic diseases, as well as the role of nutrients in gene expression.]

Baker P, Santos T, Neves PA,Machado P, Smith J, Piwoz E, Barros AJD, Victora CG, McCoy D. (2021) First-food systems transformations and the ultra-processing of infant and young child diets: The determinants, dynamics and consequences of the global rise in commercial milk formula consumption. *Matern Child Nutr*;17:e13097. [This paper describes trends and patterns in global formula sales, volumes by country, income and region and the transformation underway in first-food systems and concludes that policy and regulatory frameworks designed to protect, promote, and support breastfeeding are partially or completely inadequate in the majority of countries; hence supporting industry expansion over child nutrition.]

Banni S, Di Marzo V. (2010) Effect of dietary fat on endocannabinoids and related mediators: consequences on energy homeostasis, inflammation and mood. *Mol. Nutr. Food Res*., 54, 82–92. [An excellent review indicating that the tissue concentrations of endocannabinoids and related signaling molecules, can be modulated by the fatty acid composition of the diet, and particularly its content of long-chain PUFAs or their precursors and their potential impact on three of the major pathological conditions, that is metabolic dysfunctions, inflammation and affective disorders.]

Burdge GC, Calder PC. (2005) Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev*. Sep-Oct 2005;45(5):581-97. [A detailed description of the conversion of ALA to LC-PUFA in human adults. Overall, ALA appears to be a limited source of longer chain omega-3 PUFA in humans.]

Bazinet RP, Chu MW. (2014). Omega-6 polyunsaturated fatty acids: is a broad cholesterol-lowering health claim appropriate? *CMAJ (Can. Med. Assoc. J.)* 186 (6), 434–439. [A precise review of the effects of omega-6 PUFA on cholesterol metabolism, coronary heart disease and health claim.]

Beauchamp G, Keast R, Morel D. (2005) Ibuprofen-like activity in the extra virgin olive oil. *Nature*;437:45-46. [The firststudy to describe ibuprofen-like activity in the extra virgin olive oil.]

Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, Doyle RT Jr, Juliano RA, Jiao L, Granowitz C, Tardif JC, Ballantyne CM. (2019) Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N Engl J Med*.;380:11–22. [A seminal study on the effects of cardiovascular risk reduction with icosapent ethyl (a pharmaceutical made supplement of EPA) for the treatment of hypertriglyceridemia.]

Blasbalg TL, Hibbeln JR, Ramsden CE, Majchrzak SF, Rawlings RR. (2011) Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. *Am J Clin Nutr*. 2011 May;93(5):950-62. [A major contribution on the status of the omega-3 and omega-6 fatty acids in the US during the 20th century. The increase in LA is striking.]

Borsini A, Nicolaou A, Camacho-Muñoz D, Kendall AC, Di Benedetto MG, Giacobbe J, Su KP, Pariante CM. (2021) Omega-3 polyunsaturated fatty acids protect against inflammation through production of LOX and CYP450 lipid mediators: relevance for major depression and for human hippocampal neurogenesis. *Mol Psychiatry*. 2021 Jun 16. [The authors present and discuss their experimental studies in cells and a clinical intervention trial on the mechanisms by which EPA and DHA exert anti-inflammatory neuroprotective and anti-depressant properties in depression.]

Boskou D. Olive Oil. In: Simopoulos AP, Visioli F (Eds). (2007) More on Mediterranean Diets. *World Rev Nutr Diet*, vol 97, pp. 180-210. [A comprehensive chapter on olive oil metabolism and its various constituents.]

Carlson SE, Schipper L, Brenna JT, Agostoni C, Calder PC, Forsyth S, Legrand P, Abrahamse-Berkeveld M, van de Heijning BJM, van der Beek EM, Koletzko BV, Muhlhausler B (2021) Perspective: Moving Toward Desirable Linoleic Acid Content in Infant Formula. *Adv Nutr*. 2021 Jul 15:nmab076. [A

description of the status of the composition of infant formula, and the difficulties encountered in the determination of the desirable LA content.]

Clandinin MT, Chappell JE, Leong S, Heim T, Swyer PR, Chance GW. (1980) Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. *Early Hum Dev* 1980 Jun;4(2):121-9. [One of the very early studies on intrauterine fatty acid accretion rates in human brain that assisted in the determination of infant formula requirements.]

