Review

The role of omega-3 fatty acids in the secondary prevention of cardiovascular disease

K.W. LEE and G.Y.H. LIP

From the Haemostasis, Thrombosis and Vascular Biology Unit, University Department of Medicine, City Hospital, Birmingham, UK

Summary

It has long been recognized from epidemiological studies that Greenland Eskimos have substantially reduced rates of acute myocardial infarction (MI) compared with Western controls. From these epidemiological observations, the benefits of fatty fish consumption have been explored in cell culture and animal studies, as well as randomized controlled trials investigating the cardioprotective effects of omega-3 fatty acids. Dietary omega-3 fatty acids seem to stabilize the myocardium electrically, resulting in reduced susceptibility to ventricular arrhythmias, thereby reducing the risk of sudden death. These fatty acids also have potent anti-inflammatory effects, and may also be anti-thrombotic and anti-atherogenic. Furthermore, the recent GISSI-Prevention study of 11,324 patients showed a marked decrease in risk of sudden cardiac death as well as a reduction in all-cause mortality in the group taking a highly purified form of omega-3 fatty acids, despite the use of other secondary prevention drugs, including beta-blockers and lipid-lowering therapy. The use of omega-3 fatty acids should be considered as part of a comprehensive secondary prevention strategy post-myocardial infarction.

Introduction

It is increasingly recognized that regular consumption of fish or dietary supplementation of fish oils rich in long-chain omega-3 polyunsaturated fatty acids (n-3 PUFAs) lowers the risk of coronary heart disease (CHD) and protects against sudden cardiac death.1–3 However, this interest is not novel—the potential cardioprotective properties of fish fatty acids were first postulated by the British physiologist Hugh Sinclair in the early 1940s, when he suggested that the possibility of deficiency in some fatty acids might account for the rise in Western diseases, such as CHD.4 Indeed, Sinclair noted the freedom of Eskimos from any trace of arcus senilis and their liability to epistaxis—his views were set out in a letter to the Lancet in 1956, entitled ‘Deficiency of essential fatty acids and atherosclerosis, etcetera’.4

Interestingly, Greenland Eskimos have a low mortality rate from CHD,5–7 despite a high intake of fat (about 40% of their total caloric intake) in their diet. This so-called ‘Eskimos paradox’8 led to a series of epidemiological studies in the late 1970s by Danish investigators Bang and Dyerberg, which suggested a close correlation between the observed low incidence of CHD amongst the Inuit and their high consumption of fish and fish-eating mammals.
Omega-3 fatty acids and human nutrition

PUFAs are important fatty acids in human nutrition and can be divided into two subcategories: omega-3 (n-3) and omega-6 (n-6), depending on the location of their first double bond: the n-3 PUFAs having their first double bond located at the third carbon molecule, and the n-6 PUFAs at the sixth.

Linoleic acid (LA, 18:2n-6) is the predominant n-6 PUFA in humans, and they can be elongated and desaturated to arachidonic acid, whereas alpha-linolenic acid (α-LNA; 18:3n-3) is elongated and desaturated into longer chain PUFA EPA (Figure 1).

Omega-3 and omega-6 PUFAs are not interconvertible in the human body—they are essential fatty acids that are important components of practically all cell membranes. The metabolisms of fatty acids of the n-3 family and of the n-6 family (arachidonic acid [20:4(n-6)]) are of particular interest because of the biological actions of their metabolites (eicosanoids) in vivo. For example, eicosanoids derived from arachidonic acid are pro-inflammatory and pro-aggregatory agonists, whereas those derived from n-3 PUFAs tend to inhibit platelet aggregation and be anti-inflammatory.

The ratio between LA and α-LNA, rather than the absolute amounts of α-LNA in the diet, may be critical for disease prevention, due to the...
‘competition’ between the two essential PUFAs for their entry into the elongation and desaturation pathways, leading to the synthesis of their respective eicosanoids. Indeed, a high ratio of n-6 to n-3 PUFAs tends to accentuate the dietary deficit in α-LNA, while low ratios increase endogenous conversion of α-LNA to EPA. However, nowadays many vegetable oils are greatly enriched in n-6 PUFAs (mainly as LA in corn, sunflower, safflower and soybean oils). The blood ratio of LA:α-LNA in populations with a typical Western-diet with high intake of LA has been reported to be as high as 100:1—the optimal ratio should perhaps be 4 to 1.

The current recommendation of optimal dietary intake of α-LNA should be about 2 g/day or 0.6–1% of total energy intake. Canola oil, nuts (especially butternuts or English walnuts), seed oils (e.g. ground linseeds or flaxseeds), mustard oil, leeks and green leafy vegetables (such as purslane) are the main sources of α-LNA for the European population. In addition, α-LNA is highly sensitive to oxidation (due to its three double bonds), a high intake of α-LNA should be balanced with a high intake of antioxidants (for example, in vegetables and fruits), to protect it from oxidation.

On the other hand, fish or fish oil supplements rich in EPA and DHA are the main source of long chain n-3 PUFAs in the Western diet. Mackerel, herring, salmon and trout are among the richest sources of EPA and DHA (Table 1). Fish consumed 2.5–3 times per week would provide thus a combined intake of about 500 mg EPA and DHA per day. However, the current average daily intake of EPA and DHA combined in a typical Western diet is only about one fish serving every 10 days (that is, about 150 mg per day), which is approximately 0.15% of total dietary fat intake. Fresh, frozen, canned and smoked versions of oil-rich fish can also provide EPA and DHA, except for canned tuna since the oils are removed during processing. Concentrated formulations of fish oils supplements rich in these compounds are commercially available from industrial processing of the body fat from fish.

