SYMPOSIUM: ROLE OF POULTRY PRODUCTS IN ENRICHING THE HUMAN DIET WITH N-3 PUFA

Human Requirement for N-3 Polyunsaturated Fatty Acids

A. P. Simopoulos[1]

The Center for Genetics Nutrition and Health, Washington, DC 20009

ABSTRACT The diet of our ancestors was less dense in calories, being higher in fiber, rich in fruits, vegetables, lean meat, and fish. As a result, the diet was lower in total fat and saturated fat, but contained equal amounts of n-6 and n-3 essential fatty acids. Linoleic acid (LA) is the major n-6 fatty acid, and alpha-linolenic acid (ALA) is the major n-3 fatty acid. In the body, LA is metabolized to arachidonic acid (AA), and ALA is metabolized to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The ratio of n-6 to n-3 essential fatty acids was 1 to 2:1 with higher levels of the longer-chain polyunsaturated fatty acids (PUFA), such as EPA, DHA, and AA, than today's diet. Today this ratio is about 10 to 1:20 to 25 to 1, indicating that Western diets are deficient in n-3 fatty acids compared with the diet on which humans evolved and their genetic patterns were established. The n-3 and n-6 EPA are not interconvertible in the human body and are important components of practically all cell membranes. The N-6 and n-3 fatty acids influence eicosanoid metabolism, gene expression, and intercellular cell-to-cell communication. The PUFA composition of cell membranes is, to a great extent, dependent on dietary intake. Therefore, appropriate amounts of dietary n-6 and n-3 fatty acids need to be considered in making dietary recommendations. These two classes of PUFA should be distinguished because they are metabolically and functionally distinct and have opposing physiological functions; their balance is important for homeostasis and normal development. Studies with nonhuman primates and human newborns indicate that DHA is essential for the normal functional development of the retina and brain, particularly in premature infants. A balanced n-6/n-3 ratio in the diet is essential for normal growth and development and should lead to decreases in cardiovascular disease and other chronic diseases and improve mental health. Although a recommended dietary allowance for essential fatty acids does not exist, an adequate intake (AI) has been estimated for n-6 and n-3 essential fatty acids by an international scientific working group. For Western societies, it will be necessary to decrease the intake of n-6 fatty acids and increase the intake of n-3 fatty acids. The food industry is already taking steps to return n-3 essential fatty acids to the food supply by enriching various foods with n-3 fatty acids. To obtain the recommended AI, it will be necessary to consider the issues involved in enriching the food supply with n-3 PUFA in terms of dosage, safety, and sources of n-3 fatty acids.

(*Key words*: n-6 and n-3 essential fatty acid requirements, n-6/n-3 ratio, biological metabolism, gene expression, n-3-enriched foods)

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INTRODUCTION

Over the past 20 yr, many studies and clinical investigations have been carried out on the metabolism of polyunsaturated fatty acids (PUFA) in general and on n-3 fatty acids in particular. Today we know that n-3 fatty acids are essential for normal growth and development and may play an important role in the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, other inflammatory and autoimmune disorders, and cancer (Simopoulos et al., 1986; Galli and Simopoulos, 1989; Simopoulos, 1991; Simopoulos et al., 1991; Galli et al., 1994a,b; Salem et al., 1996). Research has been done with animal models, in tissue cultures, and on humans. The original observational studies have given way to controlled clinical trials. Great progress has taken place in our knowledge of the physiologic and molecular mechanisms of the various fatty acids in growth and development and in health and disease. Specifically, their beneficial effects have been shown in the prevention and management of coronary heart disease (Burr et al., 1989; de Lorgeril et al., 1994, 1996, 1999), hypertension (Appel et al., 1993, 1994; Morris et al., 1994), type 2 diabetes (Connor

[1]To whom correspondence should be addressed: cgnh@bellatlantic.net.

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Abbreviation Key: AA = arachidonic acid; AI = adequate intake; ALA = alpha-linolenic acid; DPA = docosapentaenoic acid; EPA = eicosapentaenoic acid; LA = linoleic acid; PUFA = polyunsaturated fatty acids.

