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Dossier: Polyunsaturated fatty acids in biology and diseases

The importance of the ratio of omega-6/omega-3 essential fatty acids

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Abstract

Several sources of information suggest that human beings evolved on a diet with a ratio of omega-6 to omega-3 essential fatty acids (EFA) of ~ 1 whereas in Western diets the ratio is 15/1–16.7/1. Western diets are deficient in omega-3 fatty acids, and have excessive amounts of omega-6 fatty acids compared with the diet on which human beings evolved and their genetic patterns were established. Excessive amounts of omega-6 polyunsaturated fatty acids (PUFA) and a very high omega-6/omega-3 ratio, as is found in today's Western diets, promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune diseases, whereas increased levels of omega-3 PUFA (a low omega-6/omega-3 ratio) exert suppressive effects. In the secondary prevention of cardiovascular disease, a ratio of 4/1 was associated with a 70% decrease in total mortality. A ratio of 2.5/1 reduced rectal cell proliferation in patients with colorectal cancer, whereas a ratio of 4/1 with the same amount of omega-3 PUFA had no effect. The lower omega-6/omega-3 ratio in women with breast cancer was associated with decreased risk. A ratio of 2–3/1 suppressed inflammation in patients with rheumatoid arthritis, and a ratio of 5/1 had a beneficial effect on patients with asthma, whereas a ratio of 10/1 had adverse consequences. These studies indicate that the optimal ratio may vary with the disease under consideration. This is consistent with the fact that chronic diseases are multigenic and multifactorial. Therefore, it is quite possible that the therapeutic dose of omega-3 fatty acids will depend on the degree of severity of disease resulting from the genetic predisposition. A lower ratio of omega-6/omega-3 fatty acids is more desirable in reducing the risk of many of the chronic diseases of high prevalence in Western societies, as well as in the developing countries, that are being exported to the rest of the world.

Keywords: Omega-6/omega-3 fatty acid balance; Genetic patterns; Chronic disease; Dietary recommendations

1. Introduction

The interaction of genetics and environment, nature, and nurture is the foundation for all health and disease. In the last two decades, using the techniques of molecular biology, it has been shown that genetic factors determine susceptibility to disease and environmental factors determine which genetically susceptible individuals will be affected [81,82,84,87]. Nutrition is an environmental factor of major importance. Whereas major changes have taken place in our diet over the past 10,000 years since the beginning of the Agricultural Revolution, our genes have not changed. The spontaneous mutation rate for nuclear DNA is estimated at 0.5% per million years. Therefore, over the past 10,000 years, there has been time for very little change in our genes, perhaps 0.005%. In fact, our genes today are very similar to the genes of our ancestors during the Paleolithic period 40,000 years ago, at which time our genetic profile was established [21]. Genetically speaking, humans today live in a nutritional environment that differs from that for which our genetic constitution was selected. Studies on the evolutionary aspects of diet indicate that major changes have taken place in our diet, particularly in the type and amount of essential fatty acids (EFA) and in the antioxidant content of foods [21,64,75-77] (Table 1, Fig. 1). Using the tools of molecular biology and genetics, research is defining

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the mechanisms by which genes influence nutrient absorption, metabolism and excretion, taste perception, and degree of satiation, and the mechanisms by which nutrients influence gene expression.

Whereas evolutionary maladaptation leads to reproductive restriction (or differential fertility), the rapid changes in our diet, particularly the last 100 years, are potent promoters of chronic diseases such as atherosclerosis, essential hyper

Table 1
Characteristics of hunter-gatherer and western diet and lifestyles

Characteristic	Hunter-gatherer diet and lifestyle	Western diet and lifestyle
Physical activity level	High	Low
Diet		
Energy density	Low	High
Energy intake	Moderate	High
Protein	High	Low-moderate
Animal	High	Low-moderate
Vegetable	Very low	Low-moderate
Carbohydrate	Low-moderate (slowly absorbed)	Moderate (rapidly absorbed)
Fiber	High	Low
Fat	Low	High
Animal	Low	High
Vegetable	Very low	Moderate to high
Total long-chain ω6 + ω3	High (2.3 g/d)	Low (0.2 g/d)
Ratio ω6/ω3	Low (2.4)	High (12.0)
Vitamins, mg/d	*Paleolithic period*	*Current US intake*
Riboflavin	6.49	1.34–2.08
Folate	0.357	0.149–0.205
Thiamin	3.91	1.08–1.75
Ascorbate	604	77–109
Carotene	5.56	2.05–2.57
(Retinol equivalent)	(927)	–
Vitamin A	17.2	7.02–8.48
(Retinol equivalent)	(2870)	(1170–429)
Vitamin E	32.8	7–10

Modified from [75].

