Review Article

Omega-3 fatty acids in health and disease and in growth and development[1-4]

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ABSTRACT Several sources of information suggest that man evolved on a diet with a ratio of ω6 to ω3 fatty acids of ~1 whereas today this ratio is ~10:1 to 20–25:1, indicating that Western diets are deficient in ω3 fatty acids compared with the diet on which humans evolved and their genetic patterns were established. Omega-3 fatty acids increase bleeding time; decrease platelet aggregation, blood viscosity, and fibrinogen; and increase erythrocyte deformability, thus decreasing the tendency to thrombus formation. In no clinical trial, including coronary artery graft surgery, has there been any evidence of increased blood loss due to ingestion of ω3 fatty acids. Many studies show that the effects of ω3 fatty acids on serum lipids depend on the type of patient and whether the amount of saturated fatty acids in the diet is held constant. In patients with hyperlipidemia, ω3 fatty acids decrease low-density-lipoprotein (LDL) cholesterol if the saturated fatty acid content is decreased, otherwise there is a slight increase, but at high doses (32 g) they lower LDL cholesterol; furthermore, they consistently lower serum triglycerides in normal subjects and in patients with hypertriglyceridemia whereas the effect on high-density lipoprotein (HDL) varies from no effect to slight increases. The discrepancies between animal and human studies most likely are due to differences between animal and human metabolism. In clinical trials eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the form of fish oils along with antirheumatic drugs improve joint pain in patients with rheumatoid arthritis; have a beneficial effect in patients with ulcerative colitis; and in combination with drugs, improve the skin lesions, lower the hyperlipidemia from etretinates, and decrease the toxicity of cyclosporin in patients with psoriasis. In various animal models ω3 fatty acids decrease the number and size of tumors and increase the time elapsed before appearance of tumors. 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. Because ω3 fatty acids are essential in growth and development throughout the life cycle, they should be included in the diets of all humans. Omega-3 and ω6 fatty acids are not interconvertible in the human body and are important components of practically all cell membranes. Whereas cellular proteins are genetically determined, the polyunsaturated fatty acid (PUFA) composition of cell membranes is to a great extent dependent on the dietary intake. Therefore appropriate amounts of dietary ω6 and ω3 fatty acids need to be considered in making dietary recommendations, and these two classes of PUFAs should be distinguished because they are metabolically and functionally distinct and have opposing physiological functions. Their balance is important for homeostasis and normal development. Canada is the first country to provide separate dietary recommendations for ω6 and ω3 fatty acids. *Am J Clin Nutr* 1991;54:438–63.

KEY WORDS Polyunsaturated fatty acids, ω3 fatty acids, ω6 fatty acids, lipids, α-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid, essentiality in growth and development, cardiovascular disease, hypertension, inflammation, arthritis and other autoimmune disorders, psoriasis, cancer, prostaglandins, leukotrienes, interleukins, platelet-derived growth factor, endothelium-derived relaxing factor

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Introduction

In the 1950s many investigators studied the effects of corn oil and fish oil in humans primarily by determining their effects on serum cholesterol concentrations in patients with atherosclerosis (1–5). Corn oil (ω6 fatty acid), an odorless, clear oil, was found to lower cholesterol, particularly when it replaced butter or lard in the diet. Although sardine oil (ω3 fatty acid) had similar effects and in addition lowered serum triglyceride concentrations, it was not given the attention it deserved (2). Vegetable oils rich in ω6 fatty acids displaced other fats in the US diet and eventually in the diet of Western Europeans, on the evidence of their hypocholesterolemic properties. Omega-3 fatty acids were not considered as important agents in the control of cardiovascular disease (CVD) despite experimental and clinical work pointing to their importance (6–9). With the emphasis on the lipid hypothesis, the lowering of serum cholesterol became the dominant factor for the control of coronary heart disease (CHD). As a result the primary contributions of inflammation and thrombosis in the development of CHD were not adequately investigated until the late 1970s and 1980s and now.

In the 1970s Bang and Dyerberg (10–12) reported their findings that Eskimos had low rates of CHD and cancer despite their high-fat diet. Bang and Dyerberg (13, 14) emphasized the importance of eicosapentaenoic acid (EPA) in the prevention of heart attacks because of its antithrombotic effects, the increase in bleeding time, and its effect in lowering serum cholesterol concentrations. Subsequently other epidemiologic studies confirmed these findings and showed that fish-eating populations other than the Eskimos had less CVD than did those who consumed less fish (15–20). Even as little as 30–40 g of fish twice a week made a difference (17). In two other studies this effect of higher fish intake was not seen, most likely because of simultaneous high saturated fatty acid intake (21, 22).

Additional clinical investigations and experimental studies confirmed the initial observations: when diets are supplemented with ω3 fatty acids, the latter partially replace the ω6 fatty acids in the membranes of practically all cells (ie, erythrocytes, platelets, endothelial cells, monocytes, lymphocytes, granulocytes, neuronal cells, fibroblasts, retinal cells, hepatic cells, and neuroblastoma cells); ω3 fatty acids modulate prostaglandin metabolism and decrease triglycerides; and in high doses ω3 fatty acids lower cholesterol and have antithrombotic and anti-inflammatory properties. These studies were extensively reviewed and reported (23–28).

The 1980s were a period of expansion in our knowledge about polyunsaturated fatty acids (PUFAs) in general and ω3 fatty acids in particular. Today we know that ω3 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, arthritis, other inflammatory and autoimmune disorders, and cancer. Research has been carried out in animal models, tissue cultures, and humans. The original observational studies have given way to controlled clinical trials. A new arena for ω3 fatty acids has emerged as adjuvants to drug treatment leading to synergism (potentiating the effects of drugs) or to decreasing their toxicity. This immense expansion in our knowledge is shown by the increase in the number of publications from 110 in 1984 to 319 in 1989 worldwide (based on January 10, 1990, NIH-MEDLINE search output), amounting to 1541 for the 5-year period (**Fig 1**) (29). In September 1989 the US National Library of Medicine published a selective bibliography on the health benefits of fish oils that included publications from January 1985 to May 1989 but was intentionally limited to human studies. Cited were 576 articles published in 155 journals from around the world. The bibliography is indicative of the explosive and expanding interest in the health benefits of fish oils and ω3 fatty acids.

Although a number of important conferences had been held before 1985, such as the Reading conference held in 1984, the expansion of this impressive growth in our knowledge can almost be dated from the 1985 conference Health Effects of Polyunsaturated Fatty Acids in Seafoods, held June 24–25, 1985, in Washington, DC (24). The 1985 conference was the first major international conference to establish the fact that ω3 fatty acids of marine origin, EPA and docosahexaenoic acid (DHA), play important roles in prostaglandin metabolism, thrombosis and atherosclerosis, immunology and inflammation, and membrane function. The 1985 conference participants recommended *1*) the support of research on the role of ω3 fatty acids in growth and development and in health and disease and on the mechanisms involved and *2*) the establishment of a test-materials program to specifically define nutritional requirements throughout the life cycle, and dose and type of ω3 fatty acid in intervention studies and in clinical trials.

After the conference the National Institutes of Health (NIH) published a series of program announcements inviting applications for research on the role of ω3 fatty acids in growth and development and in health and disease (**Table 1**) (29). To support the research, in December 1986 the US Department of Commerce developed a special program, the Biomedical Test Materials (BTM) program, which provides standardized test materials of known composition of EPA and DHA nationally and internationally. These test materials contain 0.2 mg tertiary butylhydroquinone (TBHQ)/g as an antioxidant and 2 mg tocopherols/g (30).

The response of the scientific community made it obvious from the very beginning that research with ω3 fatty acids would develop along two avenues: *1*) studies on the essentiality of the ω3 fatty acids that would define their role in growth and development throughout the life cycle based on the deficiency model and improvement in various functions upon supplementation with ω3 fatty acids and *2*) studies involving mechanisms in the

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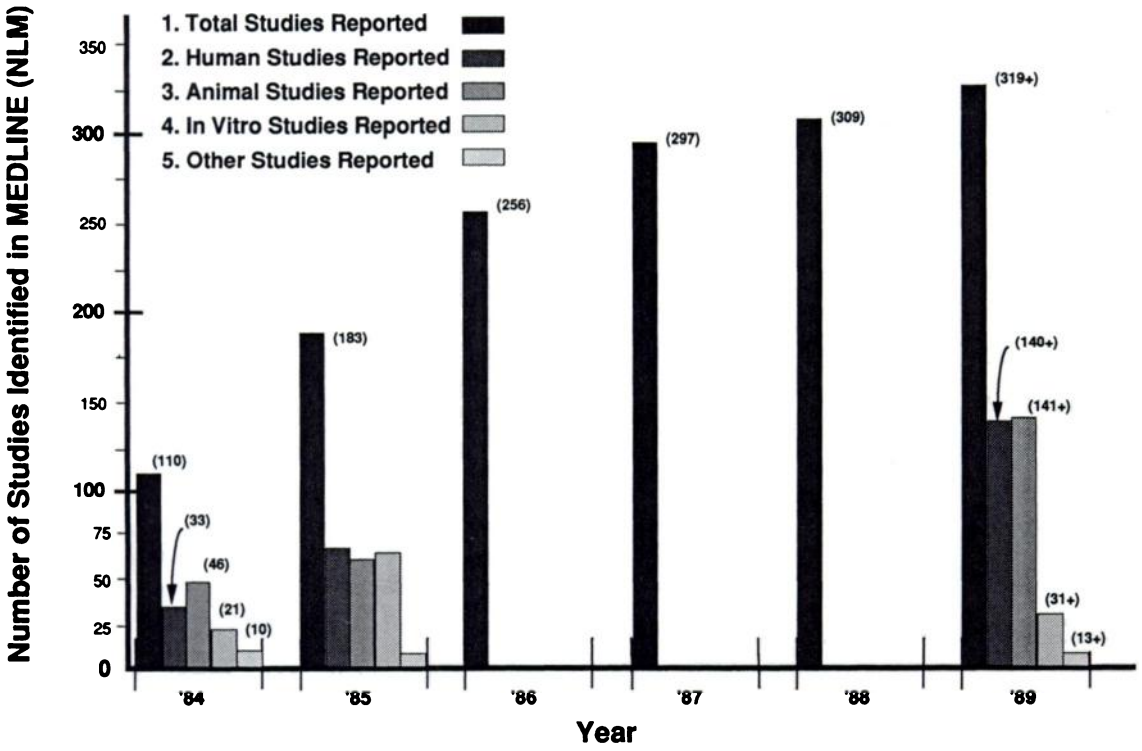

FIG 1. Publications of marine oil and fish oil and ω3 fatty acid studies retrieved from MEDLINE (National Library of Medicine, National Institutes of Health) from 1984 to 1989. (Data as of January 10, 1990, from MEDLINE. By June 1989 the total number of publications for 1989 was 386.) Reproduced with permission from reference 29.

understanding of chronic diseases that would use the supplementation approach by increasing the amount of fish in the diet, substituting fish for meat, or using fish oils.

Since 1985 many conferences have been held in various parts of the world to review progress in the field, define gaps in the knowledge, and develop a research agenda (24, 25, 31–34). In addition, major reviews and commentaries appeared in leading medical and nutrition journals (27, 28, 35–37). The most recent research advances were extensively discussed at the Second International Conference on the Health Effects of Omega-3 Polyunsaturated Fatty Acids in Seafoods, held March 20–23, 1990, in Washington, DC (25).

This paper presents the state of the art in ω3 fatty acid research, drawn from the published literature, the NIH database Computer

TABLE 1
Requests for applications (RFAs) and program announcements (PAs) by NIH and ADAMHA: December 6, 1985, to April 17, 1987*

Date	Title	Type	Institute
December 6, 1985	Biological Mechanisms of ω3 Fatty Acids in Health and Disease States	PA	NCC, (NIADDK, NINCDS, NIAID NICHD, NIGMS, NEI, NIEHS, NIA, NIAAA, NIMH)
June 1986	Studies of ω3 Polyunsaturated Fatty Acids in Thrombosis and Cardiovascular Disease	RFA	NHLBI
August 22, 1986	The Role of ω3 Polyunsaturated Fatty Acids in Cancer Prevention	PA	NCI
April 17, 1987	The Role of ω3 Polyunsaturated Fatty Acid in Cancer Prevention (reissued)	PA	NCI
October 22, 1987	Fatty Acid Derived Mediators of Inflammation	RFA	NIAID

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NIH: National Institutes of Health; ADAMHA: Alcohol, Drug Abuse and Mental Health Administration; NCC: Nutrition Coordinating Committee; NIADDK: National Institute of Diabetes and Digestive and Kidney Diseases; NINCDS: National Institute of Neurological and Communicative Disorders and Stroke; NIAID: National Institute of Allergy and Infectious Diseases; NICHD: National Institute of Child Health and Human Development; NIGMS: National Institute of General Medical Sciences; NEI: National Eye Institute; NIEHS: National Institute of Environmental Health Sciences; NIA: National Institute on Aging; NIAAA: National Institute on Alcohol Abuse and Alcoholism; NIMH: National Institute of Mental Health; NHLBI: National Heart, Lung, and Blood Institute; NCI: National Cancer Institute; and NIAID: National Institute of Allergy and Infectious Diseases.