Covas MI, Nyyssönen K, Poulsen HE, Kaikkonen J, Zunft HF, Kiesewetter H, Gaddi A, de la Torre R, Mursu J, Baumler H, Nascetti S, Salonen JT, Fito M, Virtanen J, Marrugat J. (2006) The effect of polyphenols in olive oil on heart disease risk factors: a randomized trial. *Ann Intern Med*;145:333-41. [One of the best, randomized trials on the effects of olive oil constituents – in this case polyphenols on heart disease risk factors.]

Crawford MA, Hassam AG, Stevens PA. (1981) Essential fatty acid requirements in pregnancy and lactation with special reference to brain development. *Prog Lipid Res*.;20:31-40. [One of the best early studies on the determination of essential fatty acids in pregnancy and lactation focusing on brain development.]

Dyall SC, Michael-Titus AT. (2008). Neurological benefits of omega-3 fatty acids. Neuromol Med.;10:219–235. [An extensive review of the beneficial neurological effects of omega-3 fatty acids, with emphasis on neurodegenerative conditions and acute neurological injury.]

Eaton SB, Eaton SB III, Sinclair AJ, Cordain L, Mann NJ. (1998) Dietary intake of long-chain polyunsaturated fatty acids during the Paleolithic. *World Rev Nutr Diet* 83:12–23. [Investigations on the evolutionary aspects of diet relative to LC-PUFAs during the Paleolithic period.]

Endres S, Ghorbani R, Kelley VE, Georgilis K, Lonnemann G, van der Meer JW, Cannon JG, Rogers TS, Klempner MS, Weber PC, Schaefer EJ, Wolff SM, Dinarello C. (1989) The effects of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N Engl J Med* 320:265-271. [One of the best early studies on the anti-inflammatory effects of omega-3 LC-PUFAs.]

European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies (NDA); Scientific Opinion on the substantiation of health claims related to eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA) and maintenance. *EFSA J*. 2010;8:1796.

FDA Health Messages – 1) Fish Oil and 2) Olive Oil. FDA Allows Cardiovascular Health On Olive Oil Labels. FDA, November 26, 2018.

FDA Announces New Qualified Health Claims for EPA and DHA Omega-3 Consumption and the Risk of Hypertension and Coronary Heart Disease. June 19, 2019.

Feart C, Torres MJ, Samieri C, Jutand MA, Peuchant E, Simopoulos AP, Barberger-Gateau P. (2011) Adherence to a Mediterranean diet and plasma fatty acids: data from the Bordeaux sample of the Three-City study. *Br J Nutr*;106:149–58. [A high score Mediterranean diet is associated with higher levels of EPA and DHA.]

Gu M., Li Y., Tang H., Zhang C., Li W., Zhang Y., Li Y., Zhao Y., Song C. (2018). Endogenous omega (N)-3 fatty acids in fat-1 mice attenuated depression-like behavior, imbalance between microglial M1 and M2 phenotypes, and dysfunction of neurotrophins induced by lipopolysaccharide administration. *Nutrients* 10:E1351. [An excellent experimental study using the fat-1 mice, which increases the endogenous production of omega-3 fatty acids, led to reductions in depression like behavior and a reduction of the dysfunction of neutrophils, induced by LPS administration.]

Guasch-Ferré M, Liu G, Li Y, Sampson L, Manson JE, Salas-Salvadó J, Martínez-González MA, Stampfer MJ, Willett WC, Sun Q, Hu FB. (2020) Olive Oil Consumption and Cardiovascular Risk in U.S. Adults. *J Am Coll Cardiol*. 2020 Apr 21;75(15):1729-1739. [One of the recent studies on olive oil consumption in decreasing cardiovascular disease risks in the US, thus further confirming the importance of Mediterranean diets in coronary heart disease.]

Guiterrez-Rosales F, Rios J, Gomez-Rey M. (2003) Main polyphenols in the bitter taste of virgin olive oil. Structural confirmationby online HPLC electrospray ionization mass spectrometry. *J Agric Food*

Chem;51:6021-6025. [The bitter taste of olive oil, which contributes to its taste is due to polyphenols, confirmed in this study by online HDLC electrospray ionization mass spectrometry.]