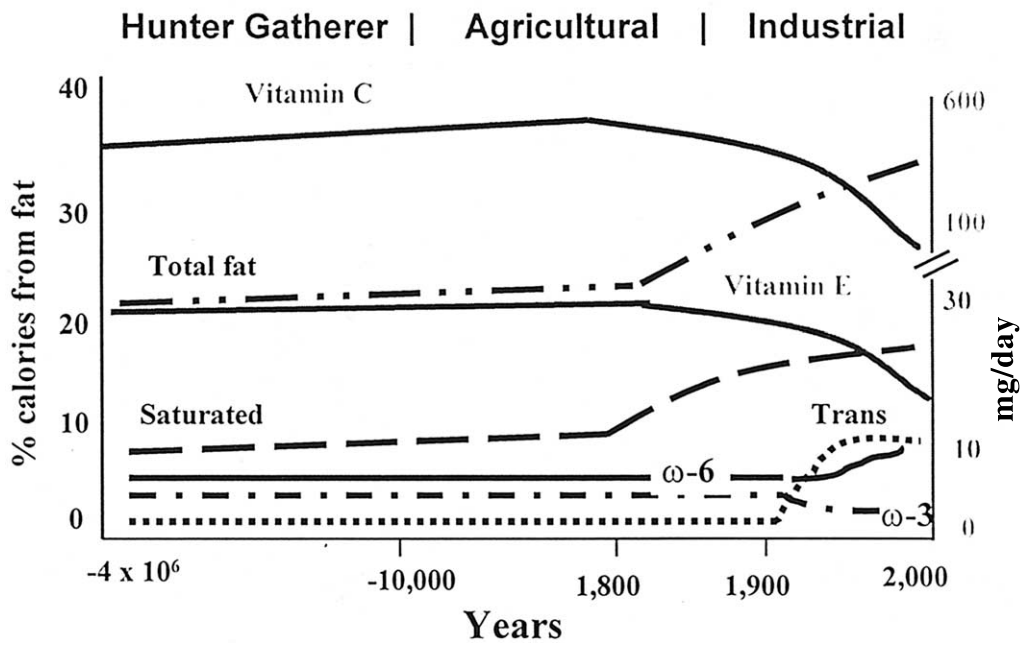

Fig. 1. Hypothetical scheme of fat, fatty acid (ω6, ω3, *trans* and total) intake (as percentage of calories from fat) and intake of vitamins E and C (mg/d). Data were extrapolated from cross-sectional analyses of contemporary hunter-gatherer populations and from longitudinal observations and their putative changes during the preceding 100 years [75].

tension, obesity, diabetes, and many cancers. In addition to diet, sedentary lifestyles and exposure to noxious substances interact with genetically controlled biochemical processes leading to chronic disease. This paper reviews the scientific evidence for a balanced intake of omega-6 and omega-3 EFA, focusing on the evolutionary aspects of diet, the biological and metabolic functions, and the health implications. Appendix A is a portion of the summary of the workshop on the essentiality of and recommended dietary intakes (RDIs) for Omega-6 and Omega-3 Fatty Acids, held at the National Institutes of Health (NIH) in Bethesda, Maryland, USA, April 7–9, 1999, which provides recommendations for adequate intakes (AI) of EFA for adults and infants [83].

2. Evolutionary aspects of diet with emphasis on omega-6 and omega-3 EFA

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. Cereal grains as a staple food are a relatively recent addition to the human diet and represent a dramatic departure from those foods to which we are genetically programmed and adapted [13,67,78]. Cereals did not become a part of our food supply but only very recently, 10,000 years ago, with the advent of the Agricultural Revolution. Prior to the Agricultural Revolution, humans ate an enormous variety of wild plants, whereas today about 17% of plant species provide 90% of the world's food supply, with the greatest percentage contributed by cereal grains [13,67,78]. Three cereals: wheat, maize, and rice together account for 75% of the world's grain production. Human beings have become entirely dependent upon cereal grains for the greater portion of their food supply. The nutritional implications of such a high grain consumption upon human health are enormous. Cereal grains are high in carbohydrates and omega-6 fatty acids, but low in omega-3 fatty acids and in antioxidants, particularly in comparison to green leafy vegetables. Recent studies show that low-fat/high-carbohydrate diets increase insulin resistance and hyperinsulinemia, conditions that increase the risk for coronary heart disease, hypertension, diabetes and obesity [26,65,66,88]. And yet, for the 99.9% of mankind's presence on this planet, humans rarely or never consumed cereal grains. It is only since the last 10,000 years that humans consume cereals. Up to that time, humans were non-cereal eating hunter-gatherers since the emergence of Homo erectus 1.7 million years ago. There is no evolutionary precedent in our species for grass seed consumption [13,21]. Therefore, we had little time (< 500 generations) since the beginning of the Agricultural Revolution 10,000 years ago to adapt to a food type which now represents humanity's major source of both calories and protein. A number of anthropological, nutritional and genetic studies indicate that human's overall diet, including energy intake and energy expenditure, has changed over the past 10,000 years with major changes occurring during the past 150 years in the type and amount of fat and in vitamins C and E intake [21-23,40,67,68,74,75,79] (Tables 1 and 2, Fig. 1).

Table 2
Estimated n-3 and n-6 fatty acid intake in the late Paleolithic period (g/d)[a,b]

Plants	
LA	4.28
ALA	11.40
Animals	
LA	4.56
ALA	1.21
Total	
LA	8.84
ALA	12.60
Animal	
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
AA + DTA/EPA + DPA + DHA	1.79
Total ω6/ω3	0.79 [b]

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

[a] Data from Eaton et al. [23].

[b] Assuming an energy intake of 35:65 of animal:plant sources [23].

Eaton and Konner [21] have estimated higher intakes for protein, calcium, potassium and ascorbic acid and lower sodium intakes for the diet of the late Paleolithic period than the current US and Western diets. Most of our food is calorically concentrated in comparison with wild game and the uncultivated fruits and vegetables of the Paleolithic diet. Paleolithic man consumed fewer calories and drank water, whereas today most drinks to quench thirst contain calories. Today industrialized societies are characterized by (1) an increase in energy intake and decrease in energy expenditure; (2) an increase in saturated fat, omega-6 fatty acids and *trans*-fatty acids, and a decrease in omega-3 fatty acid intake; (3) a decrease in complex carbohydrates and fiber; (4) an increase in cereal grains and a decrease in fruits and vegetables; and (5) a decrease in protein, antioxidants and calcium intake [21-23,74,75] (Tables 1-3). The increase in *trans*-fatty acids is detrimental to health as shown in Table 4 [70]. In addition, *trans*-fatty acids interfere with the desaturation and elongation of both omega-6 and omega-3 fatty acids, thus further decreasing the amount of arachi-

Table 3
Late Paleolithic and currently recommended nutrient composition for Americans

	Late Paleolithic	Current recommendations
Total dietary energy (%)		
Protein	33	12
Carbohydrate	46	58
Fat	21	30
Alcohol	~ 0	–
P/S ratio	1.41	1.00
Cholesterol (mg)	520	300
Fiber (g)	100–150	30–60
Sodium (mg)	690	1100–3300
Calcium (mg)	1500–2000	800–1600
Ascorbic acid (mg)	440	60

Modified from Eaton et al. [23].
P/S = polyunsaturated to saturated fat.