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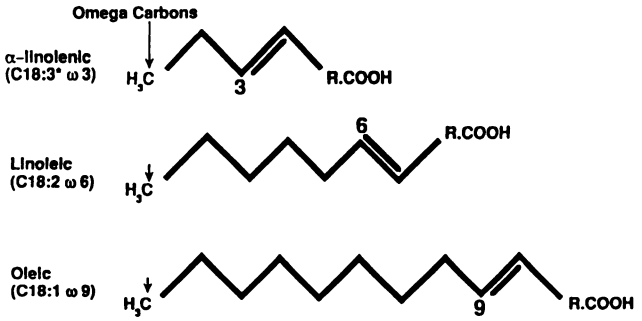

FIG 2. Structural formulas for ω3 (α-linoleic), ω6 (linoleic), and ω9 (oleic) fatty acids. The first number (before the colon) gives the number of carbon atoms in the molecule and the second gives the number of double bonds. ω3, ω6, and ω9 indicate position of the first double bond in a given fatty acid molecule.

Retrieval of Information on Scientific Projects (CRISP), and presentations at the 1990 conference.

Omega-3 and ω6 fatty acids: sources, elongation, and desaturation

Unsaturated fatty acids consist of monounsaturates and polyunsaturates. There are two classes of PUFAs, ω3 and ω6. The distinction between ω3 and ω6 fatty acids is based on the location of the first double bond, counting from the methyl end of the fatty acid molecule. Monounsaturates are represented by oleic acid, which can be synthesized by all mammals including humans. Its double bond is between the 9th and 10th carbon atoms (**Fig 2**).

Omega-3 and ω6 fatty acids are also known as essential fatty acids (EFAs) because humans, like all mammals, cannot make them and must obtain them in their diet. Omega-6 fatty acids are represented by linoleic acid (LA) and ω3 fatty acids by α-linolenic acid (LNA).

LA is plentiful in nature and is found in the seeds of most plants except for coconut, cocoa, and palm. LNA on the other hand is found in the chloroplast of green leafy vegetables. Both EFAs are metabolized to longer-chain fatty acids of 20 and 22 carbon atoms. LA is metabolized to arachidonic acid (AA) and LNA, to EPA and DHA, increasing the chain length and degree of unsaturation by adding extra double bonds to the carboxyl group (**Fig 3**).

Humans and animals except for carnivores such as lions and cats can convert LA to AA and LNA to EPA and DHA (38). This conversion was shown by using deuterated LNA (39). There is competition between ω3 and ω6 fatty acids for the desaturation enzymes. However, both Δ-4 and Δ-6 desaturases prefer ω3 to ω6 fatty acids (38, 40, 41). There is some evidence that Δ-6 desaturase decreases with age (38). Premature infants (42), hypertensive individuals (43), and some diabetics (44) are limited in their ability to make EPA and DHA from LNA. These findings are important and need to be considered when making dietary recommendations. EPA and DHA are found in the oils of fish, particularly fatty fish (**Table 2**) (24). AA is found predominantly in the phospholipids of grain-fed animals.

LA, LNA, and their long-chain derivatives are important components of animal and plant cell membranes. In mammals and birds the ω3 fatty acids are distributed selectively among lipid classes. LNA is found in triglycerides, in cholesteryl esters, and in very small amounts in phospholipids. EPA is found in cholesteryl esters, triglycerides, and phospholipids. DHA is found mostly in phospholipids. In mammals, including humans, the cerebral cortex (45), retina (46), and testis and sperm (47) are particularly rich in DHA. DHA is one of the most abundant components of the brain's structural lipids. DHA, like EPA, can be derived only from direct ingestion or by synthesis from dietary EPA or LNA.

Evolutionary aspects: the ω3 and ω6 fatty acid balance

On the basis of estimates from studies in paleolithic nutrition and modern-day hunter-gatherer populations, humans evolved on a diet that was much lower in saturated fatty acids than is today's diet. Furthermore, the diet contained small but roughly equal amounts of ω6 and ω3 PUFAs (**Fig 4**) (48–50).

Linoleate series		Linolenate series	
C18:2w6	Linoleic acid	C18:3w3	Alpha-linolenic acid
	Δ^6 desaturase		Δ^6 desaturase
	↓		↓
C18:3w6	Gamma-linolenic acid	C18:4w3	
	↓		↓
C20:3w6	Dihomo-gamma-Linolenic Acid	C20:4w3	
	Δ^5 desaturase		Δ^5 desaturase
	↓		↓
C20:4w6	Arachidonic acid	C20:5w3	Eicosapentaenoic acid
	↓		↓
C22:4w6		C22:5w3	Docosapentaenoic acid
	Δ^4 desaturase		Δ^4 desaturase
	↓		↓
C22:5w6	Docosapentaenoic acid	C22:6w3	Docosahexaenoic acid

FIG 3. Essential fatty acid metabolism desaturation and elongation of ω6 and ω3.

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TABLE 2
Content of ω3 fatty acids and other fat components in selected fish*

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

* Per 100 g edible portion, raw. Dashes denote lack of reliable data for nutrient known to be present; Tr, trace (< 0.05 g/100 g food). Adapted from the United States Department of Agriculture Provisional Table on the Content of Omega-3 Fatty Acids and Other Fat Components in Seafoods as presented by Simopoulos et al (24).

Large-scale production of vegetable oils

The increased consumption of ω6 fatty acids in the last 100 y 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 ω6 fatty acids) (51). The invention of the continuous screw press, named Expeller® by VD Anderson, and the steam-vacuum deodorization process by D Wesson made possible the industrial production of cottonseed oil and other vegetable oils for cooking (51). 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 LNA content of the oil while leaving a high concentration of LA. LNA content was reduced because LNA in soybean oil caused many organoleptic problems. It was recently documented 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 (52, 53).

As stated in the introduction, since the 1950s, research on the effects of ω6 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 (1) and subsequent work by Keys et al (2) firmly established the ω6 fatty acids as the important fatty acids in the field of CVD. 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

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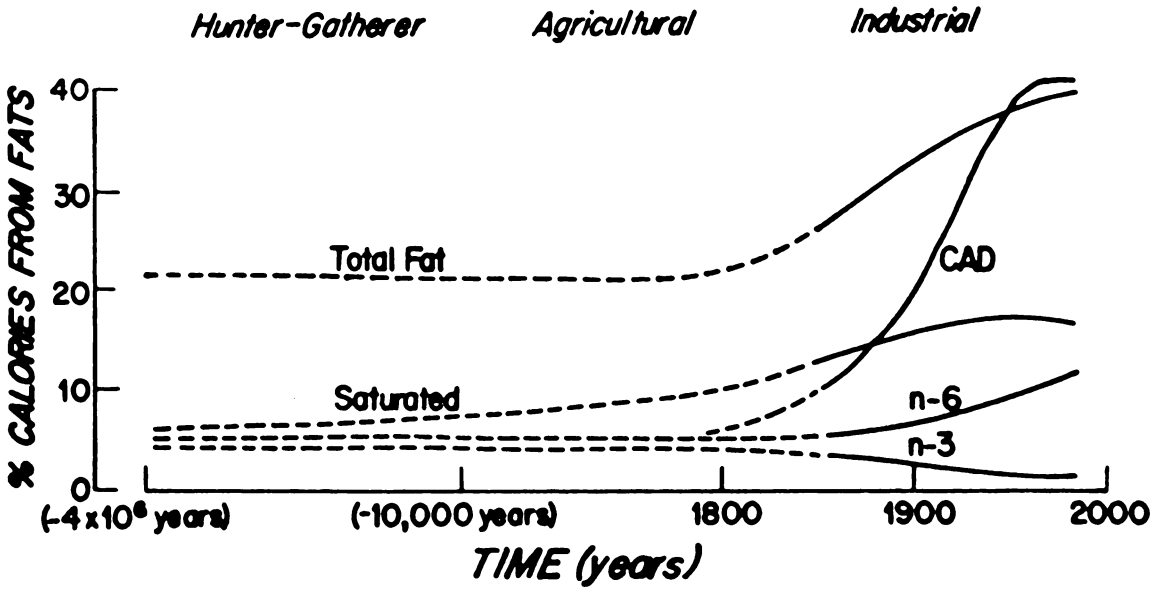

FIG 4. Scheme of the relative percentages of different dietary fatty acids (saturated fatty acids and ω6 and ω3 unsaturated fatty acids) and possible changes subsequent to industrial food processing, involving fattening of animal husbandry and hydrogenation of fatty acids. Reproduced from reference 48.

diet and the greater increase in vegetable oils rich in ω6 fatty acids.

Agribusiness and modern agriculture

Agribusiness contributed further to the decrease in ω3 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% (54), and contain about five times more PUFAs per gram than is found in domestic livestock (55, 56). Most importantly, 4% of the fat of wild animals contains EPA. Domestic beef contains very small or undetectable amounts of LNA because cattle are fed grains rich in ω6 fatty acids and poor in ω3 fatty acids (57) whereas deer that forage on ferns and mosses contain more ω3 fatty acids (LNA) in their meat.

Modern agriculture with its emphasis on production has decreased the ω3 fatty acid content in many foods: green leafy vegetables, animal meats, eggs, and even fish (58–61). Foods from edible wild plants contain a good balance of ω6 and ω3 fatty acids (**Table 3**) (58). Modern aquaculture produces fish that contain less ω3 fatty acid than do fish grown naturally in the ocean, rivers, and lakes (**Table 4**) (60). As can be seen from **Table 5** comparing the fatty acid composition of egg yolk from free-ranging chickens and the standard US Department of Agriculture (USDA) egg, the former has an ω6-ω3 ratio (ω6:ω3) of 1.3 whereas the USDA egg has an ω6:ω3 of 19.4 (59).

Imbalance of ω6:ω3

Before the 1940s cod-liver oil was ingested mainly by children as a source of vitamins A and D with the usual dose being a teaspoon. Once these vitamins were synthesized consumption of cod-liver oil was drastically decreased. Thus an absolute and relative change of ω6:ω3 in the food supply of Western societies has occurred over the last 100 y (**Fig 4**) (48). A balance existed between ω6 and ω3 for millions of years during the long evolutionary history of the genus *Homo*, and genetic changes occurred partly in response to these dietary influences (49). However, rapid dietary changes over short periods of time as have occurred over the past 100–150 y is a totally new phenomenon in human evolution.

Homo sapiens made his appearance ~ 40 000 y ago and the human genetic constitution has remained relatively unchanged. Then, 10 000 y ago, agriculture began to bring changes slowly in food consumption. It is only since the industrial revolution that changes in food consumption have occurred rapidly. These changes are reflected in increased consumption of animal fat and in imbalances in ω6:ω3. The ratio that was ~1 from vegetable and animal sources during the evolutionary period for humans is now estimated by Hunter (61) to be 10–11:1 from vegetable sources. From evidence that the per capita consumption of major foods in 1987 was 61.4 kg red meat, 28.6 kg chicken, and 6.8 kg fish plus the increases in ω6 fatty acids from vegetable oils, the ratio is closer to 20–25:1 from vegetable and

TABLE 3
Fatty acid content of plants*

Fatty acid	Purslane	Spinach	Buttercrunch lettuce	Red leaf lettuce	Mustard
			mg/g wet wt		
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	8.50	1.70	0.60	0.702	1.101

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TABLE 4
Fat content and fatty acid composition of wild and cultured trout, eel, and salmon*

	Trout (*Salmo gairdneri* and *Salmo trutta* fario)		Eel (*Anguilla anguilla*)		Salmon (*Salmo salar*)	
	Wild (n = 2)	Cultured (n = 9)	Wild (n = 4)	Cultured (n = 4)	Wild (n = 2)	Cultured (n = 2)
Fat (g/100 g)	5 ± 3	6 ± 1	21 ± 6	30 ± 2†	10 ± 0.1	16 ± 0.6‡
Fatty acids (g/100 g fatty acid)						
18:3ω3	3 ± 2	1 ± 0.3‡	2 ± 2	1 ± 0.3	1 ± 0.1	1 ± 0.1
20:5ω3	7 ± 0.6	4 ± 1‡	4 ± 2	3 ± 0.6	5 ± 0.2	5 ± 0.1
22:6ω3	15 ± 2	13 ± 1†	4 ± 2	6 ± 0.4	10 ± 2	7 ± 0.1†
Other ω3§	5 ± 0.6	2 ± 0.7‡	3 ± 1	2 ± 0.2†	3 ± 0.5	4 ± 0.1
18:2ω6	4 ± 3	9 ± 2‡	2 ± 2	5 ± 0.3‡	1 ± 0.1	3 ± 0.1
Other ω6‖	1 ± 0.4	0.6 ± 0.1‡	2 ± 0.3	0.4 ± 0.1‡	0.2 ± 0.1	0.5 ± 0.1
Total ω3	30 ± 0.2	20 ± 3‡	14 ± 3	12 ± 1	20 ± 2	17 ± 0.2
Total ω6	5 ± 3	9 ± 2†	3 ± 1	6 ± 0.3‡	2 ± 0.1	3 ± 0.1‡
ω3:ω6	7 ± 5	2 ± 0.6‡	5 ± 2	2 ± 0.3†	11 ± 2	6 ± 0.1†

* Reproduced from reference 60. $\bar{x}$ ± SD; n, number of lots; each lot consisted of about six trout or eel or one or two salmon.
†‡ Significantly different from wild: †$P < 0.05$, ‡$P < 0.01$.
§ 18:4ω3 + 20:3ω3 + 22:5ω3.
‖ 20:4ω6 + 22:4ω6.

animal sources. 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 ~50 mg·capita^{-1}·d^{-1} and the amount of DHA is 80 mg·capita^{-1}·d^{-1}. The two main sources are fish and poultry (62).

