

## REVIEW

# Polyunsaturated fatty acids n-3: new data on heart disease, cancer, immune resistance and mental depression

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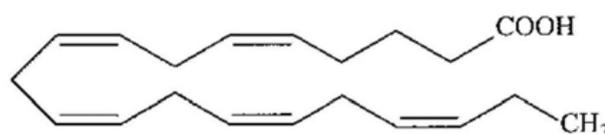
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**Abstract:** Attention to the role of n-3 long-chain polyunsaturated fatty acids from fish (n-3) in human health has remarkably increased during recent decades. Many clinical and epidemiologic studies have shown a positive role for n-3 in cardiovascular diseases, cancer, infant development and more recently, in various mental illnesses, especially in depression. These fatty acids are known to have pleiotropic effects, especially against hypertriglyceridemia and platelet aggregation. These may be mediated through several distinct mechanisms, including alterations in cell membrane composition and function, gene expression, or eicosanoid production. A number of authorities have recently recommended an increase in intakes of n-3 by the general population, because “western” diets are deficient in n-3 and have excessive amounts of n-6 fatty acids. The target EPA + DHA consumption was recommended to be at least 500 mg/day for individuals without heart disease and at least 800 to 1,000 mg/day for patients with known coronary heart disease. To comply with this recommendation, a variety of food products, most notably eggs, yogurt, milk and spreads have been enriched with n-3. Additional controlled clinical trials are needed to document whether long-term consumption or supplementation with eicosapentaenoic (EPA) or docosahexaenoic (DHA) acids results in better quality of life (Fig. 4, Ref. 47). Full Text in free PDF [www.bmj.sk](http://www.bmj.sk).

**Key words:** n-3 polyunsaturated fatty acids, heart disease, cancer, mental illnesses, inflammation, lipoproteins, plant substitutes.

n-3 PUFA (n-3) are a family of polyunsaturated fatty acids that have in common a carbon–carbon double bond in the n-3 position; that is, the third bond from the methyl end of the fatty acid. n-3 from fish contribute the nutritionally essential eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Their biological effect consists to large degree in their specific molecular structure: the carboxy and methyl terminals are positioned close to each other (Fig. 1). The incorporation of EPA and DHA into cell membranes alters the physical characteristics of the membrane. Altered membrane properties and the presence of n-3 released by the action of phospholipid lipases results in antiinflammatory eicosanoid production. n-3 improve biological functions such as ligand binding to nuclear receptors, ion channelling and signal transduction.

Although n-3 have been known as essential to normal growth and health since the 1930s, awareness of their health benefits has increased in the past decades (1). The cardiovascular benefits of DHA and EPA were first observed in the Greenland, Alaskan and Canadian Inuits who consume large amounts of fat from seafood while being afflicted with virtually no cardiovascular



**EPA (5,8,11,14,17-Eicosapentaenoic Acid)**

**Fig. 1. Chemical structure of EPA.**

disease (CVD) (2). Lately it has been revealed that n-3 have also immunosuppressive effects, may protect against cancer by inhibiting the cyclooxygenase 2 (COX-2) enzyme and have a beneficial effect in mental depressive disorders.

n-3 belong to prebiotics, dietary bioactive compounds. These influence the gut microbiota (3). Intestinal microbiota is a target for nutrition intervention and a factor influencing the biologic activity of other components ingested orally. It was disappointing for the n-3 advocates when n-3 did not prove beneficial in inflammatory bowel disease, a paradigm of impaired intestinal microbiota. There was no proven benefit of using fairly large dosage of n-3 in ulcerative colitis or for maintenance of Crohn's disease remission (4).

As with other scientific discoveries resulting in great popular expectation, the biological impact of n-3 on human health has to be subjected to constant critical update.