Table 4
Adverse effects of *trans*-fatty acids

Increase
Low-density lipoprotein (LDL)
Platelet aggregation
Lipoprotein (a) (Lp(a))
Body weight
Cholesterol transfer protein (CTP)
Abnormal morphology of sperm (in male rats)
Decrease or inhibit
Decrease or inhibit incorporation of other fatty acids into cell membranes
Decrease high-density lipoprotein (HDL)
Inhibit delta-6 desaturase (interfere with elongation and desaturation of EFA)
Decrease serum testosterone (in male rats)
Cross the placenta and decrease birth weight (in humans)

From [70].

donic acid, eicosapentaenoic acid and docosahexaenoic acid availability for human metabolism [69].

3. EFA and the omega-6/omega-3 balance

3.1. Large-scale production of vegetable oils

The increased consumption of omega-6 fatty acids in the last 100 years is due to the development of technology at the turn of the 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) [36]. The invention of the continuous screw press, named Expeller® 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 [36]. Solvent extraction of oilseeds came into increased use after World War I 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 α-linolenic (ALA) content of the oil while leaving a high concentration of linoleic acid (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 [24,98]. The effects of *trans*-fatty acids on health have been reviewed extensively elsewhere [69,72].

Since the 1950s, research on the effects of omega-6 PUFAs in lowering serum cholesterol concentrations has dominated the research support on the role of PUFAs in lipid metabolism. Although a number of investigators contributed extensively, the paper by Ahrens et al. [1] in 1954 and subsequent work by Keys et al. [34] firmly established the omega-6 fatty acids as the important fatty acid in the field of cardiovascular disease. The availability of methods for the production of vegetable oils and their use in lowering serum cholesterol concentration 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.

3.2. Agribusiness and modern agriculture

Agribusiness contributed further to the decrease in omega-3 fatty acids in animal carcasses. Wild animals and birds who feed on wild plants are very lean with a carcass fat content of only 3.9% [41] and contain about five times more PUFAs per gram than is found in domestic livestock [14,23]. Most importantly, 4% of the fat of wild animals contains eicosapentaenoic acid (EPA). Domestic beef contains very small or undetectable amounts of ALA because cattle are fed grains rich in omega-6 fatty acids and poor in omega-3 fatty acids [15] whereas deer that forage on ferns and mosses contain more omega-3 fatty acids (ALA) in their meat.

Modern agriculture with its emphasis on production has decreased the omega-3 fatty acid content in many foods. In addition to animal meats mentioned above [14,15,23,41], green leafy vegetables [85,86,89], eggs [90,91], and even fish [99] contain less omega-3 fatty acids than those in the wild. Foods from edible wild plants contain a good balance of omega-6 and omega-3 fatty acids. Table 5 shows purslane, an edible wild plant, and compares it to spinach, red leaf lettuce, buttercrunch lettuce and mustard greens. Purslane has eight times more α-linolenic acid than the cultivated plants. Modern aquaculture produces fish that contain less omega-3 fatty acids than do fish grown natu-

Table 5
Fatty acid content of plants (mg/g wet weight)

Fatty acid	Purslane	Spinach	Buttercrunch	Red leaf Lettuce	Mustard Lettuce
14:0	0.16	0.03	0.01	0.03	0.02
16:0	0.81	0.16	0.07	0.10	0.13
18:0	0.20	0.01	0.02	0.01	0.02
18:1ω9	0.43	0.04	0.03	0.01	0.01
18:2ω6	0.89	0.14	0.10	0.12	0.12
18:3ω3	4.05	0.89	0.26	0.31	0.48
20:5ω3	0.01	0.00	0.00	0.00	0.00
22:6ω3	0.00	0.00	0.001	0.002	0.001
Other	1.95	0.43	0.11	0.12	0.32
Total fatty acid content	8.50	1.70	0.60	0.702	1.101

Modified from [89].

Table 6
Fat content and fatty acid composition of wild and cultured salmon (*Salmo salar*) [a]

	Wild ($n = 2$)	Cultured ($n = 2$)
Fat (g/100 g)	10 ± 0.1	16 ± 0.6 [c]
Fatty acids (g/100 g fatty acid)		
18:3ω3	1 ± 0.1	1 ± 0.1
20:5ω3	5 ± 0.2	5 ± 0.1
22:6ω3	10 ± 2	7 ± 0.1 [b]
Other ω3 (18:4ω3 + 20:3ω3 + 22:5ω3)	3 ± 0.5	4 ± 0.1
18:2ω6	1 ± 0.1	3 ± 0.1
Other ω6 (20:4ω6 + 22:4ω6)	0.2 ± 0.1	0.5 ± 0.1
Total ω3	20 ± 2	17 ± 0.2
Total ω6	2 ± 0.1	3 ± 0.1[c]
Ratio of ω3/ω6	11 ± 2	6 ± 0.1[b]

[a] Modified from [100].
[b] Significantly different from wild, $P < 0.05$.
[c] Significantly different from wild, $P < 0.01$.

rally in the ocean, rivers and lakes (Table 6). As can be seen from Table 7, comparing the fatty acid composition of egg yolk from free-ranging chickens in the Ampelistra farm in Greece and the standard US Department of Agriculture (USDA) egg, the former has an omega-6/omega-3 ratio of 1.3 whereas the USDA egg has a ratio of 19.9 [90,91]. By enriching the chicken feed with fishmeal or flax, the ratio of omega-6/omega-3 decreased to 6.6 and 1.6, respectively [90,91]. Similarly, milk and cheese from animals that graze contain AA, EPA and DHA, whereas milk and cheese from grain-fed animals do not (Table 8) [74].

3.3. Imbalance of omega-6/omega-3

It is evident that food technology and agribusiness provided the economic stimulus that dominated the changes in the food supply [32,45]. From per capita quantities of foods available for consumption in the US national food supply in 1985, the amount of EPA is reported to be about 50 mg per capita/d and the amount of DHA is 80 mg per capita/d. The two main sources are fish and poultry [55]. It has been estimated that the present Western diet is "deficient" in omega-3 fatty acids with a ratio of omega-6 to omega-3 of 15–20/1, instead of 1/1 as is the case with wild animals and presumably human beings [14,15,21,23,41,64,75-77].