### Why are n-3 PUFAs important in human physiology?

The long-chain n-3 PUFAs are major structural components of membrane phospholipids of tissues throughout the body and in addition, they influence membrane fluidity and ion transports. These fatty acids are especially rich in the myocardium, retina, brain and spermatozoa, and are essential for proper functioning of these tissues and growth, being important modulators of many physiological processes. It is therefore of little surprise that these fatty acids have been studied extensively in a wide spectrum of human diseases (Table 2). Fatty acid analyses of serum and plasma, as well as blood cell membrane (including red blood cell, platelets or granulocytes) phospholipid levels are commonly use as indicators of n-3 PUFA intake and their physiological status. An increased intake of fish n-3 PUFAs EPA or DHA will result in a corresponding increase in blood and cellular levels of these fatty acids, as evident from both animal and human studies.

In particular, the fatty acid composition of myocardial membrane phospholipid is sensitive to the type of fatty acid consumed in the diet. Indeed, the myocardium and the myocardial membrane phospholipids are rich in n-3 PUFA after feeding fish oils.

It is of note, however, that while α-LNA supplementation can increase plasma and blood cell levels of EPA, stable isotope studies indicate that these conversion processes are slow and limited in humans, and in particular, the relative conversion of α-LNA to DHA is probably marginal at best, even in well-nourished individuals. This may

### Table 1 Some of the nutrients in some oil-rich fish

<table>
<thead>
<tr>
<th>Per 100 g (raw)</th>
<th>kcal (kJ)</th>
<th>Protein g</th>
<th>Total fat g</th>
<th>EPA g</th>
<th>DHA g</th>
<th>Vitamin D mg</th>
<th>Vitamin E mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmon</td>
<td>180 (753)</td>
<td>20</td>
<td>11</td>
<td>0.5</td>
<td>1.3</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Mackerel</td>
<td>220 (920)</td>
<td>19</td>
<td>16</td>
<td>1.1</td>
<td>0.1</td>
<td>5</td>
<td>0.4</td>
</tr>
<tr>
<td>Kippers</td>
<td>229 (958)</td>
<td>18</td>
<td>18</td>
<td>1.1</td>
<td>0.1</td>
<td>8</td>
<td>0.3</td>
</tr>
<tr>
<td>Herrings</td>
<td>190 (794)</td>
<td>18</td>
<td>13</td>
<td>1.2</td>
<td>1.1</td>
<td>19</td>
<td>0.8</td>
</tr>
<tr>
<td>Trout</td>
<td>112 (469)</td>
<td>19</td>
<td>4</td>
<td>0.2</td>
<td>0.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tuna</td>
<td>136 (569)</td>
<td>24</td>
<td>5</td>
<td>0.3</td>
<td>1.1</td>
<td>7</td>
<td>–</td>
</tr>
<tr>
<td>Sardines</td>
<td>195 (816)</td>
<td>25</td>
<td>10</td>
<td>0.9</td>
<td>1.1</td>
<td>12</td>
<td>0.3</td>
</tr>
<tr>
<td>Anchovies</td>
<td>280 (1171)</td>
<td>25</td>
<td>20</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data from reference 14.
partially account for the inability of α-LNA-rich oils to reproduce fish-oil-like effects on cardiovascular risk factors. The extent and regulation of these conversion processes in humans is still largely unclear, and therefore, the question arises as to whether plant oils rich in n-3 PUFA α-LNA have the same protective effect against CHD as marine-derived PUFAs.30,31

Potential mechanisms of anti-arrhythmic effects

How particular lipid constituents in our diets contribute to coronary risk or prevent cardiac death is unknown. The mechanisms underlying these effects are probably multifactorial and may collectively modulate the processes of atherothrombogenesis, as evident by numerous animal experiments and clinical human studies. Possible mechanisms include one or more of the following: platelet reactivity is inhibited; leukocyte chemotaxis and endothelial adhesion molecule expression is reduced; endothelial function is restored; cardiac haemodynamics are improved; and reperfusion injury due to coronary artery occlusion is reduced. In addition, n-3 PUFAs increase high-density lipoprotein-cholesterol (HDL-C) and have a potent triglyceride-lowering effect. A comprehensive review of the postulated mechanisms of action described for omega-3 fatty acids is beyond the scope of this article, but they are summarized in Table 3.33,34

The n-3 PUFAs may prevent CHD death by their effects on haemostasis (by inhibiting platelet aggregations) and serum lipids (by lowering triglycerides and/or increase HDL-C). However, the major effect of n-3 PUFA may be anti-arrhythmic rather than anti-atherothrombotic. The rapidity of onset of the beneficial effects seen in recent clinical intervention trials, and the fact that CHD mortality was reduced without significant reduction in the incidence of recurrent non-fatal MI in these trials have provided greater emphasis on the potential anti-arrhythmic effects of n-3 PUFA. Certainly, increased fish consumption or fish oil supplementation in humans seems to prevent fatal arrhythmias and sudden cardiac death.3,35,36

