

Evolutionary aspects of diet, essential fatty acids and cardiovascular disease

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Information from archaeological findings and studies from modern-day hunter-gatherers suggest that the Palaeolithic diet is the diet we evolved on and for which our genetic profile was programmed. During the Palaeolithic period the intake of omega-6 and omega-3 fatty acids was about equal. Epidemiological, experimental and clinical intervention studies have shown that omega-3 fatty acids affect the function of cells involved in atherothrombosis in numerous ways, including the modification of eicosanoid products in the cyclooxygenase and lipoxygenase pathways, the reduced synthesis of cytokines and platelet-derived growth factor by influencing gene expression and alterations in leukocyte and endothelial cell properties. In the secondary prevention of cardiovascular disease, omega-3 fatty acid supplementation leads to a decrease in cardiac deaths and total mortality. These effects are brought about without a decrease in plasma cholesterol levels, suggesting that the beneficial effects of omega-3 fatty acids in preventing sudden death are due to their antiarrhythmic properties. Because coronary

heart disease is a multigenic and multifactorial disorder, it is essential that the patients are stratified by genetic susceptibility and disease entity, as well as sex, age and severity of disease. The composition of the diet must remain constant throughout the intervention period and also the ratios of saturated fat to unsaturated fat and the ratio of omega-6:omega-3 taken into consideration. Trans fatty acids should not comprise more than 2% of energy. The dose, duration and mechanisms involved in the prevention and management of cardiovascular disease following omega-3 fatty acid ingestion or supplementation need to be further investigated by double-blind controlled clinical trials.

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Introduction

The interaction of genetics and environment, nature and nurture is the foundation for all health and disease. This concept, based on molecular biology and genetics, was originally defined by Hippocrates. In the 5th century BC Hippocrates stated the concept of positive health as follows:

Positive health requires a knowledge of man's primary constitution (which today we call genetics) and of the powers of various foods, both those natural to them and those resulting from human skill (today's processed food). But eating alone is not enough for health. There must also be exercise, the effects of which must likewise be known. The combination of these two things makes regimen, when proper attention is given to the season of the year, the changes of the winds, the age of the individual and the situation of his home. If there is any deficiency in food or exercise the body will fall sick.

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^[1,2]. 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%. Our genes today are very similar to the genes of our ancestors during the Palaeolithic period 40 000 years ago, the time at which our genetic profile was established^[3]. 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 and in the antioxidant content of foods^[3–7] (Table 1, Fig. 1). Using the tools of molecular biology and genetics, research is defining the mechanisms by which genes influence nutrient absorption,

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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)
Fibre	High	Low
Fat	Low	High
Animal	Low	High
Vegetable	Very low	Moderate to high
Total long-chain n-6+n-3 acids	High (2.3 g . day ⁻¹)	Low (0.2 g . day ⁻¹)
Ratio n-6:n-3 acids	Low (2:4)	High (12:0)
Vitamins, mg . day ⁻¹	Paleolithic period	Current U.S. 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 Reference 5.

metabolism and excretion, taste perception, and degree of satiation; it is also revealing 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 over the last 100 years, are potent promoters of chronic diseases such as atherosclerosis, essential hypertension, obesity, diabetes and many cancers. In addition to diet, sedentary life styles and exposure to noxious substances interact with

genetically controlled biochemical processes leading to chronic disease. This paper discusses evolutionary aspects of diet with emphasis on the balance of omega-6 to omega-3 fatty acids, their biological and metabolic functions, and their health implications in decreasing the risk of death in coronary heart disease. The Appendix 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, U.S.A., April 7–9, 1999^[8].

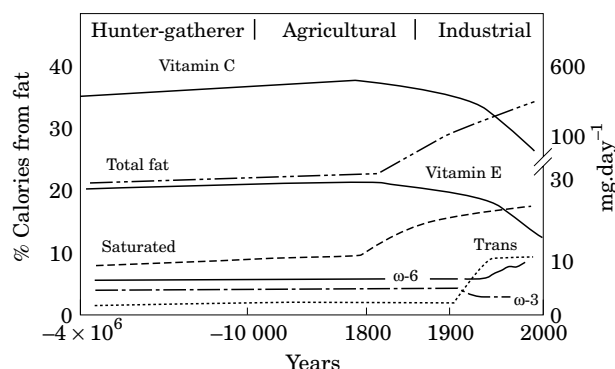


Figure 1 Hypothetical scheme of fat, fatty acid (omega (ω)-6, omega (ω)-3, trans and total) intake (as percentage of calories from fat) and intake of vitamins E and C (mg . day⁻¹). Data were extrapolated from cross-sectional analyses of contemporary hunter-gatherer populations and from longitudinal observations and their putative changes during the preceding 100 years^[5].