Biological effects of ω3 fatty acids in relation to CHD and hypertension

Eicosanoid metabolism

AA and EPA are precursors of metabolic products that consist of 20 carbon atoms and are known collectively as eicosanoids (prostaglandins, thromboxanes, and leukotrienes) (**Fig 5**) (63, 64). The discovery of prostaglandins and subsequently the recognition that AA is the precursor of the 2-series of prostanoids (prostaglandins and thromboxanes) and of leukotrienes of the 4-series expanded the horizons of research on ω6 and ω3 fatty acids because LA, the precursor of AA, is the predominant PUFA in the Western diet. EPA and DHA are precursors of the prostanoids of the 3-series and leukotrienes of the 5-series. The discovery in 1979, by Needleman et al (65), that prostaglandins derived from EPA have different biological properties than do those derived from AA stimulated further research on fish oils and on the nutritional aspects of prostaglandins.

Competition between the two different classes of PUFAs occurs in prostaglandin formation: EPA competes with AA for prostaglandin and leukotriene synthesis at the cyclooxygenase and lipoxygenase level. When humans ingest fish or fish oil, the EPA and DHA from the diet partially replace the ω6 fatty acids, especially AA, in the membranes of probably all cells but especially in the membranes of platelets, erythrocytes, neutrophils, monocytes, and liver cells. As a result, ingestion of EPA and DHA from fish or fish oil leads to *1*) a decreased production of prostaglandin E_2 (PGE_2) metabolites; *2*) a decrease in thromboxane A_2, a potent platelet aggregator and vasoconstrictor; *3*) a decrease in leukotriene B_4 formation, an inducer of inflammation and a powerful inducer of leukocyte chemotaxis and adherence; *4*) an increase in thromboxane A_3, a weak platelet aggregator and a weak vasoconstrictor; *5*) 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; and *6*) an increase in leukotriene B_5, a weak inducer of inflammation and a weak chemotactic agent (63, 64).

Molecular aspects and gene expression: beyond the eicosanoids

The phospholipid class and fatty acid composition and cholesterol content of biomembranes are critical determinants of physical properties of membranes and have been shown to influence a wide variety of membrane-dependent functions, such as integral enzyme activity, membrane transport, and receptor function. The ability to alter membrane lipid composition and function in vivo by diet, even when EFAs are adequately supplied, demonstrates the importance of diet in growth and metabolism (66).

Complex interactions and displacements of the ω3 and ω6 fatty acids take place in plasma and cellular lipids after dietary manipulations. Early steps of cell activation, such as generation of inositol phosphates, are induced by dietary fatty acids (67). The effects of dietary fatty acids on the inositol phosphate pathway indicate that diet-induced modifications of PUFAs at the cellular level affect the activity of the enzymes responsible for the generation of lipid mediators in addition to the formation of products (eicosanoids) directly derived from their fatty acid precursors. This shows that dietary fats affect key processes in cell function.

The role of ω3 fatty acids in the control of gene expression is an area that is expected to expand over the next 5 years as we begin to understand the role of nutrients in gene expression. It

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TABLE 5
Fatty acid concentrations in chicken egg yolks*

Fatty acid	Greek egg	Supermarket egg
	mg/g yolk	
Saturated		
14:0	1.10	0.70
15:0	—	0.07
16:0	77.60	56.66
17:0	0.66	0.34
18:0	21.30	22.88
Total	100.66	80.65
Monounsaturated		
16:1ω7	21.70	4.67
18:1	120.50	109.97
20:1ω9	0.58	0.68
22:1ω9	—	—
24:1ω9	—	0.04
Total	142.78	115.36
ω6		
18:2ω6	16.00	26.14
18:3ω6	—	0.25
20:2ω6	0.17	0.36
20:3ω6	0.46	0.47
20:4ω6	5.40	5.02
22:4ω6	0.70	0.37
22:5ω6	0.29	1.20
Total	23.02	33.81
ω3		
18:3ω3	6.90	0.52
20:3ω3	0.16	0.03
20:5ω3	1.20	—
22:5ω3	2.80	0.09
22:6ω3	6.60	1.09
Total	17.66	1.73
P:S†	0.4	0.44
ω6:ω3‡	1.3	19.4

* Reproduced with permission from reference 59. Fatty acid composition and lipid content were determined in hard-boiled eggs.
† Ratio of polyunsaturated fatty acids to saturated fatty acids.
‡ Ratio of ω6 to ω3 fatty acids.

is known that nutrients, like hormones, influence and control gene expression, and research is now providing more examples (68). Omega-3 fatty acids in the form of menhaden oil lower the enzyme fatty acid synthetase in the liver, presumably as a consequence of a large decrease in fatty acid synthetase mRNA concentration (69).

Omega-3 fatty acids have been extensively studied in terms of their hypolipidemic, antiatheromatous, anti-inflammatory, antithrombotic, vascular, and other effects described below. In fact, more is known about the effects of ω3 fatty acids in human metabolism than any other class of fatty acids.

Hypolipidemic effects

A review of the clinical investigations published up to February 1988 in peer-reviewed English-language journals was carried out by Harris (37) on the effects of fish oils on lipids and lipoproteins in normal volunteers and in patients with primary hyperlipidemia, isolated hypercholesterolemia (type IIa), combined hyperlipidemia (type IIb), and isolated hypertriglyceridemia (types IV and V). Hypercholesterolemia was defined as low-density-lipoprotein (LDL)-cholesterol concentrations > 4.14 mmol/L and hypertriglyceridemia was defined as plasma triglyceride concentrations > 2.26 mmol/L. There were marked variations in the design of the studies. The amount of fish oil varied from a low of 1.6 g/d to > 100 g/d and the ω3 fatty acids varied from 0.5 to 25 g/d. The length of intervention varied from 2 wk to > 2 y. The ω3 fatty acid intake was in the form of whole fish, cod-liver oil, fish-oil concentrate, fatty acid ethyl esters, or purified EPA ethyl esters.

Effects on normal subjects. Harris (37) found that ω3 fatty acids did not influence LDL cholesterol concentration, but a slight rise (~3%) occurred in high-density-lipoprotein (HDL)-cholesterol concentrations and a 25% decrease occurred in triglyceride concentrations. In other well-controlled studies using relatively lower doses of fish oil (< 20 g/d), similar findings were reported by Sanders and Hochland (70), Zucker et al (71), and Mortensen et al (72). Nagakawa et al (73) used purified EPA with no other dietary change. They found modest decreases in total cholesterol and LDL concentrations, no changes in HDL concentrations, and a marked decrease in triglyceride concentrations.

Effects on patients. In patients with type IIa hyperlipidemia, dietary ω3 fatty acids did not change total or LDL cholesterol, slightly increased HDL, and lowered triglyceride concentrations. In patients with combined hyperlipidemia, type IIb, total cholesterol concentration did not change. LDL and HDL cholesterol concentrations rose by 5–7% and triglyceride concentrations decreased by 38%. Similar results were reported in other well-controlled, low-dose, crossover trials (71, 74–76). In patients with isolated hypertriglyceridemia, total cholesterol and triglyceride concentrations decreased by 8% and 52%, respectively, and LDL and HDL increased by 30% and 10%, respectively. The decrease in total cholesterol resulted from a fall in very-low-density lipoprotein (VLDL). In patients with type IV hyperlipidemia, LDL cholesterol concentration increased by 20% (75, 77).

These studies suggest that the type of patient studied determines the hypolipidemic response to ω3 fatty acid supplementation. In patients with hypertriglyceridemia the fall in total cholesterol is due to the decrease in VLDL. Sanders (78) in an updated review reported similar findings. In addition, he found that in type III patients fish oil lowers triglycerides and cholesterol. Sanders also reviewed the studies in which ω3 fatty acids lowered triglycerides in patients with hypertriglyceridemia and found that these effects of ω3 fatty acids are indeed sustained, contrary to reports by Schectman et al (79). Schectman et al (79) suggested, on the basis of a study on a small group of patients, that triglyceride-lowering effects of fish oils cannot be sustained. This suggestion is not supported by other larger controlled trials. Miller et al (80), in a randomized controlled trial for 3 mo, showed that the triglyceride-lowering effect of 10 g MaxEPA®/d (3.2 g ω3 fatty acids) was sustained. Moreover, Saynor et al (81) showed that the effect is sustained for years. The study of Schectman et al (79) employed an ester concentrate. The failure to sustain the triglyceride-lowering effect in that study could well be related to poor patient compliance (78). Sanders found that as little as 6 g fish oil/d (2 g ω3 fatty acids) has a triglyceride-lowering effect in hypertriglyceridemic patients. The more commonly used dose is 3 g/d for EPA and DHA.

In addition to the type of patient, another factor that affects LDL concentration is whether saturated fatty acids are held constant or decreased during supplementation. In normal subjects when saturated fatty acids were held constant, LDL tended

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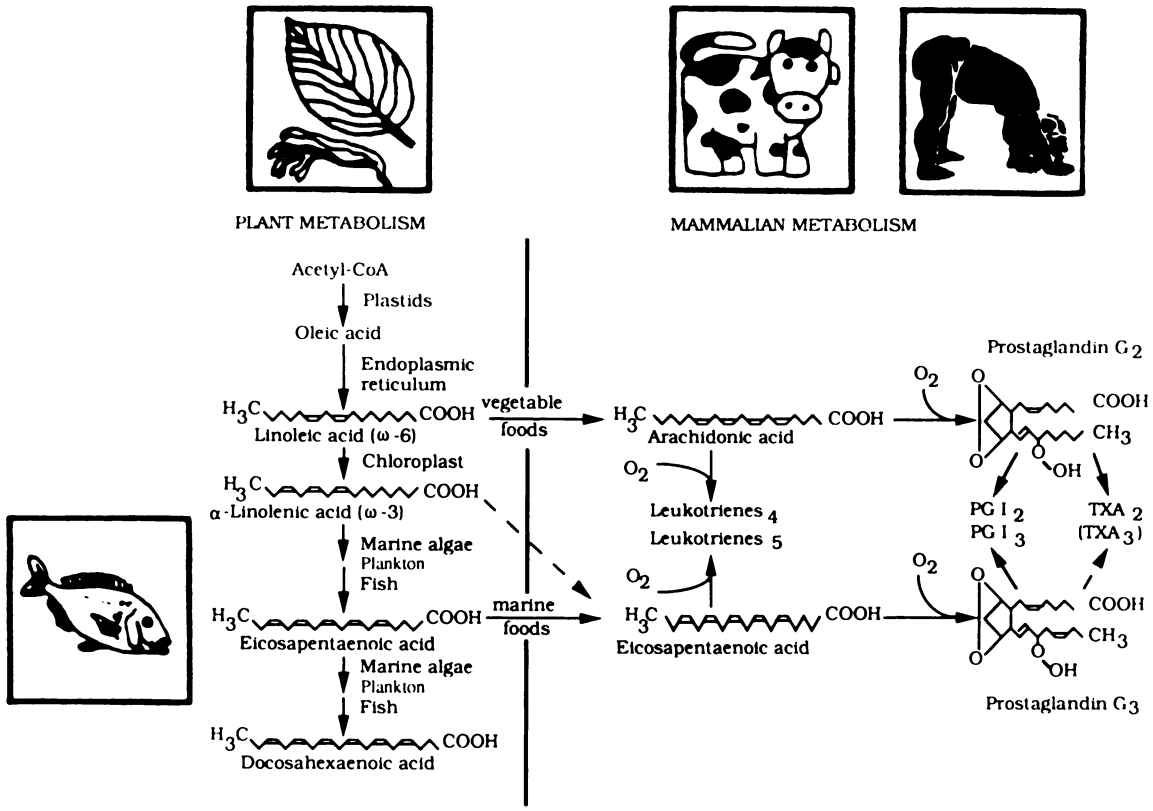

FIG 5. Origin of ω3 and ω6 unsaturated fatty acids, biosynthesis of eicosanoids from arachidonic acid (C20:4ω6) and eicosapentaenoic acid (C20:5ω3). Reproduced with permission from reference 63.

to rise but when saturated fatty acids were reduced, the LDL tended to decrease. In patients with hyperlipidemia in whom saturated fatty acids were held constant, LDL increased except in the study by Phillipson et al (82), who used a very high dose of fish oil. In general, a high dose of fish oil (10 g ω3 fatty acids/d) may lower LDL whereas lower doses do not. Whether EPA or DHA is more effective in lowering LDL is under investigation with more-purified preparations of the individual fatty acids.

