

Importance of n-3 fatty acids in health and disease¹⁻³

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ABSTRACT In the past 2 decades, views about dietary n-3 fatty acids have moved from speculation about their functions to solid evidence that they are not only essential nutrients but also may favorably modulate many diseases. Docosahexaenoic acid (22:6n-3), which is a vital component of the phospholipids of cellular membranes, especially in the brain and retina, is necessary for their proper functioning. n-3 Fatty acids favorably affect atherosclerosis, coronary heart disease, inflammatory disease, and perhaps even behavioral disorders. The 38 articles in this supplement document the importance of n-3 fatty acids in both health and disease. *Am J Clin Nutr* 2000;71(suppl):171S-5S.

KEY WORDS n-3 Fatty acids, docosahexaenoic acid, atherosclerosis, coronary heart disease, inflammatory diseases, behavioral disorders

INTRODUCTION

Interest in n-3 fatty acids began some 30 y ago and now culminates in these comprehensive proceedings of the International Conference on Highly Unsaturated Fatty Acids in Nutrition and Disease Prevention, held in Barcelona, November 4-6, 1996. The remarkable concurrence and agreement regarding n-3 fatty acids is evidenced by the several thousand papers extant in the literature.

There is little doubt that n-3 fatty acids are important in human nutrition. They are significant structural components of the phospholipid membranes of tissues throughout the body and are especially rich in the retina, brain, and spermatozoa, in which docosahexaenoic acid (DHA; 22:6 n-3) constitutes $\leq 36.4\%$ of total fatty acids (1, 2). Membrane fluidity is essential for proper functioning of these tissues. In the retina, where n-3 fatty acids are especially important, deficiency can result in decreased vision and abnormal electroretinogram results. These topics are explored extensively in the supplement.

n-3 Fatty acids are essential fatty acids, necessary from conception through pregnancy and infancy and, undoubtedly, throughout life. It is not known whether there is need in the human diet for the entire spectrum of n-3 fatty acids from the 18-carbon α -linolenic acid (ALA; 18:3n-3) with 3 double bonds to the highly polyunsaturated DHA. Considering that DHA can be synthesized from ALA, is there a need for DHA in infant formulas? Or should DHA be supplied to infant formulas in addition to ALA? DHA is certainly transferred across the placenta to the fetus during pregnancy (3) and is always present in human milk along with other n-3 fatty acids, including ALA. Also, what is the proper ratio in the diet of dietary n-6 to n-3 fatty acids? An

imbalance in this ratio can accentuate the n-3 fatty acid deficiency state, as is shown by several review articles in this supplement. The ratio of n-6 to n-3 fatty acids may have increased in industrialized societies because of increased consumption of vegetable oils rich in n-6 fatty acids, ie, linoleic acid (18:2n-6), and reduced consumption of foods rich in n-3 fatty acids. Because both n-3 and n-6 fatty acids are essential, the ratio of arachidonic acid (20:4n-6) to DHA may also be important.

Another important feature of n-3 fatty acids is their role in the prevention and modulation of certain diseases that are common in Western civilization. Evidence of such a role is firm for certain diseases, but only speculative for others. The following is a partial list of diseases that may be prevented or ameliorated with n-3 fatty acids, in descending order of the strength of the available evidence as perceived by this reviewer:

- 1) coronary heart disease and stroke;
- 2) essential fatty acid deficiency in infancy (retinal and brain development);
- 3) autoimmune disorders (eg, lupus and nephropathy);
- 4) Crohn disease;
- 5) cancers of the breast, colon, and prostate;
- 6) mild hypertension; and
- 7) rheumatoid arthritis.

Each of these disease topics and many others are discussed in the various articles in this supplement.

CARDIOVASCULAR EFFECTS OF n-3 FATTY ACIDS

The strongest evidence of a relation between n-3 fatty acids and disease is the inverse relation between the amount of n-3 fatty acids in the diet and in blood and tissues and the occurrence of coronary heart disease and its many complications. Effects of n-3 fatty acids on coronary heart disease have been shown in hundreds of experiments in animals, humans, tissue culture studies, and even clinical trials (4).