Gustafsson HC, Holton KF, Anderson AN, Nousen EK, Sullivan CA, Loftis JM, Nigg JT, Sullivan EL. (2019) Increased Maternal Prenatal Adiposity, Inflammation, and Lower Omega-3 Fatty Acid Levels Influence Child Negative Affect. *Front Neurosci*. 2019 Oct 1;13:1035. [The combination of prenatal obesity and low EPA and DHA measured at the third trimester increased the risk of child negative affect, which may lead to psychopathology later in life. This is a very important study considering the increases in obesity in the populations worldwide and the "omega-3 deficiency" that is prevalent.]

Hall KD, Ayuketah A, Brychta R, Cai H, Cassimatis T, Chen KY, Chung ST, Costa E, Courville A, Darcey V, Fletcher LA, Forde CG, Gharib AM, Guo J, Howard R, Joseph PV, McGehee S, Ouwerkerk R, Raisinger K, Rozga I, Stagliano M, Walter M, Walter PJ, Yang S, Zhou M. (2019) Ultra-Processed Diets Cause Excess Calorie Intake and Weight Gain: An Inpatient Randomized Controlled Trial of Ad Libitum Food Intake. *Cell Metab* 2019 Jul 2;30(1):226. [One of the most important studies carried out at the metabolic unit at the National Institutes of Health. The study showed that ultra-processed foods have an omega-6/omega-3 ratio of 11/1, whereas minimally processed have a much lower ratio of 5/1. Furthermore, the subjects with high ultra-processed food intake, ate faster, consumed 500 more calories a day and gained two pounds in two weeks.]

Hansen TV, Vik A, Serhan CN (2019) The Protectin Family of Specialized Pro-resolving Mediators: Potent Immunoresolvents Enabling Innovative Approaches to Target Obesity and Diabetes. *Front Pharmacol.* 2019 Jan 17;9:1582. [An excellent review applying the latest information how specialized pro-resolving mediators could be used for the management of obesity and diabetes.]

Helfer B, Leonardi-Bee J, Mundell A, Parr C, Ierodiakonou D, Garcia-Larsen V, Kroeger CM, Dai Z, Man A, Jobson J, Dewji F, Kunc M, Bero L, Boyle RJ. (2021) Conduct and reporting of formula milk trials: systematic review. *BMJ*. 2021 Oct 13;375:n2202. [This is a systematic review on the status of infant formula milk trials. The situation requires immediate attention on the part of the scientific community, national and international organizations.]

Heydari B, Abdullah S, Pottala JV, Shah R, Abbasi S, Mandry D, Francis SA, Lumish H, Ghoshhajra BB, Hoffmann U, Appelbaum E, Feng JH, Blankstein R, Steigner M, McConnell JP, Harris W, Antman EM, Jerosch-Herold M, Kwong RY. (2016) Effect of Omega-3 Acid Ethyl Esters on Left Ventricular Remodeling After Acute Myocardial Infarction: The OMEGA-REMODEL Randomized Clinical Trial. *Circulation*. 2016 Aug 2;134(5):378-91. [One of the best studies on the effects of EPA and DHA on patients following myocardial infarction at a dose of 4 gm/day in addition to standard treatment. Omega-3 PUFA ethyl esters improved ventricular remodeling after acute myocardial infarction. The higher dose of 4 grams of EPA and DHA a day indicates the need for higher doses due to excessive amounts of omega-6 PUFA in Western diets.]

Innes JK, Calder PC. (2020) Marine omega-3 (N-3) fatty acids for cardiovascular health: an update for 2020. *Int J Mol Sci*.;21:1362. [An updated review on the role of omega-3 PUFA in the Primary and Secondary prevention of coronary heart disease. Considers differential effects of EPA and DHA doses and types of supplements.]

Kang JX, Wang J, Wu L, Kang ZB. (2004) Fat-1 mice convert n-6 to n-3 fatty acids. *Nature* 427:504, 2004.71. [The original description of the fat-1 mice model, which has been used by scientists and laboratories worldwide.]

Macartney MJ, Peoples GE, McLennan P. (2020) Cardiac Arrhythmia Prevention in Ischemia and Reperfusion by Low-Dose Dietary Fish Oil Supplementation in Rats. *J Nutr*. 2020 Dec 10;150(12):3086-3093. [This is an excellent study by a pioneer in the field of omega-3 research. Macartney et al state that the failure of several randomized clinical trials to observe a beneficial effect of supplemental omega-3 PUFAs might be at least partly because those not receiving the supplements might have had a moderate/high fish consumption making difficult to obtain an effect of the supplements.]

