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Long-chain n-3 fatty acids and cardiovascular disease: further evidence and insights

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Abstract

The traditional Inuit diet was rich in long-chain n-3 polyunsaturated fatty acids (PUFAs), and this is believed to account for the low incidence of cardiovascular disease in Inuit populations. Epidemiological studies in Europe and North America demonstrate inverse relationships between consumption or status of long-chain n-3 PUFAs and cardiovascular morbidity and mortality. Long-chain n-3 PUFAs might act through modification of recognized risk factors such as hypertriacylglycerolemia and hypertension. Secondary prevention studies in post-myocardial infarction patients demonstrate that long-chain n-3 PUFAs, provided in the form of fish oil, reduce cardiovascular events and mortality, with an especially potent effect on sudden death. The anti-thrombotic and anti-arrhythmic actions of long-chain n-3 PUFAs may explain these effects. In addition, long-chain n-3 PUFAs are anti-inflammatory and so may act to increase atherosclerotic plaque stability. This may explain the observed reduction in cardiovascular events and mortality. A recent study has investigated this possibility. Patients awaiting carotid endarterectomy consumed control, sunflower oil, or fish oil capsules until surgery, when the atherosclerotic plaque was removed. The proportions of long-chain n-3 PUFAs were higher in carotid plaque lipids in patients receiving fish oil. Plaques from patients in the fish oil group were more likely to have thick fibrous caps and fewer signs of inflammation and to contain fewer macrophages. This may be indicative of increased plaque stability.

Keywords: Fatty acid; Fish oil; Atherosclerosis; Cardiovascular; Inflammation; Macrophage

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1. Long-chain n-3 polyunsaturated fatty acids protect against cardiovascular disease

The traditional Inuit diet was rich in fat, which often provided as much as 80% of dietary energy; it included regular intake of seal and whale meat and whale blubber [1]. These foods are rich in the long-chain n-3 polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Oily fish also contain these fatty acids in high proportions. Despite their high fat intake, Inuit populations consuming their traditional diet had much lower cardiovascular mortality than predicted [2,3], and the protective component in the Inuit diet was considered to be the long-chain n-3 PUFAs [4]. Japanese populations also exhibit a low cardiovascular mortality [5], and the traditional Japanese diet is rich in seafood including oily fish. Epidemiological evidence has accumulated indicating that consumption of fish or of long-chain n-3 PUFAs reduces the risk of cardiovascular mortality in Western populations [6-15] and in China [16]. Kromhout et al [6] demonstrated a significant effect of consuming fish with a 40% lower 20-year coronary heart disease mortality among men in The Netherlands who consumed 1-14 g fish per day in 1960 compared with men who did not eat fish at all in 1960. Men who consumed >30 g fish per day had a 60% lower risk of 20-year mortality from coronary heart disease compared with non -fish eaters [6]. Daviglus et al [13] reported a dose-dependent effect of fish consumption upon 30-year mortality among men in the United States. Men who ate 1-17 g fish per day in 1957 had a 10% lower risk of death from coronary heart disease or myocardial infarction compared with those who did not eat fish. Men who ate >34 g fish per day had a 40% lower risk of mortality from coronary heart disease or myocardial infarction compared with those who did not eat fish [13].