In summary, the effects of ω3 fatty acids on serum cholesterol concentrations are similar to those of other PUFAs. When ω3 fatty acids replace saturated fatty acids in the diet, they lower serum cholesterol concentrations. Omega-3 fatty acids have the added benefit of consistently lowering serum triglyceride concentrations whereas the ω6 fatty acids do not and may even increase them (82).

In considering these aspects of ω3 fatty acids on LDL-cholesterol concentrations, the issue is whether this increase in LDL is indeed significant in increasing the risk for atherosclerosis in patients with type II and IV hyperlipidemia in view of the antithrombotic, anti-inflammatory, and antivasorestrictive aspects of ω3 fatty acids. Furthermore, the possibility that this new LDL may not be atherogenic, or as atherogenic, needs to be considered because fish-oil diets have produced changes in lipoprotein composition in animal studies (83–85). Theoretically, EPA and DHA may alter the rate or form of LDL oxidation in vivo and thereby cause a reduced atherogenic potential not reflected in an actual lowering of, or even despite an increase in, LDL concentration.

Another important consideration is the finding that during chronic fish-oil feeding there is a decrease in postprandial triglyceride concentrations. Furthermore, Nestel (86) 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 VLDL triglyceride (77, 87–89). In normolipidemic subjects ω3 fatty acids prevent and reverse rapidly the carbohydrate-induced hypertriglyceridemia (87). There is also evidence from kinetic studies that fish oils increase the fractional catabolic rate (FCR) of VLDL (77, 88, 89).

Antiatheromatous actions

The antiatheromatous actions of ω3 fatty acids are supported by a number of animal studies. In dogs fed a diet high in saturated fatty acids and cholesterol, supplementation with fish oils prevented intimal hyperplasia that is induced on venous allografts inserted into their arteries (90). In hyperlipidemic swine model, dietary supplementation with cod-liver oil reduced the development of coronary atherosclerosis without any significant changes in plasma lipid concentrations between the supplemented animals and the controls (91). In the primate model, dietary fat substitution with ω3 fatty acids inhibited atherogenesis in the aorta, carotid, and femoral arteries (92). Hollander et al (93) confirmed Davis et al's (92) findings in another primate species without significant differences in serum lipid levels. Using the rabbit atherogenesis model, Thiery and Seidel (94) found that fish-oil feeding resulted in an enhancement of cholesterol-induced atherogenesis whereas Zhu et al (95) found that atherosclerosis was inhibited by fish oils in cholesterol-fed rabbits.

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These conflicting results were observed in the rabbit atherogenesis model whereas in all other models (dog, swine, and two primate species) fish oils were found to have antiatherogenic effects even if they did not lower serum lipids.

Antithrombotic effects

In addition to a prolongation of bleeding time, there is substantial agreement that 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 (24, 33, 34, 64, 96). Increased concentrations of plasminogen activator and decreased concentrations of a plasminogen inhibitor after fish-oil ingestion were reported (97). Fibrinogen also decreases after ω3 fatty acid ingestion. Although some studies failed to show a decrease in fibrinogen concentrations, a randomized, double-blind clinical trial did show a decrease after ingestion of ω3 fatty acids in adults with type IIb or IV hyperlipoproteinemia (98). A decrease in fibrinogen was also found in another double-blind trial with 64 men aged 35–40 y randomly assigned to two groups (99). In the studies that failed to show an effect, the study by Sanders et al (100) used a small dose of cod-liver oil and the study by Rogers et al (101) included healthy volunteers and was of short duration.

Although a decrease in platelet count occurs with an increase in platelet size after ingestion of ω3 fatty acids (especially in very large quantities), clinical evidence of bleeding has yet to be reported. Platelet survival has been found to be normal. Because there is an increase in platelet size with a decrease in platelet count, there is no overall decrease in platelet mass (102). When fish oils are discontinued, the platelet count returns to normal. In some cases platelet count rebounds to supernormal before returning to normal. The mechanisms of these effects of fish oil on platelets and on megakaryocytes are unknown. These effects of fish oils leading to an increase in bleeding time has raised questions: What is the clinical significance of the prolonged bleeding time? Are there any adverse effects?

The effects of different doses of fish oils on the prolongation of bleeding time were investigated by Saynor et al (81). With 1.8 g EPA there was not any prolongation in bleeding time. At 4 g the bleeding time increased and the platelet count decreased without any adverse effects. In studies in humans there has never been a case of clinical bleeding, even in patients undergoing angioplasty while they were on fish-oil supplements (103).

DeCaterina et al (104) recently reported on the preoperative use of fish oils in 13 men and 2 women who underwent coronary-artery-bypass graft surgery. The daily dose was 3 g EPA and 1.3 g DHA in purified fish oil that was taken for 28 d before surgery. The control subjects were 14 men and 1 woman perfectly matched for age and severity of disease who were scheduled for surgery by the same surgeon. The control subjects did not receive any fish oils. Despite changes in platelet function, increases in bleeding time, and increases in vascular PGI_2, the perioperative blood loss was not increased in subjects receiving fish-oil supplements. There is no evidence that the increase in bleeding time is clinically significant or has any adverse effects.

Vascular effects

It was recently shown that ω3 fatty acids inhibit the production of platelet-derived growth factor (PDGF) (105) and increase endothelium-derived relaxing factor (EDRF) (106). Omega-3 fatty acids reduce production of a PDGF-like protein in bovine endothelial cells, which leads to inhibition in the migration and proliferation of smooth muscle cells, fibroblasts, and macrophages in the arterial wall (105).

The endothelium releases an EDRF, presumably nitric oxide. When animals are fed cod-liver oil or fish oils (EPA plus DHA), they increase the release of relaxing factors, which facilitates relaxation in large arteries and in resistance vessels (106). Also in the presence of EPA, endothelial cells in culture increase the release of relaxing factors indicating a direct effect of the fatty acid on the cells. EDRF presumably contributes to antithrombotic and antiatherosclerotic effects of ω3 fatty acids by relaxing vascular smooth muscle and inhibiting platelet aggregation.

Increases in PGI_2 were shown in tissue fragments from the atrium, aorta, and saphenous vein obtained at surgery in patients treated with ω3 fatty acids (104). This finding is very important because it enhances our understanding of the effects of ω3 fatty acids on vessel walls in humans and differs from the results of some animal studies (107, 108). Rats do not form PGI_3 after dietary EPA (107, 108) whereas humans do (109). Therefore, the importance of human studies is obvious.

Antiarrhythmic effects

Sudden cardiac death is frequently a consequence of severe ventricular fibrillation or terminal cardiac arrhythmia. Experimental studies with isolated papillary muscles from either rats or marmoset monkeys indicate much less susceptibility to catecholamine-induced arrhythmia in the muscles from animals fed fish-oil supplements than from those on ω6 or low-fat diets (110). Indomethacin abolishes these effects in vitro, suggesting a mechanism operating via the eicosanoids (prostaglandins). In studies with adult marmoset monkeys fed dietary fish oil for several months, cardiac function improved, and the vulnerability of the heart to develop cardiac arrhythmia was reduced when subjected to ischemic stress. Burr et al (23) studied the effects of dietary intervention in the secondary prevention of myocardial infarction. A modest intake of fatty fish two-to-three times per week (or 3 g fish oils/d) reduced all-cause mortality by 29% over a 2-y period, possibly by preventing sudden death from arrhythmia.

Effects on restenosis

The antiatheromatous aspects of ω3 fatty acids shown in animal experiments suggested the use of ω3 fatty acids to prevent restenosis in patients undergoing angioplasty. The cause of restenosis is unknown. However platelet aggregation, proliferation of smooth muscle cells, and coronary vasospasm are considered to be important contributors to restenosis. Although the success rate of angioplasty is high, restenosis occurs in 25–40% in the dilated lesions ~ 6 mo after the procedure. Most studies showed a benefit when ω3 fatty acids supplemented the standard regimen before and after surgery (103, 111, 112). Dehmer et al (103) provided evidence that when ω3 fatty acids were given to the patients along with aspirin and dipyridamole 7 d before angioplasty and continued for 6 mo afterward, there was a reduction in the rate of restenosis on catheterization 3–4 mo after angioplasty. Others report no benefit (113, 114). There was no clinical evidence of bleeding complications in any treated patient reported in these studies. The role of ω3 fatty acids in the prevention of early restenosis after coronary angioplasty is a major area of research because percutaneous transluminal coronary angioplasty is an important treatment for selected patients with CHD.

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TABLE 6
Effects of dietary ω3 fatty acids on factors and mechanisms involved in the development of inflammation, atherosclerosis, and immune diseases*

Reduce or inhibit risk and/or precipitating factors
Arachidonic acid
Platelet aggregation
Thromboxane A_2 formation
Monocyte and/or macrophage function
Leukotriene formation (LTB_4)
Formation of platelet activating factor (PAF)
Toxic oxygen metabolites
Interleukin 1 formation (IL-1)
Formation of tumor necrosis factor (TNF)
Platelet-derived growth factor–like protein (PDGF)
Intimal hyperplasia
Blood pressure and/or blood pressure response
Very-low-density and low-density lipoproteins (VLDL, LDL)
Triglycerides
Lipoprotein (a) [Lp(a)]
Fibrinogen
Blood viscosity
Increase beneficial and/or protective factors
Prostacyclin formation (PGI_2 + PGI_3)
Leukotriene B_5 (LTB_5)
Interleukin 2 (IL-2)
Endothelial-derived relaxing factor (EDRF)
Fibrinolytic activity
Red-cell deformability
High-density lipoprotein (HDL)

* Reproduced with permission from reference 29.

Effects on lipoprotein (a)

Lipoprotein (a) [Lp(a)] is a genetically determined protein that has atherogenic and thrombogenic properties. The molecular structure of Lp(a) apoprotein is strikingly similar to that of plasminogen. Omega-3 fatty acids were reported to inhibit the inhibitor of plasminogen activator and thus contribute to fibrinolysis (97). Thus it was only natural to test the effects of ω3 fatty acids on Lp(a) concentrations (115). Herrmann et al (115) reported on such a study at the poster session of the NATO Advanced Research Workshop on Dietary ω3 and ω6 Fatty Acids: Biological Effects and Nutritional Essentiality. These investigators studied 62 male patients who had myocardial infarction 6 mo before the study. Ingestion of fish oil reduced the concentration of triglycerides, reduced blood pressure, and led to a significant reduction in Lp(a). This study provided the first evidence that ω3 fatty acids lowered Lp(a). Recently, Schmidt et al (116) showed that ω3 fatty acids lowered serum Lp(a) concentrations when Lp(a) concentrations were > 200 mg/L but had no effect < 200 mg/L.

More recently, Kostner and Herrmann (117) compared the effects of fish-oil concentrate (12 g FENICO®/d, containing 70% ω3 PUFAs) in 35 patients with coronary disease and a control group receiving an equivalent amount of rapeseed oil. In addition to measuring Lp(a), these investigators carried out standard plasma lipid and lipoprotein determinations and hemostatic indices. Plasma Lp(a) concentrations were reduced in the fish-oil group but were unaffected in the rapeseed-oil group. The total cholesterol, LDL cholesterol, and apolipoprotein B (apo B) concentrations fell significantly in both groups. HDL cholesterol increased and triglycerides decreased significantly only in the fish-oil group. Not everybody in the fish-oil group showed a decrease in plasma Lp(a) concentrations. The investigators therefore subdivided the participants in the study into two groups, responders and nonresponders. Two-thirds of the people studied were responders and they showed an average Lp(a) decrease of 24%. In this study, tissue plasminogen activator concentrations were reduced significantly in both groups by ~16%. There was a concomitant but not significant increase of plasma activator inhibitor, PAI_5.

In a recent study by Seed et al (118) on the relation of serum Lp(a) concentration and apolipoprotein A (apo A) phenotype to CHD in patients with familial hypercholesterolemia, it was shown that "the median lipoprotein(a) level in the 54 patients with CHD was 57 mg/dl, which is significantly higher than the corresponding value of 18 mg/dl in the 61 patients without CHD. According to discriminant-function analysis, the lipoprotein(a) level was the best discriminator between the two groups (as compared with all other lipid and lipoprotein levels, age, sex, and smoking status)." The authors conclude that "an elevated level of lipoprotein(a) is a strong risk factor for CHD in patients with familial hypercholesterolemia, and the increase in risk is independent of age, sex, smoking status, and serum levels of total cholesterol." In another study on apo A and ischemic heart disease in familial hypercholesterolemia, Wiklund et al (119) also concluded that Lp(a) is a genetic trait that may be useful in identifying patients with familial hypercholesterolemia at high risk for CHD (119). Clinical investigations are urgently needed to determine if lowering Lp(a) by ω3 fatty acids lowers the risk for CHD in these patients.

Additional effects

Omega-3 fatty acids have been shown in human monocytes to inhibit the production of platelet activating factor (PAF). One of the adverse effects of PAF is the activation of platelets, thus contributing to atherogenesis (120). Interleukin and tumor necrosis factor (TNF) are reduced by feeding fish-oil supplements to humans (121). Both interleukin 1 (IL-1) and TNF are considered atherogenic because they stimulate the synthesis of adhesion molecules, thus causing monocytes to adhere to endothelial cells. They also activate platelets, neutrophils, and monocytes (121).