Before the 1940s, cod-liver oil was ingested mainly by children as a source of vitamin A and D with the usual dose being a teaspoon. Once these vitamins were synthesized, consumption of cod-liver oil was drastically decreased, contributing further to the decrease of EPA and DHA intake. Table 9 shows ethnic differences in fatty acid concentrations in thrombocyte phospholipids, the ratios of omega-6/omega-3 fatty acids, and percentage of all deaths from cardiovascular disease [100].

Thus, an absolute and relative change of omega-6/omega-3 in the food supply of Western societies has occurred over the last 100 years. A balance existed between omega-6 and omega-3 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. Recent studies by Cordain et al. [12] on wild animals confirm the original observations of Crawford [14] and Sinclair et al. [92]. However, rapid dietary changes over short periods of time as have occurred over the past 100–150 years are a totally new phenomenon in human evolution (Table 10).

4. Biological effects and metabolic functions of omega-6 and omega-3 fatty acids

Mammalian cells cannot convert omega-6 to omega-3 fatty acids because they lack the converting enzyme, omega-3 desaturase. LA and ALA and their long-chain derivatives are important components of animal and plant cell membranes. These two classes of EFA are not interconvertible, are metabolically and functionally distinct, and often have important opposing physiological functions. The balance of EFA is important for good health and normal

Table 7
Fatty acid levels (mg/g yolk) in chicken egg yolks [a,b,c]

Fatty acid	Greek egg	Supermarket egg	Fishmeal egg	Flax egg
Saturates				
14:0	1.1	0.7	1.0	0.6
15:0	–	0.1	0.3	0.2
16:0	77.6	56.7	67.8	58.9
17:0	0.7	0.3	0.8	0.5
18:0	21.3	22.9	23.0	26.7
Total	100.7	80.7	92.9	86.9
Monounsaturates				
16:1ω7	21.7	4.7	5.1	4.4
18:1	120.5	110.0	102.8	94.2
20:1ω9	0.6	0.7	0.9	0.5
24:1ω9	–	–	0.1	–
Total	142.8	115.4	108.9	99.1
ω6 Polyunsaturates				
18:2ω6	16.0	26.1	67.8	42.4
18:3ω6	–	0.3	0.3	0.2
20:2ω6	0.2	0.4	0.6	0.4
20:3ω6	0.5	0.5	0.5	0.4
20:4ω6	5.4	5.0	4.4	2.6
22:4ω6	0.7	0.4	0.3	–
22:5ω6	0.3	1.2	0.2	–
Total	23.1	33.9	74.1	46.0
ω3 Polyunsaturates				
18:3ω3	6.9	0.5	4.1	21.3
20:3ω3	0.2	–	0.1	0.4
20:5ω3	1.2	–	0.2	0.5
22:5ω3	2.8	0.1	0.4	0.7
22:6ω3	6.6	1.1	6.5	5.1
Total	17.7	1.7	11.3	28.0
P/S ratio	0.4	0.4	0.9	0.9
M/S ratio	1.4	1.4	1.2	1.1
ω6/ω3 ratio	1.3	19.9	6.6	1.6

[a] Modified from Simopoulos and Salem [91].
[b] The eggs were hard-boiled, and their fatty acid composition and lipid content were assessed as described elsewhere [90].
[c] Greek eggs, free-ranging chickens; supermarket eggs, standard US Department of Agriculture eggs found in US supermarkets; fish meal eggs, main source of fatty acids provided by fish meal and whole soybeans; flax eggs, main source of fatty acids provided by flax flour.
P/S = polyunsaturates:saturates; M/S = monounsaturates:saturates.

Table 8
Fatty acid content of various cheeses (per 100 g edible portion)

	2% Milk	Cheddar	American	Swiss	Greek myzithra	Greek feta
Total saturated fat, g	1.2	21.00	19.69	16.04	9.30	7.20
12:0, g	< 1	0.54	0.48	0.57	–	–
14:0, g	< 1	3.33	3.21	2.70	1.90	1.60
16:0, g	< 1	9.80	9.10	7.19	5.40	3.90
18:0, g	< 1	4.70	3.00	2.60	2.00	1.70
Total monounsaturated fat, g	1	9.99	8.95	7.05	3.90	3.00
Total polyunsaturated						
Fat, g	0.07	0.94	0.99	0.62	0.80	0.58
18:2, g	0.04	0.58	0.61	0.34	0.38	0.29
18:3, g	0.03	0.36	0.38	0.28	0.30	0.20
Arachidonic acid, mg	–	–	–	–	14	10
Eicosapentaenoic acid, mg	–	–	–	–	18	14
Docosapentaenoic acid, mg	–	–	–	–	31	23
Docosahexaenoic acid, mg	–	–	–	–	5.5	5.1
Total fat, g	2.27	31.93	29.63	23.71	14.00	10.78

Milk, cheddar, American and Swiss from US Department of Agriculture Handbook No. 8 Greek myzithra and Greek feta from National Institute on Alcohol Abuse and Alcoholism analyses. From [74].

Table 9
Ethnic differences in fatty acid concentrations in thrombocyte phospholipids and percentage of all deaths from cardiovascular disease [a]

	Europe and United States	Japan	Greenland Eskimos
Arachidonic acid (20:4ω6) (%)	26	21	8.3
Eicosapentaenoic acid (20:5ω3) (%)	0.5	1.6	8.0
Ratio of ω6/ω3 (%)	50	12	1
Mortality from cardiovascular disease (%)	45	12	7

[a] Data modified from [100].

Table 10
Omega-6:omega-3 ratios in various populations

Population	ω6/ω3	Reference
Paleolithic	0.79	[23]
Greece prior to 1960	1.00–2.00	[74]
Current United States	16.74	[23]
Current United Kingdom and northern Europe	15.00	[61]
Current Japan	4.00	[97]

development. When humans ingest fish or fish oil, the EPA and DHA from the diet partially replace the omega-6 fatty acids, especially AA, in the membranes of probably all cells, but especially in the membranes of platelets, erythrocytes, neutrophils, monocytes, and liver cells (reviewed in [65]). Whereas cellular proteins are genetically determined, the PUFA composition of cell membranes is to a great extent dependent on the dietary intake. AA and EPA are the parent compounds for eicosanoid production (Table 11, Fig. 2).