The cardioprotective effects of long-chain n-3 PUFAs have been confirmed by studies published more recently [17-21]. Data from the Nurses' Health Study revealed that fish and long-chain n-3 PUFA intake decreased risk of coronary heart disease, fatal coronary heart disease, and nonfatal myocardial infarction [18]. In an Italian study, fish and long-chain n-3 PUFA intake were lower in patients who experienced a nonfatal myocardial infarction compared with age- and sex-matched controls [19]. Data from the Physicians' Health Study showed that the long-chain n-3 PUFA status of whole blood at study entry was strongly inversely related to the risk of sudden death over the follow-up period of 0.7-16.9 years (mean 8.7 years) [17]. The relative risk of sudden death was lower by about 50% in men in the second quartile of whole blood total long-chain n-3 PUFA content compared with those in the lowest quartile [17]. The relative risk of sudden death in the highest quartile of blood long-chain n-3 PUFA content was 70-90% lower (depending on adjustment for other factors) than in the lowest quartile [17]. A European multicenter study identified that adipose tissue DHA content, a marker of DHA intake, was inversely associated with risk of first myocardial infarction, even when toe-nail mercury level (associated with increased risk) was adjusted for [20]. The relative risk of first myocardial infarction was 40% lower in men in the highest quintile of adipose tissue DHA content compared with those in the lowest quintile [20]. In another study, the combined EPA and DHA content of plasma phospholipids was lower in cases of fatal coronary heart disease than in matched controls [21]. The authors identified that the odds ratio for fatal coronary heart disease was decreased by 70% with a 1-SD increase in plasma phospholipid EPA plus DHA content [21].

Epidemiological studies that associate decreased cardiovascular mortality with fish or long-chain n-3 PUFA consumption do not readily differentiate between protective effects toward the pathological processes leading to the disease (i.e., atherosclerosis) or toward the processes that ultimately cause death (e.g., myocardial infarction, stroke). Long-chain n-3 PUFAs favorably affect some risk factors for development of atherosclerosis, suggesting that they might slow the progression of the disease. For example, long-chain n-3 PUFAs lower fasting and post-prandial plasma triacylglycerol concentrations (for reviews, see [22-24]) and have a small blood pressure-lowering effect, as confirmed in a recent meta-analysis [25]. Intakes of long-chain n-3 PUFAs that are required to exert triacylglycerol or blood pressure lowering effects are typically >1.5 g/day [22-25]. Additionally, although blood pressure-lowering effects of long-chain n-3 PUFAs have been reported in both normotensive and hypertensive individuals, it appears that the effects are greater in the latter [25]. Despite the potential for protection against atherosclerosis, much of the interest in long-chain n-3 PUFAs has been in their potent protective effect against fatal myocardial infarction [11,13], particularly sudden death [14,17,18]. These effects suggest that long-chain n-3 PUFAs might influence acute events. Although attention has been focused on protection against fatal myocardial infarction and sudden death, several studies also report protection against nonfatal myocardial infarction [11,15,18,19], suggesting that long-chain n-3 PUFAs lower the risk of acute events, either fatal or nonfatal.

2. Secondary prevention studies in post-myocardial infarction patients

Secondary prevention studies providing long-chain n-3 PUFAs, in the form of fish oil, to patients who had already experienced a myocardial infarction demonstrate a significant reduction in mortality outcomes [26-29]. In the Diet and Reinfarction Trial, consumption of oily fish or fish oil capsules by men who had survived a previous myocardial infarction resulted in a 29% reduction in mortality (largely due to decreased mortality related to coronary heart disease) over 2 years [26]. In a more recent study, 0.885 g EPA plus DHA per day resulted in a significant decrease in mortality from cardiovascular events over 3.5 years (30% reduction), with an especially marked effect on sudden death (45% decrease) [28]. These effects occurred in the absence of lipid lowering [28]. The reduction in risk of sudden death at 3.5 years in those patients consuming long-chain n-3 PUFAs was already apparent at 4 months, and the reductions in risk of cardiovascular mortality and coronary heart disease mortality were apparent within 6-8 months of initiating fish oil treatment [29].

3. Mechanisms of protection: anti-thrombotic and anti-arrhythmic actions

Currently, two mechanisms are considered to contribute to the protection against acute cardiovascular events, especially those that are fatal. The first is an anti-thrombotic effect. This is mediated largely through changes in eicosanoid generation from the long-chain n-6 PUFA arachidonic acid. Arachidonic acid is released from cell membrane phospholipids by the increased activity of phospholipase A_2 after stimulation of platelets and endothelial cells. Metabolism of free arachidonic acid by cyclooxygenase gives rise to thromboxane A_2 (TXA_2), a potent promoter of platelet aggregation, and to prostaglandin I_2 (PGI_2;