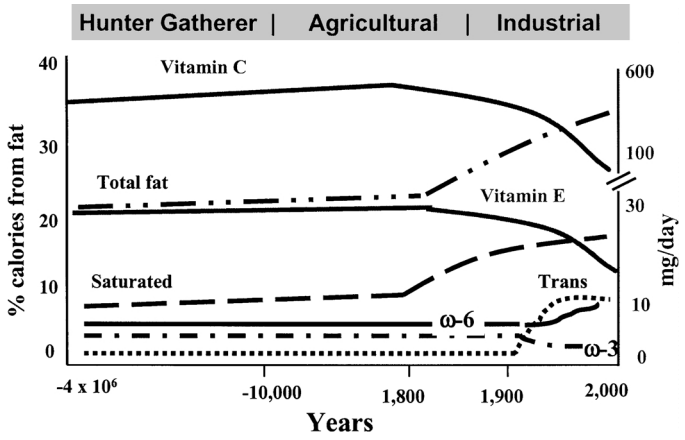

FIGURE 1. Hypothetical scheme of fat and fatty acid (n-3, n-6, *trans*, and total) intakes (as % of calories from fat) and intake of vitamins E and C (mg/d). Data were extrapolated from cross-sectional analyses of contemporary hunter-gatherer populations and from longitudinal observations and their putative changes during the preceding 100 yr (Simopoulos, 1999).

et al., 1993; Raheja et al., 1993), renal disease (De Caterina et al., 1993; Donadio, et al., 1994), rheumatoid arthritis (Kremer, 1996), ulcerative colitis (Stenson et al., 1992), Crohn's disease (Belluzzi et al., 1996), and chronic obstructive pulmonary disease (Shahar et al., 1994). This paper focuses on the evolutionary aspects of diet, biological effects; adequate intake (AI) of n-6 and n-3 fatty acids, n-3-enriched foods, and issues in terms of dosage, safety, and sources of essential fatty acids for food enrichment.

EVOLUTIONARY ASPECTS OF DIET

On the basis of estimates from studies on Paleolithic nutrition and modern-day hunter-gatherer populations, it appears that human beings have evolved while consuming a diet that was much lower in saturated fatty acids than is today's diet (Eaton and Konner, 1985). Furthermore, the diet contained small and roughly equal amounts of n-6 and n-3 PUFA (ratio of 1 to 2:1) and much lower amounts of *trans* fatty acids than does today's diet (Figure 1) (Eaton and Konner, 1985; Simopoulos, 1995). The current Western diet is very high in n-6 fatty acids (the ratio of n-6 to n-3 fatty acids is 10 to 20 to 25:1) because of the indiscriminate recommendation to substitute n-6 fatty acids for saturated fats to lower serum cholesterol concentrations (Report of the National Cholesterol Education Program, 1988). Intake of n-3 fatty acids is much lower today because of the decrease in fish consumption and the industrial production of animal feeds rich in grains containing n-6 fatty acids, leading to production of meat rich in n-6 and poor in n-3 fatty acids (Crawford, 1968). The same is true for cultured fish (van Vliet and Katan, 1990) and eggs (Simopoulos and Salem, 1989, 1992). Even cultivated vegetables contain fewer n-3 fatty acids than do plants in the wild (Simopoulos and Salem, 1986; Simopoulos et al., 1995). In summary, modern agriculture, with its emphasis on large-scale production, has decreased the n-3 fatty acid content in many foods: green leafy vegetables, animal meats, eggs, and fish.

BIOLOGICAL EFFECTS OF N-6 AND N-3 FATTY ACIDS

Linoleic acid (LA; 18:2n-6) and alpha-linolenic acid (ALA; 18:3n-3) and their long-chain derivatives are important components of animal and plant cell membranes. When humans ingest fish or fish oil, the ingested eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) partially replace the n-6 fatty acids [particularly arachidonic acid (AA; 20:4n-6) in cell membranes, especially those of platelets, erythrocytes, neutrophils, monocytes, and liver cells (reviewed in Simopoulos, 1991). As a result, ingestion of EPA and DHA from fish or fish oil leads to 1) decreased production of prostaglandin E_2 metabolites; 2) decreased concentrations of thromboxane A_2, a potent platelet aggregator and vasoconstrictor; 3) decreased formation of leukotriene B_4, an inducer of inflammation and a powerful inducer of leukocyte chemotaxis and adherence; 4) increased concentrations of thromboxane A_3, a weak platelet aggregator and vasocontrictor; 5) increased concentrations of prostacyclin PGI3, leading to an overall increase in total prostacyclin by increasing PGI3 without decreasing PGI2 (both PGI2 and PGI3 are active vasodilators and inhibitors of platelet aggregation); and 6) increased concentrations of leukotriene B_5, a weak inducer of inflammation and chemotactic agent (Lewis et al., 1986; Weber et al., 1986).