Mach F., Baigent C., Carapano A.L., Koskinas K.C., Casula M., Badimon L., Chapman M.J., de Backer G.G., Delgado V., Ference B.A., Graham I.M., Halliday A., Landmesser U., Mihaylova B., Pedersen T.R., Ricarrdi G., Richter D.J., Ssabatine M.S., Taskinen MR., Tokgozoglu L., Wiklund O. (2020) 2019 ESC/EAS guidelines for the management of dyslipidaemias: Lipid modification to reduce cardiovascular risk. *Eur. Heart J*. ;41:111–188.

Munblit D, Crawley H, Hyde R, Boyle RJ. (2020) Health and nutrition claims for infant formula are poorly substantiated and potentially harmful. *BMJ*;369:m875. [An excellent review on the problems related to infant formula, which have been recognized for years, but have not been remedied. The authors refer to several groups that have suggested that the scientific bases of claims made to consumers and health care professionals for infant formula are weak.]

Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C (2012) Health implications of high dietary omega-6 polyunsaturated Fatty acids. *J Nutr Metab*.;2012:539426.[An excellent review on the issues brought upon by the high intake of omega-6 PUFAs and the high omega-6/omega-3 ratio in view of their opposing effects.]

Piepoli MF, Hoes AW, Agewall S, Albus C, Brotons C, Catapano AL, Cooney MT, Corrà U, Cosyns B, Deaton C, Graham I, Hall MS, Hobbs F.D.R., Løchen ML, Löllgen H, Marques-Vidal P, Perk J, Prescott E, Redon J, Richter DJ, Sattar N, Smulders Y, Tiberi M, van der Worp HB, van Dis I, Verschuren W.M.M, Binno S. (2016) European Guidelines on cardiovascular disease prevention in clinical practice: The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts) developed with the special contribution of the European Association for Cardiovascular Prevention & Rehabilitation (EACPR). *Eur Heart J*. 2016;37:2315–81.

Psomiadou E, Tsimidou M. (1999) On the role of squalene in olive oil stability. *J Agric Food Chem*;47:4025-4032. [A goodreview of the effects of squalene – an olive oil constituent with anti-inflammatory and anti-carcinogenic properties.]

Ramsden CE, Zamora D, Faurot KR, MacIntosh B, Horowitz M, Keyes GS, Yuan ZX, Miller V, Lynch C, Honvoh G, Park J, Levy R, Domenichiello AF, Johnston A, Majchrzak-Hong S, Hibbeln JR, Barrow DA, Loewke J, Davis JM, Mannes A, Palsson OS, Suchindran CM, Gaylord SA, Mann JD. (2021) Dietary alteration of n-3 and n-6 fatty acids for headache reduction in adults with migraine: randomized controlled trial. *BMJ*. 2021 Jun 30;374:n1448. [An excellent clinical intervention trial using different omega-6/omega-3 ratios in the control of pain due to migraine headaches. At a lower omega-6/omega-3 ratio the pain and migraine headache improved, providing evidence for a dietary management of the pain.]

Ramsden, C.E., Zamora, D., Majchrzak-Hong, S., Faurot, K.R., Broste, S.K., Frantz, R.P., Davis, J.M., Ringel, A., Suchindran, C.M., Hibbeln, J.R., (2016). Re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from Minnesota Coronary Experiment (1968-73). *BMJ* 353, i1246. [A very important study, which reevaluated the concentrations of LA and ALA in the Minnesota Coronary Experiment, which was based on the traditional diet heart hypothesis. The reevaluation showed that LA did not contribute any benefits. The oils used were a mixture of LA and ALA. Therefore, the beneficial effects were not due to LA.]

Roche HM, Zampelas A, Knapper JM, Webb D, Brooks C, Jackson KG, Wright JW, Gould BJ, Kafatos A, Gibney MJ, Williams CM. (1998) Effect on long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism. *Am J Clin Nutr*;68:552-60. [One of the early clinical studies on the long-term effects of olive oil in lowering postprandial triacyclglycerol and factor VII metabolism.]

Simopoulos AP, Kifer RE, Martin RR (eds) (1986): *Health Effects of Polyunsaturated Fatty Acids in Seafoods,* Proceedings from the Conference, June 1985. Orlando, Academic Press,. [The first ever book on the health effects of PUFA in seafoods.]