In conclusion, many studies indicate that ω3 fatty acids appear to decrease or inhibit risk and precipitating factors in the development of CVDs. These factors are summarized in **Table 6** (29).

The new findings in relation to interleukin metabolism and gene expression indicate that, in addition to their major effects on prostaglandin metabolism, ω3 fatty acids have other far-reaching effects on intracellular cell communication. These findings indicate that it is very important to know and eventually understand the numerous inter- and intracellular factors that are influenced by ω3 fatty acids as well as the specific mechanisms involved. It is this type of information that will enable us to design appropriate clinical trials to precisely define the dose of ω3 fatty acids to be utilized and the type of fatty acid and length of intervention required for effective therapy while avoiding any possible adverse reactions.

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TABLE 7
Genetic determinants and environmental risk factors for CHD*

Genetic determinants
Family history of CHD at an early age
Total serum cholesterol, LDL, and apo B concentrations
HDL cholesterol, apo A-I, and apo A-II concentrations
Lp(a)
LDL receptor activity
Thrombosis and coagulation variables
Triglycerides and VLDL concentrations
RFLPs in DNA at the apo A-I/apo C-III and apo B loci
Other DNA markers
Blood pressure
Diabetes
Obesity
Insulin concentration and insulin response
Heterozygosity for homocystinuria
Environmental risk factors
Smoking
Sedentary lifestyle (lack of aerobic exercise)
Diet (excess energy intake)
High saturated fatty acid intake
Low ω3 fatty acid intake
Psychosocial factors
Type A personality
Social class

* Reproduced with permission from reference 123.

Cornary heart disease

Much more is known about the effects of ω3 fatty acids on CVD than on any other disease entity. CHD is a multifactorial disease with genetic determinants that interact with many environmental factors, including diet and other lifestyle changes that contribute to its development. All the hyperlipoproteinemias described so far have a significant genetic component. It has been estimated that 50% of the variance in serum cholesterol concentration and 15% of the variance in the fibrinogen concentration are due to genetic factors.

An extensive array of genes involved in normal regulation and function of the cardiovascular system have been identified with modern genetic techniques [DNA markers, restriction-fragment-length polymorphisms (RFLPs) and in situ hybridization studies]. These new genetic markers are being used to predict risk for CHD, a most important aspect for developing strategies for the prevention of CHD. The early identification of individuals in childhood and young adult life who are at high genetic risk constitutes a very powerful health care strategy for the prevention of CHD. Common genetic lipoprotein disorders associated with premature CHD include familial combined hyperlipidemia (15%), familial hypertriglyceridemia (5%), and familial hypercholesterolemia (5%) (122) (**Table 7**) (123). In the United Kingdom, 5 g MaxEPA® (18% EPA, 12% DHA) twice a day has been approved for the treatment of severe hypertriglyceridemia in patients at risk of ischemic heart disease or pancreatitis.

Atherosclerosis is a complex disease of the arteries and the arterial wall. Many cellular biochemical and physical components interact at and within the arterial wall. The cellular dynamics in atherosclerosis were reviewed by Faggiotto (124) who states that "atherosclerosis encompasses a number of pathological processes and has many of the features of degenerative disorders, inflammation and neoplasia." In 1981 Ross (125) published a most-important paper on atherosclerosis entitled "Atherosclerosis: A problem of the biology of the arterial wall cells and their interaction with blood components," in which the modern concepts of atherosclerosis were presented. Ross in 1986 (126) and Steinberg et al in 1989 (127) updated the concepts of the response to injury hypothesis in the pathogenesis of atherosclerosis, which can be summarized as follows. The first step in the formation of atherosclerosis is a nonspecific (functional) injury to endothelium followed by an accumulation of monocytes and macrophages, foam cell formation, and platelet aggregation. The platelets release growth factor, which leads to smooth muscle migration and proliferation. At this point cholesterol is deposited in the smooth muscle cells and monocyte macrophages in the vessel wall. These events further lead to the formation of ground substance and eventually to plaque formation. As seen in Table 6, ω3 fatty acid ingestion may be able to prevent the increase in cellular components generated by these cells and interfere at many steps in the development of the atherogenic process (**Fig 6**) (27, 48).

Vegetable oils rich in the ω6 LA have been promoted as lowering blood cholesterol concentrations of people in the United States. So much emphasis has been put on the lipid hypothesis and on lowering serum cholesterol concentrations through diet and drugs that the contributions of inflammation and thrombosis in the development of CHD have not been fully appreciated.

An increase in our understanding of the pathophysiology of coronary artery thrombosis has led to the hypothesis that preventing platelet activation and aggregation are essential steps in the prevention of coronary thrombotic complications. Current evidence suggests that the pathophysiology of unstable angina involves platelet recruitment and thrombosis. Burr's study referred to earlier (23) is the first prospective dietary-intervention trial for secondary prevention of CHD that demonstrates clinical benefit in those given advice to eat fatty fish or fish-oil capsules.

Omega-3 fatty acids alone clearly will not lead to the universal eradication of atherosclerosis. However, it is increasingly evident that dietary fish-oil supplementation may help in the prevention of atherosclerosis or its thrombotic complications. The favorable effects shown by DeCaterina et al (104) provide further support

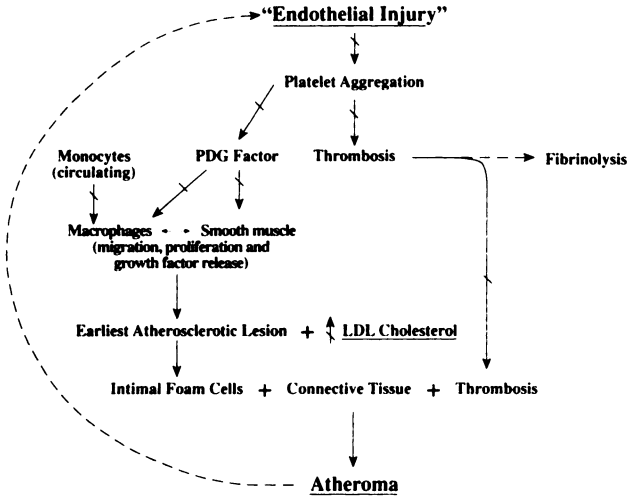

FIG 6. Sites of potential interventions for preventing development of atherosclerosis. Note that several of these possibilities would abort the disease before the concentration of plasma LDL cholesterol could contribute to the atherosclerotic process. Reproduced from reference 48.

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that ω3 fatty acids could modify the occurrence and the course of atherosclerosis.

Recently, Dolecek and Grandits (20) investigated the 24-h dietary-recall data in the usual-care group of the Multiple Risk Factor Intervention Trial and distinguished between ω3 and ω6 fatty acid intake and their relationship to four mortality categories: CHD, total CVD, all-cause mortality, and cancer. Analysis of the combined fatty acids predominantly found in fish (EPA and DHA) demonstrated significant inverse associations with CHD, CVD, and all-cause mortality groups. The benefit appeared to be in the highest intake quintile with a mean ingestion of ~664 mg/d of EPA and DHA. When compared with zero intake, mortality from CHD, CVD, and-all cause mortality was 40%, 41%, and 24% lower, respectively. An inverse association was noted between the ratio of 18:3ω3 to 18:2ω6 and cancer mortality. Thirty-three percent fewer cancer deaths occurred in the highest intake quintile when compared with the lowest.

Populations with high consumption of fish, such as the Eskimos and Japanese, have lower rates of myocardial infarction (**Table 8**) (128). The epidemiologic studies of Kromhout et al (17) and the intervention studies of Burr et al (23) showed a decrease in CHD mortality in people consuming relatively small amounts of fish (0.5 g ω3 fatty acids/d or 1.5 g fish oil/d) over a long period of time (19 y and 2 y, respectively). This suggests that small doses over long periods of time may have beneficial effects, possibly by reducing blood pressure and other risk factors. Certainly more studies are needed to define the precise dose of ω3 fatty acids based on the total long-term diet of the subjects in terms of ω3, ω6, ω9, and saturated fatty acids. The unique pharmacokinetics of ω3 fatty acids in terms of time and dose-dependent accumulation in cell membranes should help define the optimum amount of ω3 fatty acids and length of time of the intervention.

The vast majority of survivors of myocardial infarction have one or more of four lipoprotein abnormalities. These include increased LDL-cholesterol concentrations, decreased HDL-cholesterol concentrations usually accompanied by increased triglyceride or VLDL concentrations, increased concentration of chylomicron remnants and intermediate-density lipoprotein (IDL), and the presence in plasma of increased concentrations of Lp(a). The exact mechanism whereby each of these abnormalities causes CHD is an area of active investigation and the genetic contribution to each of these abnormal lipoprotein phenotypes is coming into focus (129). As we begin to unravel the genetics of atherosclerosis and identify individuals with genetic susceptibility to CHD, modification of diet early in life and the provision of increased amounts of ω3 fatty acids should be beneficial in the prevention of CHD.

TABLE 8
Ethnic differences in fatty acid concentrations in thrombocyte phospholipids and frequency of cardiovascular disorders*

	Europe, United States	Japan	Greenland Eskimos
Arachidonic acid, C20:4ω6 (%)	26	21	8.3
Eicosapentaenoic acid, C20:5ω3 (%)	0.5	1.6	8.0
ω6:ω3	50	12	1
Cardiovascular mortality (%)†	45	12	7

* Adapted from reference 128.
† Percent of all deaths.

Hypertension

Hypertension is also a multifactorial disorder involving gene-nutrient interactions and other factors (130). Different mechanisms appear to be involved, operating at variable proportions based on the organ involved or the cause of hypertension. Changes were reported in eicosanoid metabolism, renin concentrations, vascular reactivity, blood viscosity, loss of sodium, increase in potassium in cells, and a decrease in intracellular calcium, among others (130).

In 1983 two groups of investigators, Singer et al (131) and Lorenz et al (132), were the first to show that adding mackerel to the diet of patients with mild hypertension lowered the blood pressure. Many studies since then have used ω3 fatty acids in the form of fish oils with similar results in normal and hypertensive subjects (72, 133–137) but not in all intervention trials (138, 139).

More recently Knapp and FitzGerald (135) reported on a controlled study of PUFA supplements in essential hypertension. These investigators studied blood pressure and eicosanoid production during supplementation of dietary fat for 4 wk in 32 men with mild essential hypertension. Groups of eight subjects received either 3 or 15 g ω3 fatty acids/d in the form of 10 or 50 mL MaxEPA®, 39 g ω6 fatty acids/d in the form of 50 mL safflower oil, or 50 mL/d of an oil mixture that approximated the types of fat present in the American diet. Urinary metabolites were measured to assess biosynthesis of eicosanoids. The men who received the high dose of fish oil had a mean decrease in systolic blood pressure of 6.5 mm Hg and a decrease of 4.4 mm Hg in diastolic blood pressure. The group receiving the low dose of fish oil (10 mL/d) did not have any significant change in blood pressure from baseline during the supplementation period nor did the ω6-supplemented or the control groups. In the group receiving the high dose of fish oil, the formation of vasodilatory prostacyclins (PGI_2 and PGI_3) increased initially but this increase was not maintained as blood pressure fell. The concentration of thromboxane A_2 metabolites fell and metabolites of thromboxane A_3 were detected in the groups receiving fish oil. The formation of PGE_2 increased during supplementation with safflower oil and tended to decrease with fish oil but no PGE_3 metabolite was detected. These data indicate that high doses of fish oil can reduce blood pressure in men with essential hypertension.

Bonaa et al (140), in a population-based intervention trial from Tromsø, recently reported decreases of 6 mm Hg in systolic blood pressure and 3 mm Hg in diastolic blood pressure with fish-oil supplementation. These investigators monitored diet and assessed concentrations of plasma phospholipid fatty acids to determine the relation among diet, fatty acids, and blood pressure. Dietary supplementation with fish oil did not change mean blood pressure in the subjects who ate fish three or more times per week as part of their usual diet or in those who had a baseline concentration of plasma phospholipid ω3 fatty acids > 175.1 mg/L, suggesting that a relationship may exist between plasma phospholipid ω3 fatty acid concentration and blood pressure. There was a lower blood pressure at baseline in subjects who habitually consumed larger quantities of fish, suggesting that supplementation with fish oils would be important from the primary prevention standpoint. In another study, three cans of

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mackerel per week (equivalent to 1.2 g ω3 fatty acids/d or 1.2 × 3 = 3.6 g of fish oil/d) for 8 mo led to lowering of blood pressure (133). This amount of fish oil could be considered acceptable for a daily intake by the general population.