Because of the increased amounts of n-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 n-3 fatty acids, specifically EPA. The eicosanoids from AA are biologically active in small quantities, and if they are formed in large amounts, they contribute to the formation of thrombi and atheromas; the development of allergic and inflammatory disorders, particularly in susceptible people; and cell proliferation. Thus, a diet rich in n-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 less in groups of patients with hypercholesterolemia (Brox et al., 1983), hyperlipoproteinemia (Joist et al., 1979), myocardial infarction, other forms of atherosclerotic disease, type 2 diabetes, obesity, and hypertriglyceridemia. Atherosclerosis is a major complication in type 2 diabetes patients. Bleeding time is longer in women than in men and in younger than in older persons. There are ethnic differences in bleeding time that appear to be related to diet. As shown in Table 1, as the ratio of n-6 to n-3 fatty acids in platelet phospholipids increases, the death rate from cardiovascular disease increases (Weber, 1989). As the ratio of n-6 PUFA to n-3 PUFA increases, the prevalence of type 2 diabetes also increases (Raheja et al., 1993; Figure 2).

TABLE 1. Ethnic differences in fatty acid concentrations in thrombocyte phospholipids and percentage of all deaths from cardiovascular disease[1]

	Europe and United States	Japan	Greenland Eskimos
	(%)		
Arachidonic acid (20:4n-6)	26	21	8.3
Eicosapentaenoic acid (20:5n-3)	0.5	1.6	8.0
n-6:n-3	50	12	1
Mortality from cardiovascular disease	45	12	7

[1]Data modified from Weber, 1989.

The hypolipidemic, antithrombotic, and antiinflammatory effects of n-3 fatty acids have been studied extensively with animal models, in tissue cultures, and in cells (Table 2; Weber and Leaf, 1991). As expected, earlier studies focused on mechanisms that involve eicosanoid metabolites. More recently, however, the effects of fatty acids on gene expression have been investigated, and this focus of interest has led to molecular studies (Tables 3 and 4). Previous studies have shown that fatty acids, whether released from membrane phospholipids by cellular phospholipases or made available to the cell from the diet or other aspects of the extracellular environment, are important cell-signaling molecules. They can act as second messengers or substitute for the classic second messengers of the inositide phospholipid and cyclic adenosine monophosphate signal transduction pathways (Graber et al., 1994). It has been shown that fatty acids rapidly and directly alter the transcription of specific genes (Clarke and Jump, 1994).

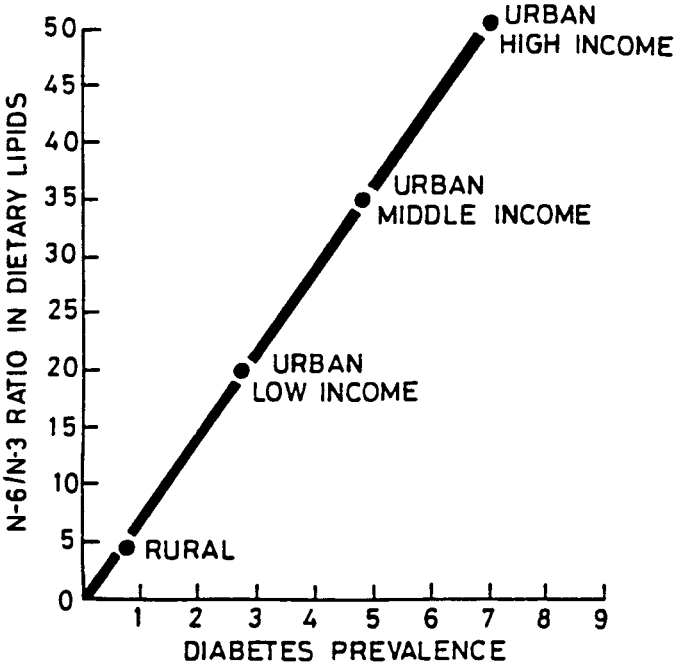

FIGURE 2. Relation between the ratio of n-6 to n-3 fatty acid in dietary lipids in the Indian diet and the prevalence of type 2 diabetes (Raheja et al., 1993).