The putative anti-arrhythmic properties of n-3 PUFAs (in particular, EPA and DHA) have been explored in experimental studies on canine models, and laboratory studies on isolated neonatal rat cardiac myocytes.35,37,38 For example, by intravenous infusion of an emulsion of fish oils and free fatty acids just before coronary artery obstruction (with an inflatable cuff placed around the left circumflex artery) in exercising conscious dogs with prior surgically-induced, large myocardial infarctions, ischaemia-induced sudden cardiac death was reduced by preventing ventricular fibrillation.37,38 Similar results were obtained with intravenous pure free EPA, DHA, and α-LNA given
sarcolemma has gained much attention. During the conductances of specific ion channels in their stabilization cardiomyocytes electrically by modulating at low (micromolar) concentrations are able to besides the sodium and L-type calcium channels; PUFAs are able to block many specific ion currents, without incorporating into myocyte membrane, n-3 PUFA at low dose as exogenous fatty acids, and DHA is the most potent agent. Whether the incorporation of the long chain n-3 PUFAs (DHA or EPA) into membrane phospholipids has the same immediate anti-arrhythmic effects conferred by exogenous free PUFA is unclear. The differences in study design or the experimental models used (for example, the induced ischaemia model vs. ischaemia-reperfusion model, ex vivo vs. in vitro models, isolated working heart model vs. isolated cultured cardiac myocytes, etc.) may partially explain this. For example, Weylandt et al. demonstrated that only the free unesterified DHA or EPA (both at 15 μmol) were able to protect the cultured neonatal rat myocytes against induced arrhythmias, whereas there was no anti-arrhythmic effect due to an increased fraction of EPA or DHA in membrane phospholipids. However, McLennan reported that the anti-arrhythmic effect of dietary fish oil appears to depend more on the accumulation of DHA in myocardial cell membranes than EPA, indeed, even at low dietary intakes (0.4–1.1% of energy), DHA but not EPA inhibits ischaemia-induced cardiac arrhythmias. Although fish oils often contain mainly EPA, the myocardium, including mitochondrial membranes, accumulates DHA as the principal n-3 PUFA, even after feeding purified EPA. Ventricular fibrillation induced under many conditions, including ischaemia, reperfusion, and electrical stimulation, and even arrhythmias induced in vitro with no circulating fatty acids, are prevented by prior dietary consumption of fish oil in animals. On the other hand, Pound et al. studied the in vitro effects of PUFA on the contractions of isolated cardiac myocytes and the conductances of their sarcolemmal ion channels. Using human RBC ghosts as a model for the plasma membrane, they reported that exogenous free fatty acids (DHA and EPA) at low micromolar concentrations (5–15 μmol) were unlikely to affect the packing of phospholipid within cell membranes as their mechanism of modulating changes in cell membrane ion currents and in preventing arrhythmias. The ability of high-dose n-3 PUFAs to reduce ischaemia/reperfusion-induced arrhythmias and cellular damage, is apparently due to the incorporation of n-3 PUFA into membrane phospholipids. On the other hand, n-3 PUFA at much lower doses and as exogenous free fatty acids are able to block sarcolemmal sodium and calcium ion channels without incorporating into membrane phospho-
lipids. This latter mechanism seems more likely to explain the n-3 PUFA on CHD mortality reduction seen in clinical trials, as a human intake of one to two meals including fish per week is cardioprotective in primary prevention when compared with no fish intake.

In contrast, the role of α-LNA per se in the prevention of ischaemic-induced arrhythmia or arrhythmia during reperfusion is also largely unclear. Although the metabolic conversion of α-LNA to the longer chain n-3 PUFAs EPA is thought to mediate any possible cardioprotective effects of dietary α-LNA, a specific anti-arrhythmic effect of α-LNA itself has been reported, at least in animal studies. More basic mechanistic studies are therefore needed to delineate the potential cardioprotective mechanisms of α-LNA, bearing in mind that the extent and the regulation of the conversion processes from α-LNA to EPA or DHA in humans are still unclear.

Nonetheless, it has recently been shown in an animal model that at higher molar concentrations of the n-3 PUFA EPA and DHA (25–100 μmol) may inhibit sarcolemmal sodium/hydrogen (Na+/H+) exchange, and thereby protect the myocardium from arrhythmias and cell death after ischaemia-reperfusion injury. This appeared to be a specific effect of these PUFAs, because 50 μmol linoleic acid or linolenic acid had no significant effect on Na+/H+ exchange. One of the most potent ways in protecting the myocardium from ischaemic-reperfusion injury is inhibition of the Na+/H+ exchange mechanism. The transmembrane Na+/H+ exchanger maintains myocardial cell pH integrity during myocardial ischaemia, but may paradoxically precipitate cell necrosis. One clinical trial of the specific Na+/H+ exchange inhibitor caporide showed a potential benefit in preventing the risk of cardiac death, providing that the drug was present prior to the index ischaemic event.

Other indirect anti-arrhythmic effects of long-chain n-3 PUFAs may include lowering of the non-esterified fatty acid (NEFA) concentrations in plasma and cell membranes. Indeed, concentrations of circulating non-esterified fatty acids are increased in ischaemic conditions and these have multiple pro-arrhythmic effects that are responsible for ventricular tachyarrhythmias and possibly an increased risk of sudden death, although not of fatal MI. A high intake of fish fatty acids may lower risk of cardiac arrhythmia and sudden death by improving the activity of the cardiac autonomic nervous system. For example, reduced heart rate variability is a strong risk predictor for cardiac arrhythmias and sudden cardiac death in patients with prior MI, and a high intake of n-3 PUFAs has been related to increased heart rate variability in a dose-dependent manner, both in healthy men and in patients with prior MI. As expected, the content of the n-3 PUFAs (both as DHA and EPA) in platelets was highest among those eating the most fish, and when the patients were divided according to the content of DHA in their platelets, there was a significant, positive correlation between DHA in platelets and heart rate variability.

Fish or fish oil supplements and cardiovascular disease

Epidemiological studies

Several, but not all, prospective cohort studies have found an inverse association between fish consumption and risk of CHD. Early important cohort studies include the Zutphen and Western Electric studies, performed in non-Mediterranean countries.

In the Zutphen study, men who ate at least 30 g of any fish every day had half the CHD-related mortality rate of individuals who ate no fish at all. In the 30-year follow-up Chicago Western Electric Study, men who consumed at least 35 g of fish daily had 40% lower risk of fatal CHD (RR 0.62, 95% CI 0.4–0.94), compared to those who consumed no fish at all. With 20 years of follow-up data, the Seven Countries Study also showed a 50% reduction in CHD mortality in men who consumed 30 g of fish daily, compared to men who rarely ate fish. The Multiple Risk Factor Intervention Trial (MRFIT) in the USA also reported that progressively higher intakes of marine n-3 PUFAs (up to about 665 mg/day) over 10.5 years were associated with a progressive reduction in CHD mortality in men who consumed 30 g of fish daily, compared to men who rarely ate fish. The Multiple Risk Factor Intervention Trial (MRFIT) in the USA also reported that progressively higher intakes of marine n-3 PUFAs (up to about 665 mg/day) over 10.5 years were associated with a progressive reduction in CHD and total mortality, with no associated increase in total cancer-related mortality.