prostacyclin), a potent inhibitor of platelet aggregation (Fig. 1). One of the characteristic features of increased availability of long-chain n-3 PUFAs, especially EPA, is a reduction in the content of arachidonic acid in membrane phospholipids in platelets [30-32] and most likely also in endothelial cells. This decreases the amount of substrate available for eicosanoid synthesis. Therefore, long-chain n-3 PUFAs are associated with a decrease in production of TXA_2 and PGI_2 (Fig. 2). Furthermore, EPA, which is readily incorporated into cell membrane phospholipids, is released by the action of phospholipase A_2 and also acts as a substrate for cyclooxygenase. The products produced (e.g., TXA_3 and PGI_3) have a different structure from those produced from arachidonic acid, and this can affect their biological potency. TXA_3 has a weaker pro-aggregatory effect than does TXA_2 (Fig. 2). In contrast PGI_2 and PGI_3 have similar anti-aggregatory potencies (Fig. 2). Therefore, the effect of long-chain n-3 PUFAs is to promote a less thrombotic environment [32]. This may explain why long-chain n-3 PUFAs have an especially potent effect on fatal events, including sudden death.

The second mechanism that might be important is an anti-arrhythmic action of long-chain n-3 PUFAs. Dietary studies in rats, dogs, and marmosets demonstrate that long-chain n-3 PUFAs have anti-arrhythmic effects [33-35], which can be mimicked in cultured cardiomyocytes [36,37]. The presence of long-chain n-3 PUFAs in cardiomyocyte membrane phospholipids decreases electrical excitability and modulates the activity of ion channels [38,39], effects that may promote electrical stability in the cell and prevent arrhythmias. In addition to events mediated by modulation of ion channels, heart rate variability may be influenced by long-chain n-3 PUFAs, and low heart rate variability is believed to be associated with increased mortality post-myocardial infarction. Christensen et al [40] reported increased heart rate variability in myocardial infarction survivors given 5.2 g long-chain n-3 PUFAs/day for 12 weeks. This dose is substantially higher than those used in the secondary prevention studies demonstrating benefit [26-29].

4. Atherosclerotic plaque stabilization—a novel protective mechanim of action?

Long-chain n-3 PUFAs exert anti-inflammatory actions [41-44]. These may play an important role in the cardioprotective effects of long-chain n-3 PUFAs. Inflammation is recognized to play a key role in the progression of atherosclerosis [45,46], and so decreased

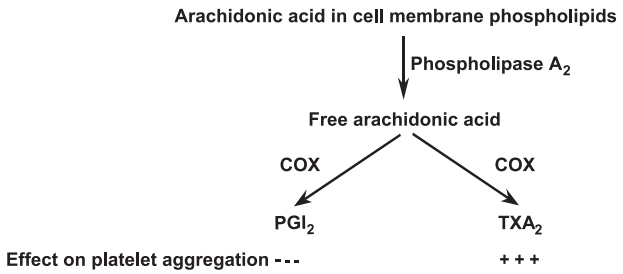

Fig. 1. Role of arachidonic acid as a precursor for eicosanoids involved in regulating platelet aggregation. PGI_3 and TXA_3, both produced from arachidonic acid, have antagonistic effects on platelet aggregation.