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### Antagonistic effects of n-6 and n-3 polyunsaturated fatty acids

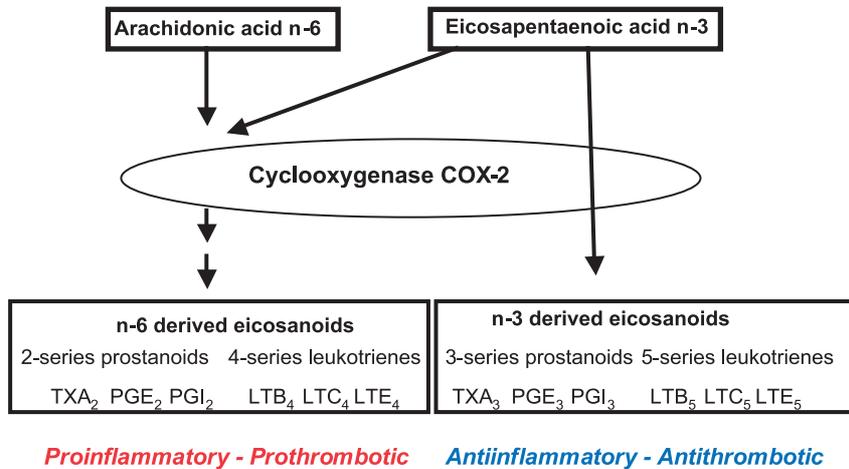


Fig. 2. Antagonistic effects of n-6 arachidonic acid and of n-3 eicosapentaenoic acid on eicosanoid formation. Prostanoids and leukotrienes formed from n-3 have the antiinflammatory and antithrombotic effects.

### DHA and EPA , cardiovascular system and diabetes

During the past three decades, the protective role of n-3, mainly EPA and DHA in prevention of CVD has been widely reported. It is anticipated that incorporation of n-3 into advanced atherosclerotic plaque increases plaque stability and ultimately leads to reduction in adverse cardiovascular events. The association between high intake of n-3 and decreased morbidity and mortality from CVD can be explained by the three main basic mechanisms:

- 1) effect on blood plasma lipoproteins
  - 2) effect on atherothrombosis
  - 3) effect on cardiac arrhythmias and blood pressure.
- 1) Most evidence suggests that n-3 reduce the synthesis and secretion of very-low-density lipoprotein (VLDL) particles and increase triglyceride removal from VLDL and also from chylomicron particles through the upregulation of enzymes, such as the lipoprotein lipase (5). The recently completed Combination of Prescription Omega-3 With Simvastatin (COMBOS) study confirmed that prescription of n-3 administered in combination with simvastatin achieve statistically significant improvements across a range of lipid indicators beyond the LDL primary target, including triglycerides and lipoprotein particle size (6). n-3 then can be used in combination with statins to achieve more global improvements in lipid profiles.
  - 2) The effect on atherothrombosis includes the modulation of the expression of pro-atherogenic genes (e.g., endothelial leukocyte adhesion molecules, inflammatory cytokines and cyclooxygenase). Cyclooxygenase-2 (COX-2) is an enzyme that is responsible for formation of important biological mediators including prostaglandins, prostacyclin and thromboxane. Inhibition of COX-2 by n-3 can provide relief from the

### n3 and sudden cardiac death

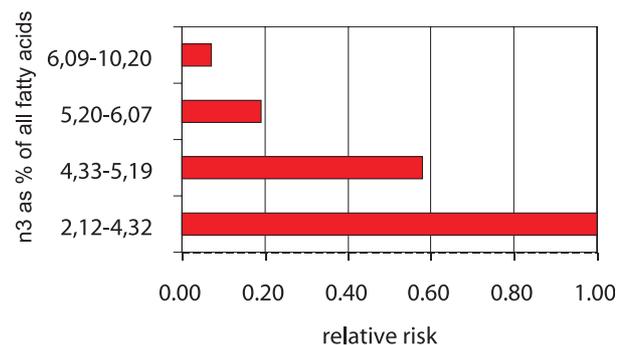
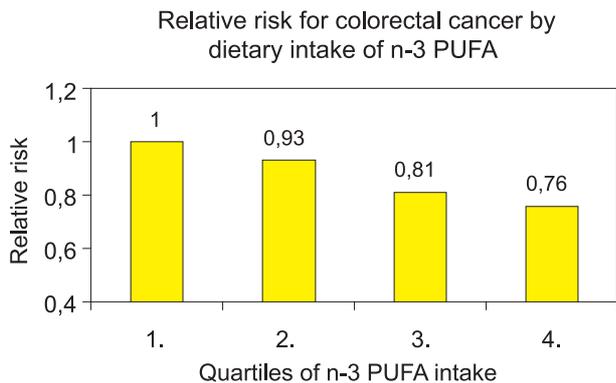