Simopoulos Artemis P., Alexander Leaf, Norman Salem, Jr. (1999) Essentiality of and Recommended Dietary Intakes for Omega-6 and Omega-3 Fatty Acids, Meeting Report, Annals of Nutrition and Metabolism; 43127-130

Simopoulos AP, Sidossis L. What is so special about the traditional diet of Greece: the scientific evidence. In:Simopoulos AP, Visioli F (Eds). (2000) Mediterranean Diets. *World Rev Nutr Diet*, vol. 87, 2000, pp. 24-42. [An extensive review of the diet of Crete that provides evidence of the importance of a balanced LA/ALA ratio of 1-2/1 in lowering coronary heart disease.]

Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 2002 Oct;56(8):365-79. [A review paper that provides the scientific evidence and critiques the importance of a balanced ratio in health.]

Simopoulos AP. (2010) Genetic variants in the metabolism of omega-6 and omega-3 fatty acids: their role in the determination of nutritional requirements and chronic disease risk. *Exp Biol Med* (Maywood). 2010 Jul;235(7):785-95. [A mini review on the role of genetic variants in the metabolism of omega-3 and omega-6 PUFAs. In determining the dietary requirement of omega-3 and omega-6 PUFA it is essential to consider the endogenous production of ARA, EPA and DHA from LA to ALA respectively. At any one time the levels of ARA, EPA and DHA in RBCs is the result of dietary intake and endogenous production.]

Simopoulos, AP, Serhan CN, Bazinet RP. (2021) The need for precision nutrition, genetic variation and resolution in Covid-19 patients. *Mol Aspects Med*. 2021 Feb;77:100943. [Genetic variants in the FADS1 and FADS2 influence the metabolism of LA to ARA and ALA to EPA and DHA. Individuals with high dietary intake of ARA and more efficient metabolism from LA to ARA have high levels of ARA and a higher Omega-6 LC-PUFA/Omega-3 LC-PUFA ratio, are in a proinflammatory state, have higher levels of IL-1, IL-6, TNF (cytokine storm) and possibly are at increased risk for Covid-19.]

Simopoulos AP. (1991) Omega-3 fatty acids in health and disease and in growth and development. *Am J Clin Nutr*. 1991 Sep;54(3):438-63. [The very first review on the subject. This review defined the field and the research areas identified by NIH that were needed to be developed. The review emphasized the need for a balanced omega-6/omega-3 ratio.]

Simopoulos AP, DiNicolantonio JJ. (2016) The importance of a balanced ω-6 to ω-3 ratio in the prevention and management of obesity. *Open Heart*. 2016 Sep 20;3(2):e000385. [Describes the scientific evidence and the mechanisms involved in increasing the risk of obesity due to high dietary omega-6/omega-3 ratio leading to opposing effects in metabolism.]

Skulas-Ray A.C., Wilson P.W.F., Harris W.S., Brinton E.A., Kris-Etherton P.M., Richter C.K., Jacobson T.A., Engler M.B., Miller M., Robinson J.G., Blum C.B., Rodriguez-Leyva D, de Ferranti S.D., Welty F.K. (2019) Omega-3 fatty acids for the management of hypertriglyceridemia: A science advisory from the American Heart Association. *Circulation*. ;140:e.

Ward ED, Thomasson K, Fischer KR. (2021) Analysis of Omega-3 Fatty Acid Content in Fish Oil Products. *J Pharm Pract*. 2021 May 6;8830738211015051. [The authors evaluated the concentration of omega-3 fatty acid supplements listed in the NIH's Dietary Supplement label data base using the search term "fish oil."]

Biographical Sketch

Artemis P. Simopoulos, M.D. is the Founder and President of The Center for Genetics, Nutrition and Health (CGNH), a nonprofit educational organization in Washington, D.C. since 1990.

Dr. Simopoulos is a graduate of Barnard College, Columbia University, with a major in Chemistry, and a graduate of the Boston University School of Medicine, Dr. Simopoulos is certified by the American Board of Pediatrics and is a member of the American Academy of Pediatrics, the Society for Pediatric Research, the American Pediatric Society, the Endocrine Society, the American Institute of Nutrition, the American Society for Clinical Nutrition, the American College of Nutrition, the North American Association for the Study of Obesity, the International Society for the Study of Fatty Acids and Lipids (ISSFAL), the International Society of Nutrigenetics/Nutrigenomics (ISNN) and the American Society of Human Genetics.

