Heart Foundation™

Review of evidence

Fish, fish oils, n-3 polyunsaturated fatty acids and cardiovascular health

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Contents

Heart Foundation recommendations

All adult Australians

To lower their risk of coronary heart disease (CHD), all adult Australians should:
1. Consume about 500 mg per day of combined docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) through a combination of the following:
- two or three serves (150 g serve) of oily fish per week
- fish oil capsules or liquid
- food and drink enriched with marine n-3 polyunsaturated fatty acid (n-3 PUFA).

2. Consume at least 2 g per day of alpha-linolenic acid (ALA).
3. Follow government advice on fish consumption regarding local safety issues.
4. Discus healthy eating and concerns about nutrition with an Accredited Practising Dietitian or a doctor.

People should look for foods and drinks with added omega-3s in their local supermarkets. For food and drinks containing ALA, see Appendix 2, Table 1.

Women who are planning pregnancy, pregnant or breastfeeding, and children

Women who are planning pregnancy, pregnant or breastfeeding, and children should:
1. Follow the Heart Foundation's recommendations for the adult Australian population.
2. Not exceed the recommended doses of fish and fish oil supplements.
3. Follow the advice from Food Standards Australia and New Zealand on mercury in fish.

Adult Australians with documented CHD

To lower their risk of further disease progression, Australian adults with documented CHD should:
1. Consume about 1000 mg per day of combined DHA and EPA through a combination of the following:
- two or three serves (150 g serve) of oily fish per week
- fish oil capsules or liquid
- food and drink enriched with marine n-3 PUFA.

2. Consume at least 2 g per day of ALA.
3. Follow government advice on fish consumption regarding local safety issues.
4. Discuss healthy eating and concerns about nutrition with an Accredited Practising Dietitian or a doctor.

People should look for foods and drinks with added omega-3s in their local supermarkets. For food and drinks containing ALA, see Appendix 2, Table 1.

Australians with lipid abnormalities

For Australian's with elevated triglyceride (TG) levels, first-line therapy could be fish oil capsules or liquid and marine n-3 PUFA enriched foods and drink. They should:

- start with a dose of 1200 mg per day of DHA and EPA; and if appropriate
- increase the dose to 4000 mg per day of DHA and EPA and check their response every 3 to 4 weeks when the dose is changed, until target TG levels are reached.

2. Discuss healthy eating and concerns about nutrition with an Accredited Practising Dietitian or a doctor.

Summary of the evidence

Evidence	Level of evidence	Studies
Individuals with a higher intake of fish have a lower risk of CHD mortality, total CHD and total stroke.	III-2	1-3
Consuming fish at least once a week is associated with a lower risk of total stroke and CHD mortality in the general population and post- myocardial infarction (MI) patients.	III-2	1-5
In secondary prevention, a diet with 2 g/day of ALA decreases the risk of CHD.	II	6-8
In secondary prevention ≥ 850 mg/day marine n-3 PUFA supplementation reduces the risk of CHD mortality, and ≥1800 mg/day reduces major coronary events.	II	9-11
In secondary prevention, there is conflicting evidence about the effect of marine n-3 PUFA supplementation on the risk of sudden death in patients.	n/a	9,12-16
Marine n-3 PUFA supplementation of 1000-4000 mg/day decreases serum triglyceride levels by 25-30% and increases high-density lipoprotein (HDL) cholesterol levels by 1-3%. A dose relationship exists between intake of marine n-3 PUFA and decreased serum triglyceride levels.	I	17-19
Marine n-3 PUFA has an additive effect to statin therapy in decreasing serum triglyceride levels and increasing HDL cholesterol.	II	20-26
Consuming fish with high levels of methylmercury may result in long-term neurological damage. Gestational exposure to methylmercury may result in neurodevelopmental deficits.	III-3	27
The consumption of oily fish twice a week promotes cardiovascular health without excessive exposure to mercury.	III-1	28,29
There is inconclusive evidence supporting a relationship between mercury exposure and incidence of cardiovascular disease.	n/a	30
Fish oil capsules available in Australia have zero or near zero methylmercury content.	IV	31
Fish oil capsules in Australia contain very low levels of dioxins (polychlorinated biphenyl (PCB)).	IV	32

Levels of evidence for clinical interventions

The following NHRMC levels of evidence have been used to rate the evidence presented in this paper.

Level of evidence	Study design
I	Evidence obtained from a systematic review of all relevant randomised controlled trials
II	Evidence obtained from at least one properly designed randomised controlled trial
III-1	Evidence obtained from well-designed pseudo-randomised controlled trials (alternate allocation or some other method)
III-2	Evidence obtained from comparative studies with concurrent controls and non-randomised allocation, cohort studies, case-control studies, or interrupted time series with a control group
III-3	Evidence obtained from comparative studies with historical control, two or more single-arm studies, or interrupted time series without a parallel control group
IV	Evidence obtained from case series, either post-test or pre-test and post-test data

Source: [300]

Background

Since the National Heart Foundation of Australia's (Heart Foundation's) *Review of the relationship between dietary fat and cardiovascular disease* in 1999, [33] new findings have been published internationally regarding the benefits of omega-3 polyunsaturated fatty acids (n-3 PUFA) in preventing and treating cardiovascular disease (CVD), particularly CHD. The American Heart Association (AHA) has found this data compelling enough to release a position statement that recommends levels of fish intake and specific marine n-3 PUFA (DHA, EPA and docosapentaenoic acid (DPA)) intake.[34,35] Similarly, in a recent report, a joint expert consultation of the United Nations Food and Agriculture Organization (FAO) and the World Health Organization (WHO) recommended an intake of 1-2 servings of fish (where each serving is defined as providing 200-500 mg/week DHA and EPA) as protective against CHD and ischaemic stroke.[36]

Guidelines released by the US Department of Health and Human Services noted that consuming two servings of fish a week (8 oz or 230 g/week) may reduce the risk of CHD mortality.[37] In 2006, the National Health and Medical Research Council (NHMRC) issued *Nutrient reference values for Australia and New Zealand Including Recommended Dietary Intakes*,[38] which recommended an intake of combined DHA, EPA and DPA of 610 mg/day for men and 430 mg/day for women to prevent chronic disease. Food Standards Australia and New Zealand (FSANZ) introduced a nutrition claim for foods containing qualifying levels of n-3 PUFA in their 2000 revision of the Food Standards Code.[39] It plans to allow a general level claim for n-3 and cardiovascular health with the introduction of the anticipated health claims policy for foods.[40]

Seafood is an excellent source of protein and oils. Seafood-derived oils are rich in marine n-3 PUFA, particularly DHA and EPA. Algae are the primary producers of DHA and EPA in the ecosystem. Fish consume algae and are therefore rich in DHA and EPA. Long chain fatty acids (FA) are defined as containing 12- to 26-carbon (possibly more) atoms in a chain.[41] Terrestrial sources rich in n-3 PUFA include flaxseed, canola, walnut and soybean oils, which contain the 18-carbon ALA as the major n-3 PUFA.

In humans, ALA is an essential PUFA because it cannot be synthesised *in vivo* or from other PUFA in the diet.[42,43] Deficiency is associated with sensory neuropathy, impaired visual activity and learning deficiency.[44] DHA and EPA can reverse n-3 PUFA deficiency. DHA is the most abundant n-3 PUFA in tissues and is generally considered to be the most essential n-3 PUFA.[42] n-3 PUFA are present in all membranes and are incorporated into phospholipids, sphingolipids and plasmogens.[45] They are also most concentrated in sperm, brain and retina. DHA is several hundred times more abundant than EPA in the brain and retina cells. In adipose tissue, ALA is the most abundant n-3 PUFA, although it comprises only about 1% of FA in this tissue.[45]

There is compelling evidence supporting the clinical benefits of fish consumption and the intake of marine n-3 PUFA. The favourable effects were attributed initially to EPA, but it is now clear that DHA has at least equally important cardio-protective effects [46,47] and may be more important than EPA for beneficial cardiovascular effects. Other n-3 PUFAs, such as the 22-carbon DPA and ALA, may also be important for cardiovascular health, but there are considerably fewer scientific studies to evaluate. Evidence supporting these benefits is derived from population studies, randomised

controlled trials and new information on the mechanisms of action of these nutrients. Additionally, there has been renewed interest in the potential toxicity of seafood (includes crustaceans, molluscs and other species) because of heavy metal or pesticide contamination, and microbial infection.

Fish consumption in Australia

Using recent databases, a recalculation of the 1995 Australian National Nutrition Survey (NNS) indicated that the Australian average intake of marine n-3 PUFA is 246 mg/day comprising 75, 71 and 100 mg/day from EPA, DPA and DHA, respectively.[48,49] Seafood is the main source of marine n-3 PUFA in the Australian diet, with lean meat also a major source—particularly of DPA.[49,50]

The consumption of fish and other seafood varies across Australia. The 1995 NNS estimated that less than 10% of the population consumed more than 500 mg/day of marine n-3 PUFA.[50] The Fisheries Research and Development Corporation (a body of the Australian Government's Department of Agriculture, Fisheries and Forestry) collected data on fish and seafood consumption in Sydney,[51] Perth [52] and Melbourne.[53] In 1998–1999, the mean adult intake of total seafood was 250–300 g/week in Sydney and Perth. In 2005, the mean intake was 10–15% less in Melbourne than the earlier estimates in Sydney and Perth. Shark and flathead, which are low in marine n-3 PUFA, were the leading fish products consumed in Sydney. The ratio of fin fish (those with fins) to seafood (crustaceans, molluscs and other marine species) consumed is about 2:1. This is equivalent to 1 ½–2 serves/week of reasonably low-level marine n-3 PUFA seafood.

Epidemiological studies

Modern industrialised societies are characterised by a high intake of saturated, omega-6 PUFA and trans fatty acids, and a low intake of n-3 PUFA. The ratio of n-6:n-3 in the Palaeolithic period has been estimated as 0.79, which is similar to that in Crete before 1960.[54,55] In contrast, in the USA and Northern Europe, the ratio is currently about 15:1.[55,56] Both the absolute amount of n-3 PUFA (especially marine n-3 PUFA) and the n-6:n-3 ratio seem to be important for health.[57]

More than a dozen studies in populations with a moderate to high prevalence of CHD have confirmed the findings from early studies of Chinese and Greenland Eskimos: a high intake of fish is associated with low CHD mortality.[58,59]

An inverse relationship between fish consumption and CHD was documented in China nearly four decades ago. The Chinese Academy of Medical Sciences in Beijing noted that the lowest incidence of CHD was in fishermen from the Choushan archipelago.[60] Nomads consuming predominantly animal fat in Xinjiang province had an eight times higher incidence of CHD than the average population.[61] The beneficial association between fish intake and low rates of CHD in China was confirmed in the prospective study from Shanghai,[62] which involved 18,224 subjects over 12 years.

At about the same time as the Chinese studies, Bang and Dyerberg's studies of Greenland Eskimos also suggested that seafood, rich in marine n-3 PUFA, prevents CHD and stroke.[63-65] Their seminal studies, coupled with the emerging understanding of prostaglandin metabolism, opened up a new research area.

The Seven Countries Study of Japan, USA, Greece, Finland, Italy, Netherlands and Yugoslavia [66] found a strong inverse association between the consumption of PUFA and CHD. The study found that people living on Crete consumed a relatively high fat intake (40% of daily energy as fat) but had a low rate of CHD. This was attributed partly to their intake of fish (and thus marine n-3 PUFA), which is 30 times higher than in the USA.[67] Other sources of n-3 PUFA in Crete are wild plants (rich in ALA) and free-range animal products (rich in ALA, DHA, DPA and EPA).

The two Japanese cohorts from the Seven Countries study had low intakes of n-6 PUFA and high intakes of n-3 PUFA from vegetables (cohort of farmers) and fish (cohort of fishermen).[68] The Cretan and Japanese cohorts had low n-6:n-3 ratios diets and extraordinarily low CHD mortality rates that were less than 5% of the rates of the cohort of railroad workers from the USA.[66,68,69] Compared with no (or a very low) intake, a modest intake of fatty fish and marine n-3 PUFA from seafood equivalent to one serve (200 g) of fish a week was associated with a 40–50% reduction in risk of sudden death.[66]

A recently published cohort study[70] compared fish and marine n-3 PUFA intakes in 4949 men and women aged 40–79 years from Norfolk, England. n-3 PUFA plasma concentration was 20% higher in fish oil consumers than in non-fish oil consumers, and was twice as high in people who consumed fatty fish than in those who consumed any type of fish. The concentration of total n-3 PUFA was significantly higher in women (7.96 mol% ± 2.46 mol%) than in men (7.81 mol% ± 2.44 mol%). Importantly, the same results were found when diet was analysed by four different methods (food frequency, two dietary recall tools, and a health and lifestyle questionnaire).