Evolutionary aspects of diet

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^[9–11]. Cereals did not become a part of our food supply until only very recently — 10 000 years ago — with the advent of the Agricultural Revolution. Before 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^[9–11]. 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. And yet, for the 99.9% of their presence on this planet, humans have rarely or never consumed cereal grains. It is only over the last 10 000 years that humans have consumed cereals. Before that, 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^[3]. Therefore, we have 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. Cereal grains are high in carbohydrates and omega-6 fatty acids, but low in omega-3 fatty acids and in antioxidants, particularly in comparison with green leafy vegetables. Recent studies show that low-fat/high-carbohydrate diets increase insulin resistance and hyperinsulinaemia, conditions that increase the risk for coronary heart disease, hypertension, diabetes and obesity^[12–14].

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 150 years, in the type and amount of fat and in vitamins C and E intake^[3,5,9,15–17] (Table 1, Fig. 1).

Eaton and Konner^[3] have estimated higher intakes for protein, calcium, potassium and ascorbic acid and lower sodium intakes for the diet of the late Palaeolithic period than the current U.S. and Western diets. Most of our food is calorically concentrated in comparison with wild game and the uncultivated fruits and vegetables of the Palaeolithic diet. Palaeolithic 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 fibre; (4) an increase in cereal grains and a decrease in fruits and vegetables; and (5) a decrease in protein, antioxidants and calcium intake^[3,5,17,18] (Tables 1 and 2).

Essential fatty acids and the omega-6: omega-3 balance

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

Table 2 Late Paleolithic and currently recommended U.S. nutrient composition

	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
Fibre (g)	100–150	30–60
Sodium (mg)	690	1100–3300
Calcium (mg)	1500–2000	800–1600
Ascorbic acid (mg)	440	60

P:S=polyunsaturated to saturated fat.

Modified from Eaton *et al.*^[18].

livestock (grains are rich in omega-6 fatty acids)^[19]. The invention of the continuous screw press, named Expeller[®] by Anderson, and the steam-vacuum deodorization process by Wesson made possible the industrial production of cottonseed oil and other vegetable oils for cooking^[19]. 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 economic. Subsequently, hydrogenation was applied to oils to solidify them. The partial selective hydrogenation of soybean oil reduced the alpha-linolenic (LNA) content of the oil while leaving a high concentration of linoleic acid (LA). LNA content was reduced because LNA 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^[20,21]. The effects of trans fatty acids on health have been reviewed extensively elsewhere^[22].

Since the 1950s, research on the effects of omega-6 polyunsaturated fatty acids (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.* in 1954^[23] and subsequent work by Keys *et al.*^[24] 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.

Agribusiness and modern agriculture

Agribusiness contributed further to the decrease in omega-3 fatty acids in animal carcasses. Wild animals

Table 3 Fatty acid content of plants ($\text{mg} \cdot \text{g}^{-1}$ wet weight)

Fatty acid	Purslane	Spinach	Buttercrunch lettuce	Red leaf lettuce	Mustard
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
18:1n-9	0.43	0.04	0.03	0.01	0.01
18:2n-6	0.89	0.14	0.10	0.12	0.12
18:3n-3	4.05	0.89	0.26	0.31	0.48
20:5n-3	0.01	0.00	0.00	0.00	0.00
22:6n-3	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 Reference 29.

and birds who feed on wild plants are very lean with a carcass fat content of only 3.9%^[25] and contain about five times more PUFAs per gram than is found in domestic livestock^[26,27]. It should be noted that 4% of the fat of wild animals contains eicosapentaenoic acid (EPA). Domestic beef contains very small or undetectable amounts of LNA because cattle are fed grains rich in omega-6 fatty acids and poor in omega-3 fatty acids^[28], whereas deer that forage on ferns and mosses contain more omega-3 fatty acids (LNA) 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^[25–28], green leafy vegetables^[29–31], eggs^[32,33], and even fish^[34] 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 3 shows the fatty acid content of purslane, a wild plant, and compares it with spinach, red leaf lettuce, buttercrunch lettuce and mustard greens. Purslane has eight times more alpha linolenic acid than the cultivated plants. Modern aquaculture produces fish that contain less omega-3 fatty acids than fish grown naturally in the ocean, rivers and lakes (Table 4). As can be seen from Table 5, comparing the fatty acid composition of egg yolk from free-ranging chickens in the Ampelistra farm in Greece and the standard U.S. 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^[32,33]. By enriching the chicken feed with fishmeal or flax, the ratio omega-6:omega-3 decreased to 6:6 and 1:6 respectively^[32,33]. Similarly, milk and cheese from animals that graze contain arachidonic acid (AA), EPA and DHA, whereas milk and cheese from grain-fed animals do not (Table 6)^[17].

Imbalance of omega-6 and omega-3 fatty acids

It is evident that food technology and agribusiness provided the economic stimulus that dominated the changes in the food supply^[35–38]. Statistics of per capita

quantities of foods available for consumption in the U.S. national food supply in 1985, indicate the amount of EPA in the diet to be about 50 mg per capita per day and the amount of DHA is 80 mg per capita per day. The two main sources are fish and poultry^[36]. 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^[3–7,25–28].