Although dietary saturated fat and cholesterol are pathogenic for coronary heart disease, n-3 fatty acids from fish are actually

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protective and, by a variety of mechanisms, prevent deaths from coronary disease, particularly cardiac arrest (5). The unique properties of these fatty acids in coronary heart disease first became apparent in the investigations of the health status of Greenland Eskimos who consumed diets very high in fat from seals, whales, and fish and yet had a low rate of coronary heart disease events (6, 7). Further studies clarified this paradox. The fat the Eskimos consumed contained large quantities of the very-long-chain and highly polyunsaturated fatty acids with 20 and 22 carbons and 5 and 6 double bonds, eicosapentaenoic acid (EPA; 20:5n-3) and DHA, which are abundant in fish, shellfish, and sea mammals and are scarce or absent in land animals and plants. EPA and DHA are synthesized by phytoplankton, which are the plants of the waters and the base of the food chain for marine life. However, the plants of the land also provide a rich source of another n-3 fatty acid, the 18-carbon ALA, from which EPA and DHA may be synthesized and which may itself confer health benefits.

Dietary n-3 fatty acids act to prevent heart disease through a variety of actions (4). They

- prevent arrhythmias (ventricular tachycardia and fibrillation),
- are prostaglandin and leukotriene precursors,
- have antiinflammatory properties,
- inhibit synthesis of cytokines and mitogens,
- stimulate endothelial-derived nitric oxide,
- are antithrombotic,
- have hypolipidemic properties with effects on triacylglycerols and VLDLs, and
- inhibit atherosclerosis.

EPA and DHA have strong antiarrhythmic action on the heart, as reviewed by Kang and Leaf (5). In experimental animals and tissue culture systems, EPA and DHA prevent the development of ventricular tachycardia and fibrillation. When EPA or DHA is given to isolated, contracting myocytes in culture (induced to ventricular fibrillation by a noxious pharmaceutical agent, ie, ouabain), the fibrillation is aborted.

Even total mortality has been improved in several studies in which the n-3 fatty acid intake was increased. In one study, men who consumed salmon ≥ 1 time/wk had a 70% less likelihood of cardiac arrest (8). In another study by Burr et al (9), overall mortality was decreased by 29% in men with overt cardiovascular disease who consumed n-3 fatty acids from fish or fish oil, probably because of the reduction in cardiac arrests. In a third study in France, coronary deaths, especially sudden deaths, were prevented by a diet high in ALA (10).

The most recent data on fish consumption and risk of sudden cardiac death were from the Physician's Health Study in the United States in 20551 male physicians (11). Consumption of ≥ 1 fish meal/wk was associated with a 52% lower risk of sudden cardiac death compared with consumption of < 1 fish meal/mo. Total mortality in this sample was also lower in those who ate fish. There did not appear to be a greater reduction in sudden death in those who ate > 1 fish meal/wk, suggesting a threshold effect. A similar threshold occurred for intake of n-3 fatty acids. Even a small intake was associated in a reduction in sudden death, from 0.3 to 2.7 g/mo. There was not a reduced risk of total myocardial infarction, nonsudden cardiac death, or total cardiovascular mortality. The limitations of this study were that the nutritional history was taken on entry to the study and then cardiovascular events, including sudden death, were followed up for 11 y. Clearly, fish intake could have varied over this period of time, especially since the study involved physi-

cians, who certainly would have been aware of the overall beneficial effects of fish consumption. There was also no measure of fish-oil supplements, which physicians might have taken in an effort to prevent coronary disease.