It was suggested that these effects on blood pressure during dietary supplementation with ω3 fatty acids are due to changes in the endogenous synthesis of vasoactive eicosanoids. Two research groups showed that dietary EPA is converted to PGI_3 in man and does not suppress formation of PGI_2 from AA (141, 142). Other possible mechanisms under consideration include effects of ω3 fatty acids on renal function, a lowering of blood viscosity, and a reduction in vascular responsiveness to systemic vasoconstrictors (143). Lorenz et al (132) observed an increase in urinary sodium and a decrease in plasma renin activity at the end of the fish-oil period in a group of men whose Western diet was supplemented with cod-liver oil. Fish-oil supplements were reported to have beneficial effects on the blood pressure of patients on hemodialysis with little residual function (144). Normal subjects do not show any change in renal function even when given pharmacologic doses of fish oil, which is encouraging from the safety standpoint (145).

Rats with reduced renal function (subjected to subtotal nephrectomy) given dietary fish oil had reduced urinary PGE and renal function along with increased proteinuria and mortality (146). As indicated earlier, studies from rats cannot be extrapolated to humans because rats do not make PGI_3 (107–109).

The effects of ω3 fatty acids on immune-mediated renal dysfunction are complex. Kelley et al (147) showed that fish oils prolong the survival in mice that develop lupus nephritis whereas Westberg et al (148) found less benefit. However, Kelley used a larger dose of fish oil. In humans the clinical course of lupus nephritis did not improve with fish-oil supplementation (149, 150). More clinical investigations are needed before any conclusions can be drawn.

Recent data on the additive effects of fish-oil supplements and propranolol were presented at the Second International Conference on the Health Effects of Omega-3 Polyunsaturated Fatty Acids in Seafoods (151). The combination of ω3 fatty acids and propranolol potentiated their blood-pressure–lowering effects. In addition, the increase in plasma triglycerides often seen during antihypertensive therapy did not occur. Therefore, ω3 fatty acid supplementation has the potential for a beneficial modification of several cardiovascular risk factors as adjuvants to the antihypertensive regimen.

Inflammatory and autoimmune disorders

The effects of supplementing the diet with ω3 fatty acids in the form of fish oils on the function of the 5-lipoxygenase pathways of peripheral blood polymorphonuclear leukocytes and monocytes has been investigated in normal subjects and in patients with diseases such as arthritis, psoriasis, ulcerative colitis, lupus erythematosus, and asthma. These studies were stimulated by the demonstration by Prickett et al (152) that the fatal spontaneous autoimmune renal disease in a genetic strain of NZB mice can be largely prevented by changing the fat in the diet from beef tallow to fish oil. In fact, the protective effect of marine lipids on autoimmune renal disease is one of the most dramatic effects of ω3 fatty acids on any pathology (152). Supplementing the diet with ω3 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 B_4 (LTB_4)-mediated function of neutrophils while increasing the production of LTB_5 (**Fig 7**) (153, 154). Studies since 1985 show that ω3 fatty acids influence interleukin metabolism by decreasing IL-1 (121, 155, 156).

Many experimental studies have provided evidence that incorporation of alternative fatty acids into tissues may modify inflammatory and immune reactions and that ω3 fatty acids in particular are potential therapeutic agents for inflammatory diseases.

Arthritis

Advances in the understanding of leukotriene metabolism and its role in inflammation and autoimmune disorders began to attract investigators who used fish oils in patients with arthritis with promising results (154). In normal volunteers, marine lipids suppress 5-lipoxygenase pathway products from both neutrophils and monocytes and they also suppress production of PAF from monocytes (120). In addition, the chemotactic response of neutrophils to transmembrane agonists is suppressed by dietary fish oils. In patients with rheumatoid arthritis, inhibition of 5-lipoxygenase products are limited to LTB_4, suggesting inhibition of the epoxide hydrolase step, and neutrophil chemotaxis is increased by the fish-oil dose rather than suppressed as in normal subjects. These differences between normal subjects and patients with arthritis could be related to specific alterations of the disease or to medications taken by patients (156). Omega-3 fatty acids were shown to decrease IL-1 in animals and in patients with rheumatoid arthritis and normal volunteers (121, 155, 156).

Kremer et al (155) carried out a prospective randomized double-blind, placebo-controlled parallel study. Three groups were studied for 24 wk with two different doses of fish oil and one dose of olive oil, and clinical and immunological indices were measured. As in previous studies there was clinical improvement and, in addition to the decrease in LTB_4 and increase in LTB_5, there was a significant decrease in IL-1 production and a nonsignificant increase in IL-2. LTB_4 exerts a positive modulating effect on the genetic control of IL-1 probably at the translational level within the cytoplasm. These findings were confirmed by a number of other investigators. Omega-3 fatty acids (fish oil), as a dietary supplement, along with nonsteroid antirheumatic drugs were shown to provide subjective relief to patients with rheumatoid arthritis.

Psoriasis

The recognition that AA metabolism is altered in psoriasis prompted attempts to inhibit the generation of proinflammatory lipoxygenase products [LTB_4 and 12-hydroxyeicosatetraenoic acid (12-HETE)], which are markedly elevated in the psoriatic lesions (157). The addition of MaxEPA® to the standard treatment produced further improvement and a decrease in LTB_4 and 12-HETE (Fig 7) (64). In other studies fish oil was successfully used in combination with etretinate to reduce the hyperlipidemia caused by that drug and with ultraviolet B (UVB), where it prolongs the beneficial effects of a course of phototherapy (158). Studies of fish oil in combination with cyclosporin are in progress in an attempt to reduce nephrotoxicity, which is the major side effect of that drug (158).

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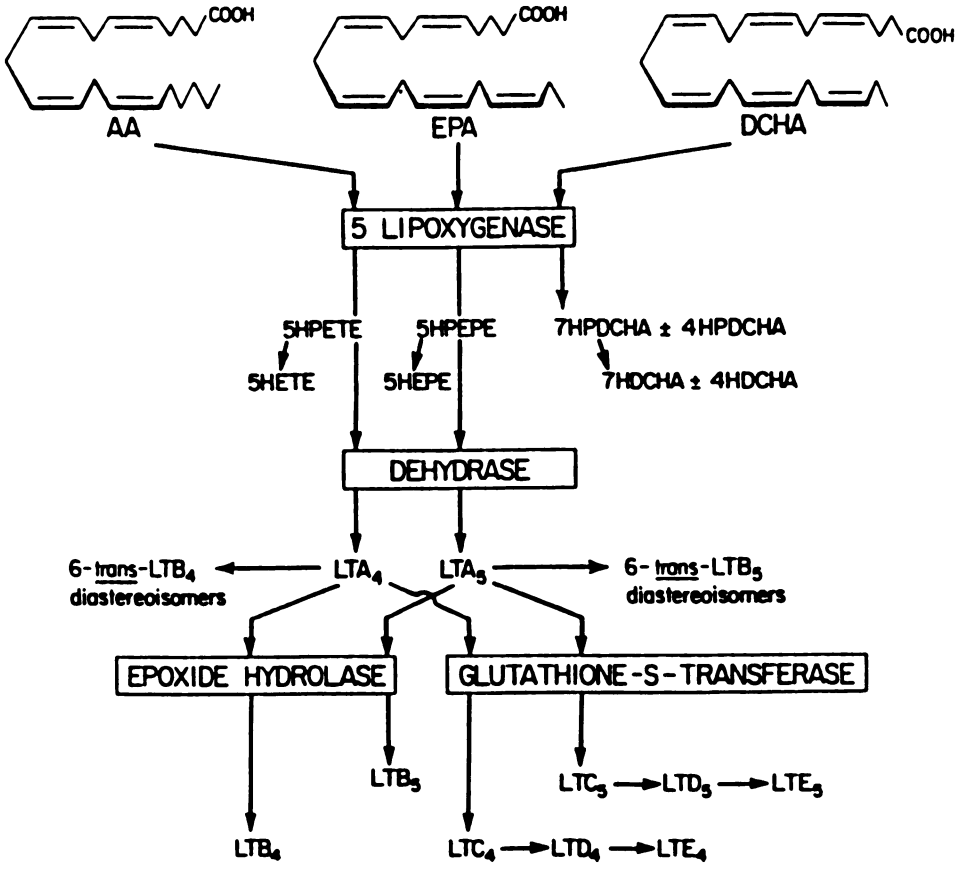

FIG 7. Oxidative metabolism of arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DCHA) by the 5-lipoxygenase pathway. 5HPETE denotes 5-hydroperoxyeicosatetraenoic acid; 5HETE, 5-hydroxyeicosatetraenoic acid; 5HPEPE, 5-hydroperoxyeicosapentaenoic acid; 5HEPE, 5-hydroxyeicosapentaenoic acid; 7HPDCHA, 7-hydroperoxydocosahexaenoic acid; 4HPDCHA, 4-hydroperoxydocosahexaenoic acid; 7HDCHA, 7-hydroxydocosahexaenoic acid; 4HDCHA, 4-hydroxydocosahexanoic acid; and LT, leukotriene. Reproduced with permission from reference 153.

Ulcerative colitis

As indicated earlier, LTB_4, a metabolite of AA, is produced by activated neutrophils, and ω3 fatty acids decrease its production. LTB_4 is an important mediator of inflammation in ulcerative colitis and it is believed to recruit additional neutrophils from the bloodstream into the mucosa. Stenson et al (159) conducted a study of the effects of fish-oil supplementation on ulcerative colitis. Preliminary analysis showed statistically significant improvement in sigmoidoscopy score and global clinical assessment after 4 mo of fish-oil–supplemented diet compared with placebo diet in active ulcerative colitis. This is the first double-blind crossover trial of fish-oil supplementation in ulcerative colitis.

Cancer

The number of publications from the use of ω3 fatty acids in cancer studies in animals has increased exponentially over the past 5 y (29). Animal tumor models in which the tumor was induced by carcinogens and animal models with transplantable tumors (breast, colon, pancreas, and prostate) have been investigated. The results have consistently shown that ω3 fatty acids delayed tumor appearance and decreased both the rate of growth and the size and number of tumors. In these models calorie restriction potentiated the effects of ω3 fatty acids (160, 161) whereas ω6 fatty acids in the form of corn oil increased tumor formation, size, and number (161, 162). It also was shown that ω3 fatty acids decrease PGE_2 production in animals fed ω3 fatty acids (162). As expected, fatty acid analysis of the transplanted tumors reflects the specific composition of the dietary fat ingested by the host (162). Furthermore, these studies indicate that the composition of dietary lipids modifies lipid metabolism and that high dietary intake of ω3 fatty acids can prevent or delay the expression of these neoplasms. In other studies involving human breast-cancer cells in nude mice, the mice fed ω3 fatty acids had fewer pulmonary metastases, decreased serum estrogen and prolactin concentrations, less PGE_2 in the tumor, and reduced *c-myc* oncogene mRNA concentrations in the tumor-tissue cells (160). The opposite occurred in the corn-oil–fed mice.

Animal studies in progress are using fish oils to elucidate the mechanisms involved, including the changes in prostaglandin production, immune function, free radical formation, membrane fluidity changes, modulation of intracellular transport systems, hormone secretion, calorie utilization, and gene expression (163).

Diabetes

Diabetes is a chronic disorder with complications including hypercholesterolemia, hypertriglyceridemia, atherosclerosis,

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CHD, and hypertension. Many of these complications are attributable to microvascular disease (164). Jensen et al (165), using a double-blind crossover design, studied the effects on endothelial permeability, blood pressure, and plasma lipids of 8-wk supplementation of a diabetic diet with cod-liver oil rich in ω3 fatty acids compared with 8-wk supplementation with olive oil in 18 insulin-treated diabetic patients with albuminuria with > 30 mg/d. The patients receiving cod-liver oil showed a significant fall in mean transcapillary escape rate of albumin compared with baseline and a reduction in mean blood pressure. No changes occurred with olive oil. Cod-liver oil was associated with a significant increase in plasma HDL cholesterol, a significant decrease in the concentration of VLDL cholesterol and triglyceride, and no change in the concentration of LDL cholesterol. Jensen et al concluded that cod-liver oil may have a direct action on vascular permeability that is independent of its beneficial effect on blood pressure and postulated that this action results from a decreased transfer of lipoproteins into the vascular wall. In this study blood glucose concentrations were unchanged. In other studies the use of fish oils in non-insulin-dependent diabetes mellitus (NIDDM) (166, 167) and in insulin-dependent diabetes mellitus (IDDM) (168) increased blood glucose, glycosylated hemoglobin, plasma total cholesterol, LDL cholesterol, and serum apo B. The magnitude of these effects is generally small and the changes in glucose metabolism are associated with increased hepatic glucose output and impaired insulin secretion but unaltered glucose disposal rates. Further studies are needed of the fatty acid composition of phospholipids in diabetic patients during ω3 supplementation under defined conditions of metabolic control, diet, and type of NIDDM and IDDM.

Omega-3 fatty acids as an adjuvant to drug therapy

Omega-3 fatty acids in combination with drugs for the treatment of diseases is an area of immense interest because it opens a new field in nutrition research, ω3 fatty acids in the control of metabolic and autoimmune disorders, that includes CVD, arthritis, lupus erythematosus, psoriasis, ulcerative colitis, and cancer. Preliminary data from animal and human studies suggest that the concurrent ingestion or administration of ω3 fatty acids with drugs leads to potentiation of drug effects, as with propranolol (151), which may lead to a decrease both in the dose of ω3 fatty acids and in the drug dose or, as with cyclosporin (158), to a decrease in toxicity of the drug. By partially replacing the fatty acids of phospholipids in the cell membranes, ω3 fatty acids modify enzymes, receptors, and other proteins (29). Additional studies suggest that the incorporation of ω3 fatty acids by cell membranes is enhanced in the presence of olive oil and linseed oil, emphasizing once again the importance of nutrient-nutrient interactions (169).