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 teaspoonful. Once these vitamins were synthesized, consumption of cod-liver oil was drastically decreased.

Thus an absolute and relative change of omega-6 versus omega-3 fatty acids in the food supply of Western societies has occurred over the last 100 years. 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. Recent

Table 4 Fat content and fatty acid composition of wild and cultured salmon (*Salmo salar*)

	Wild (n=2)	Cultured (n=2)
Fat (g per 100 g)	10 ± 0.1	16 ± 0.6**
Fatty acids (g per 100 g fatty acid)		
18:3n-3	1 ± 0.1	1 ± 0.1
20:5n-3	5 ± 0.2	5 ± 0.1
22:6n-3	10 ± 2	7 ± 0.1*
Other n-3 (18:4n-3+20:3n-3+22:5n-3)	3 ± 0.5	4 ± 0.1
18:2n-6	1 ± 0.1	3 ± 0.1
Other n-6 (20:4n-6+22:4n-6)	0.2 ± 0.1	0.5 ± 0.1
Total n-3	20 ± 2	17 ± 0.2
Total n-6	2 ± 0.1	3 ± 0.1**
Ratio of n-3:n-6	11 ± 2	6 ± 0.1*

Modified from Reference 34.

**Significantly different from wild, $P < 0.01$; *significantly different from wild, $P < 0.05$.

Table 5 Fatty acid levels ($\text{mg} \cdot \text{g}^{-1}$ yolk) in chicken egg yolks^{1,2,3}

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				
16:1n-7	21.7	4.7	5.1	4.4
18:1	120.5	110.0	102.8	94.2
20:1n-9	0.6	0.7	0.9	0.5
24:1n-9	—	—	0.1	—
Total	142.8	115.4	108.9	99.1
n-6 Polyunsaturates				
18:2n-6	16.0	26.1	67.8	42.4
18:3n-6	—	0.3	0.3	0.2
20:2n-6	0.2	0.4	0.6	0.4
20:3n-6	0.5	0.5	0.5	0.4
20:4n-6	5.4	5.0	4.4	2.6
22:4n-6	0.7	0.4	0.3	—
22:5n-6	0.3	1.2	0.2	—
Total	23.1	33.9	74.1	46.0
n-3 Polyunsaturates				
18:3n-3	6.9	0.5	4.1	21.3
20:3n-3	0.2	—	0.1	0.4
20:5n-3	1.2	—	0.2	0.5
22:5n-3	2.8	0.1	0.4	0.7
22:6n-3	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
n-6:n-3 ratio	1.3	19.9	6.6	1.6

¹Modified from Simopoulos and Salem^[33].²The eggs were hard-boiled, and their fatty acid composition and lipid content were assessed as described elsewhere^[32].³Greek eggs, free-ranging chickens; supermarket eggs, standard U.S. Department of Agriculture eggs found in U.S. 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.

studies by Cordain *et al.*^[39] on wild animals confirm the original observations of Crawford and Sinclair *et al.*^[26,40]. However, rapid dietary changes over short periods of time as have occurred over the past 100–150 years is a totally new phenomenon in human evolution.

A balance between the omega-6 and omega-3 fatty acids is a more physiological state in terms of gene expression^[41], prostaglandin and leukotriene metabolism and interleukin-1 (IL-1) production^[4]. The current recommendation to substitute vegetable oils (omega-6 fatty-acids) for saturated fats leads to increases in IL-1, prostaglandins and leukotrienes; is not consistent with human evolution, and may lead to maladaptation in those genetically predisposed.

The time has come to return the omega-3 fatty acids into the food supply. Progress in this regard is being

made^[7,42]. In the past, industry focused on improvements in food production and processing, whereas now and in the future the focus will be on the role of nutrition in product development and its effect on health and disease^[7,42].

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

Linoleic acid, LNA and their long-chain derivatives are important components of animal and plant cell membranes. When humans ingest fish or fish oil, the EPA and DHA from the diet partially replace the omega-6 fatty acids, especially arachidonic acid (AA), in the membranes of probably all cells, but especially in the membranes of platelets, erythrocytes, neutrophils, monocytes, and liver cells [reviewed in Reference 4].

Because of 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 larger 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 hypercholesterolaemia^[43], hyperlipoproteinaemia^[44], myocardial infarction, other forms of atherosclerotic disease, and diabetes (obesity and hypertriglyceridaemia). Atherosclerosis is a major complication in non-insulin dependent diabetes mellitus (NIDDM) patients. 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 7 shows that the higher the ratio omega-6:omega-3 fatty acids in platelet phospholipids, the higher the death rate from cardiovascular disease^[4,45].

The antithrombotic aspects and the effects of different doses of fish oil on the prolongation of bleeding time were investigated by Saynor *et al.*^[46]. A dose of $1.8 \text{ g} \cdot \text{day}^{-1}$ EPA did not result in any prolongation in bleeding time, but at $4 \text{ g} \cdot \text{day}^{-1}$ 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^[47].