However, the US Health Professionals Follow-up Study (with 44,895 health professionals aged 40–75 years followed-up for 6 years) showed no overall association between dietary intake of n-3 PUFAs or fish intake and the risk of CHD, although there was a non-significant trend for a lower risk of fatal CHD with increasing fish consumption. Other studies have also found no significant correlations between fish intake and the risk of CHD. Nonetheless, some of these cohorts were characterized by population with high fish consumption at baseline, and they were probably already at low risk for CHD. The discordant results may also be ascribed to differences in design, study population, duration of observation, and the dose and nature of
the n-3 PUFAs or fish oil. A recent systematic review by Marckmann and Gronbaek did suggest that higher-risk cohorts were more likely to benefit more from an increase fish intake than low-risk subjects, and it was estimated that consumption of 40–60 g of fish per day could reduce the risk of CHD death by 40–60% in these high risk groups.

The US Physicians’ Health Study was a prospective cohort study of 20,551 US male physicians (aged 40–85 years) free of cardiovascular disease who were followed-up for 11 years, which found that weekly fish consumption (up to 1 fish meal per week) was associated with a lower risk of sudden cardiac death (death within 1 h) (RR 0.48; 95% CI 0.24–0.96, p = 0.03) and a 30% reduction in total mortality, compared with consumption of less than 1 fish meal per month. Importantly, subsequent prospective, nested case-control analysis (94 men in whom sudden death occurred as the first manifestation of cardiovascular disease vs. 184 controls matched for age and smoking status) performed in the same ‘healthy’ cohort of physicians followed for up to 17 years revealed significant inverse correlation between baseline whole-blood long-chain n-3 PUFAs levels (as analysed by gas-liquid chromatography) and the risk of sudden cardiac death, even after known confounders had been controlled for. Specifically, when compared with men in the lowest quartile, those with blood levels of n-3 PUFAs in the highest quartile had an 81% lower risk of sudden death. A threshold level seems to exist, as eating fish more often than once a week did not seem to confer any additional benefit, and there was no significant risk reduction for total MI, non-sudden cardiac death, or total cardiovascular mortality. Thus, n-3 PUFAs may well be the active ingredient primarily responsible for the apparent protective effect of fish, but a causal relationship between fish consumption and n-3 PUFAs and a reduction in death is more difficult to prove.

Similar to the Physicians’ Health Study, the Nurses’ Health Study of 84,688 healthy women (aged 34–59) and 16-year follow-up concluded that a higher consumption of fish and long chain n-3 PUFAs (DHA and EPA) was associated with a lower risk of CHD and CHD deaths; after adjustment for age, smoking, aspirin use, menopausal status and other cardiovascular risk factors, the multivariable relative risks of CHD were 0.79 (95% CI 0.64–0.97) for fish consumption 1–3 times per month, and 0.66 (95% CI 0.50–0.89) for fish consumption five or more times per week (p for trend = 0.001). Further, the reduction in stroke risk was dose-dependent: the RRs for stroke were 0.93 (95% CI 0.65–1.34) for fish consumption 1 to 3 times per month, and 0.48 (95% CI 0.21–1.06) for fish consumption 5 or more times per week (p for trend = 0.001), with a major reduction in thrombotic and lacunar infarction. Importantly, there was no association between fish or long chain n-3 PUFAs and risk of haemorrhagic stroke. However, whether fish oil supplements would also help prevent strokes is unclear. The potential mechanisms that underlie the stroke/fish relationship may include inhibition of platelet aggregation, lowered blood viscosity, suppressed formation of leukotrienes (mediators of neutrophil and macrophage aggregation), and reductions in plasma fibrinogen, blood pressure, and insulin resistance. Interestingly, intakes of about 3–4 g of EPA and DHA per day have been reported to result in a moderate increase in bleeding times, although these are generally lower than those seen with aspirin therapy.

Finally, one population-based case-control study in Seattle on 334 cases of out-of-hospital primary cardiac arrest and 493 controls concluded that dietary intake of mainly EPA n-3 PUFAs equivalent to one fatty fish meal per week was associated with a 50% reduction in the risk of primary cardiac arrest, when compared with no intake of n-3 PUFAs. There was a dose-response relation between both fish consumption and the concentration of long chain n-3 PUFAs in red cell membranes phospholipids in relation to cardiac arrest. Compared with a red-cell membrane n-3 PUFAs level of 3.3% of total fatty acids, a level of 5.0% was associated with a 70% reduction in the risk of primary cardiac arrest. These observations are consistent with those from the two large secondary prevention trials, the Italian GISSI-Prevenzione Study and the Diet and Reinfarction Trial (DART) conducted in Wales, UK over a decade ago, discussed further below.

**Intervention studies**

In the DART study, 2033 Welsh men with recent MI randomized to receive at least two servings of fatty fish per week (200–400 g) or three fish oil capsules [0.5 g per day if they could not tolerate the fish] had a significant 29% reduction (RR 0.71, 95% CI 0.54–0.93) in both cardiac and total mortality within 4 months of the study compared with the non-fish supplementation groups. However, there was no significant reduction in the incidence of recurrent non-fatal MI, a puzzling feature that was also found in the subsequent GISSI-Prevenzione trial. Indeed, this early reduction in mortality observed in the DART trial has led to the hypothesis that n-3 PUFAs might have an anti-arrhythmic effect as the underlying protective...
mechanism, rather than anti-thrombotic or anti-arteriosclerotic. The subsequent results from the much larger GISSI Prevention trial (that included 11,324 recently discharged post-MI patients) found that after 3.5 years follow-up, patients randomized to daily supplement of 1 g capsule containing 850–882 mg of EPA and DHA as ethyl esters in the average ratio of EPA/DHA 1:2, on a background of a Mediterranean-type diet (which also included moderate fish consumption) had a significant relative risk reduction for the main cardiovascular end points (cardiovascular death, non-fatal MI, and stroke) by 20%, cardiovascular death by 30%, and in a subanalysis the risk of sudden death alone was reduced by as much as 45% (in absolute figures, from 3.5% to 1.9%).