Thrombosis is a major complication of coronary atherosclerosis that can lead to myocardial infarction. The n-3 fatty acids from fish oil have powerful antithrombotic actions. EPA inhibits the synthesis of thromboxane A₂ from arachidonic acid in platelets (12). This prostaglandin causes platelet aggregation and vasoconstriction. As a result, fish oil ingestion by humans increases the bleeding time and decreases the stickiness of the platelets for aggregation to glass beads (13). In addition, the administration of fish oil enhances the production of prostacyclin, a prostaglandin that produces vasodilation and less sticky platelets (12). In an in vivo baboon model, dietary fish oil prevented platelet deposition in a plastic vascular shunt (14). Injury to the intima of the carotid artery of the baboon invariably caused a marked proliferative and inflammatory lesion, greatly thickening the wall. When the animals were fed fish oil, such damage and intimal thickening were completely blocked.

The EPA and DHA contained in fish oil fed to experimental animals actually inhibited development of atherosclerosis. There is evidence in both pigs and monkeys that dietary fish oil prevents atherosclerosis by actions other than reducing plasma cholesterol concentrations (15, 16). These actions may be associated with the inhibition of monocyte migration into the plaque, with less cytokine and interleukin 1 α production, and through stimulation of the endothelial production of nitric oxide (17). What was previously known as endothelial-derived relaxing factor has been now identified as nitric oxide and the action of this beneficial substance is enhanced by the n-3 fatty acids in fish oil.

Atherosclerotic plaque formation may also be lessened by the reduction in growth factors after fish-oil consumption, particularly platelet-derived growth factor, a potent mitogen for cellular growth (18). Not only is platelet-derived growth factor diminished by fish oil consumption, but its messenger RNA is reduced. Because atherosclerosis begins with cellular proliferation in response to the influx of cholesterol-rich lipoproteins, the inhibition of this proliferation would greatly reduce the growth of the atherosclerotic plaque.

The pronounced effect of fish oil on hyperlipidemia is especially well documented and is supported by results of precise dietary studies in which the effects of a diet rich in salmon oil were compared with those of a vegetable oil and a diet high in saturated fat. Fish oil in particular was shown to lower plasma cholesterol and triacylglycerol concentrations through inhibition of triacylglycerol and VLDL synthesis in the liver (19, 20). Apolipoprotein B production is reduced by consumption of fish oil in comparison with vegetable oils such as safflower or olive oil (21). This mechanism of action is further substantiated by cultures of rabbit and rat hepatocytes in which EPA, in contrast with oleic acid, inhibited triacylglycerol synthesis and stimulated the synthesis of membrane phospholipids (22).

The occasional increase in LDL concentrations that occurs after VLDL and triacylglycerol concentrations are greatly lowered by fish oil is similar to the increase in LDL that occurs after the drug gemfibrozil is given. LDL synthesis and plasma LDL concentrations were reduced after large doses of fish oil were given (23). In contrast with the n-6 fatty acid-rich vegetable oils that lower HDL concentrations, fish oil does not decrease HDL concentrations (19).

Pronounced postprandial lipemia occurs after the fat in high-fat diets is absorbed, and postprandial lipoproteins are known to be atherogenic. They are also thrombogenic because postprandial lipemia increases activated factor VII, a procoagulant (24). Postprandial lipemia from fatty meals of different fats produced similar activation of factor VII (25). Olive oil, touted as a highly beneficial monounsaturated fat, led to just as much activated factor VII as did 4 other fats, including butter. Pretreatment with fish oil greatly lessens postprandial lipemia (26) and this effect should be considered both antiatherogenic and antithrombotic.

As indicated in the report of Hwang et al (27), it is important to ascertain whether n-6 fatty acids from vegetable oils attenuate the beneficial effects of fish and fish oil. Up to 16 g safflower oil, which supplied linoleic acid, was given along with varying amounts of fish oil (6-15 g/d) in this well-controlled metabolic feeding study. The authors documented again some of the beneficial effects of fish oil: reductions in plasma triacylglycerol and plasma fibrinogen even when the diet contained large amounts of linoleic acid-rich safflower oil. However, despite high incorporation of EPA into platelet phospholipids, platelet aggregation and thromboxane B₂ concentrations were unaffected, in contrast with many other studies.