Effects of Dietary ALA Compared with Long-Chain N-3 Fatty Acid Derivatives on Physiologic Indexes

Several clinical and epidemiologic studies have been conducted to determine the effects of long-chain n-3 PUFA on various physiologic indexes (Salem et al., 1996). Whereas the earlier studies were conducted with large doses of fish or fish-oil concentrates, more recent studies have used lower doses (Indu and Ghafoorunissa, 1992). ALA, the precursor of n-3 fatty acids, can be converted to long-chain n-3 PUFA and can, therefore, be substituted for fish oils. The minimum intake of long-chain n-3 PUFA needed for beneficial effects depends on the intake of other fatty acids. Dietary amounts of LA as well as the ratio of LA to ALA appear to be important for the metabolism of ALA to long-chain n-3 PUFA. Indu and Ghafoorunissa (1992) showed that although keeping the amount of dietary LA constant, 3.7 g ALA appears to have biological effects similar to those of 0.3 g long-chain n-3 PUFA with conversion of 11 g ALA to 1 g long-chain n-3 PUFA. Thus, a ratio of four (15 g LA:3.7 g ALA) is appropriate for conversion. In human studies, Emken et al. (1994) showed that the conversion of deuterated ALA to longer-chain metabolites was reduced by ≈50% when dietary intake of LA was increased from 4.7 to 9.3% of energy as a result of the known competition between n-6 and n-3 fatty acids for desaturation.

Indu and Ghafoorunissa (1992) further indicated that increasing dietary ALA increases EPA concentrations in plasma phospholipids after 3 and 6 wk of intervention. Dihomo-γ-linolenic acid (20:3n-6) concentrations were reduced, but AA concentrations were not altered. The reduction in the ratio of long-chain n-6 PUFA to long-chain n-3 PUFA was greater after 6 wk than after 3 wk. Indu and Ghafoorunissa (1992) were able to show antithrombotic effects by reducing the ratio of n-6 to n-3 fatty acids with ALA-rich vegetable oil. After ALA supplementation, there was an increase in long-chain n-3 PUFA in plasma and platelet phospholipids and a decrease in platelet aggregation. ALA supplementation did not alter triacylglycerol concentrations. As shown by others, only long-chain n-3 PUFA have triacylglycerol-lowering effects (Mantzioris et al., 1994).

In Australian studies, ventricular fibrillation in rats was reduced with canola oil as much or even more efficiently than with fish oil, an effect attributable to ALA (McLennan, 1993). Further studies should be able to show whether this result is a direct effect of ALA per se or occurs as a result of its desaturation and elongation to EPA and DHA.

The diets of Western countries have contained increasingly greater amounts of LA, which has been promoted for its cholesterol-lowering effect. It is now recognized that dietary LA favors oxidative modification of LDL cholesterol (Reaven et al., 1991; Abbey et al., 1993) increases platelet response to aggregation (Renaud, 1990) and stimulates the immune system (Endres et al., 1989). In contrast, ALA intake is associated with inhibitory effects on the

TABLE 2. Effects of n-3 fatty acids on factors involved in the pathophysiology of atherosclerosis and inflammation[1]

Factor	Function	Effect of n-3 fatty acid on factor concentrations
Arachidonic acid	Eicosanoid precursor, aggregates platelets, and stimulates white blood cells	↑
Thromboxane A_2	Platelet aggregation, vasoconstriction, increases intracellular Ca^{2+}	↓
Prostacyclin	Prevents platelet aggregation, vasodilator, increases cyclic AMP[2]	↑
Leukotriene B_4	Neutrophil chemoattractant increases intracellular Ca^{2+}	↓
Tissue plasminogen activator	Increases endogenous fibrinolysis	↑
Fibrinogen	Blood clotting factor	↓
Red blood cell deformability	Decreases tendency to thrombosis and improves oxygen delivery to tissues	↑
Platelet-activating factor	Activates platelets and white blood cells	↓
Platelet-derived growth factor	Chemoattractant and mitogen for smooth muscles and macrophages	↓
Oxygen free radicals	Causes cellular damage, enhances LDL uptake via the scavenger pathway, stimulates arachidonic acid metabolism	↓
Lipid hydroperoxides	Stimulates eicosanoid formation	↓
Interleukin 1 and tumor necrosis factor	Stimulates neutrophil oxygen free radical formation, lymphocyte proliferation, and platelet activating factor; expresses intercellular adhesion molecule 1 on endothelial cells; and inhibits plasminogen activator and thus is procoagulant	↓
Endothelial-derived relaxation factor	Reduces arterial vasoconstrictor response	↑
Very LDL	Related to LDL and HDL concentrations	↓
HDL	Decreases the risk of coronary heart disease	↑
Lipoprotein(a)	Atherogenic and thrombogenic	↓
Triacylglycerols and chylomicrons	Contribute to postprandial lipemia	↓