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

Table 6 Fatty acid content of various cheeses (per 100 g edible portion)

	Milk (2%)	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 acid, g	<1	0.54	0.48	0.57	—	—
14:0 acid, g	<1	3.33	3.21	2.70	1.90	1.60
16:0 acid, g	<1	9.80	9.10	7.19	5.40	3.90
18:0 acid, g	<1	4.70	3.00	2.60	2.00	1.70
Total mono-unsaturated 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 acid, g	0.04	0.58	0.61	0.34	0.38	0.29
18:3 acid, 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 data from U.S. Department of Agriculture Handbook No. 8. Greek myzithra and Greek feta data from National Institute on Alcohol Abuse and Alcoholism analyses. From Reference 17.

Table 7 Ethnic differences in fatty acid concentrations in thrombocyte phospholipids and percentage of all deaths from cardiovascular disease

	Europe and U.S.A.	Japan	Greenland Eskimos
Arachidonic acid (20:4n-6), %	26	21	8.3
Eicosapentaenoic acid (20:5n-3), %	0.5	1.6	8.0
Ratio of n-6:n-3	50	12	1
Mortality from cardiovascular disease (per 1000)	45	12	7

Data modified from Weber^[45].

reduced and erythrocyte membrane fluidity is increased^[48–51]. Fish oil ingestion increases the concentration of plasminogen activator and decreases the concentration of plasminogen activator inhibitor 1 (PAI-1)^[52]. In vitro studies have shown that PAI-1 is synthesized and secreted in hepatic cells in response to insulin, and population studies indicate a strong correlation between insulinaemia and PAI-1 levels. In patients with types IIb and IV hyperlipoproteinaemia 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, although in one a small dose of cod-liver oil was used^[54] 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 . day^{−1} of lean fish or 2 g of omega-3 EPA and DHA improve certain haematological parameters implicated in the aetiology of cardiovascular disease^[55].

In summary, the antithrombotic effects of EPA and DHA supplementation suggest that their ingestion leads to a return to a more physiological state and away from a prothrombotic and atherogenic state in both animal models and human beings.

Ingestion of omega-3 fatty acids not only increases the production of PGI₃, but also of PGI₂ in tissue fragments from the atrium, aorta and saphenous vein obtained at surgery in patients who received fish oil two weeks prior to surgery^[56]. Omega-3 fatty acids inhibit the production of platelet-derived growth factor (PDGF) in bovine endothelial cells^[57]. 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)^[58]. 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.

In animal experiments, rats fed diets high in omega-6 fatty acids developed insulin resistance. Partially substituting fish oil restored normal insulin action^[59]. LNA produced similar effects, except when the diet had high

amounts of 18:2-omega-6 and some AA, in which case it was necessary to use EPA and DHA. When diets deficient in omega-3 fatty acids were used, all fats induced insulin resistance. These findings led to the conclusion that a deficiency of EPA and DHA or a high omega-6:omega-3 ratio in dietary fats could contribute to insulin resistance. Considering that the current Western diet has an absolute and relative deficiency in omega-3 fatty acids, it is obvious that genetic predisposition in the present dietary environment is conducive to the development of NIDDM. Furthermore, in designing clinical interventions, it is evident that consideration should be given to saturated fat intake, omega-6:omega-3 fatty acid ratio, and trans fatty acid intake. The omega-6:omega-3 fatty acid ratio should be similar to the Palaeolithic diet^[3]. Although precise information is lacking, it is considered to be 1–2:1 (omega-6:omega-3) rather than the 15–20:1, which is the ratio in most Western diets.

Studies carried out in India indicate that the higher ratio of 18:2-omega-6 to 18:3-omega-3 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^[60]. A recent prospective study in the U.S.A. showed that 18:3-omega-3 intake is negatively related to the development of coronary artery disease^[61], and 18:3-omega-3 added to a Mediterranean type of diet in patients with one episode of myocardial infarction decreased mortality by 70%^[62].

The hypolipidaemic effects of omega-3 fatty acids are similar to the effects of omega-6 fatty acids provided that they replace saturated fats. Omega-3 fatty acids have the added benefit of not lowering high-density lipoprotein (HDL) and consistently lowering serum triglyceride concentrations, whereas the omega-6 fatty acids do not and may even increase them^[63].

Another important consideration is the finding that during chronic fish oil feeding there is a decrease in postprandial triglyceride concentration. Furthermore, Nestel^[64] reported that fish oil feeding blunted the expected rise in plasma cholesterol concentrations when large amounts of cholesterol were fed to humans. These findings are consistent with a reduced rate of coronary artery disease in fish-eating populations. Studies in humans have shown that fish oils reduce the rate of hepatic secretion of very-low-density lipoprotein (VLDL) triglyceride^[65–69]. In normolipidaemic subjects omega-3 fatty acids prevent and reverse rapidly the carbohydrate-induced hypertriglyceridaemia^[66,69]. There is also evidence from kinetic studies that fish oils increase the fractional catabolic rate (FCR) of VLDL^[65,67,68].