Fig. 3. The increase of n-3 level in blood cell membranes significantly lowered the relative risk of sudden cardiac death. According to Block et al (10).

symptoms of inflammation and pain (Fig. 2). Non-steroidal anti-inflammatory drugs and n-3, exert their effects through inhibition of COX-2.

- 3) Effect on cardiac arrhythmia include complex interactions with ion channels (sodium, potassium and calcium channels), typically requiring the presence of free n-3 (7). n-3 increase the production of bioactive lipid mediators (protectins and resolvins) affecting cytokine-induced signal transduction. They interfere with the generation of reactive oxygen species, responsible for the activation of the transcription nuclear factor, which controls the expression of a variety pro-inflammatory and pro-atherogenic genes. The interplay of these processes has distinct cardioprotective effects, that involve actions on blood pressure, vascular function, coagulation potential, inflammatory response and antiarrhythmic mechanisms (8, 9).



**Fig. 4. The increasing intake of n-3 PUFA significantly lowered the risk of colorectal cancer. According to the 22-year prospective study on n-3 fatty acid intake and cancer (17).**

In summary, n-3 decrease triglycerides, VLDL- and small dense LDL-cholesterol, platelet aggregation, inflammation (interleukin-6, tumor necrosis factor  $TNF_{\alpha}$ ), adiponectin, blood pressure, immune response, oxidative stress and heart rate. On the other side, n-3 increase endothelial function, fibrinolysis, HDL-cholesterol, vasodilatation and plaque stability.

Increased blood levels of n-3 have been inversely associated with a risk for sudden cardiac death and for acute coronary syndromes (10) (Fig. 3). The risk in the highest n-3 group was very small.

The most compelling evidence for cardiovascular benefits of n-3 comes from four controlled trials of nearly 40,000 participants randomized to receive EPA or DHA in studies of subjects for primary prevention and also for secondary prevention in patients after myocardial infarction and with heart failure (11). The target EPA + DHA consumption was recommended to be at least 500 mg/day for individuals without heart disease and at least 800 to 1,000 mg/day for patients with known coronary heart disease. Another report (12) analyzed 11 studies that included over 39,000 subjects who were on n-3 for secondary prevention. These patients had recent myocardial infarction, implanted defibrillators, heart failure, peripheral vascular disease and hypercholesterolemia. Dietary supplementation with n-3 significantly reduced the risk of CVD death.

n-3 are thought to help reduce the risk for cardiovascular disease in diabetes type 2. In 24 trials published between 1966 and 2008 in diabetics, n-3 supplementation decreased triglycerides, fibrinogen and ADP platelet aggregation. There was no influence on HDL-cholesterol, LDL particle size, glycemia, insulinemia, inflammatory biomarkers and blood pressure (13–14). Effect of n-3 supplementation on lipoprotein subclasses was measured by nuclear magnetic resonance in subjects with type 2 diabetes (15). n-3 administration decreased very low-density lipoprotein (VLDL) size, particle concentrations of large VLDL and increased HDL size and small low-density lipoprotein (LDL) concentration. There was no effect on oxidized LDL.