In the GISSI-Prevenzione trial, most of the patients were already on a CHD risk reduction regimen comprising aspirin, beta-blockers, angiotensin converting enzymes (ACE) inhibitors and/or lipid lowering statins. All these agents offer reduction of death in this subset of patients by 20–40%, but a 30% reduction on top of this, independent of blood cholesterol lowering, suggests that the effects of n-3 PUFAs, as secondary prevention after MI may be very potent. Furthermore, this degree of risk reduction was achieved in Italian post-MI survivors, whose dietary habit was the ‘typical’ Mediterranean diet, suggesting that greater benefits might possibly be seen with n-3 PUFAs in a Western-style diet typified by high consumption of saturated fats and low intake of n-3 PUFAs. Importantly, the intervention was well-tolerated and had no serious side-effects during the trial. It is notable that oral vitamin E 300 mg (given as one capsule of synthetic alpha-tocopherol) supplementation, which was also studied in this trial, was without any significant cardiovascular benefit.

The results from the GISSI-Prevenzione trial were further reaffirmed by the recently published time-course data re-analysis, which showed that the survival curves for patients receiving treatment began to diverge early after randomization, and that their total mortality was significantly lowered after 3 months (RR 0.59; 95%CI 0.36–0.97; p = 0.037). Survival was predominantly due to a reduction in the risk of sudden cardiac death, which became statistically significant by 4 months (p = 0.048). Hence, as in the DART trial, such an early effect of n-3 PUFAs on total mortality and sudden death ‘supports the hypothesis of an anti-arrhythmic effect’ of these fatty acids, an observation consistent with experimental and clinical studies discussed above (Table 4).

### Cardiovascular benefits of alpha-linolenic acid

#### Epidemiological studies

With the abundance of data supporting the cardiovascular protection with fish consumption and/or fish oil supplements, the association of α-LNA and cardiovascular benefits has received less attention. For those who cannot (or will not) eat fish or other seafood that are rich in long-chain n-3 PUFAs, there is some evidence to support the consumption of foods (seed, nuts or vegetable oil) containing intermediate-chain PUFA α-LNA.

Several cohort studies have reported significant inverse relationships between α-LNA (measured in the diet, plasma or adipose tissue) and the risk of CHD. Although there was no significant association between marine n-3 PUFAs intake and the risk of CHD in the Health Professional Follow-up Study, α-LNA intake was inversely related with the risk of MI and cardiac death, even after adjustment for traditional risk factors and total fat intake, giving a relative risk of 0.41 for each 1% increase in energy (p for trend <0.01). Data from the ‘usual care’ cohort of 6250 men who were followed up for 10.5 years of the Multiple Risk Factor Intervention Trial (MRFIT) also demonstrated significant inverse associations between the intake of the n-3 PUFA α-LNA (calculated from four dietary recall interviews at baseline and 1-, 2-, and 3-year follow-up) and mortality rates from CHD (p<0.04), total cardiovascular disease (p<0.03), and all-cause mortality (p<0.02). In contrast, the Zutphen Elderly Study did not observe any beneficial effect of dietary α-LNA on the 10-year risk of CHD.

In an analysis from the Nurses’ Health Study (76,283 women followed-up for 10 years), a higher intake of α-LNA was associated with a lower relative risk of fatal CHD in women who consumed this food ≥5–6 times per week, compared with those who rarely consumed foods rich in α-LNA (RR 0.46, 95% CI 0.27–0.76; p for trend = 0.001). The results were unchanged even after adjustments for intake of fish fatty acids, oleic acids, trans fatty acids, cholesterol, folate or fibres. Importantly, there were no adverse effects reported.

#### Dietary intervention trials

The Lyon Diet Heart Study from France was the first secondary prevention trial designed to test the hypothesis that a Mediterranean α-LNA-rich diet may improve prognosis in survivors of a first MI...
<table>
<thead>
<tr>
<th>Studies</th>
<th>Methods</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Animal studies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>McLennan et al. 1993</td>
<td>Programmed electrical stimulation in anesthetized marmoset monkeys pre-fed n-3 PUFAs</td>
<td>Dietary n-3 PUFAs ↓ vulnerability of normal or ischaemic myocardium to arrhythmias.</td>
</tr>
<tr>
<td>McLennan et al. 1995</td>
<td>Rats were randomized to one of four experimental diet groups for 12 weeks. Arrhythmias were induced by coronary artery occlusion and reperfusion.</td>
<td>VF, mortality and arrhythmia score were significantly ↓ with diet containing canola oil (55% oleic, 8% ALNA) relative to the other diets, as does dietary fish oil, but the effectiveness of ALNA is ↓ by high levels of LA.</td>
</tr>
<tr>
<td>Billman et al. 1999</td>
<td>i.v. administration of two major dietary n-3 PUFAs in fish oil in an exercising dog model of sudden cardiac death induced by coronary occlusion</td>
<td>Purified n-3 PUFAs can prevent ischaemia-induced VF.</td>
</tr>
<tr>
<td><strong>Isolated working heart model</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pepe &amp; McLennan 1996</td>
<td>60 adult male rats fed with fish oil diet or an isoenergetic saturated fat diet or a low fat reference diet for 16 weeks. Hearts isolated were perfused with washed porcine erythrocyte.</td>
<td>Dietary fish oil prevented the initiation and ↓ the severity of arrhythmias in the isolated hearts in response to a variety of stimuli.</td>
</tr>
<tr>
<td><strong>Isolated animal cardiac myocytes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kang &amp; Leaf 1995</td>
<td>Electrophysiology of the cardiac cycle in isolated neonatal rat cardiac myocytes was studied by exposing of myocytes to various fatty acids and strength of the depolarizing current required to elicit an action potential and the cycle length of excitability were measured.</td>
<td>Free PUFAs can ↓ membrane electrical excitability of cardiac myocytes, but neither the monounsaturated oleic acid nor the saturated stearic acid had similar effects.</td>
</tr>
<tr>
<td>Kang &amp; Leaf 1995</td>
<td>Neonatal rat cardiac myocytes for their ability to prevent the tachyarrhythmias induced by a beta-adrenergic agonist</td>
<td>PUFAs, especially from the fish oil, but not mono-unsaturated and saturated fatty acids, were able to effectively prevent and terminate the arrhythmias without affecting the cell contractility.</td>
</tr>
<tr>
<td><strong>Human studies</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sellmayer et al. 1995</td>
<td>68 out-patients with frequent VPCs (minimum of 2000 VPCs over 24 h) on Holter were randomized to fish oil or placebo (sunflower seed oil).</td>
<td>Moderate dose of fish oil has anti-arrhythmic effect, leading to a reduction in VPCs in nearly 50% of patients on fish oil supplements.</td>
</tr>
<tr>
<td>Christensen et al. 1997</td>
<td>55 survivors of MI with LV dysfunction.</td>
<td>n-3 PUFAs in platelets was closely associated with the patient’s fish-consuming habits, and a significant positive correlation was observed between DHA and HRV.</td>
</tr>
<tr>
<td>Brouwer et al. 2002</td>
<td>Cross-sectional study of 53 healthy volunteers, the association of intake of n-3 PUFAs with HRV and with length of the QTc interval.</td>
<td>Higher DHA level in blood is associated with a higher HRV.</td>
</tr>
</tbody>
</table>