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 anti-inflammatory effects of fish oils are partly mediated by inhibiting the 5-lipoxygenase pathway in neutrophils and monocytes and inhibiting the leukotriene B₄ (LTB₄)-mediated function of LTB₅ (Fig. 2)^[70,71]. Studies since 1985 show that omega-3 fatty acids influence interleukin metabolism by decreasing IL-1 level^[72–74]. Inflammation plays an important role in both the initiation of atherosclerosis and the development of atherothrombotic events^[75]. 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)^[76]. The expression of E-selectin, ICAM-1 and VCAM-1, which is relatively low in normal vascular cells, is up-regulated 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 in human studies^[77–80].

Clinical intervention studies

Over the past 15 years there have been a number of cardiac intervention studies evaluating the role of omega-3 fatty acids in the secondary prevention of cardiovascular disease^[62,81–86]. More recently, studies have focused on other aspects of cardiovascular disease such as: coronary artery by-pass grafting, the relationship of dietary intake and cell membrane levels of long-chain omega-3 PUFAs and the risk of primary cardiac arrest; serum fatty acids and the risk of coronary heart disease; the effects of dietary fish oil on ventricular premature complexes. These studies indicate that omega-3 fatty acids exert their beneficial effects in the absence of lowered serum lipid levels, suggesting a special role of omega-3 fatty acids based on their antithrombotic, antiinflammatory and antiarrhythmic properties.

Coronary artery by-pass grafting

Coronary artery by-pass grafting constitutes an important treatment alternative in the management of coronary artery disease. The Shunt Occlusion Trial was a randomized, controlled study initiated to assess the effect of dietary supplementation with fish oil rich in omega-3 fatty acids, on a 1-year graft occlusion rate, in patients undergoing coronary artery bypass grafting^[87]. A total of 610 patients were assigned either to a fish oil group, receiving 4 g . day⁻¹ of fish oil ethyl ester concentrate, or to a control group. Patients continued their

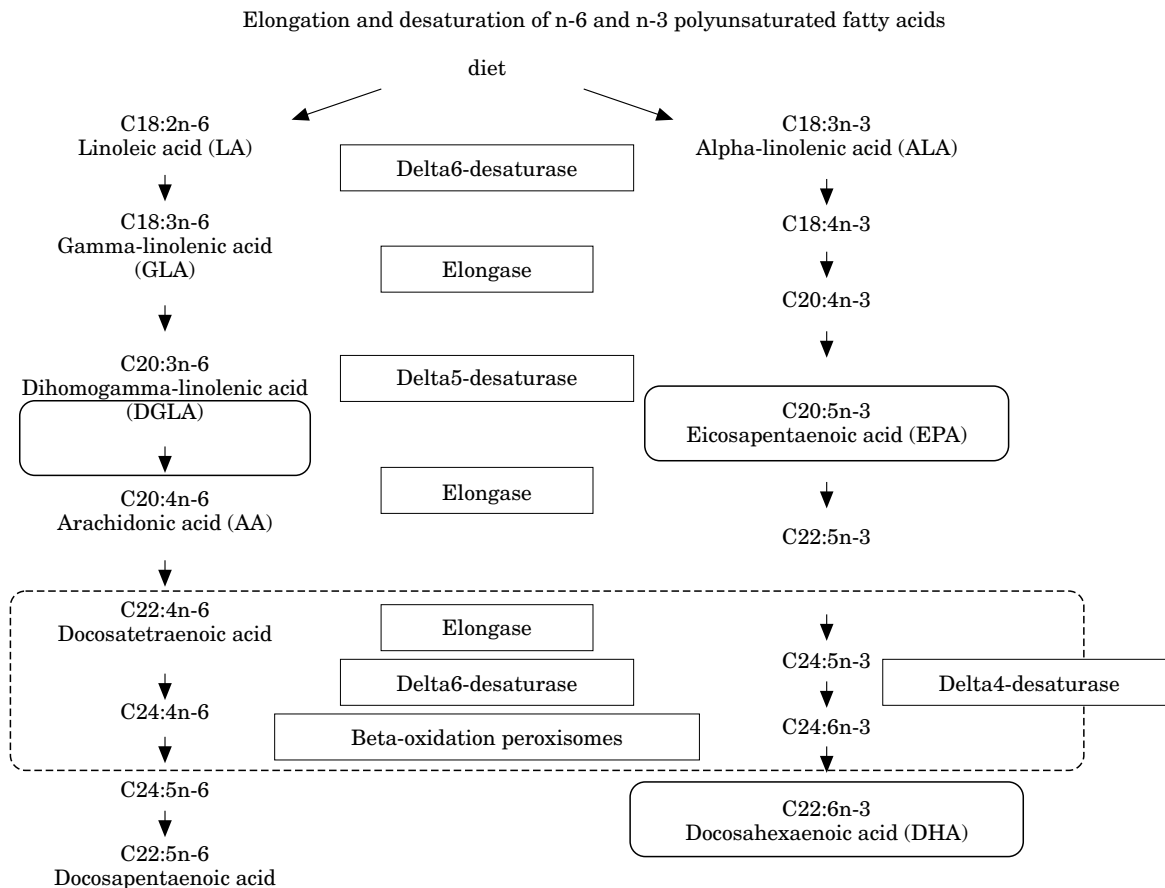


Figure 2 Elongation and desaturation of omega-6 and omega-3 polyunsaturated fatty acids.