Several recent meta analyses questioned if dietary supplementation with EPA and DHA provides an additive CVD pro-

tection beyond standard care (16). However, the heterogeneity of the studies included in these analyses suggests a reduction in validity of such negative assessment. For a more definitive answer on n-3 effects on CVD and diabetes, there is a need for more trials with defined vascular events or mortality.

#### DHA, EPA and cancer

n-3 may protect against cancer by inhibiting the cyclooxygenase 2 (COX-2) enzyme and by inhibiting the production of arachidonic acid (n-6) derived eicosanoids. Eicosanoids produced from arachidonic acid are proinflammatory, whereas those produced from n-3 are anti-inflammatory. Because n-3 and n-6 fatty acids compete for the COX-2 enzyme, which converts the longer-chain fatty acids to precursors for eicosanoid synthesis, higher n-3 intake may result in decreased production of proinflammatory eicosanoids that could play a role in the development of cancer.

n-3 were shown to attenuate growth and induce apoptosis in a variety of animal and human cancer cell lines derived from colonic, pancreatic, prostate, and breast cancer. The mechanisms underlying the anti-tumor effects of n-3 are complex. n-3 act as ligands of nuclear peroxisome proliferator-activated receptors that attenuate transcription of many genes.

Most studies on n-3 and malignant disease originate from animal experiments. Experience in humans appears contradictory. Of all malignant disorders, colorectal cancer has been mostly focused on. A study that included 22,000 men over 20 years reported a clear preventive influence of n-3 for colorectal malignancy (17) (Fig. 4). While there were other reports on benefits of n-3 in colorectal cancer, other authors denied such beneficial effect (18–23). Recent findings indicated that n-3 act synergistically with chemotherapeutic agents and may also be used to enhance tumor radiosensitivity (24).

#### DHA, EPA and the central nervous system

DHA is the predominant structural fatty acid in the central nervous system and its availability is crucial for brain development. Inadequate intake of n-3 decrease DHA in the brain. Decreased DHA in the developing brain leads to deficits in neurogenesis, neurotransmitter metabolism, and altered learning and visual function. Epidemiological studies have linked low maternal DHA to increased risk of poor child neural development. Improving maternal DHA nutrition decreased the risk of inadequate infant and child visual and neural development (25, 26).

In adult patients with symptoms of depression, the evident role of n-3 deficiency was found in the last years. Depletion of n-3 may be related to the immune and serotonergic pathophysiology of depression by alterations of membrane fluidity and modulation of membrane receptors, enzyme activities and carriers. In epidemiological studies, it has been observed that societies with high consumption of n-3 appear to have lower prevalence of depression. In case-controlled studies, lower levels of n-3 have been found in patients with depression (27). The level of n-3 correlated significantly negatively with the severity of

depressive symptoms. n-3 were shown to be more effective than placebo for depression (28, 29). Long-term fish intake was associated with less severe depressive symptoms (30, 31). A high incidence of depressive symptoms was described in pregnancy with low n-3 intake from fish (32).

DHA deficiency is associated with dysfunction of neuronal membrane stability and transmission of serotonin, norepinephrine and dopamine, which may contribute to the etiology of depression. EPA is important in balancing the immune function and physical health by reducing membrane arachidonic acid and prostaglandin E (2) synthesis, which might be linked to the physical comorbidity in depression (33). There was an interesting speculation (34) that n-3, when co-administered with anti-psychotic agents that are prone to cause arrhythmia by adversely prolonging the QT interval, may prevent these side effects via an arrhythmogenic influence of n-3.

The role of n-3 in brain development and healthy brain aging is emerging as a field of an intense scientific inquiry. The events, in which DHA fulfills its essential roles, including neurotransmission, neurogenesis, and protection from oxidative stress are relevant throughout the lifespan. Larger trials using n-3 in clinically well-defined patients with depressions will be required to demonstrate a therapeutic role for n-3 in these disorders.