Because men and women consumed the same amount of fish in the Norfolk study, the authors proposed that the lower concentration of total n-3 PUFA in men related to their larger body size and plasma volume, and possibly to hormonal differences.[70] Interestingly, only 20–25% of the variation in marine n-3 PUFA levels was explained by marine n-3 PUFA intake. The mechanism that accounts for the underlying residual concentration of n-3 PUFA in the absence of marine n-3 PUFA consumption remains unknown. The authors concluded that men should consume more marine n-3 PUFA to achieve the same blood n-3 PUFA concentration as women.

In contrast, a recent Danish epidemiological study[71] of 4513 men and 3984 women aged 30–70 years showed no relationship between fish consumption and all-cause mortality or incidence of CHD. People eating fish less than once a week had lower all-cause mortality (hazard ratio (HR), 0.88, 95% CI, 0.76–1.02) compared with those eating fish twice or more a week (HR, 1.06; 95% CI, 0.88–1.28). However, frequent fish consumption was not significantly related to death from all causes either before or after adjustment for confounding factors. In the high-risk CHD group, all-cause mortality seemed to increase with fish intake. It is unknown whether the most common modes of preparation and cooking fish, which included added fat, affected the results. The authors queried whether the study was sufficiently powered in the high-risk group in terms of outcome events. They also noted that they could not exclude an effect of other possible confounding factors correlated with fish intake, such as genetic disposition in the high-risk group or the fish preparation method.

The Japan Public Health Center-based (JPHC) Study Cohort I[72] followed 41,578 Japanese men and women aged 40–59 years who were free of clinical CVD. Data was collected from 1990 to 1992, and in 2001. The hazard ratio (HR) was 0.63 (95% CI, 0.38–1.04) for total CHD and 0.44 (95% CI, 0.24–0.81) for definite MI in people with the highest quintile of fish intake (8 times/week or median intake 180 g/day) compared with the lowest quintile (once/week or median intake 23 g/day—below the mean adult Australian intake). Dietary intake of marine n-3 PUFA was inversely correlated with the risks of MI (HR, 0.35; CI, 0.18–0.66) and non-fatal coronary events (HR, 0.33; CI, 0.17–0.63).

An epidemiological study of another Japanese cohort[73] (NIPPON DATA80 study) did not show any benefit of very high fish consumption. This 19-year study of 3945 men and 4934 women was considerably smaller than the JPHC study. Multivariate Cox analyses showed no differences between people who ate fish more than twice a day and those who ate fish 1–2 times/week in all-cause mortality (relative risk (RR), 0.99; 95% CI, 0.77–1.27), stroke (RR, 1.26; 95% CI, 0.70–2.29) or CHD mortality (RR, 0.91; 95% CI, 0.35–2.35). Few participants consumed fish less than once a week. The results of this study are consistent with previous epidemiological data, suggesting a threshold in the protective effect of fish consumption once or twice a week compared with little or no fish consumption.

The Cardiovascular Health Study[74] examined fish consumption and stroke risk in 4775 adults aged 65 years with no history of CVD. Consumption of tuna or broiled or baked fish was inversely associated with total stroke (P = 0.04) and ischemic stroke (P = 0.02). An intake of fish 1–4 times/week was associated with a 27% lower risk of ischaemic stroke (multivariate HR, 0.73; 95% CI, 0.55–0.98). Eating fish five or more times a week was associated with a 30% lower risk of stroke compared with eating fish less than once per month (HR, 0.70; 95% CI, 0.50–0.99).

A prospective population-based cohort study of the relationship between fish consumption and mortality in 3910 adults who were free of disease at the onset found a significant inverse association between fish consumption and death due to

ischaemic heart disease (IHD) and arrhythmic IHD (both $P < 0.001$).[75] However, it found no association between fish consumption and the incidence of non-fatal MI. Compared with the consumption of fish once a month or less, consumption of tuna or broiled or baked fish three or more times a week was associated with a 49% decrease in the incidence of IHD death and a 58% decrease in the incidence of arrhythmic IHD.

A 1999 study in Japan,[76] involving one of the communities in the Seven Countries Study, demonstrated an inverse relationship between carotid intima–media thickness (IMT) and intake of DHA and EPA ($P < 0.01$ for trend).

Intervention studies in healthy individuals and patients

Intervention studies in healthy individuals and in patients show several beneficial effects of fish oil supplementation or regular fish consumption, including lower rates of mortality, CHD and sudden death. They also show favourable changes in blood lipid concentrations, symptoms of CHD (for example, angina) and cardiac electro-physiological properties.

Fish oil supplementation and mortality

The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico Prevenzione (GISSI-P) trial was a high-quality study and the first large intervention CHD endpoint trial that assessed marine n-3 supplementation.[9] It involved 11,324 post-MI patients and showed clear cardiac benefits of marine n-3 PUFA supplementation. After 3 ½ years, the intake of a fish oil capsule (850–880 mg marine n-3 PUFA; EPA/DHA 2:1 ratio) was associated with a 20% reduction in mortality and 45% reduction in sudden death. Further analysis demonstrated that marine n-3 PUFA consumption was associated with a 53% decrease in sudden death during the first four months after MI.[15] The benefit was greatest in patients with a high risk of sudden death and those who adhered closely to the intervention. The dramatic reduction in sudden death in the GISSI-P trial is consistent with epidemiological studies and with the anti-arrhythmic properties of marine n-3 PUFA demonstrated in animals[77] and humans.[78,79]

The Kansas Study[80] compared 2870 individuals who took marine n-3 PUFA supplements with 27,811 who did not take marine n-3 PUFA supplements. Participants included healthy individuals and patients with CHD, who were followed from 1998 to 2005. The endpoint was all-cause mortality. During the follow-up, more patients who were not taking supplements (11%) died that those who were taking supplements (4.0%; $P < 0.001$). The same relative benefit occurred regardless of the presence or absence of CHD at baseline.

A recent analysis of the GISSI-P trial data examined the effect of marine n-3 PUFA on post-MI patients with left ventricular systolic dysfunction.[14] Marine n-3 supplementation reduced mortality in patients with (RR, 0.76; 95% CI, 0.60–0.96; $P = 0.02$) and without (RR, 0.81; 95% CI, 0.59–1.10; $P = 0.17$) left ventricular systolic dysfunction. Interestingly, marine n-3 PUFA supplementation strongly reduced the risk of sudden death in post-MI patients with left ventricular systolic dysfunction (RR 0.42; 95% CI, 0.26–0.67; $P = 0.0003$) but did not significantly reduce the risk of sudden death in patients with an ejection fraction > 50%. The authors recommended further research to examine the effect of marine n-3 PUFA on populations with left ventricular systolic dysfunction.

Fish oil supplementation and susceptibility to ventricular tachycardia/fibrillation

The lipid composition of cell membranes can be modified by diet. Marine n-3 supplementation alters myocardial responsiveness to beta-adrenoceptor receptor stimulation and therefore vulnerability to ventricular tachycardia/fibrillation.[81]

In a multi-centre trial of 200 patients, marine n-3 PUFA supplementation failed to prevent sudden death in patients with cardiomyopathy.[16] In these patients, ventricular fibrillation (VF) was related to 'irritable ventricular foci' and not ischaemia. Patients were randomised to receive either 1800 mg/day of marine n-3 PUFA or placebo and were followed up for about two years. Recurrent ventricular tachycardia (VT) or VF events occurred more frequently in patients randomised to receive marine n-3 PUFA ($P < 0.001$). Post hoc analysis of the time between the first VT and first VF showed a trend toward an increased risk of VT in patients assigned to marine n-3 PUFA but no apparent affect on the risk of VF. The authors concluded that in this study n-3 PUFA supplementation may be pro-arrhythmic in some patients.

The recently completed study on omega-3 fatty acids (FA) and ventricular arrhythmia[12,13] also did not show clear benefit of supplementation with marine n-3 PUFA on prevention of sudden death in patients with an implantable cardioverter defibrillator (ICD). After one year, 30% of patients in the marine n-3 PUFA group had a life-threatening arrhythmia compared with 33% in the placebo group. In patients with previous MI, 28% of the patients on marine n-3 PUFA had an event compared with 35% on placebo, although this difference was not significant. The absolute risk reduction of life-threatening arrhythmias is consistent with data from previous trials, such as GISSI-P and Japan EPA Lipid Intervention Study (JELIS),[10] which had adequate power to detect a risk reduction of 2% or more.

Non-invasive electrophysiological testing was performed in 10 men with ICDs who had VT at pre-implant testing and repeated episodes of VT. Seven of the 10 patients had monomorphic sustained VT induced. Five of these patients were rendered non-inducible after an intravenous infusion of 3.8 g of n-3 PUFA.[82]

Fish consumption, fish oil supplementation and atherosclerosis

Two human angiographic trials compared the effects of marine n-3 PUFA supplementation and placebo on progression of CHD, determined by repeat coronary angiography. The first study[83] involved 59 patients given either 6000 mg/day of marine n-3 PUFA or olive oil for two years. It found no benefit of marine n-3 PUFA supplementation. A larger, more recent trial[11] of 223 patients randomised to receive either placebo or 3000 mg/day of marine n-3 PUFA for three months and then 1500 mg/day for 21 months found less progression and more regression of angiographic CHD ($P = 0.04$) compared with a placebo group.

A three-year follow-up of 50 British men with coronary artery disease (CAD) demonstrated no association between dietary marine n-3 PUFA and absolute width of coronary segments examined with angiography.[84] In contrast, fish intake had a beneficial effect after 3.2 years in 229 women who participated in the estrogen replacement and atherosclerosis trial[85] and whose disease progression was determined using quantitative coronary angiography. Progression of atherosclerosis, quantified as the change in minimum luminal diameter, was less in women who consumed two or more serves of fish a week than in those who consumed less than two serves a week ($P = 0.02$). Adjusting for factors affecting CHD risk strengthened the association ($P = 0.006$). However, the benefit was confined mainly to the 52 diabetic women ($P < 0.001$).

Marine n-3 PUFA intake may influence plaque growth because supplemental marine n-3 PUFA is incorporated into the phospholipids and cholesteryl esters in atherosclerotic lesions.[86] In a recent study of patients awaiting carotid endarterectomy, specimens obtained in surgery showed that marine n-3 PUFA had

been incorporated into plaques. This was associated with a significant decrease in macrophage infiltration, which is consistent with greater plaque stability.[87] As with human trials, in animal models marine n-3 PUFA supplementation inhibits development of experimental atherosclerosis independent of plasma lipid effects.[88-91]

A serial carotid ultrasound trial[90] found no effect of two years of marine n-3 PUFA supplementation on progression of carotid atherosclerosis. Marine n-3 PUFA supplementation appears to be ineffective in preventing restenosis after angioplasty to coronary lesions.[92] However, there may be benefit in patients who have had bypass grafting in decreasing graft occlusion rates.[93] In 610 patients who had coronary bypass grafting, 3400 mg/day of marine n-3 PUFA decreased vein graft occlusion rates from 33% (control group) to 27% (P = 0.03).[93]

Fish oil supplementation and atrial fibrillation

Fish oils may prevent patients developing episodes of atrial fibrillation. In a prospective population-based cohort of 4815 elderly patients followed for 12 years, 980 cases of incident atrial fibrillation were diagnosed. Multivariate analysis of data showed an inverse association between fish consumption and incident atrial fibrillation.[94] The consumption of fish 1–4 times/week was associated with a 28% lower risk of incident atrial fibrillation (P = 0.005) than the consumption of fish less than once a month. The consumption of fish five times a week or more was associated with a 31% lower risk (P = 0.08).

A rat study has shown that marine n-3 PUFA decreases the asynchronous contractile activity in electrically stimulated atrial myocytes.[95] The decrease in asynchronous contractile activity may be explained by changes in membrane fluidity and could be the reason for the beneficial effects of marine n-3 PUFA in preventing atrial fibrillation. A recent study of rabbits[96] has shown that DHA-rich fish oils can prevent fibrillation induced by atrial stretch.