antithrombotic treatment. The primary end-point was 1-year graft patency assessed by angiography in 95% of patients. The omega-3 fatty acid dietary supplementation reduced significantly the incidence of vein graft occlusion. An inverse relation between relative change in serum phospholipid omega-3 fatty acids and vein graft occlusions was observed^[87]. This beneficial effect on vein graft patency may be due to antithrombotic as well as antiatherosclerotic properties of omega-3 fatty acids. It is unlikely that the effect is directly linked to serum lipoproteins, because serum cholesterol levels were not altered by fish oil supplementation, and there was no association between the reduction in serum triglycerides and vein graft patency. Their effects may most likely be due to the influence of omega-3 fatty acids on cellular processes locally in the vessel wall.

The effect of omega-3 supplementation on the incidence of restenosis after coronary angioplasty has been addressed in several clinical studies and the results so far are equivocal^[88,89], although the pathophysiology of coronary restenosis is different from that of vein graft occlusion. The two conditions are not analogous. The vein graft occlusion study suggests that patients undergoing coronary by-pass surgery should be encouraged to keep a high dietary intake of omega-3 fatty acids.

Dietary intake and cell membrane levels of long-chain omega-3 PUFAs and the risk of primary cardiac arrest

In a population-based control study, Siscovick *et al.*^[90] assessed the dietary intake of EPA and DHA from seafood in the risk of primary cardiac arrest. All cases and controls were free of prior clinical heart disease, major co-morbidity and use of fish oil supplements. Information on the dietary intake of omega-3 PUFAs from seafood during the previous month was obtained from the spouses of the case patients and controls. Blood specimens were analysed to determine fatty acid composition in red blood cell membranes. The data show that dietary intake of omega-3 PUFAs from seafood is associated with a reduced risk of primary cardiac arrest compared with no fish intake. Intake of 5.5 g of omega-3 fatty acids per month, or the equivalent of one fatty fish meal per week, was associated with a 50% reduction in the risk of primary cardiac arrest. A concentration of 5.0% omega-3 PUFAs in red blood cell membrane phospholipids was associated with a 70% reduction in the risk of primary cardiac arrest.

Serum fatty acids and the risk of coronary heart disease

Simon *et al.*^[91] examined the relationship between serum fatty acids and coronary heart disease (CHD) by conducting a nested case-control study of 94 men with incident CHD and 94 men without CHD who were enrolled in the usual care group of the Multiple Risk Factor Intervention Trial (MRFIT) between December 1973 and February 1976. The results are consistent with other evidence indicating that saturated fatty acids are directly correlated with CHD and that omega-3 PUFA levels are inversely correlated with CHD. Because these associations were present after adjustment for blood lipid levels, other mechanisms, such as a direct effect on blood clotting, may be involved.

Effects of dietary fish oil on ventricular premature complexes

In a prospective, double-blind and placebo-controlled study, Sellmayer *et al.*^[92] tested the potential antiarrhythmic effects of dietary supplementation of fish oil rich in DHA and EPA in patients with spontaneous ventricular premature complexes (VPCs). The patients were eligible if they had a minimum of 2000 VPCs per 24 h on a 24-h Holter monitoring. The patients had moderate-to-low-grade ventricular arrhythmias in the absence of severe myocardial pump failure and were randomly assigned to receive either fish oil (cod-liver oil) or placebo sunflower oil. Vitamin E (10 mg . ml⁻¹) of alpha-tocopherol had been added as antioxidant in both oils.

A daily dose of 0.9 g of EPA, 1.5 g of DHA, or 5 g of 18:2-omega-6 acid were taken for 16 weeks by which time tissue levels of omega-3 fatty acids would have reached steady state. Compliance was checked by telephone call after 1 week, a visit after 8 weeks and serum phospholipid fatty acid analysis after 16 weeks. Serum levels of EPA and DHA increased significantly ($P < 0.001$) more than 2- and 1.7-fold, respectively, whereas levels of AA and linoleic (18:2-omega-6) were slightly reduced. No changes in the fatty acids of the placebo groups were noted. The proportion of the reduction in VPCs was more than 70% in 44% (15 patients) after fish oil versus 15% (five patients) in the placebo group. The results showed that a moderate dose of fish oil has antiarrhythmic effects leading to a reduction of VPCs in nearly half the patients with frequent ventricular arrhythmia.

In another study by Danish investigators^[93], the patients receiving fish oil had a non-significant reduction in VPCs whereas those receiving corn oil did not.