[1]Data from Weber and Leaf (1991). ↑ increases; ↓ decreases.

[2]AMP = adenosine monophosphate, HDL = high density lipoprotein, LDL = low density lipoprotein.

clotting activity of platelets, on their response to thrombin (Renaud et al., 1986a,b), and on the regulation of AA metabolism (Budowski and Crawford, 1985). In clinical studies, ALA contributed to lowering of blood pressure (Berry and Hirsch, 1986). In a prospective study, Ascherio et al. (1996) showed that ALA is inversely related to the risk of coronary heart disease in men.

ALA is not equivalent in its biological effects to the long-chain n-3 fatty acids found in marine oils. EPA and DHA are more rapidly incorporated into plasma and membrane lipids and produce more rapid effects than does ALA. Relatively large reserves of LA in body fat, as are found in vegans or in the diet of omnivores in Western societies, would tend to slow down the formation of long-chain n-3 fatty acids from ALA. Therefore, the role of ALA in human nutrition becomes important in terms of long-term dietary intake. One advantage of the consumption of ALA over n-3 fatty acids from fish is that the problem of insufficient vitamin E intake does not exist with high intake of ALA from plant sources.

RECOMMENDED DIETARY INTAKES FOR THE N-6 AND N-3 FATTY ACIDS

On April 7 to 9, 1999, an international working group of scientists met at the National Institutes of Health in Bethesda, Maryland, to discuss the scientific evidence relative to dietary recommendations of n-6 and n-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 5 and 6 include AI for n-6 and n-3 essential fatty acids for adults and infant formula or diet, respectively.

Adults. The working group recognized that there are not enough data to determine dietary reference intakes, but these data are useful to make recommendations for AI for adults, as shown in Table 5.

Pregnancy and Lactation. For pregnancy and lactation, the recommendations are the same as those for adults (with the additional recommendation shown in footnote 1 of Table 5) that during pregnancy and lactation women must ingest at least 300 mg DHA/d.

Composition of Infant Formula and Diet. Focus on the composition of the infant formula is of utmost importance, 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 and 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 6).

One recommendation deserves explanation here. After much discussion, consensus was reached on the importance of reducing the n-6 PUFA, even as the n-3 PUFA are increased in the diets of adults and newborns for optimal brain and cardiovascular health and function. This change in the n-6:n-3 ratio is necessary to reduce adverse effects of excesses of AA 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 n-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 n-6 class, which is converted to AA, needs to be reduced. Second, and simultaneously, the n-3 PUFA 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 ALA, the parent compound of the n-3 class; each competes with the other for this desaturase. The presence of ALA in the diet can

TABLE 3. Effects of polyunsaturated fatty acids on several genes encoding enzyme proteins involved in lipogenesis, glycolysis, and glucose transport[1]

Function and gene	Reference	Linoleic acid	α-Linolenic acid	Arachidonic acid	Eicosapentaenoic acid	Docosahexaenoic acid
Hepatic cells						
Lipogenesis						
FAS	Clarke and Jump (1996) Clarke and Jump (1993) Clarke et al. (1990) Clarke et al. (1977)	↓	↓	↓	↓	↓
S14	Clarke and Jump (1996) Clarke and Jump (1993) Clarke et al. (1990) Clarke et al. (1977)	↓	↓	↓	↓	↓
SCD1	Ntambi (1991)	↓	↓	↓	↓	↓
SCD2	DeWillie and Farmer (1993)	↓	↓	↓	↓	↓
ACC	Clarke and Jump (1996)	↓	↓	↓	↓	↓
ME	Clarke and Jump (1996)	↓	↓	↓	↓	↓
Glycolysis						
G6PD	Jump and Clarke (1994)	↓				
GK	Jump and Clarke (1994)	↓	↓	↓	↓	↓
PK	Liimaatta et al. (1994)	—	↓	↓	↓	↓
Mature adiposites						
Glucose transport						
GLUT4	Tebby et al. (1994)	—	—	↓	↓	—
GLUT1	Tebby et al. (1994)	—	—	↑	↑	—

inhibit the conversion of the large amounts of LA in the diets of Western industrialized countries that contain too much dietary plant oils rich in n-6 PUFA (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.