In a study of 160 patients undergoing coronary artery bypass grafting who were randomised to receive either marine n-3 PUFA or placebo,[97] pre-operative supplementation with marine n-3 PUFA decreased incident atrial fibrillation by 50%. Not all studies have shown prevention of atrial fibrillation, and more human trials are needed to confirm and quantify the effect of high-dose marine n-3 PUFA on high heart rate activity.

A paper presented at the Heart Rhythm Society in May 2006[98] examined the effect of marine n-3 PUFA consumption on the development of atrial fibrillation in 17,679 men involved in the Physicians' Health Study. After adjusting for multiple CVD risk factors and lifestyle habits, higher consumption of fish was associated with a paradoxical increased risk of developing atrial fibrillation (OR 1.46; 95% CI, 0.94–2.28 for five meals or more a week; trend for increasing frequency of consumption, P = 0.017). From the adjusted data, the authors calculated that estimated marine n-3 PUFA consumption was also associated with an increased risk of developing atrial fibrillation (adjusted OR 1.37; 95% CI, 0.90–2.10, for the highest versus lowest quintile; trend, P = 0.017).

Fish oil supplementation and heart rate variability

Marine n-3 PUFA supplementation at the moderately low dose recommended by the AHA for secondary prevention influences prognostically important heart rate variables in patients with CHD. High resting heart rate is associated with increased cardiovascular mortality and increased risk of sudden death from MI in apparently healthy individuals.[99-102] A recent study[103] assessed the effects of 810 mg/day of marine n-3 PUFA in 18 men who had MI and left ventricular ejection fraction < 40%, and were stable. Supplementation decreased resting heart rate from 73 to 68 beats/minute ($P < 0.0001$) and improved heart rate recovery one minute after exercise (–27 to –32 beats/minute, $P < 0.01$). Heart rate variability in the high-frequency band increased ($P < 0.02$), but overall heart rate variability did not change. These observations are consistent with a favourable alteration of vagal activity. Heart rate variables, including heart rate recovery, are important predictors of CHD prognosis.[104,105]

Fish oil supplementation, infarction size and angina

In models of experimental MI, marine n-3 PUFA decreases mortality rate, creatine kinase levels and myocardial lipid peroxides.[106]

Marine n-3 PUFA may also:

- reduce infarct size and the incidence of large infarcts[107]
- be associated with a lower prevalence of myocardial lesions[108]
- reduce cardiac oxygen consumption during experimental ischaemia in a rat ischaemia–reperfusion model[109]
- decrease episodes of angina and improve exercise time to angina.[110-112]

Dietary advice about fish consumption—DART and DART-2

The Diet and Reinfarction Trial (DART)[113] and Diet and Angina Randomized Trial (DART-2)[114,115] were randomised clinical trials (RCT) in which men < 70 years of age, who had recovered from MI (DART) or who had angina (DART-2), received advice on changing their diets - they were advised to increase intake of fruits and vegetables, and decrease their intake of saturated fat. One arm of the trials included advice to eat more fatty fish.

In the DART[113], all-cause mortality over the first two years was 29% lower in men advised to eat more fish (‘fish group’) than in men who were not given advice about eating fish. The decrease in all deaths resulted from a decrease in IHD mortality. The incidence of non-fatal MI and IHD events was unchanged in the fish group.

A follow-up of the DART found no long-term benefits on risk of death for up to 10 years afterwards, although the men in the fish group continued to eat slightly more fish.[116] The follow-up showed that the early decrease in all-cause mortality in the fish group was not sustained. [113] The rates of all-cause mortality did not differ between the groups.

Interestingly, in the follow-up, investigators sent a letter to participants in the ‘non-fish’ group telling them about the results of the trial and recommending that they eat fish for cardiac benefit.[116] Over the next 5–15 years, CAD mortality and total mortality rates in the non-fish groups became congruent with former participants of the fish group. Hamazaki[117] proposed that the men in the non-fish group may have started

eating more fish during the first year of the follow-up because of the letter. It was suggested that this accounted for the decrease in mortality in the non-fish group and the slightly higher mortality in the fish group.[117] In addition, the amount of fish eaten by both groups differed by less than 7 g/day, and 19.3% of men in the non-fish group took fish oil supplements.[116]

In the DART-2, 3114 men with angina were randomly allocated to four diet groups and followed for three to nine years. Members of the first group (n = 764) were instructed to 'eat at least two portions of fish each week or up to 3 g of *MaxEpa* fish oils (18% EPA, 12% DHA)[114,115] as a partial or total substitute'. Other groups were advised to eat more fruits and vegetables, to eat more fish or take supplements, or to eat a 'sensible diet'. All-cause mortality was not reduced in the group advised to eat more fish. Risk of cardiac death was higher in men given fish advice (adjusted HR = 1.26; 95% CI, 1.00–1.58; P = 0.047) and even higher for risk of sudden cardiac death (adjusted HR = 1.54; 95% CI, 1.06–2.23; P = 0.025). The excess risk occurred in the men supplied with fish oil capsules.

The authors offered several possible explanations for these unexpected findings, such as an adverse effect of fish oils in patients with angina, interaction with certain drugs, or risk compensation or changes in the patients' health behaviour.[114,115,118] The authors proposed that fish oils are protective after MI, but increases the risk of cardiac death in men with angina. The adverse effects occurred only in men who took fish oil capsules and not in men who ate fish. The excess mortality in the fish group occurred in men who took supplements but were not taking β-blockers. Digoxin was the only drug that showed a significant interaction with advice to eat more fish. The authors proposed that fish oils have arrhythmic effects in patients with chronic disease (DART-2) but anti-arrhythmic effects in patients with acute disease (DART, GISSI-P).[118] Burr et al. proposed that certain drugs, for example β-blockers, and dihydropyridine class of L-type calcium channel inhibitors, protect against the adverse effects of fish oils in certain patients, but that digoxin (a potent inhibitor of Na+/K+ ATPase) exacerbates the arrhythmic effects of fish oils.

The DART and DART-2 have been criticised for poor patient compliance, large confidence intervals, and offering advice about the benefits of fish consumption to control groups. This is discussed further in the section on systematic reviews and meta-analysis.

Ongoing clinical trials

A number of large ongoing marine n-3 PUFA RCT are examining clinical endpoints in various patient groups. These include:

- the Outcome Reduction with Initial Glargine Intervention Trial[119]
- a new study being undertaken by the GISSI study team[120]
- the Alpha Omega Trial.[121]

Systematic reviews and meta-analyses

Two meta-analyses of case-control or cohort studies of more than 200,000 individuals, with an average follow-up of 11.8 years, confirmed the independent beneficial effect of fish intake.[1,2] Compared with individuals who never consumed fish or who ate fish less than once a month, CHD mortality was found to be:

- 11% lower for people who ate fish 1–3 times/month
- 15% lower for people who ate fish once/week
- 23% lower for people who ate fish 2–4 times/week
- 38% lower for people who ate fish five times/week or more.[1]

He and colleagues estimated that an increment of 20 g/day of fish intake could possibly lower CHD mortality rates by 7%. The relationship was not as strong for non-fatal MI.

The Harvard School of Public Health Centre for Risk Analysis convened an expert panel to quantify the benefits and potential risks of fish consumption.[29] The group noted that consuming small quantities of fish was associated with a 17% reduction in CHD mortality and that each additional serving a week was associated with a further reduction of 3.9%.[5]

A meta-analysis of nine independent cohorts from eight studies comprising 200,575 participants with an average follow-up of 12.8 years demonstrated that fish consumption protects against stroke.[3] Compared with individuals who consumed fish less than once per month, those who consumed fish at least once a week were found to have a 13% lower risk of total stroke (95% CI, 0.77 to 0.98). Those who ate fish five or more times a week were found to have a 31% lower risk of stroke (95% CI, 0.54–0.88). A systematic review also found that 'any fish consumption' confers a substantially lower stroke risk (12% on a linear model) compared with 'no fish consumption'.[4] A probable 2.0% reduction of stroke risk was calculated per serving of fish a week.

Consistent with these systematic reviews of reported fish intake is a recent case control systematic review of 25 studies of tissue n-3 and n-6 fatty acids for CHD events. Low concentrations of n-3 PUFA, especially DHA, were consistently and significantly reduced in CHD patients.[122]

In a 2004 Cochrane review, Hooper et al. reviewed 48 RCT (36,913 participants) and 41 cohort studies, published up to February 2002.[123] This meta-analysis was widely reported as showing little clinical benefit of marine n-3 PUFA intake. However, the only significant major trial added besides those included in the meta-analyses mentioned above[1-3] was the DART-2, which was discussed earlier.[116,124]

It is surprising that the DART-2 was included in the Cochrane meta-analysis, because it was published after the February 2002 cut-off and it did not fulfil the inclusion criteria for the meta-analysis by Hooper et al. The inclusion of the DART-2 led to significant heterogeneity between the included trials. Although acknowledging the heterogeneity of the DART-2 data, Hooper et al. included the DART-2 data in their formal meta-analysis. It has not been widely appreciated that the Cochrane analysis by Hooper et al. revealed that, even with inclusion of the DART-2 data, high marine n-3 PUFA intake was still associated with a significant decrease in total mortality (RR, 0.90; 95% CI, 0.83–0.98, P= 0.002). Removing the DART-2 from the

pooled analysis reveals a greater benefit of high marine n-3 PUFA intake (overall RR of death, 0.83; 95% CI, 0.75–0.91). The latter values reflect a more robust analysis.

The major source of heterogeneity between the cardiovascular outcomes of the DART-2 and the outcomes of other trials is the apparent increase in sudden death rate (but lower overall CHD death rate) of people who consumed fish and fish oils in the DART-2. In the actual Cochrane meta-analysis tables,[123] data from the two-way analysis rather than the four-way analysis was used from the DART-2 and from the GISSI-P trial. The numbers from the DART-2 are correct, but some data on deaths and events were misquoted from the GISSI-P trial.

The original meta-analysis by Hooper et al.[123] was republished[125] 17 months after its original online publication in the Cochrane database. This later version included electronic-based searches to February 2002 (although it included the DART-2 published in 2003 [115]). No new trials were added to the original review and four large cohort studies were not included (Cardiovascular Health Study,[74] Kansas study,[80] JPHC study[72] and the NIPPON DATA80 study.[73]) The large JELIS trial,[10,126,127] which confirmed the GISSI-P trial findings,[9,15,120] was also not included.

The later version has been criticised heavily[128] and generated a number of letters to the editor of the *British Medical Journal*[129-132]. The response by Hooper et al.[133] confirmed the perceived inadequacies of the Cochrane review.

Scott et al.[134] noted that up to a quarter of the reviews analysed by Hooper et al. were of poor quality and did not meet the outlined quality requirements. A paper by Kris-Etherton and Harris[135] and a report commissioned by the US Department of Health and Human Services[136] are consistent with criticism of the DART-2 data.

A systematic review by Studer et al.[137] compared different anti-lipidemic agents and diets on mortality. This review included 97 RCT of 137,140 subjects in trials using statins, resins, niacin fibrates, marine n-3 PUFA, or dietary intervention. The statins and marine n-3 PUFA trials were the most effective in decreasing mortality. Compared with control groups, risk rates for total mortality were 0.87 for statins (95% CI, 0.81–0.94) and 0.77 for marine n-3 PUFA (95% CI, 0.63–0.94). The review included 14 trials of marine n-3 PUFA supplementation. The authors noted moderate heterogeneity, which was related mainly to the inclusion of the DART-2 (whose quality has been questioned as discussed above). Studer et al. noted that the higher quality studies show a greater risk reduction. After excluding the DART-2, the risk ratio for overall mortality improved from 0.77 to 0.75 (95% CI to 0.65–0.87), and the heterogeneity decreased substantially.

The benefits and risks of fish intake on the basis of an extensive review and meta-analyses of relevant evidence unequivocally concluded that '…(f)or major health outcomes among adults, based on both the strength of the evidence and the potential magnitudes of effect, the benefits of fish intake exceed the potential risks'.[30] Concentrating on human studies from randomised trials and large prospective studies, and performing a meta-analysis on the evidence where possible, Mozaffarian and Rimm[30] found that 1–2 servings/week of 170 g of fish high in DHA and EPA reduces risk of coronary death by 36% (95% CI, 20–50%; $P < 0.001$) and total mortality by 17% (95% CI, 0–32%; $P = 0.046$). The authors estimated that an intake of 250 mg/day of DHA and EPA is sufficient for primary prevention.