Dr. Simopoulos was Director of the Newborn Nursery at the George Washington University Hospital in Washington, D.C. and Assistant Professor in both Pediatrics and Obstetrics and Gynecology from 1962 to 1967 at the George Washington University Medical School in Washington, DC. From 1968 to 1972 she did research at the Endocrinology Branch of the National Heart, Lung, and Blood Institute (NHLBI) on the Genetic and Nutritional aspects of Endocrine Disorders. Dr. Simopoulos was Executive Secretary of the Division of Medical Sciences at the National Research Council, National Academy of Sciences in Washington, D.C. from 1972 to 1977 and Project Director for the pioneering book *Genetic Screening: Programs, Principles and Research* published in 1975 by the National Academy of Sciences-National Research Council. This was the first book that analyzed in depth the scientific, legal, ethical, economic, and public policy aspects of genetic screening, and is found in all the medical libraries around the world. In 1975 Dr. Simopoulos was the Project Officer for the International Conference on Recombinant DNA Molecules and responsible for the statement of the Asilomar Conference on Recombinant DNA

Molecules. From 1977 to 1978 she was Director of the Developmental Biology and Nutrition Branch of the National Institute of Child Health and Human Development (NICHD). Dr. Simopoulos chaired the Nutrition Coordinating Committee at the National Institutes of Health (NIH) from 1978 to 1986. She is a pediatrician and endocrinologist with a long and distinguished career, whose research was originally focused on the nutritional, endocrine, and genetic aspects of growth and development in children and later on throughout the life cycle. Since 1984, her research has been on genetic variation and nutrition, the evolutionary aspects of diet, and the omega-6/omega-3 balance. In addition to her responsibilities at NIH, Dr. Simopoulos served as Consultant on Nutrition and Health to Ms. Ester Peterson, Special Assistant to The President for consumer Affairs, The White House, from 1978 to 1980. During that time she was a member of a number of White House delegations to the World Health Organization and the Food and Agriculture Organization. From 1978 to 1983 she was Cochairman and Executive Secretary of the Joint Subcommittee on Human Nutrition Research, Federal Coordinating Council on Science, Engineering, and Technology, Office of Science and Technology Policy, Executive Office of The President, The White House, and a member of its successor, the Interagency Committee for Human Nutrition Research from 1983-1986.

Dr. Simopoulos has written extensively on genetic variation and nutrition; nutrition and fitness; the characteristics of obesity and body weight standards; evolutionary aspects of diet and fatty acids; and on the role of omega-3 fatty acids in health and disease and in growth and development. She has edited over 50 books and journal supplements in addition to publishing over 360 scientific papers. She served as a consultant to the "Eat Well, Be Well" television series. She coauthored a book for the public entitled *Genetic Nutrition. Designing a Diet Based on Your Family Medical History* (Macmillan, 1993) and printed in paperback in 1995 with the new title *The Healing Diet*. Her latest book for the public *The Omega Plan* (hardcover, Harper Collins, 1998, USA; paperback, Hodder Headline Australia, 1998) is now in paperback in the United States with the title *The Omega Diet* (HarperCollins, 1999) is based on her extensive studies on the traditional diet of Greece prior to 1960 that defined "What is so special about the diet of Greece: The Scientific Evidence." The Omega diet has been translated into Dutch, Swedish, French, Greek, published in Australia, New Zealand and the U.K, Chinese, Korean, Arabic, Persian, Turkish, Bulgarian, and Taiwanese. Dr. Simopoulos was the Editor of the Karger series *World Review of Nutrition and Dietetics* from 1989-2011 (www.karger.com/wrund) and has been a member of the Editorial Boards of the International Journal for Vitamin and Nutrition Research, the Annals of Nutrition and Metabolism, Food Reviews International, Environmental Health and Preventive Medicine, Biomedicine & Pharmacotherapy, Current Food and Nutrition Science, Turkiye Klinikeri Journal of Medical Sciences, the Hellenic Journal of Nutrition and Dietetics; and the Chinese Journal of Clinicians; consulting editor to the Journal of the American Medical Association (JAMA) from 1987 to 2003 and the Journal of Lifestyle Genomics from 2017 to present; and was contributing editor to Nutrition Reviews from 1979 to 1986.