THE RETURN OF N-3 FATTY ACIDS INTO THE FOOD SUPPLY

Products

There are now many products in the market that are enriched with n-3 fatty acids. A partial list follows.

1. Oils. Oils rich in ALA such as canola, flaxseed, perilla, and soybean. In addition, avoidance of vegetable oils rich in n-6 fatty acids and the use of oils lower in LA such as olive oil, canola oil, and new vegetable oils rich in monounsaturated oils help bring about an improvement in the LA/ALA ratio (Simopoulos and Robinson, 1999).
2. Bakery products. Flaxseed flour and encapsulated fish oils (Andersen, 1995) are used in bakery products, including breads. Enrichment of almost any food with n-3 fatty acids is possible by microencapsulation: bread, breakfast cereal, pasta, biscuits and cookies, cakes, ready-made meals, sausages, fruit bars, diet powders, fruit juices (Germany), and infant formula (Spain).

TABLE 4. Effects of polyunsaturated fatty acids on several genes encoding enzyme proteins involved in cell growth, early gene expression, adhesion molecules, inflammation, β-oxidation, and growth factors[1]

Function and gene	Reference	Linoleic acid	α-Linolenic acid	Arachidonic acid	Eicosapentaenoic acid	Docosahexaenoic acid
Cell growth and early gene expression						
c-fos	Sellmayer et al., 1996	—	—	↑	↓	↓
Egr-1	Sellmayer et al., 1996	—	—	↑	↓	↓
Adhesion molecules						
VCAM-1 mRNA[2]	De Caterina et al., 1996	—	—	↓	[3]	↓
Inflammation						
IL-1β	Robinson et al., 1996	—	—	↑	↓	↓
β-oxidation						
Acyl-CoA oxidase[4]	Clarke and Jump, 1996	↑	↑	↑	↑↑	↑
Growth factors						
PDGF	Kaminski et al., 1993	—	—	↑	↓	↓

[1]VCAM = vascular cell adhesion molecule; IL = interleukin; PDGF = platelet-derived growth factor, ↓ = suppresses or decreases, and ↑ = induces or increases.

[2]Monounsaturated fatty acids (MONO) also suppress VCAM1 mRNA but to a lesser degree than does docosahexaenoic acid (DHA). Arachidonic acid also suppresses to a lesser extent than DHA.

[3]Eicosapentaenoic acid has no effect by itself but enhances the effect of DHA.

[4]MONO also induce acyl-CoA oxidase mRNA.

TABLE 5. Adequate intakes (AI) for adults[1]

Fatty acid	2,000 kcal Diet (g/d)	% Energy
LA	4.44	2.0
(Upper limit)[2]	6.67	3.0
ALA	2.22	1.0
DHA + EPA	0.65	0.3
DHA to be at least[3]	0.22	0.1
EPA to be at least	0.22	0.1
TRANS-FA		
(Upper limit)[4]	2.00	1.0
SAT		
(Upper limit)[5]	. . .	<8.0
MONO[6]	. . .	. . .

[1]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 intake 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, and MONO = monounsaturated fatty acids. From Simopoulos et al., 1999.

[2]Although the recommendation is for AI, the working group felt that there is enough scientific evidence to also state an upper limit for LA of 6.67 g/d based on a 2,000 kcal diet or of 3.0% of energy.

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

[4]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).

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

[6]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 to 40% of energy) but with special attention to the importance of weight control and reduction of obesity.