Due to the increased amounts of omega-6 fatty acids in the Western diet, the eicosanoid metabolic products from AA, specifically prostaglandins, thromboxanes, leukotrienes, hydroxy fatty acids, and lipoxins, are formed in larger quantities than those formed from omega-3 fatty acids, specifically EPA. The eicosanoids from AA 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 shifts the physiological state to one that is prothrombotic and proaggregatory, with increases in blood viscosity, vasospasm, and vasoconstriction and decreases in bleeding time. Bleeding time is decreased in groups of patients with hypercholesterolemia [8], hyperlipoproteinemia [31], myocardial infarction, other forms of atherosclerotic disease,

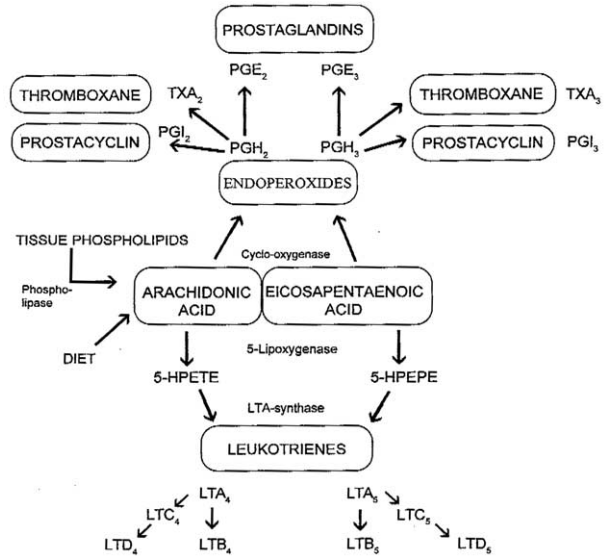

Fig. 2. Oxidative metabolism of arachidonic acid and eicosapentaenoic acid by the cyclooxygenase and 5-lipoxygenase pathways. 5-HPETE denotes 5-hydroperoxyeicosatetranoic acid and 5-HPEPE denotes 5-hydroxyeicosapentaenoic acid.

and diabetes (obesity and hypertriglyceridemia). Bleeding time is longer in women than in men and longer in young than in old people. There are ethnic differences in bleeding time that appear to be related to diet. Table 9 shows that the higher the ratio of omega-6/omega-3 fatty acids in platelet phospholipids, the higher the death rate from cardiovascular disease [65,100].

The antithrombotic aspects and the effects of different doses of fish oil on the prolongation of bleeding time were investigated by Saynor et al. [62]. 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

Table 11
Effects of ingestion of EPA and DHA from fish or fish oil

- 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

Table 12
Effects of omega-3 fatty acids on factors involved in the pathophysiology of inflammation

Factor	Function	Effect of ω3 fatty acid
Arachidonic acid	Eicosanoid precursor; aggregates platelets; stimulates white blood cells	↓
Thromboxane	Platelet aggregation; vasoconstriction; increase of intracellular Ca^{++}	↓
Prostacyclin ($PGI_{2/3}$)	Prevent platelet aggregation; vasodilation; increase camp	↑
Leukotriene (LTB_4)	Neutrophil chemoattractant; increase of intracellular Ca^{++}	↓
Fibrinogen	A member of the acute phase response; and a blood clotting factor	↓
Tissue plasminogen activator	Increase endogenous fibrinolysis	↑
Platelet activating factor (PAF)	Activates platelets and white blood cells	↓
PDGF	Chemoattractant and mitogen for smooth muscles and macrophages	↓
Oxygen free radicals	Cellular damage; enhance LDL uptake via scavenger pathway; stimulate arachidonic acid metabolism	↓
Lipid hydroperoxides	Stimulate eicosanoid formation	↓
Interleukin 1 and tumor necrosis factor	Stimulate neutrophil O_2 free radical formation; stimulate lymphocyte proliferation; stimulate PAF; express intercellular adhesion molecule-1 on endothelial cells; inhibit plasminogen activator, thus, procoagulants	↓
Interleukin-6	Stimulates the synthesis of all acute phase proteins involved in the inflammatory response: C-reactive protein; serum amyloid A; fibrinogen; α_1-chymotrypsin; and haptoglobin	↓

Adapted and modified from [101].

never been a case of clinical bleeding, even in patients undergoing angioplasty while they were on fish oil supplements [18].

There is substantial agreement that ingestion of fish or fish oils has the following effects: platelet aggregation to epinephrine and collagen is inhibited, thromboxane A_2 production is decreased, whole blood viscosity is reduced, and erythrocyte membrane fluidity is increased [5,10,43,101] (Table 12). Fish oil ingestion increases the concentration of plasminogen activator and decreases the concentration of plasminogen activator inhibitor 1 (PAI-1) [2]. In vitro studies have demonstrated that PAI-1 is synthesized and secreted in hepatic cells in response to insulin, and population studies indicate a strong correlation between insulinemia and PAI-1 levels. In patients with types IIb and IV hyperlipoproteinemia and in another double-blind clinical trial involving 64 men aged 35–40 years, ingestion of omega-3 fatty acids decreased the fibrinogen concentration [53]. Two other studies did not show a decrease in fibrinogen, but in one, a small dose of cod-liver oil was used [60] and in the other, the study consisted of normal volunteers and was of short duration. A recent study noted that fish and fish oil increase fibrinolytic activity, indicating that 200 g/d of lean fish or 2 g of omega-3 EPA and DHA improve certain hematologic parameters implicated in the etiology of cardiovascular disease [7].

