Long-chain omega 3 fatty acids, blood lipids and cardiovascular risk reduction

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Increasing evidence suggests that omega 3 fatty acids derived from fish and fish oils may play a protective role in coronary heart disease and its many complications, through a variety of actions, including effects on lipids, blood pressure, cardiac and vascular function, prostanoids, coagulation and immunological responses. Interesting differences between the effects of highly purified eicosapentaenoic acid and docosahexaenoic acid are emerging, which may be relevant in the choice of omega 3 fatty acid for incorporation into food products. On the basis of our current knowledge, we believe it is justified to recommend, particularly to high-risk populations, an increased dietary intake of omega 3 fatty acids through the consumption of fish. Curr Opin Lipidol 12:11–17.

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Abbreviations

DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid

0957-9672

Introduction

There is evidence from experimental, clinical and some epidemiological observations that omega 3 fatty acids derived from fish and fish oils protect against atherosclerotic heart disease and sudden coronary death [1,2•]. The omega 3 fatty acids have a wide range of biological effects leading to: improvements in lipid and lipoprotein metabolism [3–5]; blood pressure [6,7] and cardiac function [8]; arterial compliance [9]; endothelial function and vascular reactivity [10]; reduced neutrophil and monocyte cytokine production [11]; and potent anti-platelet and anti-inflammatory effects [1]. Recent data have also shown that the two principal omega 3 fatty acids in fish oils, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have differential effects on lipids and lipoproteins [12••,13], blood pressure [14••], heart rate [14••] and vascular reactivity [15••] in humans.

Many of the above-mentioned effects of omega 3 fatty acids have recently been reviewed [2•,16•,17•], and will not be addressed in detail in this discussion. This review will focus on the most recent research related to omega 3 fatty acids and cardiovascular disease.

Epidemiological observations and population studies

Most, but not all, population studies have shown that omega 3 fatty acids are associated with reduced rates of coronary mortality. The Zutphen Study [18] showed that men who rarely or never ate fish had a higher rate of coronary heart disease than those who ate fish more than once a week. These findings were confirmed in subsequent studies [19–22], including those by Siscovick and colleagues [23,24•], who demonstrated in a population-based case-controlled study, that patients consuming modest amounts of fish, the equivalent of one fatty fish meal per week, had a significantly lower risk of primary cardiac arrest compared with those that did not eat fish at all. The authors suggested that alterations in cell membrane composition after omega 3 fatty acid intake were partly responsible. Such studies contrast with others that have failed to demonstrate benefits from increased fish consumption [25••]. In a systematic review, Marckmann and Gronbaek [25••] concluded that the discrepancy between studies may be related to differences in the populations studied, with only high-risk individuals benefiting from increasing their fish consumption. It was proposed that, in high-risk popula-

tions, an optimum fish intake estimated at 40–60 g a day, would lead to approximately a 50% reduction in death from coronary heart disease.

The benefits of increased omega 3 fats in the secondary prevention of heart disease have been assessed in several prospective, randomized, controlled trials. Burr *et al.* [26] in the Diet and Reinfarction Trial showed that overall mortality was decreased by 29% in men who ate fish or consumed fish oils and had had a previous myocardial infarction. The largest trial to date is the GISSI Study [27••], which involved over 11 300 patients who had survived a myocardial infarction within the previous 3 months. Patients were randomly assigned in a factorial, open-label, controlled design to receive either fish oil (1 g a day), vitamin E (300 mg a day), both interventions, or no treatment. After 3.5 years, the omega 3 fatty acid group had a statistically significant 20% reduction in total mortality, a 30% reduction in cardiovascular death and a 45% decrease in sudden death. Non-fatal myocardial infarction and stroke were not significantly influenced by omega 3 fatty acids. Interestingly, the combination of fish oil with vitamin E did not increase the benefit compared with fish oil alone.

Possible mechanisms underlying cardiovascular benefits

The omega 3 fatty acids have been demonstrated to modify key cardiovascular risk factors. The following describes recent literature addressing the possible mechanisms of action of the omega 3 fatty acids benefiting cardiovascular disease.