The interactions of dietary saturated fatty acids and fish oil with both thrombotic factors and hyperlipidemia is of interest and was evaluated in healthy men (28). The effects of n-3 fatty acids, principally EPA and DHA, were similar in all diets regardless of variable intakes of saturated fat. The presence of dietary n-3 fatty acids in both the high- and low-saturated-fat diets significantly lowered plasma total cholesterol, VLDL cholesterol, HDL cholesterol, total triacylglycerol, and VLDL triacylglycerol. Because the low-saturated-fat diet decreased total-, LDL-, and HDL-cholesterol concentrations, these results indicated that dietary saturated fats and n-3 fatty acids had independent mechanisms of actions on plasma lipids and lipoproteins. The diet low in saturated fatty acids and high in n-3 fatty acids produced optimal plasma lipid concentrations. The most favorable outcome on platelet function and platelet vascular interactions was obtained when a low-fat diet was supplemented with n-3 fatty acids. A significantly longer bleeding time occurred when n-3 fatty acids were added to a low-saturated-fat diet than when they were added to a diet rich in saturated fats (24). Apparently, a diet high in saturated fats may counteract the beneficial effects of n-3 fatty acids on platelet-vessel wall interactions.

Ideally, the diet best designed to produce the optimal action to prevent cardiovascular disease would be low in saturated fatty acids and high in EPA and DHA from fish or fish oil (28). The low-saturated-fat diet would lower total cholesterol and LDL and the fish oil would lower triacylglycerol and VLDL and have antithrombotic action. However, as already emphasized, the most powerful action of the n-3 fatty acids from fish and fish oil in cardiovascular disease is to prevent ventricular fibrillation and sudden death.

THE ESSENTIALITY OF n-3 FATTY ACIDS AS COMPONENTS OF MEMBRANE PHOSPHOLIPIDS IN INFANCY

There are 2 critical periods for the acquisition of these essential n-3 fatty acids: during fetal development and after birth until the biochemical development in the brain and retina is completed. As already noted, the n-3 fatty acid DHA is an important constituent of the membrane phospholipids of these neural

structures, usually occupying the *sn*-2 position. A typical example is phosphatidylethanolamine, which is especially rich in the brain and retina. DHA occupies the 2 position on the glycerol backbone and stearic acid occupies the 1 position of this molecule. Other phospholipids in which DHA is a prominent feature include phosphatidylcholine, or lecithin; phosphatidylinositol; phosphatidylserine; cerebrosides; and sphingomyelin. There are dozens of different molecular species in the brain and retina, as was denoted in several publications on this subject (29, 30).

n-3 Fatty acid deficiency is manifested in both the blood and in tissue biochemistry (1). Of note is a strikingly low concentration of DHA, which may fall to as much as one-fifth of the normal amount. In addition, the body attempts to replace the deficient DHA with another highly polyunsaturated fatty acid of the n-6 series, docosapentaenoic acid (22:5n-6). Thus, the total polyunsaturated fatty acid content of the membranes may be quite similar, even with a deficiency of DHA, because of its replacement with docosapentaenoic acid. In rhesus monkeys, n-3 fatty acid-deficient diets fed to pregnant animals and then continued after birth induce profound functional changes such as reduced vision, abnormal electroretinograms, impaired visual evoked potential, polydipsia, more stereotypic behavior (eg, pacing), and, perhaps, disturbances of cognition (31, 32). Some of these findings have been replicated in infants fed formulas deficient in n-3 fatty acids (eg, corn- and coconut-oil formulas). However, in human infants the results have been more variable and, obviously, the experimental protocols less rigorous because of ethical considerations. Even so, most studies of premature infants have shown visual impairment and abnormal electroretinograms. In full-term infants the results have been more ambiguous. However, a recent study in full-term infants, in which a standard infant formula was compared with human milk and with formulas enriched with DHA, provided unequivocal evidence of considerable differences in visual evoked potential (33). In all of the human studies, the biochemical evidence in plasma, red blood cells, and, occasionally, in tissues from autopsied infants has substantiated the n-3 fatty acid deficiency state. The lower concentrations of DHA in plasma and erythrocytes are mirrored by lower concentrations in the brain and retina (1). Formula-fed infants have lower concentrations of brain DHA than do infants fed human milk (34, 35). They also have lower intelligence quotients (36).