Mechanisms responsible for the protective effects of marine n-3 PUFA on CHD

Fish and fish oils are thought to decrease the risk of CHD by several possible mechanisms (see Table 1).

Table 1. Mechanisms of action for CHD protection

Mechanisms by which fish and fish oils decrease the risk of CHD
• decreases triglyceride and remnant lipoprotein levels • alters metabolism of n-6 PUFA eicosanoids to inhibit inflammatory processes • increases HDL cholesterol levels (variable response) • improves heart rate variability and lower heart rate • elevates ventricular fibrillation threshold • decreases risk of thrombosis and anti-platelet effects • slows progression of atherosclerotic plaques • improves endothelial function • modestly reduces blood pressure • lowers plasma leptin levels

The protective effect of fish and marine n-3 PUFA intake may be confounded by the likelihood that regular fish consumption is a marker for a healthy diet high in vegetables, pulses, fruits and red wine.[138] High fish consumers in Western countries also tend to have a higher socioeconomic position and lower prevalence of depressive symptoms—both significant independent risk factors for CHD.[139-141]

Membrane effects

Fatty acids (FA), particularly marine n-3 PUFA, are incorporated into cell membranes. Increasing the amount of PUFA in the membrane increases its fluidity and deformability. DHA is the most unsaturated FA and is particularly effective in transitional changes associated with transmembrane protein activation.[142-144]

DHA, EPA, ALA and arachidonic acid (AA) compete for the sn-2 position on membrane phospholipids. The relative proportion of these FA determines their relative availability as substitutes for cyclo-oxygenases and lipoxygenases, and the balance of eicosanoid and docosanoic mediators, such as resolvins.[145,146]

Marine n-3 PUFA are ligands for nuclear receptors such as peroxisome proliferator-activated receptors and retinoid X receptors. Therefore they may influence gene regulation.[147-149]

Effect on lipid profile

Marine n-3 PUFA has well-known effects in decreasing serum TG concentration. A review of marine n-3 PUFA studies[17] reported that 4000 mg/day of marine n-3 PUFA decreases TG concentration by 25–30% and increases HDL cholesterol concentration by 1–3%. The review confirmed that there is a dose-response relationship between intake of marine n-3 PUFA and TG concentration: the higher

the baseline TG level the greater the response. The dose-response relationship has now been confirmed in a single clinical trial by Milte et al.[18] For patients with severe hypertriglyceridaemia (TG concentration 5.65–22.60 mmol/L), marine n-3 PUFA may decrease TG concentration by 45% ($P < 0.00001$).[150] In patients with extremely high concentrations of TG (> 22.60 mmol/L), marine n-3 PUFA may lower TG concentration by 50% or more.

Animal and in vitro studies show that consumption of marine n-3 PUFA increases HDL cholesterol receptors and the turnover of HDL cholesterol.[151,152] However, a human study showed no relationship between marine n-3 PUFA intake and HDL cholesterol level.[153] In the INTERLIPID study, marine n-3 PUFA intake was positively correlated with serum HDL cholesterol concentration in Japanese men in Japan and Hawaii[154] A 1% kcal increment of marine n-3 PUFA intake was associated with a 4.6 mg/dL higher HDL cholesterol ($P = 0.011$). The relationship was not significant in women after adjustment for hormone-replacement therapy, although the reason for this sex difference is unclear.

One clinical trial[155] showed for the first time the cholesterol-lowering effects of a diet supplemented with high-dose marine n-3 PUFA and plant sterols. The trial comprised four phases, each lasting 29 days. All participants received an olive oil-based, weight-maintaining diet during the first month. During the other three phases, a small amount of olive oil was replaced daily by marine n-3 PUFA only, marine n-3 PUFA plus plant sterols, or plant sterol only. Plasma TG concentrations were markedly lower in the groups fed either of the diets with marine n-3 PUFA ($P = 0.0001$) compared with the olive oil or sunflower oil plus plant sterol diets. Low-density lipoprotein (LDL) cholesterol level concentration was significantly lower after the fish oils plus plant sterol, or the sunflower oil plus plant sterol diets, than after the olive oil diet. HDL cholesterol concentration was not changed by the diets. In this study, the combination of plant sterols and marine n-3 FA had an additive effect on plasma lipid concentrations.

In a study of children with hyperlipidemia, supplementation with DHA significantly increased the concentration of the less-atherogenic LDL subclasses 1 and 2 by 91% and 14%, respectively. It also decreased the concentration of the more atherogenic LDL subclass 3 by 48%.[156] Another recent trial[157] measured the effect of DHA supplementation (as microalgae oil, DHA 940 mg/day) in vegetarians who ate no meat and who did not consume more than one fish meal per month. Supplementation with DHA achieved a beneficial Omega-3 Index (defined later in this document) of ≥ 8% in 69% of the participants with previously low concentrations of marine n-3 PUFA.[157]

Marine n-3 PUFA is effective in lowering the concentrations of postprandial TG-rich lipoprotein particles, chylomicrons, chylomicron remnants, and very low-density lipoprotein (VLDL) cholesterol.[158] It appears that chylomicron clearance is accelerated with marine n-3 PUFA therapy, probably because of reduced competition with VLDL cholesterol for hepatic receptor uptake after remnant particles are partly metabolised and acquire apolipoprotein E.[158] Marine n-3 PUFA supplementation has no significant effect on total serum cholesterol or LDL cholesterol concentrations if the TG level is not elevated.[17]

The most recent systematic review of the effects of consumption of marine n-3 PUFA and ALA on serum CVD risk factors combined 21 trials that evaluated lipid outcomes and performed a meta-analysis on the relevant data.[19] Marine n-3 PUFA consumption decreased TG level by 0.30 mmol/L (95% CI, –0.37 to –0.22 mmol/L) and increased the concentrations of HDL cholesterol by 0.04 mmol/L (95% CI, 0.02–

0.06 mmol/L) and LDL cholesterol by 0.15 mmol/L (95% CI, 0.08–0.21 mmol/L). Higher marine n-3 PUFA dose and higher baseline levels were associated with a greater reduction in TG concentration. There is no significant evidence that ALA alters the concentrations of blood lipids, HbA1c or fasting blood glucose, or that marine n-3 PUFA intake alters high-sensitivity C-reactive protein (hs-CRP) concentration. The authors concluded that marine n-3 PUFA has a dose-dependent beneficial effect of on TG concentration, particularly in individuals with high levels. In contrast, a very small study of 15 healthy vegetarian men found that supplementation with flaxseed oil (ALA) for 4 weeks (51–55% ALA) elevated TG concentration from 1.40 ± 0.17 mmol/L to 1.98 ± 0.30 mmol/L (P = 0.035) [159].

Marine n-3 PUFA can regulate TG metabolism through at least four nuclear receptors: liver X receptor (LXR), hepatocyte nuclear factor-4α (HNF-4α), farnesol X receptor (FXR), and peroxisome proliferator activated receptor (α, β and γ PPAR). These nuclear receptors regulate expression of various genes and influence various aspects of lipoprotein metabolism.

Activation of PPAR-α reduces TG levels mainly by decreasing apolipoprotein CIII expression, which inhibits lipoprotein lipase, a key enzyme of TG catabolism. Marine n-3 PUFA and its eicosanoid metabolites are natural ligands with high affinity for binding to PPAR-α.[160]

Marine n-3 PUFA significantly lowers chylomicron levels probably by decreasing secretion of VLDL cholesterol from the liver. Marine n-3 PUFA also decreases chylomicron size, which increases clearance and possibly increases lipoprotein lipase activity. DHA and EPA appear equally effective in improving chylomicron clearance.[161]

Marine n-3 PUFA modulates several other lipid and carbohydrate metabolic enzymes by regulating the expression of their genes.[162,163] This results in a decrease in TG synthesis[164,165] and an increase in mitochondrial β-oxidation of FA,[166-168] which subsequently decreases the formation of VLDL cholesterol.[169,170]

EPA directly induces the oxidation of mitochondrial FA deposits, which helps reduce tissue lipid content.[171] The effects of DHA are unclear.[172] A recent in vivo study supports the hypothesis that the hypolipidaemic effect of EPA is primarily liver mediated. The authors hypothesised that increasing β-oxidation rates should gradually reduce fat storage in adipocytes.[173] Unlike treatment with fibrate drugs, the TG-lowering action of marine n-3 PUFA appears to take place without increasing the activity of lipoprotein lipase.[174,175]

Table 2 is adapted from Davidson's 2006 review[176] and conveniently summarises the lipid mechanisms of marine n-3 PUFA.

Table 2. Summary of effects of marine n-3 PUFA on nuclear receptors involved in regulation of lipogenesis

Metabolic nuclear receptor	Expected changes		
	Triglycerides	High-density lipoprotein cholesterol	Low-density lipoprotein cholesterol
Peroxisome proliferator–activated receptor	↓↓	↑	↓
Liver X receptor	↓↓	↓	↓
Farnesol X receptor	↓↓	↑	↑
Hepatocyte nuclear factor–4α	↓↓	↓	↔
Overall effects	↓↓↓↓	↔	↔

↑: increase; ↓: decrease; ↔: neutral effect
Adapted from a table in source[176]

Interaction effects of fish oil supplementation with fibrates and statins

Theoretically, the combination of marine n-3 PUFA and fibrates to treat high TG concentration should have an additive or synergistic effect.[175] There is anecdotal evidence from clinicians and some animal studies that supports this theory.[163] We are unaware of any clinical trials in humans of combination therapy of marine n-3 PUFA and fibrates.

Marine n-3 PUFA is effective in altering lipid levels when given in combination with atorvastatin, simvastatin or pravastatin.[20,21,26] Combined treatment redistributes LDL particles to a less dense form and may decrease the total number of LDL particles beyond the response to statin-only therapy. Addition of marine-3 PUFA to statin therapy has an additive effect in decreasing TG concentration[22,26] and may increase HDL cholesterol concentration.[23,24,177] Moreover, while n-3 supplementation alone tends to raise total cholesterol, it may have a cholesterol-lowering effect when superimposed on statin therapy.[26]

In JELIS,[10,126,127,178] 18,645 hypercholesterolaemic people were given low-dose statins and randomised to receive a high concentration of 1800 mg/day EPA, or placebo. They were then followed up for 4 ½ years. This population had a high background intake of fish, and EPA intake was four times higher than in the GISSI-P study mentioned on page 13. In the statin-only group, 3.5% had a cardiac event compared with 2.8% in the statin plus fish oils group, representing a 19% reduction in the HR (95% CI, 0.69–0.95; P = 0.048). The rate of unstable angina in the statin-only group was 6.7% and 4.9% in the statin plus EPA group, a 28% risk reduction (95% CI, 0.55–0.95; P = 0.019). There was no difference in sudden death rate (0.2% of the population). In patients with known CHD, the composite CHD endpoint occurred in 10.7% of the statin-only group and in 8.7% in the statin plus EPA group. This was a significant 19% relative risk reduction and 2% absolute risk reduction. The composite endpoint rate in patients with stable heart disease was considerably lower than that in the previous statin–placebo-controlled trials. In this study, about 80% of the patients in the primary prevention arms and 26% in the secondary prevention arm smoked.

In a recent Western Australian study, 40 mg of atorvastatin increased HDL cholesterol concentration from 1.00 mmol/L to 1.04 mmol/L.[25] A daily dose of 4000

mg/day of marine n-3 PUFA (45% EPA) increased HDL cholesterol concentration from 0.99 mmol/L to 1.00 mmol/L. In the group given both 40 mg/day atorvastatin and 4000 mg/day marine n-3 PUFA, HDL cholesterol concentration increased 14% from 1.10 mmol/L to 1.25 mmol/L. This study suggests that the combination of statin and marine n-3 PUFA treatment synergistically increases HDL cholesterol concentration. Marine n-3 PUFA did not significantly alter fasting concentrations of plasma glucose, insulin, tumour necrosis factor-α (TNF-α) or hs-CRP.

Inflammation

Marine n-3 PUFA supplementation inhibits nuclear transcription factor κB (NF-κB), a key transcription factor in cytokine gene expression, cellular adhesion, inflammation and carcinogenesis.[179] In a murine model, marine n-3 PUFA decreases NF-κB activation and TNF-α expression by 46% in lipopolysaccharide-stimulated macrophages.[180] It is thought that marine n-3 PUFA may decrease production of nitric oxide (NO) and its reactive products, especially in macrophages.[181,182] This is a favourable action when there is chronic inflammation or a tumour.[183] In endothelial cells, NO is important for normal function. Marine n-3 PUFA supplementation is associated with improved endothelial function and presumably does not adversely influence NO production in the endothelium.