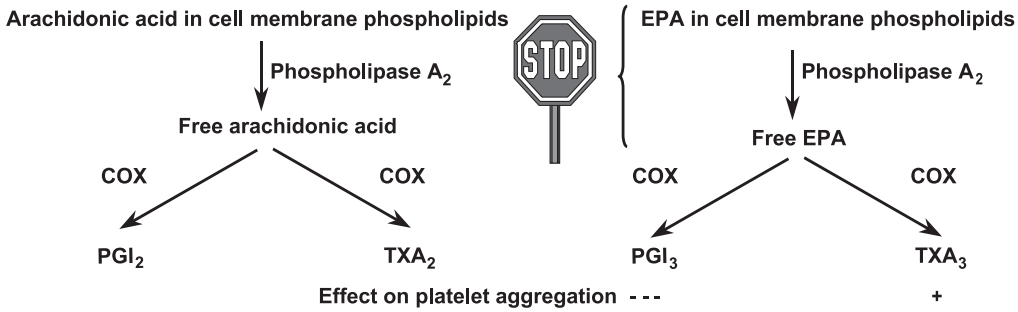

Fig. 2. Mechanism of action of eicosapentanoic acid (EPA) upon platelet aggregation. EPA decreases the amount of arachidonic acid in cell membranes, so making less substrate available for synthesis of PGI_2 and TXA_2. Furthermore, EPA inhibits arachidonic acid metabolism by cyclooxygenase (COX) (see Refs. [41-44]). Thus, EPA results in decreased synthesis of PGI_2 and TXA_2. EPA is a substrate for COX, giving rise to PGI_3 and TXA_3. PGI_3 is a potent inhibitor of platelet aggregation, whereas TXA_3 is a relatively weak promoter.

inflammatory activity as a result of dietary exposure to long-chain n-3 PUFAs could alter the progression of the disease. Furthermore, the rupture of an atherosclerotic plaque, which is the acute event that exposes the plaque contents to the highly pro-thrombotic environment of the blood vessel lumen, is essentially an inflammatory event [47]. The characteristics of an atherosclerotic plaque that make it vulnerable to rupture include a thin fibrous cap and increased numbers of inflammatory cells such as macrophages [48-50]. Long-chain n-3 PUFAs might act to stabilize atherosclerotic plaques by decreasing infiltration of inflammatory and immune cells into the plaques and/or by decreasing the activity of those cells once in the plaque.

A recent study attempted to address for the first time the question of whether long-chain n-3 PUFAs influence atherosclerotic plaque stability [51]. Patients destined to undergo carotid endarterectomy (surgical removal of an advanced atherosclerotic plaque from the carotid artery) were randomized to consume placebo, sunflower oil, or fish oil until surgery. Patients consumed 6 g oil per day. The placebo was an 80:20 mix of palm and soybean oils; this mix has a fatty acid composition that is very similar to that of the average diet in the United Kingdom. Patients in the fish oil group consumed an extra 1.4 g EPA plus DHA per day. This is not dissimilar to the amount used in the secondary prevention trials [26-28], and is an amount that could be included in the diet through regular consumption of oily fish or fish oil capsules. About 60 patients were randomized to each treatment arm, and patients continued with other medications. After exclusions and dropouts, data for 57, 52, and 53 patients were available in the placebo, sunflower oil, and fish oil groups, respectively. Duration of treatment was 7-189 days, with a median of 42 days, and did not differ among the three groups. The proportions of EPA and DHA were higher in carotid plaque lipids in the fish oil group than in either of the other two groups (Table 1). Thus, even when provided at a modest dose and for a relatively short duration, long-chain n-3 PUFAs are able to enter advanced atherosclerotic plaques. Whether they enter as components of lipoproteins or of cells is not clear from the study. A higher content of EPA and DHA in atherosclerotic plaques from patients consuming fish oil had been reported previously [52]. However, that study used a very high dose of fish oil (48-64 g/day providing 16-21 g EPA plus DHA per day), was not placebo-controlled or

Table 1
Outcomes in the control, sunflower oil, and fish oil groups studied by Thies et al [51]

Outcome	Units	Placebo	Sunflower Oil	Fish Oil
EPA in plaque phospholipids*	g/100 g total fatty acids	0.6 ± 0.4	0.6 ± 0.5	1.1 ± 0.6[a]
EPA in plaque cholesteryl esters*	g/100 g total fatty acids	1.1 ± 0.5	1.1 ± 0.9	1.5 ± 0.5[a]
DHA in plaque phospholipids*	g/100 g total fatty acids	3.3 ± 1.2	2.9 ± 1.0	3.6 ± 1.2[b]
DHA in plaque cholesteryl esters*	g/100 g total fatty acids	1.5 ± 0.6	1.6 ± 0.6	2.0 ± 0.8[a]
Plaque morphology				
AHA type IV	% plaques	60	61	72[a]
AHA type V	% plaques	30	32	15[a]
Macrophage infiltration				
Moderate	% plaques	13	19	38[a]
Heavy	% plaques	84	81	62[a]