Cardiac function and antiarrhythmic actions

Experimental animal, human and cell culture studies have reported anti-arrhythmic actions of omega 3 fatty acids [8]. Such studies suggest that omega 3 fatty acids alter electrophysiological function in a manner that reduces the vulnerability to ventricular fibrillation. It is thought that omega 3 fatty acids induce changes in fatty acid composition of the myocardial phospholipids, which may affect cellular transport processes or enzyme activities, leading to alterations in cardiac function. In support of this, Ku *et al.* [28•] showed that the recovery of cardiac function after cold storage was impaired in hyperlipidaemic rats fed a high-fat diet, but was restored in the presence of omega 3 fatty acids. Moreover, EPA was more effective than DHA. In contrast, McLennan *et al.* [29] reported that DHA, but not EPA, inhibited ischaemia-induced cardiac arrhythmias in rats. In humans, we [14••] and others [30] have also shown that compared with placebo, heart rate was significantly reduced by DHA and increased by EPA.

Decreased heart rate variability is a powerful predictor of mortality, sudden cardiac death and arrhythmic events in post-myocardial infarction patients [31]. Christensen *et al.* [32] reported that omega 3 fatty acids significantly increased heart rate variability in human survivors of myocardial infarction, suggesting an anti-arrhythmic effect. Furthermore, the beat-to-beat variability correlated directly with the DHA content of platelet membranes in these patients [33]. The same authors [34•] demonstrated a beneficial dose–dependent effect of omega 3 fatty acids on heart rate variability in healthy men and women randomly assigned to receive either 6.6 or 2.0 g omega 3 fatty acids, or olive oil, daily for 12 weeks. Basal DHA levels in granulocytes and platelets were also positively associated with all indices of heart rate variability in men.

Blood pressure and vascular reactivity

Improvements in blood pressure [6,7,35] after omega 3 fatty acid intake are partly related to changes in vascular function [10], as well as possible cardiac effects [8]. In a placebo-controlled trial, Goodfellow *et al.* [36•] showed a significant improvement in flow-mediated dilation of the brachial artery after a 4-month treatment with 4 g per day of omega 3 fatty acids, in individuals with hyperlipidaemia. The improvement was confined to endothelial-dependent responses.

Our group has reported that ambulatory blood pressure [14••] and vascular reactivity of the forearm microcirculation [15••] in hyperlipidaemic men, were significantly improved by DHA but not EPA supplementation. Furthermore, the improvement in vascular function after DHA supplementation was evident in both endothelial and smooth muscle responses [15••].

There are several mechanisms by which omega 3 fatty acids may influence vascular function, including the suppression of vasoconstrictor prostanoids, enhanced production or the release of nitric oxide, reduced plasma noradrenaline, changes in calcium flux and increased membrane fluidity [10,15••]. Hashimoto *et al.* [37•] showed that DHA had a greater effect than EPA in increasing membrane fluidity of endothelial cells cultured from rat thoracic aortas. These findings may be pertinent to our results in hyperlipidaemic humans, demonstrating that DHA had a greater effect on maintaining vascular function than EPA [15••].

Platelet aggregation and haemostasis

The omega 3 fatty acids have potent anti-platelet effects [1,38]. Wensing *et al.* [39•], compared the effects of a mixture of EPA/DHA or olive oil or α-linolenic acid, for 6 weeks in elderly volunteers. They found that EPA/DHA, but not α-linolenic acid, significantly reduced ex-vivo platelet aggregation as measured with a filtragometer. However, none of the diets affected ADP- or collagen-induced platelet aggregation and thromboxane

B_2 *in vitro* in either platelet-rich plasma or whole blood. In an uncontrolled study, Lund *et al.* [40] showed that the maximum platelet aggregation to ADP was significantly reduced after a daily supplement of approximately 1.5 g omega 3 fatty acids in 17 healthy volunteers, for 6 weeks. Nordoy *et al.* [41•] also showed that in patients with combined hyperlipidaemia taking a statin, omega 3 fatty acids significantly reduced activated factor VII concentrations during postprandial hyperlipidaemia compared with corn oil, indicating that fish oils could reduce the thrombotic potential associated with an intake of fat-rich meals.

Effects on glucose and insulin

Controlled trials in healthy volunteers [42], in hypertensive individuals [43] and in dyslipidaemic patients [44] have shown no adverse effects on glucose tolerance. However, the effects of omega 3 fatty acids on glycaemic control remain controversial, particularly in patients with type 2 diabetes [42,45,46]. A meta-analysis by Friedberg *et al.* [46], comprising 26 controlled trials in types 1 and 2 diabetic patients, reported that omega 3 fatty acids did not lead to any deleterious effects on glycaemic control in diabetic patients, and concluded that doses of up to 3 g per day were safe and effective.