A number of marine n-3 PUFA-derived products are generated by action of cyclooxygenase-2 (COX-2). These products include oxygenated bioactive products termed 'resolvins', which inhibit the inflammatory response by decreasing leukocyte exudate cell number. This may explain part of the beneficial actions of marine n-3 PUFA on cardiovascular and chronic immune diseases, inflammation and neoplasia.[145,146]

A recent trial[184] investigating the effect of short-term (48 hours) intravenous infusion of marine n-3 PUFA on immunological function found rapid interference with monocyte-related immunological and pro-inflammatory functions. For example, suppression of the production of pro-inflammatory cytokines interleukin 1 (IL-1) and TNF-α, monocyte adhesion to the endothelium, and trans-endothelial monocyte migration. Howe et al.[47] found that DHA- and EPA-rich fish oils had equivalent effects on IL-1 production.

A recent placebo-controlled, double-blind study investigated the effects of marine n-3 PUFA-enriched food on inflammatory markers in middle-aged men and women with normal to mildly elevated TG concentrations.[185] One group was fed an EPA-rich drink (600 mg/day EPA, 260 mg/day DHA) for 12 weeks. The other group took a placebo (control group). EPA concentrations in the total red blood cell phospholipid fraction increased significantly by 79% in the EPA group. The inflammatory markers measured (hs-CRP and soluble TNF receptors 1 and 2) did not change in either group. More trials are needed to clarify these counterintuitive results. Hill et al.[186] also found no effects of DHA-rich oil on cytokine production but noted that it suppressed superoxide production in stimulated blood neutrophils.

Two papers in the *Proceedings of the National Academy of Science of the United States of America* demonstrated an in vitro mechanism for the inverse relationship between marine n-3 PUFA intake and markers of inflammation. In 2003, Bagga et al.[187] demonstrated that successful replacement of n-6 PUFA with marine n-3 PUFA in cell membranes decreases the cellular response to mitogenic and inflammatory stimuli (in particular COX-2 and IL-6 production). Massaro et al.[188] studied the effects

of DHA on COX-2 expression and activity in human saphenous vein endothelial cells stimulated by the pro-inflammatory cytokine IL-1. Exposure to DHA for more than 24 hours reduced COX-2 expression and activity induced by IL-1, without affecting cyclo-oxygenase-1 expression. The DHA effect depended on the NF-κB binding site in the COX-2 promoter. Western blots showed that DHA blocks nuclear p65 NF-κB subunit translocation by decreasing cytokine-stimulated reactive oxygen species and ERK1/2 activation. Finally, inhibition of various enzymes involved in PUFA metabolism demonstrates that 15-lipoxygenase-1 products may mediate part of the DHA effects.

Leptin

Leptin is a major cytokine secreted by adipose tissue. Plasma concentrations of leptin reflect adipose tissue mass, and a high leptin level is associated with obesity in humans.[189] Elevated plasma concentration of leptin is independently associated with adverse cardiovascular risk.[190-192] The mechanism responsible for the increased risk associated with elevated leptin concentration is not clear. Marine n-3 PUFA supplementation inhibits leptin gene expression in an animal model.[193] In a tribal population in Tanzania, a diet rich in fish and high in marine n-3 PUFA is associated with a low plasma leptin concentration independent of body fat level.[194]

Platelets and coagulation

Marine n-3 PUFA has a mild anti-platelet effect but no significant effect on bleeding time. Pre-operative supplementation does not significantly decrease post-operative bleeding following coronary artery bypass surgery.[97] In combination with aspirin, high doses of marine n-3 PUFA (> 3000 mg/day DHA and EPA) may lower the fibrinolytic response to venous occlusion.[195] Interestingly, marine n-3 PUFA protects rather than worsens the gastric mucosa against ulcers induced by non-steroidal anti-inflammatory drugs.[196] Marine n-3 PUFA supplementation does not change INR levels in patients on Warfarin. There have been a few case reports of an idiosyncratic increase in INR after marine n-3 PUFA was commenced.

Alpha-linolenic acid (ALA)

Similarly to linoleic acid (LA, n-6 PUFA), ALA is an essential fatty acid in humans that must be supplied in the diet. ALA is partly metabolised in the body to EPA, although, because only 10% is metabolised to EPA, ALA does not contribute significantly to total body EPA content. It is not clear whether ALA prevents recurrent coronary events, although there are trends suggesting that this may be the case. Due to the paucity of high-quality conclusive data, the Heart Foundation's previous recommendation of at least 2 g/day of ALA[6,33] remains unchanged.

Some epidemiological studies show that a high intake of ALA is associated with a low rate of CHD.[197] It is not clear whether this reflects a specific protective effect of ALA or a surrogate effect of healthy eating patterns, although dietary LA has beneficial effects on carotid wall thickness.[198]

A 2004 meta-analysis of five prospective cohort studies and three clinical trials in patients with CHD found that high ALA intake is associated with a 21% reduction in fatal CHD, but this did not reach clinical significance (95% CI, 0.60–1.04).[199]

A 2005 18-year follow-up from the Nurse's Health Study[200] suggested that, after accounting for coronary risk factors and other FA, the intake of ALA was inversely associated with the risk of sudden cardiac death (P = 0.02) especially in women with high ALA intake. ALA intake was not related to the risk of other CHD deaths or non-fatal MI. The inverse association between ALA and sudden cardiac death was linear and remained significant in women who also consumed high amounts of marine n-3 PUFA. The authors concluded that the specificity of this inverse association with ALA 'supports the hypothesis that these n-3 PUFA may have anti-arrhythmic properties'. This inverse association between ALA intake and CHD may not be as strong in men.[199]

In the 1960s, a number of trials were conducted to determine whether ALA supplementation or added unsaturated oil rich, particularly in ALA, prevents CHD. In 412 patients with a history of MI,[201] a high-ALA diet (soybean oil) had no significant effect on mortality, although there was a 43% reduction in the rates of fatal MI in the experimental group (95% CI, 0.21–0.89; P = 0.004).

The Norwegian vegetable oil experiment[202] is the largest ALA supplementation trial and involved 13,000 men, mostly without known CHD. Participants were randomised to receive 10 ml/day of flaxseed oil (equivalent to 500 mg of ALA) or a control diet containing sunflower oil, which is also rich in ALA (65%). The rates of all-cause mortality and coronary events such as MI did not differ between dietary groups. The sunflower oil used as a control appeared to have had a cardioprotective effect because the rate of cardiac events was lower in all participants than the background population rate.

The Medical Research Council Soya-Bean trial[203] found no effects of soybean oil supplementation on cardiovascular events or all-cause mortality. The Lyon Diet Heart Study[204] is a randomised prospective study that assessed an ALA-enriched diet. About 600 post-MI patients were randomised to two diets: a Mediterranean diet high in ALA and the AHA prudent diet. Patients who consumed the Mediterranean diet had 50% lower rates of mortality and CHD events (P < 0.001).

A randomised clinical trial[205] assessed the effects of ALA supplementation on the progression of carotid and femoral IMT and inflammatory markers in 103 men and women with moderate hypercholesterolemia. ALA supplementation had no effect on two-year progression rates of mean carotid and femoral IMT or blood cytokine concentrations. However, it did lower CRP concentration.

Docosapentaenoic acid (DPA)

DPA is an n-3 elongation product of EPA. It may also be formed by the retro-conversion of DHA. At present, there is insufficient evidence on the role of DPA in preventing CVD. The Heart Foundation has not made any recommendations about DPA.

DPA may comprise a significant proportion of n-3 PUFA intake, particularly in non-fish eaters, and may account for 30% of n-3 PUFA intake in high meat eaters.[50,206]

In the Kuopio heart study, men in the top 20% intake of n-3 DPA and DHA had a 44% risk reduction of incident CHD, compared with those in the lowest 20%.[207] A similar benefit associated with high serum levels of n-3 DPA was seen in men with and without incident CHD in the USA Multiple Risk Factor Intervention Trial.[208] Serum n-3 DPA concentration was associated with a 42% risk reduction (OR, 0.58; 95% CI, 0.38–0.89) and DHA concentration with a 43% risk reduction (OR, 0.57; 95% CI, 0.36–0.90).

Seal oil, which is rich in n-3 DPA, lowers tumour necrosis factor-α (TNF-α) concentration in healthy volunteers.[209] Another study showed that n-3 DPA is more effective than DHA or EPA in inhibiting platelet aggregation by inhibiting the cyclooxygenase (COX) pathway and by stimulating the lipoxygenase pathway.[210] n-3 DPA is more potent than EPA in suppressing vascular growth factor (VEGF)-induced angiogenesis.[211] VEGF is thought to be the most important stimulator of plaque angiogenesis, which contributes to atherosclerotic plaque progression and instability.[212]

Marine n-3 PUFA and other conditions

The Heart Foundation supports the 2006 position statement from The Cancer Council of New South Wales on *Omega-3 fatty acids, fish and cancer prevention*.[213] In addition, a review concluded that marine n-3 PUFA inhibits carcinogenesis.[214] Altering the n-6:n-3 ratio in human tumour cells by gene transfer inhibits cancer cell proliferation and increases apoptotic cell death in the tumour cells.[215] A large, 14-year epidemiological prospective study [199] of the relationship between intake of various FA and cancer found a relationship between the intake of DHA and EPA and a lower risk of prostate cancer.[216] A meta-analysis found only a weak relationship between a high intake of ALA and the risk of prostate cancer, and concluded that more research is needed.

Reviews of trials of marine n-3 PUFA supplementation indicate some clinical benefits in the treatment of rheumatoid arthritis.[217-220] Marine n-3 PUFA appear to have anti-inflammatory effects, such as decreasing the levels of inflammatory markers, including leukotriene B4, interleukin-1 (IL-1) and CRP.[221-223]

In the Framingham Heart Study, individuals with plasma DHA concentration in the upper quartile had a 47% lower risk of dementia. The average fish intake in this group was 2.7 servings a week.[224] Supplementation with n-3 PUFA improves cognitive function in patients with and without dementia.[225]

The Heart Foundation considers depression, the most common mental disorder, an independent risk factor for CHD.[141] Epidemiological studies indicate a relationship between low fish consumption and increased prevalence of depression, and an inverse relationship between blood or tissue marine n-3 PUFA content and depression. Depressed patients with acute coronary syndrome (acute MI or unstable angina) have significantly lower serum n-3 PUFA concentration and a lower ratio of n-3:n-6 than controls.[226] Marine n-3 PUFA supplementation improves mild to moderately severe depression with efficacy similar to standard drug therapy.[227]

Risks and cautions associated with fish consumption and fish oil supplementation

Fish, like any food, has the potential to cause illness in some individuals. Allergy to seafood occurs in a small percentage of the population. Most often this is because of an anaphylactic-type reaction to proteins in prawns and other crustaceans, and less commonly, in fin fish. Seafood may harbour a number of biological, chemical and physical hazards including viruses, bacteria and biotoxins. In Australia, FSANZ allows the general sale of food and food products if they fulfil the requirements of being 'safe and suitable foods'.[228] Specifically, there has been concern regarding mercury and pesticides in fish. There is also a growing awareness of the risk of ciguatera, an under-recognised form of fish poisoning.

A recent survey of seafood-related illness in Australia from 1990 to 2000 demonstrated that the risk of becoming ill as a result of consuming seafood is very low.[229] A total of 2158 cases of seafood-borne illness were reported in Australia during this time.[229] More than 80% of these cases came from three outbreaks of viral illness associated with oyster intake. All of these outbreaks resulted from contamination of oyster leases by human sewage during heavy rainfall. The most prevalent and enduring seafood illness is predictable with high precision and is preventable. There is an estimated 48 cases of ciguatoxicosis in Queensland each year.[230,231]

FSANZ recommends that pregnant women avoid eating raw fish and oysters to reduce the risk of listeria.[232]

Mercury

Exposure to high concentrations of mercury in various forms is toxic. Exposure to mercury in utero and after the birth can cause developmental neurotoxicity.[233,234] Fish is the major source of mercury in food. The most important inorganic contaminant in fish is methylmercury (MeHg). Fish absorb MeHg from water, and it binds to tissue protein. Cooking does not reduce MeHg content.