[a] Significantly different from placebo and sunflower oil.
[b] Significantly different from sunflower oil.
* Mean ± SD.

blinded, studied a heterogeneous group of atherosclerotic plaques (carotid, femoral, aorta, iliac) removed from 11 patients, and provided no structural details of the plaques. In contrast, Thies et al [51] used a modest and achievable dose of fish oil in a placebo-controlled, double blind, randomized trial. They studied one type of plaque (carotid) removed from a large number of patients (>50 per treatment group) and provided some highly novel detail about plaque structure. The morphology of plaque sections was characterized according to the American Heart Association (AHA) classification [48] and a modification of this [53]. About 90% of plaques were of the AHA type IV or type V classification. These types relate to the "fibrous cap atheroma" (well formed necrotic core with an overlaying thick fibrous cap) and the "thin fibrous cap atheroma" (thin fibrous cap infiltrated by macrophages and lymphocytes) of the modified classification [53], respectively. Type IV or fibrous cap atheromas are considered to be more stable than type V or thin fibrous cap atheromas. Plaques from patients treated with fish oil were more likely to be type IV than those from the other two groups (Table 1). Conversely, plaques from patients treated with fish oil were less likely to be type V than those from the other two groups (Table 1). Thus, there were more plaques with a well formed fibrous cap, rather than a thin inflamed cap, in the fish oil group than in either of the other groups. Across all patients, the EPA and DHA contents of type IV plaques were higher than those of type V plaques, which in turn were higher than those of type VI plaques, further suggesting that long-chain n-3 PUFAs are associated with plaque stability. Infiltration by macrophages was investigated using immunohistochemistry. It was found that plaques from patients given fish oil were less heavily infiltrated with macrophages (Table 1). Across all patients, plaques with a higher content of EPA and DHA showed lower macrophage infiltration [51].

The relatively rapid effects of long-chain n-3 PUFAs on plaque morphology and macrophage infiltration observed by Thies et al [51] are consistent with the time course of the reported effects of these fatty acids on mortality in secondary prevention studies. In the study of Burr et al [26], the survival curves of the control and long-chain n-3 PUFA groups

began to diverge after about 60 days, although it is not clear when the groups became significantly different. In the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI) Prevenzione study, the survival curve for patients receiving fish oil diverged from that of the controls also after about 60 days, and statistically significant differences in total mortality and sudden death were apparent by 3 and 4 months, respectively [29]. These studies support the idea that atherosclerotic plaques are dynamic and responsive to dietary modification that can affect plaque stability, as suggested by the results of Thies et al [51].

Because it is the vulnerability of the plaque to rupture rather than the degree of atherosclerosis that is the primary determinant of thrombosis-mediated acute cardiovascular events [50], it is likely that the findings of Thies et al [51] are clinically relevant. If carotid plaques are stabilized by fish oil, then the risk of neurological events (e.g., transient ischemic attacks) in patients with advanced carotid atherosclerosis may be reduced. Furthermore, if these effects occur early in atherosclerosis (rather than just in advanced disease as examined in [51]), then it might be possible to significantly slow the development of unstable plaques. If a similar stabilizing effect of long-chain n-3 PUFAs occurs in coronary plaques, then these too might be stabilized. This might explain the significant protective effects of long-chain n-3 PUFAs toward both fatal and nonfatal cardiovascular events, which are so far unexplained.