#### DHA and EPA in white adipose tissue

The concept of white adipose tissue as an endocrine organ originated from the discovery of leptin. To maintain normal body functions, each adipocyte secretes diverse cytokines and bioactive substances. n-3 become incorporated into the cell membrane of the adipocytes, profoundly affecting the adipocyte function, due to the specific molecular structure of n-3. In mammals, high n-3 content of membrane phospholipids is linked to improved exercise performance due to increased membrane fluidity that accelerates transmembrane lipid transport. n-3 ameliorate a low-grade inflammation of adipose tissue associated with obesity and induce changes in the pattern of secreted adipokines.

n-3 affect the adipocytes by binding to the peroxisome proliferator-activated receptor (PPAR). The binding of n-3 to PPAR activates genes regulating lipid metabolism. Ligand binding causes the dimerization of PPAR with the retinoid-X-receptor (RXR). This complex activates target genes, which include the  $\beta$ -oxidation enzymes, hydroxyacyl dehydrogenase, fatty acid binding proteins, the transmembrane fatty acid transporters and many other enzymes.

n-3 have well-documented protective effects that are attributed to the formation of novel biologically active lipid mediators, such as resolvins and protectins. These compounds are made in the human body from the n-3. Experimental evidence indicates that resolvins reduce cellular inflammation by inhibiting the production and transportation of inflammatory cells and chemicals to the sites of inflammation. The resolvins and protectins are highly stereospecific lipids, which are endogenous local mediators with strong anti-inflammatory effects, in addition to some immunoregulatory activities at picomolar to nanomolar concentrations (35).

There is also evidence that an increased intake of n-3 can reduce body fat, but studies in men are relatively rare. Body mass index (BMI), waist circumference and hip circumference were inversely correlated with n-3, EPA and DHA in the obese group. Obese individuals had significantly lower plasma concentrations of n-3, compared to healthy-weight individuals (36). The dietary transition leading to reduced consumption of fish among younger Canadian aborigines may compound the effects of obesity and emerging insulin resistance (37).

Therefore, n-3 may play an important role in weight status and abdominal adiposity in man. Increased intake of n-3 may improve body composition, but longer-term human studies are needed to confirm efficacy and determine whether increased intake of n-3 might be an effective strategy to combat obesity. Treatments affecting adipose tissue by multiple mechanisms, such as combining n-3 with either caloric restriction or antidiabetic/anti-obesity drugs, should be explored (38). n-3 consumption during energy reduction improves the insulin resistance in young overweight individuals (39). In diabetic women, a moderate dose of n-3 PUFAs reduced adiposity and atherogenic markers without improvement or deterioration of insulin sensitivity (40). Diets in the "Western" part of the world are deficient in n-3 and have excessive amount of n6 fatty acids, compared with the diets on which humanity developed and established its genetic patterns (41).

#### Dietary intake of n-3: From deficient to adequate to excessive

Current intakes of n-3 are low in most individuals living in countries of the "Western" world. Reduced tissue levels of n-3 were documented by the erythrocyte EPA + DHA. This value was suggested as an omega 3 index, a risk marker (42). n-3 are readily incorporated from natural sources or from fish oil capsules into transport (blood lipids), functional cells and storage in the adipose tissue. This incorporation is dose dependent and follows a kinetic pattern characteristic for each pool (43).

In a typical US pharmacy, there is a variety of over the counter (non-prescription) n-3 products, all in softgel form, based on fish oil, some declaring being odorless, cleared of mercury contamination and some in the form of lemonade flavored gums. There is 800–2400 mg of fish oil per soft gel. Some declare only the total of n-3 of 200 to 720 mg per softgel, others are more specific claiming EPA content between 52 to 720 mg and DHA from 52 mg to 400 mg in two softgels (the recommended daily dose). Some products also declare the n6 fatty acids, from 67 to 448 mg in two softgels.