MeHg contamination varies according to geographic location and fish species. Some species of fish may contain significant levels of MeHg, PCB, dioxins and other environmental contaminates.[235] Larger predatory fish with longer lifespan have greater concentrations of MeHg. Swordfish and shark have the highest levels, but also contain high levels of marine n-3 PUFA. However, MeHg levels can vary enormously within a single species by up to 150 fold.[236] Mercury content is high in fish where there is volcanic soil runoff (as in some parts of New Zealand)[237] and where there is industrial waste contaminating fresh water and lakes (as in some parts of Northern Europe). In the mass poisoning in Minamata Bay, Japan, 50 years ago, industrial waste from a factory contaminated fish with MeHg levels 100 times the usual level. The severe mercury toxicity was linked to 900 deaths and two million people have suffered long-term neurological damage.[238]

Recommendations about the limit of MeHg have relaxed from the first recommendation of the NHRMC in 1971[239] (0.03 μg/kg of body weight/week* of

*Consumption of MeHg in fish is measured in μg/kg of body weight/week.

MeHg) to the 2004 FSANZ recommendation (3.3 μg/kg of body weight/week of MeHg). The recommendations of international bodies (such as the Joint Expert Committee for Food Additives and Contaminants) and scientific studies examining the effect of MeHg intake have influenced the Australian upper limit recommendation.[240]

Recently FSANZ revised its recommendation[241] limiting certain fish species to minimise the risk of MeHg toxicity. This update was based on a report from the June 2003 meeting of the Joint Expert Committee for Food Additives and Contaminants (JECFA) of the WHO and the FAO.[234] The JECFA reduced the general population upper limit MeHg intake recommendation from 3.3 to 1.6 μg/kg body weight/week. The main reason for this reduction was the adoption of a new safety factor margin to decrease MeHg intake, particularly among pregnant women.

Japanese researchers were among the first to analyse sections of their own population in the light of the recent JECFA revision. Yasutake et al.[242] recently found that 25% of Japanese females of childbearing age consume MeHg over the JECFA recommended limit. The authors noted, however, that food habits and the possible benefits of fish consumption should be considered when determining an appropriate regulatory standard for fish.

The Japanese government has responded to these findings by issuing advice to limit consumption of several kinds of fish, dolphins and whales that exhibit high levels of mercury.[243]

No international consensus has formed regarding upper limit recommendations for mercury intake.[244] FSANZ recommends an upper limit of MeHg intake by pregnant women of 1.6 μg/kg body weight/week, in line with JECFA. However, it has maintained the older recommendation of 3.3 μg/kg body weight/week for the general population (excluding pregnant women). The reason for maintaining the older recommendation is due to the need to offset the potential harm of MeHg contamination with the benefits of eating fish.[245]

A recent study.[246] of 50–70-year-olds found that blood mercury level and neurobehavioural function were not significantly related. The median blood mercury level of 2.1 μg/L (range, 0–16 μg/L) was lower than the 'acceptable' level for children and women of childbearing age, as established by the US Environmental Protection Agency (US EPA) and National Research Council.[247,248] A recent study in the Seychelles[249] rebutted the findings of a number of other earlier studies[250-253] and did not find any link between ocean fish consumption with relatively high mercury intake (up to 12 times that of the average intake of people in the USA) and neurodevelopment risk. Thus, the intake and blood mercury concentrations advocated by the US EPA may be unnecessarily restrictive.

The tables in Appendix 1 compare MeHg content and recommendations of fish intake in commonly available fish species in Australia and the USA, based on the MeHg limits established under the aforementioned regulatory guidelines (FSANZ, the WHO and US EPA) on the basis of available data. Table 3 summarises the advice for pregnant women, women planning pregnancy and children who should limit their consumption of fish with higher levels of mercury based on FSANZ recommendations.[241] There is no need to restrict intake of fish with lower levels of mercury (not listed in the table).

Table 3. Advice for pregnant women, women planning pregnancy and young children

FSANZ recommendation	Fish
Limit to one serve per fortnight. If consumed, no other fish should be eaten	Swordfish, shark, broadbill, marlin
Consume no more than one serve a week, with no other fish being consumed during that week	Orange roughy (sea perch), catfish (imported basa)

Table adapted from FSANZ advice on mercury in fish[241]

Two recent studies on toxic contaminants in salmon[254,255] concluded that even the most contaminated salmon has unequivocal health benefits that far outweigh any potential worst-case scenario (for example 92 patients with CHD would avoid death for every one person who would develop cancer as a result of contaminants in fish[255]).

The Kuopio Ischaemic Heart Disease Risk Factor Study and the EURAMIC study demonstrated that high mercury level is a significant risk factor for CHD events.[28,256-258] The data suggested that consumption of oily fish twice a week is sufficient for benefit without excessive exposure to toxic contaminants such as mercury.[28]

The Harvard Centre for Risk Analysis expert panel[29] summed up the most pessimistic scenario of lifetime exposure to organic chemicals through consumption of contaminated farmed salmon of 4–16 oz/month (113–454 g/month) over a lifetime. The calculated loss of quality-adjusted life years for the total population of the USA is 600 per year, while the quality-adjusted life years gain from eating this fish is 120,000 per year. The net benefit of fish consumption is clear and unequivocal.

Mozaffarian and Rimm[30] published the most recent review of the evidence weighing the benefit of fish consumption against the risks to human health. The review focused on the relationships between intake of fish or marine n-3 PUFA and cardiovascular risk and early neurological development. The authors concluded that women of childbearing age and breastfeeding mothers should consume no more than two fish servings a week and should limit the intake of selected species high in MeHg.

Mercury in fish oil supplements

Fish oil capsules available in Australia have zero or near-zero MeHg content. This is to be expected because MeHg is bound to protein in fish and manufacturers are required by the Therapeutic Goods Administration (TGA) to have virtually no detected mercury in supplements sold in Australia (< 0.5 Hg/L).[31]

A recent investigation of five fish oil brands from the USA showed that all five contained insignificant concentrations of mercury. Three brands contained a level of mercury roughly equivalent to that found in human blood.[259]

Dioxins and PCBs

Dioxins (which include PCBs) are groups of toxic organic chemicals[260] that are widespread in the environment and that can accumulate in body fat and remain unchanged over long periods. Overall dietary exposure seems to have declined in Australia and throughout the world in the past 10 years.[261]

A pilot study of PCB levels in farmed and wild salmon from Vancouver, Canada, found that farmed fish have up to 10 times more PCBs compared with wild caught salmon.[262] A recent study from Norway examined the effect of the intake of salmon exposed to selected organic and inorganic contaminants.[263] The levels of dioxins in all the salmon fillets was less than the mandated European Union limit, although fillets of salmon fed with fish oils had the highest concentration of dioxins, arsenic and related contaminants. The authors concluded that less reliance on fish oils as a form of fish feed should decrease the amount of dioxins and contaminants in fish consumed by humans while still maintaining a good dietary source of marine n-3 PUFA.

The mean levels of dioxins and PCB are very low in Australian fish: 1/15 of the FSANZ maximum level (ML) of PCB and 1/17 of the European Commission ML for dioxin in bluefin tuna,[264] and about 30% of the FSANZ ML of mercury in farmed and wild caught tuna.[265] In commercial aquaculture-produced yellowtail kingfish and mulloway from the Spencer Gulf in South Australia sampled from 2003 to 2004,[266] the mean levels of mercury were 4–6% of the FSANZ limit. Dioxin levels were similarly low.

A 2004 sample of commercial and wild yellowtail kingfish and mulloway found mean mercury levels to be 5.8% of the FSANZ limit in commercial yellowtail kingfish, 16.8% in wild yellowtail kingfish, and 7.4% in mulloway. Dioxin levels were 17% of the EC limit for dioxins in farmed yellowtail, 3% in wild yellowtail, and 4% in farmed mulloway.

As part of the National Dioxins Program, FSANZ found that foods including fish in Australia and New Zealand have a low level of dioxins.[32] The Australian TGA requirements are consistent with the WHO recommendations of dioxin intake (< 10 pg/kg of body weight/day),[267] and it is unlikely that the highest therapeutic doses of fish oils (up to 10 capsules/day) would exceed the upper limit for PCBs.

The recently developed supercritical extraction technique, combined with adsorbent techniques, removes nearly 100% of the total toxicity from fish oils.[268] Importantly, these methods do not change the marine n-3 PUFA content of fish oil supplements. Emerging purification technologies such as these will serve to allay consumer fears and ensure compliance to regulatory guidelines for dioxin and PCB content.

At the levels of fish and fish oils commonly consumed in Australia, there is no evidence of the harmful effects of dioxins. However, there is uncertainty regarding consumption of dioxins at the highest therapeutic doses in high-risk groups such as pregnant women and young children.

Ciguatera

Ciguatera fish poisoning is the most frequently reported intoxication resulting from fish consumption in Australia. However, it is often unrecognised, and significantly under-diagnosed and under-reported.[269] Ciguatoxins are produced by the unicelluar *Gambierdiscus toxicus* component of plankton typically associated with bleached coral reef. Toxins are passed along the food chain and are concentrated in the flesh of progressively larger fish.[269,270] The toxin is tasteless and has no odour. One large serve of an infected fish can cause the illness.

The most common symptoms of ciguatera fish poisoning are diarrhoea, vomiting, abdominal pain and myalgia. More serious symptoms can include weakness, myalgia, aching joints, cramping, palpitations, pruritus and sweating.[230]

In Australia, fish that produce ciguatera are found predominantly in Queensland and Northern Territory waters.[231] The fish species most likely to harbour *Gambierdiscus toxicus* are coral trout and Spanish mackerel. There are no known clinical cases of ciguatera from taking fish oil products.

Variability of marine n-3 PUFA content in fish

Unlike mammals, fish do not store lipids in adipose tissue, but deposit lipids in the liver, muscles, perivisceral area and subcutaneous tissues.[271,272] On the basis of muscle fat per body weight, fish may be classified as:

- lean < 1% fat (for example Atlantic cod or rainbow trout)
- intermediate 1–10% fat (for example Eastern gemfish, Barramundi cod)
- fatty > 10% fat (for example Atlantic salmon, herring, swordfish).

It should also be noted that the fat content of wild species of fish can vary dramatically during the year[273] and that there are differences between the nutritional values of farmed and wild fish.[274]

The Commonwealth Scientific and Industrial Research Organisation (CSIRO) conducted an Australian-wide study of the oil content and composition of Australian fish. It concluded that Australian fish generally have higher relative levels of DHA than fish from the northern hemisphere.[275] The tables in Appendix 1 compare the content of DHA, EPA and total marine n-3 PUFA in commonly available fish species in Australia and the USA. These tables also estimate the number of servings each week of these species needed to achieve the recommended weekly intake of total marine n-3 PUFA. Appendix 1, Table 1 reports the considerable disparity between oil content in Australian fish and the published levels.

The type of oil fed to fish may influence the effects of fish consumption in humans. A recent double-blind intervention study fed different types of fish oils to Atlantic salmon. It then measured lipid concentration and markers of inflammation in humans who consumed the different fish.[276] The levels of total marine n-3 PUFA and the n-3:n-6 ratio were significantly higher in participants consuming Atlantic salmon fed the 100% marine n-3 PUFA feed (fish oils) than in those consuming fish fed rapeseed oil or combined rapeseed and salmon oil.

The CSIRO recently initiated a study into the marine n-3 PUFA content of potential new farmed seafood species. It discovered that sampled specimens of the Australian striped trumpeter (*Latris lineata*) contained the highest concentrations of n-3 PUFA globally recorded for an aquaculture species (up to 10,200 mg/100g marine n-3 PUFA).[277]

A study in Tasmania investigated the effects of replacing marine-3 PUFA in the diet of Atlantic salmon with stearidonic acid (18:4n-3, a biosynthetic precursor of marine n-3 PUFA) derived from plant sources.[278] Atlantic salmon fed stearidonic acid had marine n-3 PUFA levels comparable to fish fed with a standard diet high in marine n-3 PUFA.