Three recent studies provided further insight into the issue relating to omega 3 fatty acids and glycaemia. In type 2 diabetic patients, Dunstan *et al.* [47] found that a daily fish meal impaired glucose control over 2 months, but the effect could be prevented by a conventional exercise programme. In overweight hypertensive individuals, Mori *et al.* [48••] showed that the combination of a daily fish meal and a weight loss programme improved glucose and insulin metabolism. Patients were randomly allocated to either a daily fish meal, a weight-loss regimen, the two regimens combined, or a control group, for 16 weeks. Although there was no significant effect of fish in participants in the non-energy-restricted groups, the combination of the two regimes improved fasting glucose and insulin levels, as well as responses after an oral glucose load. In another report in mildly hyperlipidaemic men [12••], we demonstrated that although both 4 g a day of EPA and DHA significantly increased fasting insulin levels, EPA, but not DHA, was responsible for increasing fasting glucose levels. We also found that in type 2 diabetic hypertensive patients, 4 g a day of EPA or DHA both increased fasting glucose levels, whereas self-monitored blood glucose levels increased more with EPA than DHA (R. Woodman *et al.*, personal communication).

Influence on lipids, postprandial lipaemia and peroxidation

There is now overwhelming evidence that lipids play a major role in atherosclerosis, and the modification of lipids by omega 3 fatty acids is most likely to represent a major anti-atherogenic mechanism of action. The effects of omega 3 fatty acids likely to play a key role are summarized below.

Lipids and lipoproteins

Studies in animals and humans have shown that omega 3 fatty acids reduce serum triglyceride levels by approximagely 25–30% [3–5,49•]. The primary mechanisms involve the suppression of hepatic VLDL and triglyceride production. Lu *et al.* [50•] also suggested that omega 3 fatty acids increase the catabolism of VLDL to LDL, via enhanced binding of omega 3 fatty acid-enriched VLDL to lipoprotein lipase.

In a meta-analysis of human trials, Harris [4] concluded that omega 3 fatty acids have no clinically substantial effect on total cholesterol levels. LDL-cholesterol levels increased by approximately 3% in normolipidaemic individuals and 7% in various types of hyperlipidaemic individuals in the short term, but returned to baseline levels with time. The effect on HDL-cholesterol levels was minimal and inconsistent, although in our own carefully controlled studies [42] 3.5–4 g a day of fish oils consistently increased HDL-cholesterol levels, primarily as a result of a significant increase in the HDL_2-cholesterol subfraction. HDL_2-cholesterol levels increased by 24% after a weight-loss regimen that included a daily fish meal in overweight hypertensive individuals [48••]. At the same time, triglyceride levels were reduced by 38%, whereas total cholesterol and LDL-cholesterol levels were unaffected.

The differential effects of highly purified EPA and DHA on HDL and LDL metabolism have been shown in overweight, hyperlipidaemic men randomly assigned to receive 4 g a day of EPA, DHA or olive oil [12••]. DHA significantly increased HDL_2-cholesterol levels by approximately 29%, without changing HDL-cholesterol levels, whereas EPA reduced HDL_3-cholesterol levels but had no effect on LDL-, HDL- or HDL_2-cholesterol levels. DHA significantly increased LDL-cholesterol levels by 8%, but the LDL particle size was significantly increased, a finding that might be considered to be anti-atherogenic. This was the first report of a specific effect of pure DHA on LDL particle size, although others [51] showed an increase after omega 3 fatty acid intake. This effect may be related to the reduced activity of the cholesterol ester transfer protein, which mediates the exchange of triglycerides and cholesterol esters between VLDL, LDL and HDL. Reduced cholesterol ester transfer protein activity has been shown after omega 3 fatty acid intake [52]. Both EPA and DHA reduced triglyceride levels to a similar extent as also reported by Grimsgaard *et al.* [13].

Postprandial lipaemia

Omega 3 fatty acids also reduce triglyceride levels after a postprandial challenge [53•], in normal individuals [54], hypertriglyceridaemic patients [55], and in patients with combined hyperlipaemia taking a statin [41•]. In addition, Emken *et al.* [56•] in a stable isotope study in humans, reported that DHA enhanced postprandial triglyceride clearance.

A pronounced lowering of postprandial chylomicrons [54] and chylomicron remnants [57] has also been described after omega 3 fatty acid intake. It is thought that omega 3 fatty acids accelerate chylomicron lipid clearance by facilitating lipoprotein lipase-mediated lipolysis [58]. Westphal *et al.* [59], in an uncontrolled study in hypertriglyceridaemic men, showed that omega 3 fatty acids reduced postprandial chylomicrons and VLDL at 4–8 h, but both small and large chylomicron remnants were reduced only in the late postprandial phase.