3. Eggs. Changes in chicken feed lead to enrichment of n-3 fatty acids in eggs. n-3-Enriched eggs are found in many markets around the world. The chicken diets are enriched with fishmeal, flaxseed, or DHA from algae (Simopoulos and Salem, 1989, 1992; Abril and Barclay, 1998; Van Elswyk, et al., 1998). These eggs have a lower n-6:n-3 ratio and contain significant amounts of AA and DHA and are modeled after the "natural egg" (Ampelistra, Table 7), which is the egg obtained under completely natural conditions (Simopoulos and Salem, 1989, 1992).
4. Infant formula. Human milk contains AA, DHA, and EPA, whereas infant formula based on cow milk does not. In Europe and the Far East, infant formula is now enriched with AA and DHA from various sources (Koletzko, 1995; Kyle, 1998). However, in the United States, infant formula does not yet contain AA and DHA.
5. Milk. Research shows promising results in increasing DHA in cow milk (Wright et al., 1998).
6. Mayonnaises, margarines, and salad dressings. Hydrogenated fish oils and canola oils were used in the preparation of mayonnaise, margarines, and salad dressings (Andersen, 1995).
7. Meat and poultry products. Research on how best to titrate the amount of fish oils in animal feeds without affecting stability and organoleptic properties is advancing in many parts of the world. Poultry, cattle, and pigs are being studied, and the consumption of n-3-enriched meats is not far in the future (Abril and Barclay, 1998; Howe, 1998; Mandell et al., 1998).
8. Farmed fish. There is a need to further improve the fatty acid composition of fish in aquaculture (Van Vliet and Katan, 1990).

Enriched processed foods are a feasible way to increase EPA and DHA intake and bring about beneficial changes (Lovegrove et al., 1997). Consumption of the new products modulates tissue fatty acid composition, decreases triglycerides, platelet aggregation, and reduces low-density lipoprotein particle density, making it less atherogenic (Van Elswyk et al., 1998).

Issues

Advances in research are solving a major problem of the deficiency of n-3 fatty acids in Western diets. Yet, many questions need to be answered. How much n-3 fatty acids should be in each serving? How much LNA and how much EPA, docosapentaenoic acid (DPA; 22:5n-3), and DHA? What should be the ratio of total n-6:n-3, how much LA:ALA, and what should be the proportions of the n-3 fatty acids EPA, DPA, and DHA? What are the best models—animals in the wild, fish in the wild, mother's milk from women on a Paleolithic diet, or a Greek/Mediterranean diet (Simopoulos and Visioli, 2000)?

TABLE 6. Adequate intake (AI) for infant formula or diet[1]

Fatty acid	Fatty Acids (%)
LA[2]	10.00
ALA	1.50
AA[3]	0.50
DHA	0.35
EPA[4]	
(Upper limit)	<0.10

[1]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 = alpha-linolenic acid, AA = arachidonic acid, DHA = docosahexaenoic acid, EPA = eicosapentaenoic acid, TRANS-FA = trans fatty acids, SAT = saturated fatty acids, and MONO = monounsaturated fatty acids. From Simopoulos et al., 1999.

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

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

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

TABLE 7. Fatty acid levels (mg/g 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[4]	0.4	0.4	0.9	0.9
M:S ratio[5]	1.4	1.4	1.2	1.1
n-6:n-3 ratio	1.3	19.9	6.6	1.6

[1]Modified from Simopoulos and Salem, 1992.

[2]The eggs were hard-boiled, and their fatty acid composition and lipid content were assessed as described elsewhere (Simopoulos and Salem, 1989).

[3]Greek eggs were from free-range chickens; supermarket eggs were standard US Department of Agriculture eggs found in US supermarkets; fishmeal eggs had main source of fatty acids provided by fishmeal and whole soybeans; flax eggs had main source of fatty acids provided by flax flour.

[4]P:S = Polyunsaturates:saturates.

[5]M:S = Monounsaturates:saturates.

What can we learn from the composition of edible wild plants? We have been saying that edible wild plants contain more ALA than LA, but Guil et al. (1996) showed that many of the edible wild plants along the Mediterranean contain in addition AA, EPA, DPA, and DHA (Table 8). Table 9 shows that certain plants are higher in AA than EPA, DPA, and DHA. The findings of Guil et al. are provocative and important. Although their findings need to be confirmed, they point to a need to have precise information on the fatty acid composition of traditional

TABLE 8. Edible wild plants (% fatty acid content)[1]

Species	Arachidonic acid	Eicosapentaenoic acid	Docosapentaenoic acid	Docosahexaenoic acid
Wild beet (*Beta maritima L.*)	0.52	0.54	0.49	0.65
Hoary cress (*Cardaria draba L.*)	0.56	2.16	0.00	0.00
Goosefoot (*Ch. opulifolium Schrader*)	0.00	3.06	0.74	2.30
Rock samphire (*Crithmum maritimum L.*)	0.00	0.76	0.76	0.00
Plantain (*Plantago major L.*)	1.02	1.27	0.00	1.47
Hedge mustard (*Sisymbrium irio L.*)	0.32	0.55	0.21	0.83

[1]Data are from Guil et al. (1996).