Sustainability of fish stocks

There has been widespread concern at the decline and collapse of major fishing stocks throughout the world because of a near doubling of fish consumption in the last 40 years.[36] The large predatory fish biomass today is estimated to be only 10% of pre-industrial levels.[279]

Depletion of the world's fish stocks and rising fish prices have reduced the affordability of fish for many people throughout the world.[279] Aquaculture now accounts for > 25% of the total marine and freshwater harvest.[280] Although the conversion of feed to protein is more efficient in fish than in land animals,[281] aquaculture has significant potential environment impacts.[282]

Limited fishing stocks are driving the need to find new food sources of marine n-3 PUFA. Recent technological advances have made it possible for marine n-3 PUFA to be incorporated in various foods, including odourless varieties of marine n-3 PUFA supplements.[283]. Howe et al[284] pioneered the use of fishmeal, a waste product of fish processing, to enrich pork, poultry and eggs with DHA. Novel marine n-3 PUFA can be used to enrich everyday foods, such as margarine, milk, luncheon meat, sausages and dips.[283] Foods enriched with marine n-3 can increase plasma concentrations of marine n-3 PUFA to a similar extent as fish oil capsules.[285] In the future, these enriched foods are likely to play a greater role as an alternative to fish and fish oil supplementation to help us to increase our marine n-3 PUFA intake.[286] A recent study showed a favourable TG-lowering effect (16–18% over two three week periods) of eggs enriched with marine n-3 PUFA (240–1280 mg/serve DHA and EPA).[287]

A sustainable source of marine n-3 PUFA is needed to supplement medicines, food additives and aquaculture. Direct algae sources of marine n-3 PUFA have been used in some clinical trials.[288] In the near future, non-fish sources of marine n-3 PUFA will become readily available.[289] Companies are already producing DHA from *Crypthecodinium* algae through fermentation. Another alternative is genetically engineered plants that accumulate marine n-3 PUFA in their seeds.[290]

CSIRO scientists are exploring the potential of genetically modified plants as sources of marine n-3 PUFA. If successful, cost-effective plant-based sources of marine n-3 PUFA could solve problems associated with the sustainable management of fish stocks, affordability sources of marine n-3 PUFA, and the dietary requirements of vegetarians and those allergic to fish.[277] Initial reports are encouraging. In 2005, a CSIRO team metabolically engineered DHA and EPA into the seed oil of *Arabidopsis thaliana.* The team has further plans to use genetic engineering to increase the amount of n-3 long-chain PUFA in these plants.[291]

Cooking

Fish is usually eaten after cooking. Because fat may be added during cooking, the cardiac benefits of fish consumption depend on how it is cooked. Consumption of fried fish, low in marine n-3 PUFA, and typically eaten from take-away shops, is not associated with lower prevalence of CHD.[75] In the Cardiovascular Health Study, people who consumed fried fish more than once a week had a 44% higher risk of ischemic stroke than those who consumed fried fish once per month (HR, 1.44; 95% CI, 1.12–1.85).[74] In this study, broiled or baked fish was associated with lower stroke and CHD rates.

Daily requirements of marine n-3 PUFA

Australian recommendations

In 2006, the NHMRC published its *Nutrient Reference Values for Australia and New Zealand*, which included recommendations on ALA, DHA, EPA and DPA intake for the first time.[38]

The following adequate intake (AI) (see Table 4) values were set.

- ALA: 1.3 g/day for men and 0.8 g/day for women
- DHA + EPA + DPA: 160 mg/day for men and 90 mg/day for women.

The upper limit (see Table 4) for children, adolescents and adults was set at 3000 mg/day for combined DHA, EPA and DPA. This upper limit is unlikely to be met by the consumption of seafood alone.

No upper limit was set for ALA because there is no known level at which adverse effects occur.

To prevent chronic disease, dietary intakes for DHA, EPA and DPA have been set at the current 90th centile in the population,[50] values that are known to be safe and to provide potential benefit.[292] The suggested dietary target (see Table 4) to reduce chronic disease is 610 mg/day for men and 430 mg/day for women. The NHMRC report suggests achieving this by replacing energy-dense low-nutrient foods with marine n-3 PUFA-rich foods, such as oily fish.

The acceptable distribution range of ALA intake to reduce chronic disease risk equates to 0.4–0.5% of total dietary energy at the lower end, and 1% of total dietary energy at the upper end, as relevant for the age- and sex-specific AIs. These values were based on intakes to optimise the reduction in chronic disease risk, notably CHD.

Table 4. Australian terms for nutrient recommendations

Suggested dietary targets	These are higher intakes of nutrients that may prevent chronic diseases such as heart disease, certain cancers or high blood pressure.
Adequate intake (AI)	AI represents the average daily nutrient intake level that is assumed to be adequate to prevent a deficiency.
Upper limit	This is the highest average daily intake likely to pose no adverse health effects to almost all individuals in the general population.

USA recommendations

A workshop sponsored by the National Institutes of Health (NIH) made recommendations for the dietary intake of marine n-3 and n-6 PUFA. The working group recognised that there is sufficient data to recommend an AI for adults of 650 mg/day of combined DHA and EPA, assuming a 2000 kcal (8400 kJ) diet. This is approximately 0.3% of an adult's daily energy intake.[293] The recommendation was based on the benefit observed at this intake level in the MRFIT study.[294] This group

also recommended an AI for adults of 2.22 g/day of ALA and an upper limit of 6.67 g/day of LA. No recommendation was made for DPA.

The US Food and Drug Administration (FDA) concluded that daily intake of marine n-3 PUFA should not exceed 3000 mg/day from combined conventional food and dietary supplement sources. The FDA also suggested that dietary supplements should not exceed 2000 mg/day of DHA and EPA.[295]

Further research: Omega-3 Index

The FA composition of red blood cell membranes reflects the FA composition of the cardiac myocyte membrane and long-term marine n-3 PUFA intake. This biomarker has been termed the 'Omega-3 Index'. It is calculated as the total EPA + DHA content of the red blood cell membrane as a percentage of the total FA in the membrane. The Omega-3 Index was recently established as a graded risk factor for death from CHD. The gradient of risk associated with the Omega-3 Index is steeper than for established risk factors, such as serum concentrations of cholesterol and HDL cholesterol, and the emerging risk factors homocysteine and CRP levels.[296] Reviews of marine n-3 PUFA dosing, and epidemiological and clinical studies strongly suggest that an Omega-3 Index of 8% or more provides a relative CHD risk reduction of 90% compared with an index of less than 4%.[297]

To be consistent with current terminology, this new putative risk factor should be classified as an emerging risk factor.[298] It provides a useful assessment of baseline risk and the efficacy of dietary or supplementary marine n-3 PUFA therapy. For example, in a dosing study, the intake of 1 g of marine n-3 PUFA supplement for five months increased the omega-3 index from 5% to 13%.[298]

The Index reflects the intake, absorption and metabolism of marine n-3 PUFA, which can vary considerably between individuals. A recent multivariate population analysis showed that the number of fish servings, age, body mass index and diabetes are independent factors that influence the omega-3 index.[299] However, given the relatively small size of the sample (n = 163), the authors cautioned that further studies are needed to identify which non-dietary factors influence red blood cell marine n-3 PUFA content.

Conclusions

The evidence presented in this review has demonstrated that fish consumption and fish oil supplementation reduce the risk of CVD and CHD. While the review has acknowledged the risks and cautions associated with eating fish, there is sufficient evidence to recommend fish consumption and fish oil supplementation for cardiovascular health for all Australians.

Epidemiological studies

Large epidemiological studies show lower incidence of CHD in populations with high fish consumption. Studies have shown that fish consumption is inversely associated with incidence of CHD, stroke and MI. It is also associated with lower CHD mortality. Blood n-3 PUFA concentration appears to be higher in women than in men, when fish consumption is similar.

Intervention studies

Evidence shows beneficial effects of fish consumption or fish oil supplementation in patients, including:

- less progression of atherosclerosis
- further decrease in the risk of cardiac events and unstable angina when combined with statins.

The Heart Foundation's recommendations on ALA are based on the evidence gathered in the 1999 review paper on dietary fat and CVD.[33]

Systematic reviews and meta-analyses

The robust evidence from meta-analyses shows beneficial effects of fish consumption in reducing the risk of total mortality, CHD mortality and stroke.[1-5]

Risks and cautions

Some fish may have relatively high methylmercury content. Consuming these fish should be restricted, particularly in pregnant women and children, who are at the greatest risk of toxicity. Marine n-3 PUFA supplements are, for practical purposes, methylmercury and dioxin free. Dioxin and other PCB levels in Australian fish are very low. The recommended intake of fish with the highest levels of methylmercury or dioxins and PCBs is associated with a very small potential risk. However, it provides significant health benefits beyond reasonable doubt. The absolute risk of seafood-borne illness is extremely low in the type of fish commonly consumed in Australia.

The recommendations from this review are based on compelling scientific evidence. They represent achievable goals and at the same time aim to minimise potential risk associated with seafood intake in certain individuals. The recommendations are consistent with, and extend the, recommendations of national and international organisations including FSANZ,[32,241] the NHRMC (Australia),[38] the AHA (USA),[34,35] the NIH (USA),[37] and the WHO (International).[36]

Terminology and abbreviations

AA	Arachidonic acid, omega-6 fatty acid with 20-carbon chain C20:4n-6
AHA	American Heart Association
AI	Adequate intake
ALA	Alpha-linolenic acid, omega-3 fatty acid with 18-carbon chain C18:3n-3
CAD	Coronary artery disease
CHD	Coronary heart disease
CI	Confidence interval
COX-2	Cyclooxygenase-2
CRP	C-reactive protein
CSIRO	Australian Commonwealth Scientific and Industrial Research Organisation
CVD	Cardiovascular disease
DHA	Docosahexaenoic acid, omega-3 fatty acid with 22-carbon chain C22:6n-3
DPA	Docosapentaenoic acid, omega-3 fatty acid with 22-carbon chain C22:5n-3
EPA	Eicosapentaenoic acid, omega-3 fatty acid with 20-carbon chain C20:5n-3
FA	Fatty acid
FAO	Food and Agriculture Organization
FDA	US Food and Drug Administration
Fish oil	Oil derived from fish rich in DHA and EPA
FSANZ	Food Standards Australia and New Zealand
GISSI-P	Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico Prevenzione
HDL	High-density lipoprotein
HR	Hazard ratio
hs-CRP	High-sensitivity C-reactive protein
ICD	Implantable cardioverter defibrillator
IHD	Ischaemic heart disease
IL-1, IL-6	Interleukin 1, interleukin 6
IMT	Intima-media thickness
JECFA	Joint Expert Committee for Food Additives and Contaminants
JELIS	Japan EPA Lipid Intervention Study
JPHC	Japan Public Health Center-based Study
LA	Linoleic acid, omega-6 fatty acid with 18-carbon chain C18:2n-6
LDL	Low-density lipoprotein
Marine n-3 PUFA	Combination of EPA and DHA
MeHg	Methylmercury
MI	Myocardial infarction
ML	Maximum level
n-3 PUFA	Omega-3 polyunsaturated fatty acids with first double bond between 3rd and 4th carbon atoms from terminal methyl group (ALA, EPA, DPA, DHA)
n-6 PUFA	Omega-3 polyunsaturated fatty acids with first double bond between 6th and 7th end carbon
NF-κB	nuclear transcription factor κB, a key transcription factor
NHMRC	National Health and Medical Research Council

NIH	National Institutes of Health (USA)
NNS	National Nutrition Survey
NO	Nitric oxide
Omega-3 index	EPA + DHA as a percentage of total fatty acids in the red blood cell membrane
OR	Odds ratio
PCB	Polychlorinated biphenyl
PUFA	Polyunsaturated fatty acids
RCT	Randomised clinical trial
RR	Relative risk
Secondary prevention	Long-term treatment to prevent recurrent cardiac morbidity and mortality and to improve quality of life in people who have either had a prior acute MI or are at high risk of cardiac events for other reasons
TEQ	Toxic equivalent
TG	Triglycerides
TGA	Therapeutic Goods Administration
TNF-α	Tumour necrosis factor-α
US EPA	United States Environmental Protection Agency
VEGF	Vascular endothelial growth factor
VF	Ventricular fibrillation
VLDL	Very low-density lipoprotein
VT	Ventricular tachycardia
WHO	World Health Organization

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Appendix 1

Table 1. Australian comparison of mercury in fish with national and international recommended levels