(Continued)
### Table 4  Continued

<table>
<thead>
<tr>
<th>Studies</th>
<th>Methods</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Case-control study</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Siscovick et al. 1995</td>
<td>334 cases of primary cardiac arrest and 493 controls</td>
<td>Dietary intake of n-3 PUFAs from seafood is associated with a 50% reduction in the risk of primary cardiac arrest compared with no intake of n-3 PUFAs</td>
</tr>
<tr>
<td><strong>Randomized clinical trials</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Burr et al. 1989</td>
<td>2033 Welsh men with recent MI randomized to ≥ 2 servings of fatty fish/week or 3 fish oil capsules/day; followed up for 2 years.</td>
<td>Significant 29% reduction in cardiac and total mortality within 4 months of the study, compared with the non-fish groups</td>
</tr>
<tr>
<td>Singh et al. 1997</td>
<td>360 suspected MI patients randomized to fish oil (EPA 1 g/day) or mustard seed oil (ALNA 2.9 g/day) or placebo; followed-up for 1 year</td>
<td>Fish oil and mustard oil groups had significantly less total cardiac events and total cardiac arrhythmias than placebo.</td>
</tr>
<tr>
<td>de Lorgeril et al. 1999</td>
<td>4 years follow-up results of about 233 survivors of first MI</td>
<td>50–70% lower risk of recurrent risk in different combinations of outcome measures: cardiac death and non-fatal MI in the group who had Mediterranean diet supplemented with ALNA</td>
</tr>
<tr>
<td>GISSI Prevenzion 1999</td>
<td>11 324 post-MI patients randomized to 1 g Omacor capsule/day together with a Mediterranean-type diet vs. a ‘prudent Western-diet’; 3.5 years follow-up</td>
<td>Significant risk reduction of CV death, non-fatal MI and stroke by 20%, CV death by 30%, and risk of sudden death alone by 45%</td>
</tr>
<tr>
<td>GISSI Prevenzion 2002</td>
<td>Time-course re-analysis of the 1999 data.</td>
<td>Total mortality was significantly lowered after 3 months, and risk reduction of sudden death became significant by 4 months</td>
</tr>
</tbody>
</table>

ALNA, alpha-linolenic acid; CV, cardiovascular; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; HRV, heart rate variability; LA, linoleic acid; LV, left ventricular; MI, myocardial infarction; n-3, omega-3; PUFAs, polyunsaturated fatty acids; VF, ventricular fibrillation; VPCs, ventricular premature complexes.

(with 204 control and 219 experimental subjects, mean age 53 years). This study reported a 50–70% lower risk of recurrent risk (after a mean follow-up of 46 months per patient), as measured by different combinations of outcome measures, including cardiac death and non-fatal MI in the active group who received a Mediterranean diet (with more fish, more fibre with cereals, bread, fresh vegetables and fruits, but less animal fat) supplemented with ω-LNA (in particular, olive and canola oils for salad and food preparation, and canola-oil-based margarine to spread on bread) when compared to controls who received ‘usual care’. Control patients consumed about 0.7 g of ω-LNA per day, compared with about 1.8 g in the experimental group, with a ratio of LA:ω-LNA of about 10:1 in controls, compared to 4:1 in the experimental group, resulting in significant differences in the fatty acid composition of both circulating plasma lipids and cell membrane phospholipids. The investigators found that ω-LNA plasma concentrations 2 months after randomization were significantly and inversely associated with the risk of CHD recurrence, and in particular, with fatal recurrences, including the prevention of sudden death. However, it is uncertain whether it was the high intake of ω-LNA or other ingredients from the Mediterranean diet that were responsible for the reduction in sudden cardiac death, although the study investigators suggest that most of the risk reduction was from the ω-LNA supplementation.

Another randomized controlled trial, the Indian Diet Heart Study, randomized 505 patients with a recent MI (within 48 h of event) to either a low-fat diet (about 28% of the total calories) or an even lower-fat diet (about 24% of the total calories). This trial also reported a significant reduction in the risk of cardiac events: a 42% reduction in cardiac mortality and a 45% reduction in all-cause mortality after one year of follow-up. In fact, CHD events were reduced in the experimental group as early as 6 weeks after randomization. In this trial, patients in the experimental group with...
a lower-fat diet were encouraged to eat a healthy diet rich in vegetables, fruits, nuts and grain products, which are the main sources of α-LNA. The observations from the Indian Diet Heart study are consistent with earlier studies on the Seventh Day Adventists\(^6^6\) and American nurses,\(^6^7\) which also suggested that eating nuts was associated with a lower risk of CHD.