Ingestion of omega-3 fatty acids not only increases the production of PGI_3, but also of PGI_2 in tissue fragments from the atrium, aorta, and saphenous vein obtained at surgery in patients who received fish oil 2 weeks prior to surgery [17]. Omega-3 fatty acids inhibit the production of platelet-derived growth factor (PDGF) in bovine endothelial cells [27]. PDGF is a chemoattractant for smooth muscle cells and a powerful mitogen. Thus, the reduction in its production by endothelial cells, monocytes/macrophages, and platelets could inhibit both the migration and proliferation of smooth muscle cells, monocytes/macrophages, and fibroblasts in the arterial wall. Insulin increases the growth of smooth muscle cells, leading to increased risk for the development of atherosclerosis. Omega-3 fatty acids increase endothelium-derived relaxing factor (EDRF) [63]. EDRF (nitric oxide) facilitates relaxation in large arteries and vessels. In the presence of EPA, endothelial cells in culture increase the release of relaxing factors, indicating a direct effect of omega-3 fatty acids on the cells.

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 (3.2 g EPA and 2.2 g DHA) in normal subjects increased the EPA content in neutrophils and monocytes more than sevenfold without changing the quantities of AA and DHA. The antiinflammatory effects of fish oils are partly mediated by inhibiting the 5-lipoxygenase pathway in neutrophils and monocytes and inhibiting the leukotriene B_4 (LTB_4)-mediated function of LTB_5 (Fig. 2) [37,42]. Studies show that omega-3 fatty acids influence interleukin metabolism by decreasing IL-1β and IL-6 [25,35,38,58]. Inflammation plays an important role in both the initiation of atherosclerosis and the development of atherothrombotic events [59]. An early step in the atherosclerotic process is the adhesion of monocytes to endothelial cells. Adhesion is mediated by leukocyte and vascular cell adhesion molecules (CAMs) such as selectins, integrins, vascular cell adhesion molecule 1 (VCAM-1), and intercellular adhesion molecule 1 (ICAM-1) [95]. The expression of E-selectin, ICAM-1 and VCAM-1, which is relatively low

in normal vascular cells, is upregulated in the presence of various stimuli, including cytokines and oxidants. This increased expression promotes the adhesion of monocytes to the vessel wall. The monocytes subsequently migrate across the endothelium into the vascular intima, where they accumulate to form the initial lesions of atherosclerosis. Atherosclerotic plaques have been shown to have increased CAM expression in animal models and human studies [16,50,52,57]. A balance between the omega-6 and omega-3 fatty acids is a more physiologic state in terms of gene expression [71], eicosanoid metabolism and cytokine production.

Further support for the need to balance the omega-6/omega-3 EFA comes from the studies of Ge et al. [28] and Kang et al. [33]. The study by Ge et al. clearly shows the ability of both normal rat cardiomyocytes and human breast cancer cells in culture to form all the omega-3s from omega-6 fatty acids when fed the cDNA encoding omega-3 fatty acid desaturase obtained from the roundworm *C. elegans*. The omega-3 desaturase efficiently and quickly converted the omega-6 fatty acids that were fed to the cardiomyocytes in culture to the corresponding omega-3 fatty acids. Thus, omega-6 LA was converted to omega-3 ALA and AA was converted to EPA, so that at equilibrium, the ratio of omega-6 to omega-3 PUFA was close to 1/1 [33]. Further studies demonstrated that the cancer cells expressing the omega-3 desaturase underwent apoptotic death whereas the control cancer cells with a high omega-6/omega-3 ratio continued to proliferate [28].

5. Clinical intervention studies and the omega-6/omega-3 EFA balance

The Lyon Heart Study was a dietary intervention study in which a modified diet of Crete (the experimental diet) was compared with the prudent diet or Step I American Heart Association Diet (the control diet) [19,20,56]. The experimental diet provided a ratio of LA to ALA of 4/1. This ratio was achieved by substituting olive oil and canola (oil) margarine for corn oil. Since olive oil is low in LA whereas corn oil is high, 8% and 61%, respectively, the ALA incorporation into cell membranes was increased. Cleland et al. [11] have shown that olive oil increases the incorporation of omega-3 fatty acids whereas the LA from corn oil competes. The ratio of 4/1 of LA/ALA led to a 70% decrease in total mortality at the end of 2 years [19].

The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI) Prevenzione Trial participants were on a traditional Italian diet plus 850–882 mg of omega-3 fatty acids at a ratio of 2/1 EPA to DHA [29]. The supplemented group had a decrease in sudden cardiac death by 45%. Although there are no dietary data on total intake for omega-6 and omega-3 fatty acids, the difference in sudden death is most likely due to the increase of EPA and DHA and a decrease of AA in cell membrane phospholipids. Prostaglandins derived from AA are proarrhythmic, whereas the corresponding prostaglandins from EPA are not [44]. In the Diet and Reinfarction Trial (DART), Burr et al. [9] reported a decrease in sudden death in the group that received fish advice or took fish oil supplements relative to the group that did not. Similar results have been obtained by Singh et al. [93,94].

Studies carried out in India indicate that the higher ratio of 18:2ω6 to 18:3ω3 equalling 20/1 in their food supply led to increases in the prevalence of NIDDM in the population, whereas a diet with a ratio of 6/1 led to decreases [54].

Except for the Lyon Heart Study, most of the cardiovascular disease omega-3 fatty acid supplementation trials did not attempt to modify the consumption of other fat components, and specifically did not seek to reduce the intake of omega-6 fatty acids despite the fact that there is convincing support for such studies. However, James and Cleland [30] have reported beneficial effects in patients with rheumatoid arthritis and Broughton et al. [6] have shown beneficial effects in patients with asthma by changing the background diet. James and Cleland evaluated the potential use of omega-3 fatty acids within a dietary framework of an omega-6/omega-3 ratio of 3-4/1 by supplying 4 g of EPA + DHA and using flaxseed oil rich in ALA. In their studies, the addition of 4 g EPA and DHA produced a substantial inhibition of production of IL-1β and TNF when mononuclear cell levels of EPA were equal or greater than 1.5% of total cell phospholipid fatty acids which correlated with a plasma phospholipid EPA level equal to or greater than 3.2%. These studies suggest the potential for complementarity between drug therapy and dietary choices that increased intake of omega-3 fatty acids and decreased intake of omega-6 fatty acids may lead to drug-sparing effects. Therefore, future studies need to address the fat composition of the background diet, and the issue of concurrent drug use. A diet rich in omega-3 fatty acids and poor in omega-6 fatty acids provides the appropriate background biochemical environment in which drugs function.