Lipid peroxidation

Concern remains with respect to the potential for increased lipid peroxidation after long-chain polyunsaturated omega 3 fatty acid intake [60]. To date, however, the data *in vivo* are inconclusive, perhaps because of limitations in the methodologies employed. Our data do not support previous literature suggesting adverse effects of omega 3 fatty acids on lipid peroxidation. Fish meals, providing approximately 4 g per day of omega 3 fatty acids to type 2 diabetic patients, reduced rather than increased in-vivo oxidant stress, as assessed by a reduction in urinary F_2-isoprostanes [61••]. These findings were confirmed in a trial in overweight, mildly hyperlipidaemic men [62•] randomly assigned to receive 4 g a day of purified EPA, DHA, or olive oil, where relative to the olive oil group, post-intervention urinary F_2-isoprostanes were reduced equally by EPA (27%) and DHA (26%). We suggested that these results might be explained by the anti-inflammatory effects of omega 3 fatty acids, with reduced free radical formation by white cells.

These findings are further supported by reports of omega 3 fatty acids leading to significantly reduced malondialdehyde levels in unstimulated platelets in an elderly population [63], and reduced the susceptibility of LDL to in-vitro metal ion oxidation [64].

Other recent developments

Studies employing omega 3 fatty acids [1] have shown that the cell membrane composition of EPA and DHA is increased at the expense of omega 6 fatty acids. Recently, Lemaitre-Delaunay *et al.* [65•] examined the distribution of ^{13}C-DHA into plasma, platelets and erythrocytes of healthy men as a function of time, and demonstrated that the metabolic fate of DHA differed significantly when ingested in triglyceride or phospholipid form, both in terms of the bioavailability in plasma and accumulation in target organs.

Prevention and regression of atherosclerosis

Omega 3 fatty acid supplements have now been shown to be effective in reducing the progression of atherosclerotic lesions in humans. In patients with angiographically confirmed coronary heart disease randomly assigned to receive either 1.5 g per day of fish oil or placebo, patients taking fish oil had less progression and more regression after 2 years [66••].

Studies in pigs [67] and monkeys [68] have shown that the development of atherosclerosis can be significantly inhibited by fish oils, via mechanisms not entirely dependent on lipid lowering. These mechanisms include the suppression of cell growth factors and the inhibition of smooth muscle cell proliferation, less macrophage infiltration into the vessel wall, attenuation of cytokine and IL-1α production and stimulation of endothelial nitric oxide [16•,69]. Recent literature [70••] has shown that fish oils prevented neointima formation in rabbits fed a non-hypercholesterolaemic diet and subjected to balloon injury of the carotid artery, by reducing medial and adventitial cell activation. Pakala and Benedict [71•] also showed that both EPA and DHA at low concentrations inhibited thromboxane A_2-stimulated canine aortic smooth muscle cell proliferation *in vitro*. Omega 3 fatty acids also increased induced and spontaneous apoptosis, by increasing the generation of lipid peroxides and mediators of apoptosis [72•]. In other studies, omega 3 fatty acids reduced LDL binding to fibroblast receptors [73] and reduced the uptake of LDL by macrophages [63].

Conclusion

Recent developments confirm and extend the concept that omega 3 fatty acids are beneficial in the prevention of cardiovascular disease and sudden cardiac death. There appear to be no clinically significant adverse effects at doses up to 3 g per day. Indeed, relatively small doses of 1 g per day, achievable with two to three fish meals per week, may be protective. Omega 3 fatty acids do not appear to have adverse interactions with medications (e.g. statins, anytihypertensive agents, lipid-lowering drugs, etc.) used for the treatment or prevention of coronary heart disease.

Recent reports also suggest that DHA may be more important than EPA as the principal omega 3 fatty acid in fish and fish oils responsible for some of the observed benefits. These findings, which will need further verification in different study populations, may have an important impact on the choice of omega 3 fatty acid

supplements, and on the relative use of EPA and DHA in food nutrition in the form of incorporation into animal feeds or foodstuffs. A mild impairment of glucose tolerance in type 2 diabetic patients continues to be of some concern at high doses of omega 3 fatty acids, and emphasizes the need for conventional lifestyle changes such as weight control and increased physical activity in these patients.

On the basis of the available literature, omega 3 fatty acids should be considered as important components of a healthy diet and as a potential therapeutic modality in patients with coronary artery disease, particularly in populations at heightened risk of cardiovascular disease.

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