The observations of fewer macrophages within the plaque and fewer plaques with thin inflamed fibrous caps in the fish oil group [51] might be linked, since it is primarily macrophages that contribute to plaque inflammation and instability [54]. Therefore, the primary effect of long-chain n-3 PUFAs might be on macrophages. It is not clear how macrophage numbers within the plaque might be decreased. This might be due to fewer monocyte/macrophages entering the plaque. This could occur as a result of decreased adhesion molecule expression on endothelial cells and/or the monocyte/macrophage itself, which would act to limit movement of monocyte/macrophages into the plaque. Cell culture studies have shown that long-chain n-3 PUFAs can decrease the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule -1 (VCAM-1) on the surface of endothelial cells [55-57] and monocytes [58]. Furthermore, feeding fish oil decreased the expression of several adhesion molecules, including ICAM-1, on the surface of rat lymphocytes [59], mouse macrophages [60], and human monocytes [61]. However, Thies et al [51] reported no reduction in staining of ICAM-1 or VCAM-1 in plaques after fish oil treatment, suggesting that this is not the mechanism by which the reduction in macrophage numbers occurs. A second mechanism by which monocyte/macrophage entry into the plaque might be decreased is through decreased generation of chemoattractants. There is evidence that dietary fish oil decreases the production of a range of chemoattractants including leukotriene B_4 [62,63], platelet-derived growth factor [64], platelet activating factor [63], and monocyte chemoattractant protein -1 [65]. This mechanism was not investigated by Thies et al [51] and so cannot be excluded. An alternative means by which macrophage numbers within the plaque could be decreased is by more monocyte/macrophages leaving the plaque. That material, perhaps including cells, can leave atherosclerotic plaques is indicated by a study that demonstrated regression of coronary atherosclerosis in some patients with angiographically documented coronary heart disease who were given fish oil [66]. One other

means by which the number of monocyte/macrophages in the plaques of patients given fish oil might be decreased is through an increased rate of cell death by either apoptosis or necrosis. Feeding fish oil to mice was shown to increase the level of expression of fas [67], a cell surface protein that acts to sensitize cells to apoptotic signals, on lymphocytes, and to increase lymphocyte apoptosis [68]. However, there is little published information about dietary n-3 PUFAs and monocyte/macrophage apoptosis, although both EPA and DHA have been shown to increase apoptosis of human monocytes and monocytic cell lines in culture [69,70]. Activation of peroxisome proliferator activated receptor- γ (PPAR γ) can result in monocyte/macrophage apoptosis [71], and studies suggest that n-3 PUFAs can induce PPAR γ activation. PPAR γ is found in atherosclerotic plaques [72], and dietary fish oil might result in activation of PPAR γ in plaque monocyte/macrophages, driving them toward apoptosis. Cell culture studies indicate that activation of PPAR γ in human monocytes also results in inhibition of production and activity of matrix metalloproteinase -9 [72]. Since matrix metalloproteinases are a major contributor to plaque instability [54], this might provide a mechanism by which n-3 PUFAs improve plaque stability. However, although apoptosis might decrease the numbers of monocyte/macrophages in the plaque, apoptosis could enhance plaque inflammation due to release of mediators from the apoptosed cell [73,74]. Future studies will need to examine the relationship between n-3 PUFA incorporation into plaques, PPAR γ, and inflammatory mediator expression in the plaque, plaque monocyte/macrophage apoptosis, and plaque morphology.

5. Summary

The traditional Inuit diet was rich in long-chain n-3 PUFAs, and this is believed to account for the low incidence of cardiovascular disease in Inuit populations. Epidemiological studies in Europe and North America have demonstrated inverse relationships between consumption or status of long-chain n-3 PUFAs and cardiovascular morbidity and mortality. Long-chain n-3 PUFAs might act through modification of recognized risk factors such as hypertriacylglycerolemia and hypertension. Secondary prevention studies in post-myocardial infarction patients have shown that long-chain n-3 PUFAs, provided in the form of fish oil, decrease cardiovascular events and mortality, with an especially protective potent effect on sudden death. The anti-thrombotic and anti-arrhythmic actions of long-chain n-3 PUFAs may explain these effects. In addition, long-chain n-3 PUFAs are anti-inflammatory and so may act to increase atherosclerotic plaque stability. This may contribute to the observed reduction in cardiovascular events and mortality. More information is needed about the molecular and cellular effects of long-chain n-3 PUFAs within atherosclerotic plaques to understand fully how these nutrients exert their benefits.

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