In another secondary prevention, placebo-controlled trial, the Indian Experiment of Infarct Survival,\(^6^8\) 4360 patients less than 1 day after MI were randomized to one of three arms: a group receiving fish oil capsules (EPA 1.08 g/day and DHA 0.72 g/day), a group receiving mustard seed oil, 20 g/day (α-LNA 2.9 g/day), and a control group (aluminum hydroxide 100 mg/day). After 1 year, total cardiac events (total cardiac deaths and non-fatal MI) were significantly fewer in the fish oil and mustard oil groups compared with the placebo group (24.5% and 28.0%, respectively, vs. 34.7%; \(p<0.01\)). Interestingly, this study also revealed that, compared to placebo, patients who received fish oil capsules or mustard oil had significantly lower risk for total cardiac arrhythmias (28.7% vs. 13.1% and 13.3%, respectively).

For many years, the apparent beneficial effects of eating more vegetables and fruits was attributed to their antioxidant content, such as beta-carotene, vitamin E and C. However, recent large clinical trials with vitamin E supplements in high and low doses for the prevention of CHD have been disappointing, all failing to show any significant benefit.\(^5^9,6^9–7^1\) One suggestion was that the cardioprotective effects of vegetables, fruits and nuts seen in many epidemiological studies are due to the fact that these foods were also rich in α-LNA, the precursor for the long-chain fatty acids EPA and DHA. Certainly, there were no additional significant benefits seen in the vitamin E treatment arms of the mega-trials.

### Omega-3 fatty acids: a place in secondary prevention

Data from epidemiological studies and the recent randomized clinical trials of n-3 PUFAs in the form of fish or fish oil concentrates, particularly EPA and DHA, have suggested a role for these agents for the secondary prevention of CHD. In particular, benefits of n-3 PUFAs compare favourably with those seen in landmark secondary prevention trials with lipid-lowering statins, beta-blockers, ACE inhibitors and aspirin (Table 5). However, the

<table>
<thead>
<tr>
<th>Study (drug)</th>
<th>Mean follow-up duration (year)</th>
<th>Clinical events</th>
<th>Active treatment (%)</th>
<th>Placebo/ control (%)</th>
<th>RRR (%)</th>
<th>ARR (%)</th>
<th>NNT</th>
<th>NNT/year</th>
</tr>
</thead>
<tbody>
<tr>
<td>GISSI–Prevenzione trial (fish oil capsules)(^5^9)</td>
<td>3.5</td>
<td>CHD death and MI</td>
<td>6.9</td>
<td>9.2</td>
<td>25.0</td>
<td>2.3</td>
<td>43</td>
<td>152</td>
</tr>
<tr>
<td>DART (fish or fish oil capsules)(^3^6)</td>
<td>2</td>
<td>Total mortality</td>
<td>8.3</td>
<td>10.4</td>
<td>20.0</td>
<td>2.1</td>
<td>48</td>
<td>167</td>
</tr>
<tr>
<td>Lyon Heart Study (ALNA-rich diet)(^8^9)</td>
<td>3.8</td>
<td>CHD death and MI</td>
<td>1.24</td>
<td>4.07</td>
<td>69.5</td>
<td>2.83</td>
<td>35</td>
<td>134</td>
</tr>
<tr>
<td>Indian Experiment of Infarct Survival (fish oil or ALNA-rich mustard oil)(^6^8)</td>
<td>1</td>
<td>CHD death and MI</td>
<td>24.5</td>
<td>34.7</td>
<td>29.4</td>
<td>10.2</td>
<td>9.8</td>
<td>9.8</td>
</tr>
<tr>
<td>4S (simvastatin)(^7^2)</td>
<td>5.4</td>
<td>Total mortality</td>
<td>8.2</td>
<td>11.5</td>
<td>28.8</td>
<td>3.3</td>
<td>30</td>
<td>164</td>
</tr>
<tr>
<td>LIPID (pravastatin)(^7^3)</td>
<td>6.1</td>
<td>Total mortality</td>
<td>11.0</td>
<td>14.1</td>
<td>22.0</td>
<td>3.1</td>
<td>32</td>
<td>197</td>
</tr>
<tr>
<td>CARE (pravastatin)(^9^0)</td>
<td>5</td>
<td>CV death, MI</td>
<td>10.2</td>
<td>13.2</td>
<td>22.7</td>
<td>3.0</td>
<td>33</td>
<td>165</td>
</tr>
<tr>
<td>AIRE (ramipril)(^9^1)</td>
<td>1.2</td>
<td>Total mortality</td>
<td>16.8</td>
<td>22.4</td>
<td>25.1</td>
<td>5.6</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>BHAT (propanolol)(^9^2)</td>
<td>2.1</td>
<td>Total mortality</td>
<td>7.2</td>
<td>9.8</td>
<td>26.4</td>
<td>2.6</td>
<td>38</td>
<td>81</td>
</tr>
<tr>
<td>Juul-Moller et al. (aspirin)(^9^3)</td>
<td>4.2</td>
<td>CV death, MI</td>
<td>8.0</td>
<td>12.1</td>
<td>33.6</td>
<td>4.1</td>
<td>24</td>
<td>102</td>
</tr>
</tbody>
</table>

\(^*\)\(p>0.05\) between groups; NNT, number of patients needed to treat to prevent one clinical event; ARR, absolute risk reduction; RRR, relative risk reduction; CHD, coronary heart disease; MI, non-fatal myocardial infarction; CV, cardiovascular.
mechanism(s) underlying the prevention of sudden death from cardiac causes seems different from that required for the prevention of atherosclerotic heart disease.