Antiarrhythmic effects of omega-3 fatty acids

The antiarrhythmic aspects of omega-3 fatty acids are supported by two clinical intervention trials. The results

of the DART study strongly support the role of fish or fish oil in decreasing total mortality and sudden death in patients with one episode of myocardial infarction^[83]. The estimated dose of EPA was about 0.3 g . day⁻¹ in the fish advice group and 0.1 g . day⁻¹ in the control group^[83]. In the de Lorgeril *et al.* study^[62] the estimated intake of 18:3-omega-3 acid was 2 g . day⁻¹ in the intervention group and 0.6 g . day⁻¹ in the control group. Experimental studies suggest that intake of 3–4 g . day⁻¹ of 18:3-omega-3 is equivalent to 0.3 g . day⁻¹ of EPA in its effect on the EPA content of plasma phospholipids^[94].

The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione trial was a multicentre open-label design secondary prevention for cardiovascular disease trial of 3.5 years duration^[86]. The addition of 850–882 mg of EPA + DHA at a 2:1 EPA to DHA ratio to their regular (Mediterranean) diet and drug treatment led to a 20% decrease in risk of death, 30% decrease of cardiovascular death and 45% decrease for sudden death.

The studies of McLennan^[95] and McLennan *et al.*^[96] showed that omega-3 fatty acids, more so than omega-6 PUFAs, can prevent ischaemia-induced fatal ventricular arrhythmia in experimental animals. Kang and Leaf^[97,98] showed that omega-3 fatty acids make the heart cells less excitable by modulating the conductance of the sodium and other ion channels. The clinical studies of Burr *et al.*^[83] and de Lorgeril *et al.*^[62] further support the role of omega-3 fatty acids in the prevention of sudden death due to ventricular arrhythmias which in the U.S.A. account for 50–60% of the mortality from acute myocardial infarction and claim 250 000 deaths per year. The intervention studies of Sellmayer *et al.*^[92] and Christensen *et al.*^[93], showing a decrease in the rate of PVCs and the case-control study by Siscovick *et al.*^[90] reporting an inverse relationship between fish consumption and sudden death, provide further evidence for the antiarrhythmic effects of fish oil ingestion.

Conclusions and recommendations

The studies described above provide further support on the beneficial effects of omega-3 fatty acids in the prevention and management of cardiovascular disease. The effects of omega-3 fatty acids and the mechanisms involved are not operating in changing lipid levels. Most likely their effects are at the level of the vessel wall affecting blood clotting^[87,91] or their action is antiarrhythmic^[62,83,90,92]. Of further interest is the fact that effects of omega-3 fatty acids appear to occur within the first 4 months^[62], whereas such an effect was not apparent in the Scandinavian Simvastatin Survival Study Group^[99] or the Scottish trial^[100] for 6 months to 2 years using simvastatin and pravastatin, respectively. Furthermore, a recent study on the cost-effectiveness of statins showed a great variation between different risk groups^[101]. Dietary intervention with omega-3 fatty acids is cheaper and free of side-effects. Clearly there is a

Table 8 Genetic determinants and environmental risk factors for coronary heart disease

Genetic determinants	Environmental risk factors
Family history of CHD at an early age	Smoking
Total serum cholesterol, LDL and Apo B levels	Sedentary lifestyle (lack of aerobic exercise)
HDL cholesterol, Apo A-I and Apo A-II levels	Diet
Apo A-IV-1/1	Excess energy intake
Apo E polymorphism	High saturated fat intake
Lipoprotein (a)	High trans fatty acids intake
LDL receptor activity	High omega-6 fatty acid intake
Thrombosis-coagulation parameters	Low omega-3 fatty acid intake
Triglyceride and VLDL concentrations	Psychosocial factors
RFLPs in DNA at the Apo A-I/Apo C-II and Apo B loci	Type A personality
Other DNA markers	Social class
Blood pressure	
Diabetes	
Obesity	
Insulin level and insulin response	
Heterozygosity for homocystinuria	

need to carry out large randomized double-blind controlled clinical trials to confirm the effects of omega-3 fatty acids in the prevention of sudden death and decrease in total mortality.

Omega-3 fatty acids have been part of our diet since the beginning of time. They have been shown to be essential for normal growth and development^[4]. For the primary prevention of cardiovascular disease the dose should be consistent with estimates derived from knowledge on the evolutionary aspects of diet, namely an omega-6:omega-3 ratio of 1^[6,17]. Epidemiological studies suggest that one to two fish meals per week, or as little as 30–35 g . day⁻¹ of fish throughout life, decrease the risk of coronary heart disease relative to those who do not eat any fish^[90,102]. Furthermore, in the study by von Schacky *et al.*, patients with coronary artery disease who ingested approximately 1.5 g of omega-3 fatty acids per day for 2 years had less progression and more regression of coronary artery disease on coronary angiography than did comparable patients who ingested a placebo^[103]. For the secondary prevention of coronary heart disease, 300 g of fatty fish providing 2.5 g EPA from fish or fish oil per week decreased the risk of sudden death by 29%^[83]. Overall diet most definitely influences the dose of omega-3 fatty acids, since 2 g of 18:3-omega-3 acid added to a Mediterranean type diet decreased the rate of sudden death by 70% beginning at 4 months after the change^[62]. In the GISSI study, the dose of omega-3 fatty acids was 850–882 mg of EPA+DHA at a ratio of 2:1 in addition to a Mediterranean type diet. For the reduction of VPCs the dose was 0.9 g of EPA and 1.5 g of DHA, whereas for the prevention of occlusions following coronary by-pass grafting the dose was 4 g . day⁻¹ of fish oil concentrate.