Cyclosporin is used widely in organ transplantation and in many individuals its use leads to impairments in renal function (170) and increased thromboxane formation (171). It was noted that the use of fish oil instead of olive oil as the vehicle for its administration in rats led to attenuation of the cyclosporin nephrotoxicity (172) without affecting thromboxane synthesis (173). These findings led to the use of 3.6 g EPA/d and 2.4 g DHA/d in patients with psoriasis who were taking cyclosporin; there was a decrease in the decline in renal function without cyclosporin pharmacokinetics being affected (174). In another patient who had received a renal transplant, a low dose of ω3 fatty acids (1.5 g EPA/d) did not lead to the expected increase in blood pressure that is produced by cyclosporin and, in fact, a favorable reduction in thromboxane A_2 occurred (175). There were not any adverse effects attributable to the supplements. In a randomized controlled study van der Heide et al (176) investigated the effects of fish-oil supplements on cyclosporin therapy in renal-transplant patients. The fish-oil supplements caused a significant decrease in renal vascular resistance, increased glomerular-filtration rates, and lowered mean arterial pressure. This is another example of the beneficial effects of ω3 fatty acids combined with drugs in which ω3 fatty acids decrease drug toxicity and also improve the hemodynamic aspects of illness.

Essentiality: the role of ω3 fatty acids in growth and development

In parallel with the studies investigating the role of ω3 fatty acids in disease states, an outstanding group of scientists turned their attention to the essentiality of ω3 fatty acids throughout the life cycle.

Animal studies

Studies in rats and rhesus monkeys showed that dietary restriction of ω3 fatty acids (primarily LNA) during pregnancy and lactation interferes with normal visual function and may even impair learning ability in offspring (177). Connor et al (178) concluded that ω3 fatty acid deficiency in rhesus monkeys is characterized by reduced vision, abnormal electroretinograms (ERGs), and polydipsia (29). In this model, abnormalities of the ERG induced by ω3 fatty acid deficiency during development appear to be irreversible. In 1987 Rotstein et al (179) studied the effects of aging on the composition and metabolism of DHA-containing lipids of the retina in rats. They concluded that an impairment of the Δ-4 desaturase enzyme system is probably responsible for the decreased concentrations of 22:6ω3 (and 22:5ω6) observed in retinal lipids as a consequence of aging. Because DHA is required for normal function of photoreceptors in rats and primates, it is quite possible that a decrease in DHA plays an important role in visual impairments that accompany old age. Therefore, dietary DHA rather than LNA would be the appropriate ω3 fatty acid to use in studies aiming to influence the development of visual impairments and even improve visual function in elderly people.

Human studies

Pregnancy. The role of ω6 and ω3 fatty acids in pregnancy has been examined only recently in humans. Over the past 50 y the influence of maternal nutrition on fetal growth has been extensively studied in the context of protein-calorie malnutrition. In 1981 Crawford et al (180) calculated the LA and LNA requirements for pregnancy to be ~1% of the nonpregnant woman's dietary energy and the AA and DHA requirements to be another 0.5%.

Fetal development, human milk, and infant feeding. As far back as 1973, Crawford et al (181) analyzed 32 samples of human milk and found that it contained LA, AA, LNA, EPA, and DHA and recommended their inclusion in infant formula. Infant formula does not contain AA, EPA, or DHA (**Table 9**) (35).

EPA and DHA are higher in the erythrocytes of breast-fed infants than in those of bottle-fed infants (182). The milk of vegan women contains lower concentrations of DHA than does the milk of omnivores and this difference is reflected in the

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TABLE 9
Fatty acid composition of human milk and formulas (molar percent)*

Fatty acid†	Human milk (n = 11)	Portagen® ‡	Enfamil Premature® ‡	Similac Special Care® §
8:0 (caprylic acid)	0.3	60	24.5	24.1
10:0 (capric acid)	1.4	24	14.1	17.7
12:0 (lauric acid)	7.0	0.42	12.2	14.9
14:0 (myristic acid)	8.0	Trace	4.7	5.8
16:0 (palmitic acid)	19.8	0.19	7.5	6.8
16:1 (palmitoleic acid)	3.2		0.1	0.2
18:0 (stearic acid)	5.9	0.47	1.7	2.3
18:1 (oleic acid)	34.8	4.1	12.4	10.0
18:2ω6 (linoleic acid)	16.0	8.1	22.4	17.4
18:3ω3 (α-linolenic acid)	0.6	Trace	0.6	0.9
20:1 (gondoic acid)	1.1		0.3	0.1
20:2ω6	0.6			
20:3ω6 (dihomo-y-linolenic acid)	0.4			
20:4ω6 (arachidonic acid)	0.6			
20:5ω3 (eicosapentaenoic acid)	0.0			
22:1 (docosenoic acid)	0.1			
22:4ω6 (docosatetraenoic acid)	0.2			
22:5ω6 (docosapentaenoic acid)	0.2			
22:5ω3 (docosapentaenoic acid)	0.1			
22:6ω3 (docosahexaenoic acid)	0.2			

* Reproduced with permission from reference 35.
† Common name in parentheses.
‡ Values from Pediatric Products Handbook, 1983 Edition, Mead Johnson Nutritional Division, Evanston, IN.
§ Ross Laboratories, Columbus, OH.

erythrocytes of their infants. After birth there is a decrease in the DHA content of erythrocytes of full-term and premature infants (42, 183). Infants born at term and fed mother's milk had approximately twice as much DHA in erythrocyte phospholipids as did infants fed formula containing LNA but not DHA. Because the greatest amount of DHA accumulation occurs during the last trimester of pregnancy, the amount of DHA available to premature infants assumes critical importance. In 1987 Liu et al (184) determined that 11 mg DHA $\cdot kg^{-1} \cdot d^{-1}$ added to formula resulted in 0.2% DHA in the total dietary fatty acids in the formula, which is within the range of 0.1–0.3% found in human milk. The inclusion of 0.2% DHA in the formula did not decrease plasma AA and appeared to be a physiological amount that could prevent declines in membrane DHA of premature infants.

A major question remaining is to what degree the fatty acid pattern in erythrocytes reflects the neural status of ω6 and ω3 metabolites in humans.

The effects of PUFA deficiency on the developing brain have been widely documented in experimental animals whereas information from humans is scarce. However, work by Martinez et al (185–188), Innis (189), Carlson (190), Neuringer et al (191, 192), and Bourre et al (193) has added considerably to our knowledge, much of which was pioneered by Lamptey and Walker (194), Walker (195), Wheeler et al (196), Crawford et al (197), and Clandinin et al (198).

During 18:3ω3 dietary deprivation, DHA is replaced by 22:5ω6 in the retina and brain of animals. Replacement with 22:5ω6, the fatty acid most closely resembling DHA, suggests activation of a cellular compensatory mechanism. The ω3 fatty acids are required by the membranes of photoreceptor cells and synapses for *1*) synaptogenesis and photoreceptor membrane biogenesis during the perinatal period, *2*) normal functioning of tissues, and *3*) response to injury to the nervous system (ischemia and convulsions) and also during retinal stimulation, both of which trigger the release of DHA from membrane phospholipids; some of this DHA may be peroxidated or lost through washout to the blood and may need to be replenished (199).

Bourre et al (193) showed that the brain of the ω3-deficient rat is more susceptible to environmental toxins and alcohol.

It is now well recognized that nutrition during the first weeks of life can have a decisive influence on brain development. Because fatty acid patterns of all organs change during development, it is necessary to know the normal profiles during the various stages of development to understand the role of nutritional influences.

Martinez (185) studied the composition of ω3 and ω6 fatty acids in brain, liver, and retina in human fetuses during the last trimester of pregnancy. After 30 wk gestation there is a preferential desaturation of the long-chain ω3 fatty acids in the brain. The liver shows a similar profile. In both tissues, 22:6ω3 increases quadratically and 20:4ω6 and 18:1ω9 decrease linearly in phosphatidylethanolamine (PE). In the retina, as in the forebrain and the liver, the proportion of ω3 fatty acids increases whereas that of ω6 fatty acids decreases throughout development. These changes can be clearly illustrated by using the ratio of 22:6ω3 to 20:4ω6. In the human retina this ratio doubles between 24 wk of gestation and term and continues to increase with age. These findings should be the guidelines for the feeding of prematurely born infants.

Martinez and Ballabriga (187) also investigated the liver and forebrain of infants who had received total parenteral nutrition high in linoleate (Intralipid®) for 4–12 d. At autopsy a significantly lower-than-normal proportion of 22:6ω3 was found in liver phosphoglycerides. There were a number of other changes in long-chain PUFAs and high concentrations of 18:2ω6 that

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were not consistent with the values noted in normal fetal development.

Martinez's (185) findings in the retinas of two postnatally malnourished infants were similar to those described in the liver of children receiving high intravenous doses of 18:2ω6. One of the malnourished children had mucoviscidosis. Both children had unusually high concentrations of 22:5ω6 in retina and phosphatidylcholine as a sign of DHA deficiency. One premature infant (25 wk gestation) had received commercial milk formulas with ω6:ω3 varying between 18:1 and 66:1 during the first 4 mo of life. The retina of this infant was very deficient in 22:6ω3.

It can be concluded that diets with a high ω6:ω3 can be considered unbalanced relative to human breast milk and that these diets are damaging to the PUFA composition of the developing central nervous system in humans. Martinez (185) stated that "when high doses of 18:2ω6 are given intravenously, the inhibiting effect on the ω3 series is very strong, even with a theoretically correct omega-6:omega-3 ratio, probably because substrate inhibition adds to competition between families of fatty acids for the desaturase systems. This should serve as a warning against manufacturing such unphysiological fatty acid mixtures for use in pediatric nutrition."

Carlson (190) showed that membrane 22:6ω3 was highest at birth and declined with time in formula-fed infants and that fish oil could be used as a source of 22:6ω3 in the range found in human-milk–fed infants. One month after delivery, preterm infants not fed human milk had plasma phospholipid 22:6ω3 more like that of monkeys fed safflower oil, and the low concentrations seen in premature infants are analogous to those at which demonstrable deficits in visual acuity occur in infant monkeys.

Uauy et al (200) showed that premature infants fed formulas with a high ratio of LA to LNA (30:1) have poorer ERG responsiveness early in infancy than do those fed human milk or formula with a lower ratio of LA to LNA (9:1). The addition of fish oil further improved some ERG responses. Visual-acuity development was improved by fish-oil supplementation of formula during the first half of infancy compared with formula containing 1.5–2.5% of energy as LNA. These data strongly suggest that DHA is essential for the functional development of the eye and brain of premature infants.

Children, adults, and elderly adults. Omega-3 fatty acid deficiency was originally reported by Holman et al (201) in a young child. Subsequently Bjerve et al (202) reported ω3 fatty acid deficiency in a child and in a group of elderly patients in nursing homes who were fed orally for several years by gastric tube with diets containing very low amounts of ω3 fatty acids. Studies based on clinical findings and determinations of plasma and erythrocyte lipids after dietary supplementation with soya and cod-liver oil strongly suggest that the patients had ω3 fatty acid deficiency. These patients represent the first adults and the second child described with ω3 fatty acid deficiency. The results indicate that ω3 fatty acids are essential for normal growth and cell function in humans in ways similar to those in several animal species.

Assuming linear relationships between dietary intake of ω3 fatty acids and the measured concentrations of ω3 fatty acids in plasma and erythrocyte lipids, the optimal intake of 18:3ω3 has been estimated to be 800–1100 mg/d whereas the optimal intake of very-long-chain ω3 fatty acids was estimated to be 300–400 mg/d (115). It is suggested that the dietary requirements of ω3 and ω6 fatty acids should be stated in milligrams or grams per day and not only as a percent of energy.

Dietary implications

Omega-3 and ω6 PUFAs are two classes of EFAs that are not interconvertible and that constitute a significant part of practically all cell membranes. Whereas cellular proteins are genetically determined and control important cellular functions independently of dietary intake, the lipid composition of cell membranes is dependent to a great extent on the composition of the diet. When ingested, fatty acids such as EPA and DHA are incorporated into the sn-2 position in cell membrane phospholipids, displacing AA. Because the fatty acid composition of cell membranes modulates important cell functions and because the fatty acids in membranes are dependent on dietary intake, it is obvious that in referring to PUFAs it is essential to distinguish between ω3 and ω6 fatty acids in making dietary recommendations. Simply using the P-S ratio of polyunsaturated fatty acids to saturated fatty acids (P:S) ratio is inappropriate and inadequate on the basis of the knowledge we have today.