Fish species	Total mercury range (μg/150 g serve)	Range of max 150 g serves/wk (FSANZ)	Range of max 150 g serves/wk (US EPA)	Range of max 150 g serves/wk (WHO)	Average mercury level (μg/150 g serve)	Max average 150 g serves/wk (FSANZ)	Max average 150 g serve/wk (US EPA)	Max average 150 g serves/wk (WHO)
Swordfish (f)	4.50–210.00	1.10–51.33	0.23–10.89	0.53–24.89	85.50	2.70	0.57	1.31
Swordfish (h)	69.00–277.50	0.83–3.35	0.18–0.71	0.40–1.62	147.75	1.56	0.33	0.76
Shark [301]	6.00–375.00	0.62–38.50	0.13–8.17	0.30–18.67	98.31	2.35	0.50	1.14
Shark [302]	9.00–825.00	0.28–25.67	0.06–5.44	0.14–12.44	129.09	1.79	0.38	0.87
Shark (f)	4.50–643.50	0.36–51.33	0.08–10.89	0.17–24.89	127.50	1.81	0.38	0.88
Shark (t) [303]	19.50–511.50	0.45–11.85	0.10–2.51	0.22–5.74	172.50	1.34	0.28	0.65
Shark [304]	4.50–738.00	0.31–51.33	0.07–10.89	0.15–24.89	147.00	1.57	0.33	0.76
Cod (f)	1.50–142.50	1.62–154.00	0.34–32.67	0.79–74.67	45.00	5.13	1.09	2.49
Cod (h)	0.75–195.00	1.18–308.00	0.25–65.33	0.57–149.33	24.00	9.63	2.04	4.67
Fresh tuna (f)	24.00–199.50	1.16–9.63	0.25–2.04	0.56–4.67	48.00	4.81	1.02	2.33
Fresh tuna (h)	0.75–510.00	0.45–308.00	0.10–65.33	0.22–149.33	33.00	7.00	1.48	3.39
Fresh tuna (m)	19.50–120.00	1.93–11.85	0.41–2.51	0.93–5.74	55.50	4.16	0.88	2.02
Wild southern bluefin tuna	42.00–63.00	3.67–5.50	0.78–1.17	1.78–2.67	45.00	5.13	1.09	2.49
Farmed southern bluefin tuna	27.00–67.50	3.42–8.56	0.73–1.81	1.66–4.15	46.50	4.97	1.05	2.41
Local canned tuna	6.00–36.00	6.42–38.50	1.36–8.17	3.11–18.67	18.00	12.83	2.72	6.22

Imported canned tuna	1.50–58.50	3.95–154.00	0.84–32.67	1.91–74.67	13.50	17.11	3.63	8.30
Fresh salmon (f)	12.75–13.50	17.11–18.12	3.63–3.84	8.30–8.78	12.00	19.25	4.08	9.33
Fresh salmon (m)	0.75–7.50	30.80–308.00	6.53–65.33	14.93–149.33	3.75	61.60	13.07	29.87
Fresh salmon (h)	0.75–42.00	5.50–308.00	1.17–65.33	2.67–149.33	3.75	61.60	13.07	29.87
Local canned salmon [305]	22.50–51.00	4.53–10.27	0.96–2.18	2.20–4.98	36.00	6.42	1.36	3.11
Imported canned salmon [305]	0.75–61.50	3.76–308.00	0.80–65.33	1.82–149.33	6.00	38.50	8.17	18.67
Snapper [306]	1.50–82.50	2.80–154.00	0.59–32.67	1.36–74.67				
Snapper (t)					39.42	5.86	1.24	2.84
Snapper [307]					79.50	2.91	0.62	1.41
Red emperor [306]	13.50–72.75	3.18–17.11	0.67–3.63	1.54–8.30				
Red emperor [307]					44.70	5.17	1.10	2.51
Barramundi [306]	4.50–63.75	3.62–51.33	0.77–10.89	1.76–24.89				
Barramundi [307]					67.35	3.43	0.73	1.66

Total mercury range is the highest and lowest sample reported in the particular species.
Range of max 150 g serves/wk is calculated as the maximum number of 150 g fish servings a 70 kg person should consume each week. It is assumed that reported mercury content is methylmercury.
Sources: FSANZ[241]; US EPA[233,248,308]; FAO/WHO.[234]
Swordfish (f): Fabiansson 2006, personal communication.
Swordfish (h): Hambridge 2006, personal communication.
Shark (f): Fabiansson 2006, personal communication**.**
Shark (t): Reference range based on taking the lowest (*Squatina Australis*) and highest (*Isurus oxyrinchus*) mean figures for Australian shark species examined to date as shown in Table 3.[303] This table compares the mean of all previous studies on mercury content in shark with the data collected by Turoczy et al.[303] The extremely high mean level of mercury found in *Cephaloscyllium laticeps* of the continental shelf adjacent to Tasmania (2100 μg/200g wet weight) has not been taken into consideration here because its inclusion would have a distorting effect. The value was calculated by adding together the individual mean mercury contents of all of the shark species surveyed in the study (except for *Cephaloscyllium laticeps* found on the continental shelf adjacent to Tasmania) and divided by the number of surveys.
Cod (f): Fabiansson 2006, personal communication.
Cod (h): Hambridge 2006, personal communication.
Fresh tuna (f): Fabiansson 2006, personal communication.
Fresh tuna (h): Hambridge 2006, personal communication.
Fresh tuna (m): Miller 2006, personal communication. Data collected by the federal government's National Residue Survey. Data approved for release by the Tasmanian Salmonid Growers Association.

Fresh salmon (f): Fabiansson 2006, personal communication.
Fresh salmon (h): Hambridge 2006, personal communication.
Fresh salmon (m): Miller 2006, personal communication. Data collected by the federal government's National Residue Survey. Data approved for release by the Tasmanian Salmonid Growers Association.
Snapper (t): Tinggi 2006, personal communication.

Table 2. Australian comparison of marine n-3 PUFA content in fish with recommended intake

Fish species (manufacturer)	EPA (μg/150 g serve)	DHA (μg/150 g serve)	Total n-3 PUFA (μg/150 g serve)	Serves/wk required for 7000 μg of n-3 PUFA
Swordfish	560	810	1590	4.40
Gummy shark	40	330	440	15.91
Coral cod	20	150	180	38.89
Southern bluefin tuna	30	300	340	20.59
Southern bluefin tuna [309]	350	1210	1730	4.05
Skipjack tuna	20	140	170	41.18
Yellowfin tuna	20	150	180	38.89
Canned tuna (Farmland) [310]	80	440	560	12.50
Canned tuna (Rex)	80	310	420	16.67
Canned tuna (Greenseas)	40	310	360	19.44
Canned tuna (Ayam)	30	270	320	21.88
Canned tuna (Safcol)	30	230	270	25.93
Atlantic salmon	260	570	1030	6.80
Atlantic salmon [309]	710	1720	3210	2.18
Australian salmon [309]	140	770	980	7.14
Pink salmon (Farmland)	410	660	1400	5.00
Pink salmon (John West)	390	920	1670	4.19
Pink salmon (Paramount)	830	1430	2850	2.46
Pink salmon (Rex)	400	660	1360	5.15
Australian salmon (Greenseas)	100	570	750	9.33
Australian salmon (Savings)	250	1460	1910	3.66
Australian salmon (Safcol)	480	880	1630	4.29
Red salmon (Farmland)	440	740	1440	4.86

Red salmon (John West)	1330	1950	4110	1.70
Red salmon (Paramount)	930	1480	3200	2.19
Silver salmon (Safcol)	240	1010	1430	4.90
Snapper	40	250	330	21.21
Red emperor	20	150	180	38.89
Freshwater barramundi	20	80	130	53.85
Saltwater barramundi	20	110	150	46.67

EPA, DHA, total n-3 PUFA Source [304]

Table 3. USA comparison of mercury and marine n-3 PUFA content in fish with recommendations

Fish species	Total mercury range (μg/150 g serve)	Range of max 150 g serves/ wk (FSANZ)	Range of max 150 g serves/ wk (USEPA)	Range of max 150 g serves/ wk (WHO)	Average mercury level (μg/150 g serve)	Max average 150 g serves/ wk (FSANZ)	Max average 150 g serve/wk (USEPA)	Max average 150 g serves/ wk (WHO)	EPA (μg/ 150 g serve)	DHA (μg/ 150 g serve)	Total n-3 (μg/ 150 g serve)	Serves/ wk req'd for 7000 μg of total n-3 PUFA
Swordfish	15–483	0.48–15.4	0.1–3.27	0.23–7.47	145.50	1.59	0.34	0.77	160	800	1240	5.65
Shark	ND–681	0.34–NUL	0.07–NUL	0.16–NUL	148.50	1.56	0.33	0.75	470	790	1520	4.61
Cod	ND–63	3.67–NUL	0.8–NUL	1.76–NUL	16.50	14.00	3.07	6.72				
Atlantic cod									100	180	300	23.33
Pacific cod									120	200	330	21.21
Fresh tuna	ND–195	1.18–NUL	0.25–NUL	0.57–NUL	57.00	4.05	1.13	1.95				
Bluefin tuna									420	1340	2010	3.48
Fresh tuna [305]									110	280	410	17.07
Fresh tuna (m)									60	270	370	18.92
Canned albacore tuna	ND–127.5	1.81–NUL	0.38–NUL	0.88–NUL	52.50	4.40	0.93	2.13	70	340	440	15.91
Canned light tuna	ND–127.5	1.81–NUL	0.38–NUL	0.88–NUL	18.00	12.83	2.72	6.22	350	940	1500	4.67
Fresh salmon	ND–28.5	8.11–NUL	1.72–NUL	3.93–NUL	1.50	154.00	32.67	74.67				
Farmed Atlantic salmon									930	1940	3240	2.16
Wild Atlantic									480	1670	3510	1.99

salmon												
Chinook salmon									1510	1420	3800	1.84
Farmed coho salmon (USA species)									580	1230	2090	3.35
Wild coho salmon (USA species)									640	980	2390	2.93
Pink salmon									630	880	1840	3.80
Sockeye salmon									780	980	2110	3.32
Chum salmon (USA species)									350	590	1210	5.79
Canned salmon	ND	NUL	NUL	NUL	ND	NUL	NUL	NUL				
Canned pink salmon									1270	1210	2840	2.46
Canned sockeye salmon									740	1000	2110	3.32
Canned chum salmon									710	1050	2080	3.37
Snapper	ND–205.50	1.12–NUL	0.24–NUL	0.55–NUL	28.50	8.11	1.72	3.93	80	390	580	12.07

Fresh tuna (m): Miller 2006, personal communication.
Data collected by the federal government's National Residue Survey. Data approved for release by the Tasmanian Salmonid Growers Association.
ND: not detectable. Mercury concentration below the level of detection of 0.01ppm.
NUL: no upper limit.

Appendix 2

Table 1: ALA content of some common foods

Food group	Food	Serving size	ALA mg per serve
Nuts	Walnut	30 g	1890
	Pecan nut	30 g	180
	Hazelnut	30 g	30
	Tahini	30 g	30
	Peanuts	30 g	0
Fats and oils	Canola oil	1 tbs (20 g)	2000
	Soybean oil	1 tbs (20 g)	1600
	Oil, poly blend	1 tbs (20 g)	1380
	Margarine spread, canola-based	1 tbs (20 g)	915
	Corn oil	1 tbs (20 g)	400
	Peanut oil	1 tbs (20 g)	400
	Margarine spread, unsaturated	1 tbs (20 g)	250
	French dressing, commercial	1 tbs (20 g)	120
	Coleslaw dressing, commercial	1 tbs (20 g)	20
	Italian dressing, commercial	1 tbs (20 g)	20
	Mayonnaise, commercial	1 tbs (20 g)	20
	Dressings, fat free	1 tbs (20 g)	trace
Dairy products	Cheddar cheese	40 g slice	100
	Cheddar cheese, reduced fat	40 g slice	50
	Cottage cheese	40 g slice	40
	Milk and yoghurt (low fat)		0.32
Meat and poultry	Lamb, lean	100 g	48
	Beef, lean	100 g	27
	Chicken breast, no skin	100 g	7
Fruit and vegetables	Mushrooms	75 g	430
	Spinach	75 g	190
	Lettuce	75 g	150
	Green beans	75 g	45
Breads and cereals	Bread, soy and linseed	2 slices (80 g)	1600
	Wheat breakfast biscuits	2 slices (56 g)	56
	Bread, wholemeal	2 slices (56 g)	56

Sources: Morgan W. 2007, personal communication [311]
Bread and cereals: Source [312]
Data correct as at November 2007.

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