Coronary heart disease is a multigenic and multifactorial disease. Table 8 lists a number of genetic and environmental factors that contribute to its development^[104]. Double-blind controlled clinical trials are the

‘gold standard’ to demonstrate a cause and effect relationship. In the planning of such trials, it is essential that the patients are stratified by genetic susceptibility and disease entity, as well as sex, age and severity of disease. The composition of the diet must remain constant throughout the intervention period, the ratios of saturated fat to unsaturated fat and the omega-6:omega-3 ratio must be taken into consideration^[105]. Trans fatty acids should not comprise more than 2% of energy. The exact dose of omega-3 fatty acids and length of treatment prior to surgical procedures such as angioplasty appears to be a critical one. Judging from the beneficial effects in the study by Bairati *et al.*^[106] it appears that omega-3 fatty acid supplementation should be given at least 3 weeks prior to surgery. Many of the studies reported thus far were not controlled for many of the above factors. Common protocols need to be established, but modified according to prevailing genetic, dietary and other environmental factors.

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Appendix

Recommended dietary intakes for omega-6 and omega-3 fatty acids

On April 7–9, 1999, an international working group of scientists met at the National Institutes of Health in Bethesda, Maryland (U.S.A.) to discuss the scientific evidence relative to dietary recommendations of omega-6 and omega-3 fatty acids (Simopoulos *et al.*, 1999). The latest scientific evidence based on controlled intervention trials in infant nutrition, cardiovascular disease, and mental health was extensively discussed. Tables 1 and 2 include the Adequate Intakes (AI) for omega-6 and omega-3 essential fatty acids for adults and infant formula/diet respectively.

Table 1 Adequate Intakes (AI)* for adults

Fatty acid	Intake, g . day ⁻¹ (2000 kcal diet)	Energy, %
LA	4.44	2.0
(Upper limit) ⁽¹⁾	6.67	3.0
LNA	2.22	1.0
DHA + EPA	0.65	0.3
DHA to be at least ⁽²⁾	0.22	0.1
EPA to be at least	0.22	0.1
Trans-FA		
(Upper limit) ⁽³⁾	2.00	1.0
Sat		
(Upper limit) ⁽⁴⁾	—	<8.0
Monos ⁽⁵⁾	—	—

⁽¹⁾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 . day⁻¹ based on a 2000 kcal diet or of 3.0% of energy.

⁽²⁾For pregnant and lactating women, ensure 300 mg . day⁻¹ of DHA.

⁽³⁾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).

⁽⁴⁾Saturated fats should not comprise more than 8% of energy.

⁽⁵⁾The Working Group recommended that the majority of fatty acids are 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 15–40% of energy) but with special attention to the importance of weight control and reduction of obesity.

*If sufficient scientific evidence is not available to calculate an Estimated Average Requirement, a reference intake called an Adequate Intake (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; LNA=alpha-linolenic acid; DHA=docosahexaenoic acid; EPA=eicosapentaenoic acid; Trans-FA=trans fatty acids; Sat=saturated fatty acids; Monos=monounsaturated fatty acids.

Table 2 Adequate Intake (AI)* for infant formula/diet

Fatty acid	Percentage of fatty acids
LA ⁽¹⁾	10.00
LNA	1.50
AA ⁽²⁾	0.50
DHA	0.35
EPA ⁽³⁾ (Upper limit)	<0.10

⁽¹⁾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.

⁽²⁾The Working Group endorsed the addition of the principal long-chain polyunsaturates, AA and DHA, to all infant formulas.

⁽³⁾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.

*If sufficient scientific evidence is not available to calculate an Estimated Average Requirement, a reference intake called an Adequate Intake (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; LNA=alpha-linolenic acid; AA=arachidonic acid; DHA=docosahexaenoic acid; EPA=eicosapentaenoic acid.

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 AIs for Adults as shown in Table 1.

Pregnancy and lactation

For pregnancy and lactation, the recommendations are the same as those for adults with the additional recommendation seen in footnote (1) (Table 2), that during

pregnancy and lactation women must ensure a DHA intake of 300 mg . day⁻¹.

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 2).

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 (AA) and its eicosanoid products. Such excesses can occur when too much linoleic acid (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 AA 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 AA and the enzyme, Δ -6 desaturase, necessary to desaturate it, is the same one necessary to desaturate alpha-linolenic acid (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.