Calculations from the DART data suggest that an intake of two fish meals per week results in an absolute risk reduction of 3.5%, with a number needed to treat (NNT) to prevent one death of 28 during the 2-year trial. Similarly, the data derived from the GISSI-Prevenzione trial show that up to 5.7 lives can be expected to be saved per 1000 patients treated with one n-3 PUFAs capsule (Omacor) daily per year. In terms of NNT per year, this is comparable to statin treatment alone, as seen in the Scandinavian Simvastatin Survival Study72 and the Long-Term Intervention with Pravastatin in Ischaemic Disease (LIPID) trial73 (Table 5). Accordingly, in the LIPID trial, 5.2 lives could be saved per 1000 hypercholesterolaemic CHD patients treated with pravastatin for one year. In terms of cost-effectiveness, n-3 PUFAs capsule (Omacor) is probably comparable to simvastatin, but less costly than pravastatin.74

Increasing fish intake is the most obvious way to increase n-3 PUFAs. However, what is the ideal fish diet for secondary prevention? The reduction in coronary risk appears to be greatest when adding a modest amount of dietary fish to population that consumed little fish. However, despite government recommendations to increase intake of oil-rich fish, for many people this advice is not a readily accepted means of consuming n-3 PUFAs, as attaining the proposed recommended combined EPA and DHA intake will require a ~ 4- to 10-fold increase in fish consumption in the USA and UK, respectively. The long chain n-3 PUFAs supplementation in the GISSI-Prevenzione trial3 corresponds to an intake of approximately 100 g of fish per day.

As an alternative to n-3 PUFAs capsules, American Heart Association (AHA) has recommended at least two servings of fish per week, especially fatty fish (equivalent to an intake of long chain n-3 PUFAs approaching at least 1 g per day), which may have cardioprotective effects in patients with established CHD.75,76 In 1992, the British Nutrition Foundation Task Force on Unsaturated Fatty Acids77 suggested a desirable population intake for very-long-chain n-3 PUFAs of 0.5% of energy, which equates to about 8 g EPA/DHA per week for women and 10 g per week for men. In food terms, using the latest data on oil-rich fish composition, this is broadly equivalent to 2–3 medium servings of oil-rich fish per week. However, the current intake of oil-rich fish, even among regular consumers of this food in the UK (about a third of the population) equates to only one small portion per week. Indeed, the average intake in the population as a whole is currently very small (total fish intake is only 144 g/person/week, most of which is white fish,78 and the typical Western-diet only provides about 1–3 g of α-LNA per day and 0.1–0.15 g of EPA plus DHA per day (Table 6). The current mean adult combined intake of EPA and DHA is also far too low, approaching only 13–20% of recommended target levels. It is important to note that some types of fish may contain significant levels of mercury, polychlorinated biphenyls, dioxins and other environmental contaminants. Levels of these substances are generally highest in older, larger, predatory fish and marine mammals. Thus, consumption of a variety of fish is recommended, to minimize any potentially adverse effects due to environmental pollutants and, at the same time, achieve desired cardiovascular disease health outcomes.76

For vegetarians, or those who dislike fish or fish oil supplements, foods rich in α-LNA may be an alternative. Data derived from the Lyon Heart Study indicated that the NNT to prevent a major cardiac event is 134—a figure comparable with other established secondary prevention regimens such as statins (Table 5). The data also support inclusion of vegetable oils (e.g. soybean, canola, walnut, flaxseed) and food sources (e.g. walnuts, flaxseeds) high in α-LNA in a healthy diet for the general population (AHA statement). Nevertheless, one can argue that it is not α-LNA per se that is important...
cardioprotective but the long-chain fatty acids derived from α-LNA, such as EPA. Furthermore, components in the Mediterranean or Indian diet tested in the trials (as described above) were different from the control diet in many other aspects; potentially protective constituents could be due to more vegetable proteins, various antioxidants, folates, arginine, etc. The definite demonstration of a specific cardioprotective effect for α-LNA would require a trial specifically designed for that purpose. However, given that a healthy diet with more vegetables and fruits forms an important component of a healthy lifestyle in either patients or healthy persons, such a trial may not be necessary.

Future perspectives and clinical trials

Few prospective randomized placebo-controlled double-blind clinical trials have already been or will be launched to study the possible effects of n-3 PUFAs in preventing malignant cardiac arrhythmias which are considered to be one of the main causes of cardiovascular death, especially in the ‘high risk’ groups, such as those with an implantable cardiac defibrillator (ICD), severe left ventricular hypertrophy and severe heart failure.79

Dietary intervention trials, such as DART, the Lyon Heart Study and the Indian Experiment of Infarct Survival, are limited by the multiple and complex dietary changes in the trials that do not permit easy differentiation of which specific components or certain combinations of these diets are most beneficial. It is also unclear what difference the usual dietary patterns of the particular populations (with different dietary habits and background risk for heart disease) studied have on a particular outcome. Further trials at multinational level are therefore needed, not only to confirm the pharmacological approach of GISSI-Prevenzione in countries with a different background habits and risk, but also to explore in parallel the various mechanistic hypotheses. Such studies would need more in-depth experimental sub-studies, which could complement each other, to define easily measurable markers of n-3 PUFA intake and/or their effects, which could then be used as surrogate outcome measures for studies of shorter duration.

While a cardioprotective diet rich in α-LNA should be included in a comprehensive strategy to decrease cardiovascular morbidity and mortality, further randomized control trials are clearly required to identify the effects of α-LNA in high risk patients. For example, the Carolina Diet Heart Study is timely—this is a prospective, randomized, multi-centre, secondary prevention trial in post-AMI patients, comparing two ‘proven’ cardioprotective diets, the AHA endorsed Step II diet vs. an American-Mediterranean diet, with the main dependent variable being the amount and type of unsaturated fat.80 Such a trial should help to clarify the evidence suggesting a beneficial effect of α-LNA on the secondary prevention of CHD, or otherwise.

While we wait for primary prevention trials before recommending more extensive changes in the diet focusing on increasing n-3 PUFAs levels, it seems safe to follow the recommendations by the AHA and British Nutrition Foundation, in those at high risk of developing CHD. Conversely, clinical trials are needed in ‘high risk’ groups, such as those with congestive heart failure, who are at high risk of sudden death, to ascertain the benefits of n-3 PUFAs in such patients.

Acknowledgements

GYHL has received funding for meetings, educational symposia and research projects from Solvay, who promote Omacor.

References


Omega-3 fatty acids


46. Christensen JH, Gustenhoff P, Korup E, Aaroe J, Toft E, Moller J, Rasmussen K, Dyerberg J, Schmidt EB. Effect of fish oil on...