Many dietary studies and interventions have been carried out and dietary recommendations have been made in relation to saturated fatty acids and cholesterol. However, the amount of ω3 fatty acids in the diet and their effects on health and disease have not yet been considered in the development of dietary guidelines by national governments except for the most recent Canadian Nutrition Recommendations (**Table 10**) (203). Because ω3 fatty acids have different metabolic effects than do ω6 fatty acids and because ω3 fatty acids are essential for normal growth and development and for overall health, accurate knowledge of the amount and type of ω3 fatty acids in foods is essential. Both terrestrial and marine sources of ω3 fatty acids are important in this regard.

It is now accepted that it is important to consider the functions of the different types of fatty acids (ω3, ω6, and ω9) rather than simply total fat (percentage of calories from fat) or the amount of polyunsaturates. The question is not simply about the P:S in the diet but about the concentrations of the ω3, ω6 polyunsaturated, and ω9 monounsaturated fatty acids relative to saturated fatty acids in the diet. Fatty acids should be considered in terms of their overall metabolic effects in growth and development and for their effects on serum lipids, inflammation, thrombus formation, and tumor development. *Trans* fatty acids were recently shown to elevate serum cholesterol (52). Stearic acid formed during hydrogenation does not raise cholesterol but it increases the risk of thrombosis (204). Clearly there is a need to define precisely the functions of the various fatty acids.

Because of the increased amounts of ω6 fatty acids in our 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 ω3 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 atheroma; to allergic and inflammatory disorders, particularly in those who are susceptible; and to proliferation of cells. Thus a diet rich in ω6 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 (205), hyperlipoproteinemia (206), myocardial infarction (207, 208) and other forms of atherosclerotic disease (209), and diabetes (obesity and hypertriglyceridemia). Bleeding time is longer in women than in men and longer

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TABLE 10
Summary of examples of recommended nutrients based on energy expressed as daily rates*

Age and sex	Energy	Thiamin	Riboflavin	Niacin	ω3 PUFAs	ω6 PUFAs
	MJ (kcal)	*mg*	*mg*	*NE*†	*g*	*g*
0–4 mo (M, F)	2.51 (600)	0.3	0.3	4	0.5	3
5–12 mo (M, F)	3.77 (900)	0.4	0.5	7	0.5	3
1 y (M, F)	4.60 (1100)	0.5	0.6	8	0.6	4
2–3 y (M, F)	5.44 (1300)	0.6	0.7	9	0.7	4
4–6 y (M, F)	7.53 (1800)	0.7	0.9	13	1.0	6
7–9 y						
M	9.20 (2200)	0.9	1.1	16	1.2	7
F	7.95 (1900)	0.8	1.0	14	1.0	6
10–12 y						
M	10.5 (2500)	1.0	1.3	18	1.4	8
F	9.20 (2200)	0.9	1.1	16	1.1	7
13–15 y						
M	11.7 (2800)	1.1	1.4	20	1.4	9
F	9.20 (2200)	0.9	1.1	16	1.2	7
16–18 y						
M	13.4 (3200)	1.3	1.6	23	1.8	11
F	8.79 (2100)	0.8	1.1	15	1.2	7
19–24 y						
M	12.6 (3000)	1.2	1.5	22	1.6	10
F	8.79 (2100)	0.8	1.1	15	1.2	7
25–49 y						
M	11.3 (2700)	1.1	1.4	19	1.5	9
F	8.37 (2000)	0.8	1.0	14	1.1	7
50–74 y						
M	9.62 (2300)	0.9	1.3	16	1.3	8
F	7.53 (1800)	0.8‡	1.0‡	14‡	1.1‡	7‡
75+ y						
M	8.37 (2000)	0.8	1.0	14	1.0	7
F§	7.11 (1700)	0.8‡	1.0‡	14‡	1.1‡	7‡
Pregnancy (additional)						
1st trimester	0.42 (100)	0.1	0.1	0.1	0.05	0.3
2nd trimester	1.26 (300)	0.1	0.3	0.2	0.16	0.9
3rd trimester	1.26 (300)	0.1	0.3	0.2	0.16	0.9
Lactation (additional)	1.88 (450)	0.2	0.4	0.3	0.25	1.5

* From reference 203.
† Niacin equivalents.
‡ Value below which intake should not fall.
§ Assumes moderate physical activity.

in young than in old people. There are ethnic differences in bleeding time that appear to be related to diet. The increase in bleeding time brought on by the ingestion of fish or fish oils is an attempt to return to a more physiological state.

Bleeding time is determined by platelet function, local tissue factors, and components of the coagulation system. Rodgers and Levin (210) carried out a critical reappraisal of the bleeding time and concluded that there is no evidence that bleeding time is a predictor of hemorrhage risk and summarized their findings as follows: "The pathophysiology of an abnormal bleeding time remains poorly understood. The bleeding time is affected by a large number of diseases, drugs, physiologic factors, test conditions, and therapeutic actions, not all of them platelet-related. The test is likely to remain widely used for the diagnosis of inherited disorders of platelet function, such as von Willebrand's syndrome, despite the lack of clear criteria for its use in this context. There are no data that support use of the bleeding time to predict bleeding: there is no evidence that the test changes sufficiently in advance of serious bleeding to allow successful intervention, that the risk of bleeding for a given bleeding time is independent of the cause of the prolongation, or that bleeding from the skin can predict bleeding elsewhere in the body (for example, duration of bleeding from a skin wound does not correlate with duration of bleeding from a gastric biopsy site). There is no evidence that the bleeding time is useful for monitoring the effects of hemodialysis or transfusion therapy."

Evidence that long-chain ω3 fatty acids protect against the development of CVD continues to accumulate from epidemiologic surveys, animal-feeding studies, biochemical research, and human clinical trials and intervention studies. Most investigators advise that addition of fish to the diet several times weekly may be of benefit in preventing CHD. There is insufficient evidence to quantify the exact prophylactic benefit. Yet the epidemiologic evidence from the Eskimo (13), the Japanese (15), the Oslo studies (16) and from population-intervention studies and clinical trials are highly suggestive and support the hypothesis that ω3 fatty acids are contributing factors in the prevention of CHD and in the control of blood pressure.

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In considering dietary implications it is necessary to distinguish among the various roles of ω3 fatty acids:

1) Omega-3 fatty acids are essential for normal growth and development throughout the life cycle and they must be included in the diet of pregnant women, premature infants, full-term infants, children, young adults, and elderly adults. As indicated in the previous section, the optimal intake of 18:3ω3 was estimated to be 800–1100 mg/d and that of very-long-chain ω3 fatty acids to be 300–400 mg/d; the current amount in the US population is 50 mg EPA and 80 mg DHA per capita per day, indicating that the present diet does not provide adequate amounts.

2) Increased intake of fish or fish oils may be necessary over and above the amount determined for their essentiality, particularly in those who have a family history or other evidence of susceptibility to CHD, hypertension, arthritis, psoriasis, and cancer.

3) Omega-3 fatty acids are potentially valuable as adjuvants to treatment of some of these diseases.

It is interesting to consider the progress that has taken place in dietary recommendations from 1985 to 1990. At the Conference on the Health Effects of Polyunsaturated Fatty Acids in Seafoods in 1985, it was noted that in the United States the per capita consumption was 5.9 kg fish/y, which is equivalent to about one fish meal per week (24). A recommendation was made to increase this amount to two to three fish meals per week. It appears that the American public is responding because the current per capita consumption has increased to 6.8 kg fish per year.

It was further recommended at the 1985 conference that total fat intake should be 30% of total calories, with 10% being saturated fatty acids, 10% monounsaturated fatty acids, and 10% PUFAs, the latter being equally divided between ω6 and ω3. Therefore physicians should recommend the substitution of fish for meat so that fish is eaten at least twice per week. Alternatively, individuals who do not like fish and cannot get their ω3 fatty acids from their diet can get them from supplements.

An ideal supplement would contain high amounts of EPA and DHA but little or no cholesterol or vitamins A and D. Vitamin E should be added to prevent oxidation of these highly unsaturated fatty acids. Products that conform to most of these requirements are produced from fish oils pressed or extracted from the flesh of the fish.

In the lay press there is confusion between fish-oil supplements and cod-liver oil. Cod-liver oil extracted from the liver is rich in vitamins A and D. Before the 1940s cod-liver oil was ingested mainly by children as a source of vitamins A and D. In the United Kingdom cod-liver oil is standardized by its vitamin content rather than its fish-oil content. In contrast, fish-oil supplements from the flesh of fish contain only negligible amounts of vitamins A and D.

At the most recent conference, the Second International Conference on the Health Effects of Omega-3 Polyunsaturated Fatty Acids in Seafoods (March 20–23, 1990), the following statement was released:

- Based upon clear evidence of an essential role for ω3 fatty acids in human development and health, the scientists recommended that all infant formula and diets for humans should include ω3 fatty acids, and they expressed concern that steps be taken to stop marketing enteral and parenteral formulas that fail to include any ω3 fatty acids.
- The researchers urged that the appropriate government agencies officially recognize the vitally important differences between ω3 and ω6 polyunsaturated fatty acids. Estimates of the average ω3 nutrition consumption in the U.S. presented by USDA scientists agreed with new nutrient measurements reported from a NHLBI study, with both studies indicating inadequate supplies of ω3 fatty acids in the typical American diet.
- New evidence with an extremely high level of statistical precision, from the National Heart, Lung and Blood Institute study, suggests that the daily dietary intake of 0.5 to 1.0 grams of long chain ω3 fatty acids per day reduces the risk of cardiovascular death in middle aged American men by about 40%, and some new data suggests that ω3 fatty acids may also decrease cancer mortality.
- The research reports make it increasingly evident that eating ω3 fatty acids can have beneficial effects on chronic inflammatory and cardiovascular diseases.

Recently Canada published its 1990 nutrition recommendations (203). As can be seen from Table 10, the Canadian nutrition recommendations include separate recommendations for the two classes of PUFAs. The amounts of ω3 and ω6 fatty acids are given in grams based on energy expressed as daily rates for the various age groups from birth to 75+ y. For pregnancy additional ω3 and ω6 fatty acids are recommended in amounts that increase from the first to the second trimester. There is no increase between the second and third trimester. Additional ω3 and ω6 fatty acids are recommended during lactation.

Conclusions

Omega-3 fatty acids, LNA, EPA, and DHA, have been part of the human diet throughout evolution. Modern agriculture and aquaculture and the industrial revolution have led to changes in the production of both plants and animals and to marked changes in the composition of the food supply of Western societies. Specifically, the amount of ω6 fatty acids in the food supply has increased and that of ω3 fatty acids has decreased during human evolution from an estimated ratio of 1:1 for ω6:ω3 to 10:1 or 20–25:1, based on various estimates.

Omega-3 fatty acids are found in human milk. Earlier animal studies with rodents and nonhuman primates and recent studies with premature infants have shown that DHA is essential for the normal function of retina and brain. Additional studies indicate that ω3 fatty acids are essential throughout the life cycle and many scientific groups have recommended establishing recommended dietary allowance so that ω3 fatty acids will be included in infant formulas and in enteral and parenteral solutions. Furthermore, current data support recommendations for the general public. The 1990 Canadian nutrition recommendations already include specific amounts for ω3 and ω6 fatty acids (in g/d) for the various age groups, with additional amounts recommended during pregnancy and lactation. Thus, the great progress that has been made in nutrition research on the role of ω3 fatty acids in health and disease is already incorporated into nutrition policy in Canada.

Many investigators worldwide have examined the role of ω3 fatty acids in disease states. About 2000 studies involving animal models, tissue cultures, clinical investigations, and randomized double-blind clinical trials have been reported in the past 6 y in the world literature. Such studies include normal subjects and

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patients with atherosclerosis; CHD; hypertension; inflammatory and autoimmune disorders such as arthritis, psoriasis, and ulcerative colitis; and a number of animal models for research on cancer.

The majority of studies have been carried out in patients with CVD. The role of ω3 fatty acids, particularly EPA and DHA, has been investigated in terms of their hypolipidemic, antithrombotic, antiarrhythmic, antihypertensive, and anti-inflammatory aspects. Mechanisms include studies on eicosanoid production and metabolism, cytokine production and suppression, plasminogen activator production, and gene expression. Many of these studies indicate that ω3 fatty acids appear to decrease or inhibit risk and precipitating factors in the development of CVD.

Although an increase in consumption of ω3 fatty acids alone clearly will not eradicate CVD, it is increasingly evident that increasing the amount of ω3 fatty acids in the Western diet by eating fish or supplementing the diet with fish oils may help in the prevention of heart disease as well as in the prevention or amelioration of other disease states.

Addendum

Since this paper was completed in November 1990, a paper was published in the December 1990 issue of *The American Journal of Clinical Nutrition* entitled "Erythrocyte fatty acids, plasma lipids, and cardiovascular disease in rural China" by Wenxun et al (211). In this paper the authors concluded that within China neither plasma total cholesterol nor LDL cholesterol was associated with CVD. A strong inverse correlation between erythrocyte oleate concentrations and CVD was observed. However, erythrocyte oleate concentrations were not associated with plasma cholesterol but were strongly negatively associated with arachidonate concentrations, suggesting potential diminution of CVD by oleate through reduced platelet aggregability. This study then implicates arachidonate in contributing to CVD.

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