During pregnancy, both maternal stores and dietary intake of n-3 fatty acids are of importance in insuring that the fetus has adequate amounts of n-3 fatty acids at the time of birth. All the polyunsaturated fatty acids, including DHA, are transferred across the placenta into fetal blood (3). In addition, EPA and DHA in maternal adipose tissue can be mobilized as free fatty acids bound to albumin and be made available to the developing fetus via placenta transport. Several studies in monkeys have indicated that when the maternal diet is deficient in n-3 fatty acids, the infant at birth is likewise deficient as evidenced by low DHA concentrations in their plasma and red blood cells (31). In humans, it was shown that the administration of fish oil or sardines to pregnant women led to higher DHA concentrations in both maternal plasma and red blood cells and in cord blood plasma and red blood cells at the time of birth (37). Once membrane phospholipids have adequate concentrations of DHA, there is an avid retention of these fatty acids in the brain and the retina, even though the diet may subsequently be deficient. Several articles in this supplement illustrate clearly the effects of n-3 deficiency in both animals and humans.

A crucial question for the scientific community and regulatory health bodies throughout the world relates to the amount and kinds of n-3 fatty acids that should be included in infant formulas. It is, of course, completely accepted that infant formulas should contain adequate amounts of n-6 fatty acids. In this context the history of infant formulas is of interest. For many years, infants whose mothers could not feed them human milk were given cow milk diluted with water and reinforced with added sugar (38). These modified cow milk formulas are still used in many parts of the world where poverty or the habits of life prevent the use of commercial infant formulas. The essential fatty acid status of cow milk is questionable, even though the ratio of the n-6 fatty acid linoleic acid to the n-3 fatty acid ALA is appropriate and is in the 2 to 1 range; however, the percentage of energy of essential fatty acids falls far short of World Health Organization recommendations. Modified cow milk has 2% of energy as n-6 and 1% as n-3 fatty acids. Only linoleic acid and ALA are found in cow milk. There are few, if any, detailed studies of the biochemistry and the function of infants fed cow milk.

As noted in the prominent text by Fomon (38) on infant nutrition, infant formulas have undergone many changes since their genesis. A typical corn- and coconut-oil formula has a plethora of linoleic acid and little of ALA. Such formulas were still being marketed up to several years ago in Mexico (32). In the United States, such formulas were changed in the 1980s and soybean oil, which has a good ratio of linoleic acid to ALA (7:1), was introduced. The use of soybean oil has greatly improved the n-3 fatty acid status of currently marketed formulas. The debate now is whether infant formulas could be further improved by the addition of the highly polyunsaturated fatty acids of both the n-3 (DHA) and n-6 (arachidonic acid) categories. This potential improvement in infant formulas, which would make them similar in fatty acid content to human milk, is examined in several articles in this supplement.

CONCLUSION

In summary, n-3 fatty acids have important roles in the modulation and prevention of human diseases, particularly coronary heart disease. Their preventive effects on the brain later in life against disorders such as Alzheimer disease are unknown but are certainly worthy of study. Certainly, the evidence is now strong that n-3 fatty acids are essential for human development in utero and in infancy and are likely to have a role throughout life. The antiarrhythmic effect of n-3 fatty acids is a discovery that has great relevance to the prevention of sudden death from ventricular fibrillation. Further clinical trials in this area are indicated. 

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