Asthma is a mediator driven inflammatory process in the lungs and the most common chronic condition in childhood. The leukotrienes and prostaglandins are implicated in the inflammatory cascade that occurs in asthmatic airways. There is evidence of airway inflammation even in newly diagnosed asthma patients within 2–12 months after their first symptoms [39]. Among the cells involved in asthma are mast cells, macrophages, eosinophils, and lymphocytes. The inflammatory mediators include cytokines and growth factors (peptide mediators) as well as the eicosanoids, which are the products of AA metabolism, which are important mediators in the underlying inflammatory mechanisms of

asthma (Fig. 2, Table 10). Leukotrienes and prostaglandins appear to have the greatest relevance to the pathogenesis of asthma. The leukotrienes are potent inducers of bronchospasm, airway edema, mucus secretion, and inflammatory cell migration, all of which are important to the asthmatic symptomatology. Broughton et al. [6] studied the effect of omega-3 fatty acids at a ratio of omega-6/omega-3 of 10/1 to 5/1 in an asthmatic population in ameliorating methacholine-induced respiratory distress. With low omega-3 ingestion, methacholine-induced respiratory distress increased. With high omega-3 fatty acid ingestion, alterations in urinary 5-series leukotriene excretion predicted treatment efficacy and a dose change in > 40% of the test subjects (responders) whereas the non-responders had a further loss in respiratory capacity. A urinary ratio of 4-series to 5-series of < 1 induced by omega-3 fatty acid ingestion may predict respiratory benefit.

Bartram et al. [3,4] carried out two human studies in which fish oil supplementation was given in order to suppress rectal epithelial cell proliferation and PGE_2 bio synthesis. This was achieved when the dietary omega-6/omega-3 ratio was 2.5/1 but not with the same absolute level of fish oil intake and an omega-6/omega-3 ratio of 4/1. More recently, Maillard et al. [49] reported their results on a case control study. They determined omega-3 and omega-6 fatty acids in breast adipose tissue and relative risk of breast cancer. They concluded, “our data based on fatty acid levels in breast adipose tissue (which reflect dietary intake) suggest a protective effect of omega-3 fatty acids on breast cancer risk and support the hypothesis that the balance between omega-3 and omega-6 fatty acids plays a role in breast cancer”.

Psychologic stress in humans induces the production of proinflammatory cytokines such as interferon gamma (IFNγ), TNFα, IL-6 and IL-10. An imbalance of omega-6 and omega-3 PUFA in the peripheral blood causes an overproduction of proinflammatory cytokines. There is evidence that changes in fatty acid composition are involved in the pathophysiology of major depression. Changes in serotonin (5-HT) receptor number and function caused by changes in PUFA provide the theoretical rationale connecting fatty acids with the current receptor and neurotransmitter theories of depression [47,48,51]. The increased C20:4ω6/C20:5ω3 ratio and the imbalance in the omega-6/omega-3 PUFA ratio in major depression may be related to the increased production of proinflammatory cytokines and eicosanoids in that illness [47]. There are a number of studies evaluating the therapeutic effect of EPA and DHA in major depression. Stoll and colleagues [46,96] have shown that EPA and DHA prolong remission, that is, reduce the risk of relapse in patients with bipolar disorder.

The above clinical studies in patients with cardiovascular disease, arthritis, asthma, cancer, and mental illness clearly indicate the need to balance the omega-6/omega-3 fatty acid intake for prevention and during treatment. The scientific evidence is strong for decreasing the omega-6 and increasing the omega-3 intake to improve health throughout the life cycle. The scientific basis for the development of a public policy to develop dietary recommendations for EFA, including a balanced omega-6/omega-3 ratio is robust [73,80]. What is needed is a scientific consensus, education of professionals and the public, the establishment of an agency on nutrition and food policy at the national level, and willingness of governments to institute changes. Education of the public is essential to demand changes in the food supply.

6. Conclusions

In conclusion:

- Human beings evolved on a diet in which the ratio of omega-6/omega-3 EFA was about 1, whereas in the Western diets, the ratio is 15/1 to 16.7/1. Such evidence comes from studies on the evolutionary aspects of diet, modern day hunter-gatherers, and traditional diets. Agribusiness and modern agriculture have led to decreases in omega-3 fatty acids and increases in omega-6 fatty acids. Such practices have led to excessive amounts of omega-6 fatty acids, upsetting the balance that was characteristic during evolution when our genes were programmed to respond to diet and other aspects of the environment.
- LA and ALA are not interconvertible and compete for the rate-limiting Δ6-desaturase in the synthesis of long-chain PUFA.
- AA (omega-6) and EPA (omega-3) are the parent compounds for the production of eicosanoids. Eicosanoids from AA have opposing properties from those of EPA. An increase in the dietary intake of omega-6 EFA changes the physiological state to a prothrombotic, proconstrictive, and proinflammatory state.
- Many of the chronic conditions, cardiovascular disease, diabetes, cancer, obesity, autoimmune diseases, rheumatoid arthritis, asthma and depression, are associated with increased production of thromboxane A_2 (TXA_2), leukotriene B_4 (LTB_4), IL-1β, IL-6, tumor necrosis factor (TNF), and C-reactive protein. All these factors increase by increases in omega-6 fatty acid intake and decrease by increases in omega-3 fatty acid intake, either ALA or EPA and DHA. EPA and DHA are more potent, and most studies have been carried out using EPA and DHA.
- The optimal dose or ratio of omega-6/omega-3 varies from 1/1 to 4/1 depending on the disease under con-

sideration. Since many of the chronic diseases prevalent in Western cultures are multigenic and multifactorial, it is not surprising that the dose or the ratio differs.

- Studies show that the background diet when balanced in omega-6/omega-3 decreases the drug dose. It is, therefore, essential to decrease the omega-6 intake while increasing the omega-3 in the prevention and management of chronic disease. Furthermore, the balance of omega-6 and omega-3 fatty acids is very important for homeostasis and normal development. The ratio of omega-6 to omega-3 EFA is an important determinant of health. Therefore, appropriate amounts of dietary omega-6 and omega-3 fatty acids at a ratio of about 1-2/1 consistent with the recommended adequate intakes (AI) found in Tables 13 and 14 of Appendix A, need to be considered in making dietary recommendations, and these two classes of PUFA should be distinguished in food labels because they are metabolically and functionally distinct.