TABLE 9. Edible wild plants high in arachidonic acid (% fatty acid content)[1]

Species	Arachidonic acid	Eicosapentaenoic acid	Docosapentaenoic acid	Docosahexaenoic acid
Goosefoot (*Chenopodium album L.*)	1.30	0.36	0.00	0.00
Goosefoot (*Ch. murale L.*)	1.01	0.41	0.00	0.00
Common Mallow (*Malva sylvestris L.*)	5.30	0.00	0.00	0.00
Sow-Thistle-of-the-Wall (*Sonchus tenerrimus L.*)	1.83	0.00	0.38	0.00

[1]Data are from Guil et al. (1996).

diets. Obviously, there is a need to further investigate edible wild plants in order to determine the terrestrial and marine sources of the 20- to 22-carbon PUFA.

CONCLUSIONS AND RECOMMENDATIONS

Essential fatty acids, n-6 (LA) and n-3 (ALA), have been part of our diet since the beginning of human life. Before the agricultural revolution 10,000 yr ago humans consumed about equal amounts of both. Over the past 150 yr this balance has been upset. Current estimates in Western cultures suggest a ratio of n-6 to n-3 fatty acids of 10 to 20 or 25:1 instead of 1 to 2:1.

The n-6 and n-3 fatty acids are the parent fatty acids for the production of eicosanoids, e.g., prostaglandins, thromboxanes, and leukotrienes. Eicosanoids derived from n-6 fatty acids have opposite metabolic properties to those derived from n-3 fatty acids. A balanced intake of both n-6 and n-3 fatty acids is essential for health. There is competition among the enzymes involved in the elongation and desaturation of LA and ALA. A ratio of LA to ALA of 4:1 or less has been shown to be optimal for the elongation of 11 g of ALA to 1 g EPA. This relationship is important for vegetarians because their diets are typically rich in LA and poor in ALA. Because EPA is biologically more active than ALA and high amounts of LA decrease the conversion of ALA to EPA, the optimal intake of LA relative to ALA is crucial for normal metabolism.

Clinical interventions provide further support for the beneficial effects of n-3 fatty acids in the prevention and management of cardiovascular disease, hyperinsulinemia, and possibly type 2 diabetes. n-3 Fatty acids affect coronary heart disease beneficially not by changing blood cholesterol concentrations, although EPA and DHA do lower triacylglycerol concentrations, but by reducing blood clotting in vessel walls (Simon et al., 1995; Eritsland, et al., 1996) and ventricular arrhythmias (Burr et al., 1989; de Lorgeril et al., 1994, 1996, 1999; Sellmayer et al., 1995; Siscovick et al., 1995).

n-3 Fatty acids must become incorporated into foods rather than used solely as dietary supplements, which is a quasi-pharmaceutical approach. Furthermore, the development of a variety of n-3-rich foodstuffs would allow increased dietary intakes with little change of dietary habits.

The n-3 fatty acids maintain preventive and therapeutic properties when packaged in a food other than fish. Efficient use of dietary n-3 fatty acids will require the simultaneous reduction in the food content of n-6 fatty acids and their substitution with monounsaturated oils. Dietary n-3 fats give rise to higher tissue levels of EPA when the "background" diet is low in n-6 fats. Compared with n-6 fatty acids, olive oil increases the incorporation of n-3 fatty acids into tissues.

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 (Simopoulos, 1989, 1998). This change in focus will necessitate the development of research for nutritional evaluation of the various food products and educational programs for professionals and the public (Simopoulos, 1998). The definition of food safety will have to expand in order to include the adverse effects of nutrient structural changes (i.e., *trans* fatty acids) and food composition (i.e., ratio of n-6:n-3 fatty acids) (Simopoulos, 1998). The 21st century will enhance the scientific base for product development and will expand collaboration among agricultural, nutritional, and medical scientists. This enhancement should bring about a greater involvement of nutritionists and dietitians in industrial research and development to respond to an ever-increasing consumer interest in the health attributes of food.

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