Supplementation of maternal diets with large amounts of n-3 recently started to gain acceptance. However, it is possible that both over- and under-supplementation with n-3 can harm fetal development. Pregnant rats were administered diets with either a control n-3/n6 ratio of 0.14 or an excess with a ratio of 14.5 (44). Offsprings from the excess maternal n-3 feeding had persistent sensory and neurological abnormalities, lower body weight and shorter life span. Although studies like this send a warning message on potential nutritional toxicity of n-3, the dosage used

is not consistent with conditions in humans. The target n-3 consumption was recommended to be at least 500 mg/day for individuals without heart disease and at least 800 to 1,000 mg/day for patients with known coronary heart disease.

### Food products as vehicles for n-3 supplementation

The n-3 that have received most attention are those derived from the fish source. American consumers influenced by popular reports perceive red meat as a source of too much saturated fat, too high in n-6 fatty acids. Concerns on overfishing due to progressively increasing fish consumption, as well as reports on sea pollution (for example mercury) resulted in an urgent need to develop alternate sources of EPA and DHA. The plant n-3, alpha linoleic acid (ALA) can be converted to EPA, but the conversion to DHA is insufficient in humans. But even the conversion of ALA to EPA seems limited. Moderately increased consumption of ALA appears to be of little benefit compared to direct intake of EPA and DHA.

Of the many different strategies considered to develop alternative sources of EPA and DHA, one of the most promising is plant engineering, transgenic plant “reverse engineered” to produce fish oils (45). Oil seed crops are being studied to produce n-3 for cost effective, large scale food supplementation.

Pigs were fed canola mixed with DHA added in the form of alga biomass, 1 g DHA to 1 kg of feed, in order to obtain bacon with more favorable n-3/n-6 index. Bacon DHA increased to 97 mg/100 g. Unfortunately, a taste panel criticized the off-odor and the off-taste of the product. Milk industry is eager to substitute the saturated fatty acids by oleic acid and/or n-3 from fish oil (46). Nine controlled intervention studies with enriched milk have reported effects on healthy volunteers and patients with CVD. The main effect consisted in reduction of blood lipids, mainly cholesterol, LDL-cholesterol and triglycerides. In another study (47), an n-3 supplement was incorporated into pasta sauce, oatmeal and spreads served to cancer patients and to non-patients. The taste panel concluded that the n-3 fortified meals were highly accepted by patients with cancer.

### Conclusion

The interest in the cardiovascular protective effect of n-3 rapidly gained momentum during the past 35 years since publication of the original reports, which described a low cardiovascular morbidity in Greenland, Canadian and Alaskan Eskimos. Since then, the protective role of n-3, mainly of EPA and DHA in the primary prevention in asymptomatic subjects and also in the secondary prevention in patients with existing heart disorders has been amply documented. Lately, it was also revealed that n-3 has the immunosuppressive effects and also may protect against diabetes and cancer by inhibiting the cyclooxygenase 2 (COX-2) enzyme. n-3 have a prominent role in fetal development and in childhood, being important for brain growth and at a later age, having beneficial effect in depressive disorders. An excessive intake of n-6-fatty acids promotes pathogenesis of cardiovascular

disease, inflammatory and autoimmune diseases and cancer. The increased intake and tissue level of n-3 (a low n-6/n-3 ratio) supposedly protect via a suppressive effect on immune mechanisms.

As the importance of n-3 has gained more awareness, the number of food products enriched in n-3 has also increased. Food industry frequently adds fish oil into their final product to enrich it in n-3. Milk and eggs can be naturally enriched in EPA and DHA by feeding animals a diet that is rich in n-3.

The immense popularity of n-3, fueled by thousands of predominantly positive scientific reports, has further increased the appetite of our society for fish. Heavier requirements have been placed on overfishing the oceans. A solution may be in plants, yet the plant-derived n-3, alpha-linoleic acid (ALA) only poorly converts to DHA. One of the most promising strategies appears in metabolic engineering of oil seed crops that may serve as alternative source of EPA and DHA.

Additional controlled trials are required to document whether long-term consumption or supplementation with EPA and DHA will result in healthier mankind and a better quality of life.

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