Appendix A

RDIs for omega-6 and omega-3 fatty acids

Between April 7 and 9, 1999, an international working group of scientists met at the National Institutes of Health in Bethesda, Maryland (USA) to discuss the scientific evidence relative to dietary recommendations of omega-6 and omega-3 fatty acids [83]. The latest scientific evidence based on controlled intervention trials in infant nutrition, cardiovascular disease, and mental health was extensively discussed. Tables 13 and 14 include the AI for omega-6 and omega-3 EFA for adults and infant formula/diet, respectively.

Adults: The working group recognized that there are not enough data to determine dietary reference intakes (DRI), but there are good data to make recommendations for AI for adults as shown in Table 13.

Pregnancy and lactation: For pregnancy and lactation, the recommendations are the same as those for adults with the additional recommendation seen in footnote (a) (Table 14), that during pregnancy and lactation women must ensure a DHA intake of 300 mg/d.

Composition of infant formula/diet: It was thought of utmost importance to focus on the composition of the infant formula considering the large number of premature infants around the world, the low number of women who breastfeed, and the need for proper nutrition of the sick infant. The composition of the infant formula/diet was based on studies that demonstrated support for both the growth and neural development of infants in a manner similar to that of the breastfed infant (Table 14).

Table 13
AI [a] for adults

Fatty acid	g/d (2000 kcal diet)	(%) Energy
LA	4.44	2.0
(*Upper limit*) [b]	6.67	3.0
ALA	2.22	1.0
DHA + EPA	0.65	0.3
DHA to be at least [c]	0.22	0.1
EPA to be at least	0.22	0.1
TRANS-FA		
(*Upper limit*) [d]	2.00	1.0
SAT		
(*Upper limit*) [e]	–	< 8.0
MONOs [f]	–	–

[a] AI = adequate intake. If sufficient scientific evidence is not available to calculate an estimated average requirement, a reference intake called an AI is used instead of a recommended dietary allowance. The AI is a value based on experimentally derived intake levels or approximations of observed mean nutrient intakes by a group (or groups) of healthy people. The AI for children and adults is expected to meet or exceed the amount needed to maintain a defined nutritional state or criterion of adequacy in essentially all members of a specific healthy population; LA = linoleic acid; ALA = α-linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; *TRANS*-FA = *trans*-fatty acids; SAT = saturated fatty acids; MONOs = monounsaturated fatty acids.

[b] Although the recommendation is for AI, the working group felt that there is enough scientific evidence to also state an upper limit (UL) for LA of 6.67 g/d based on a 2000 kcal diet or of 3.0% of energy.

[c] For pregnant and lactating women, ensure 300 mg/d of DHA.

[d] Except for dairy products, other foods under natural conditions do not contain *trans*-FA. Therefore, the working group does not recommend *trans*-FA to be in the food supply as a result of hydrogenation of unsaturated fatty acids or high temperature cooking (reused frying oils).

[e] Saturated fats should not comprise more than 8% of energy.

[f] The working group recommended that the majority of fatty acids is obtained from monounsaturates. The total amount of fat in the diet is determined by the culture and dietary habits of people around the world (total fat ranges from 15% to 40% of energy) but with special attention to the importance of weight control and reduction of obesity.

One recommendation deserves explanation here. After much discussion, consensus was reached on the importance of reducing the omega-6 polyunsaturated fatty acids (PUFAs) even as the omega-3 PUFAs are increased in the diet of adults and newborns for optimal brain and cardiovascular health and function. This is necessary to reduce adverse effects of excesses of arachidonic acid and its eicosanoid products. Such excesses can occur when too much LA and AA are present in the diet and an adequate supply of dietary omega-3 fatty acids is not available. The adverse effects of too much arachidonic acid and its eicosanoids can be avoided by two interdependent dietary changes. First, the amount of plant oils rich in LA, the parent compound of the omega-6 class, which is converted to AA, needs to be reduced. Second, simultaneously the omega-3 PUFAs need to be increased in the diet. LA can be converted to arachidonic acid and the enzyme, Δ-6 desaturase, necessary

Table 14
AI [a] for infant formula/diet

Fatty acid	Percentage of fatty acids
LA [b]	10.00
ALA	1.50
AA [c]	0.50
DHA	0.35
EPA [d] (*Upper limit*)	< 0.10

[a] AI = adequate intake. If sufficient scientific evidence is not available to calculate an estimated average requirement, a reference intake called an AI is used instead of a recommended dietary allowance. The AI is a value based on experimentally derived intake levels or approximations of observed mean nutrient intakes by a group (or groups) of healthy people. The AI for children and adults is expected to meet or exceed the amount needed to maintain a defined nutritional state or criterion of adequacy in essentially all members of a specific healthy population; LA = linoleic acid; ALA = α-linolenic acid; AA = arachidonic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; *TRANS*-FA = *trans*-fatty acids; SAT = saturated fatty acids; MONOs = monounsaturated fatty acids.

[b] The working group recognizes that in countries like Japan, the breast milk content of LA is 6–10% of fatty acids and the DHA is higher, about 0.6%. The formula/diet composition described here is patterned on infant formula studies in Western countries.

[c] The working group endorsed the addition of the principal long chain polyunsaturates, AA and DHA, to all infant formulas.

[d] EPA is a natural constituent of breast milk, but in amounts more than 0.1% in infant formula may antagonize AA and interfere with infant growth.

to desaturate it, is the same one necessary to desaturate ALA, the parent compound of the omega-3 class; each competes with the other for this desaturase. The presence of ALA in the diet can inhibit the conversion of the large amounts of LA in the diets of Western industrialized countries which contain too much dietary plant oils rich in omega-6 PUFAs (e.g. corn, safflower, and soybean oils). The increase of ALA, together with EPA and DHA, and reduction of vegetable oils with high LA content, are necessary to achieve a healthier diet in these countries.

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