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Dietary *n*-3 PUFA and CVD: a review of the evidence

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Many clinical and epidemiological studies have shown that the polyunsaturated *n*-3 fatty acids EPA and DHA from fish and fish oils, provide cardiovascular protection, particularly in the setting of secondary prevention. *n*-3 Fatty acids beneficially influence a number of cardiometabolic risk factors including blood pressure, cardiac function, vascular reactivity and lipids, as well as having anti-platelet, anti-inflammatory and anti-oxidative actions. They do not appear to adversely interact with other medications such as statins and other lipid-lowering drugs or antihypertensive medications. *n*-3 Fatty acids have gained widespread usage by general practitioners and clinicians in a number of clinical settings such as pregnancy and infant development, secondary prevention in CHD patients, treatment of dyslipidaemias and haemodialysis patients. Small doses are achievable with consumption of two to three oily fish meals per week or via purified encapsulated preparations now readily available. *n*-3 Fatty acids, particularly when consumed as fish, should be considered an important component of a healthy diet. The present paper reviews the effects of *n*-3 fatty acids on cardiometabolic risk factors, concentrating particularly on the evidence from randomised controlled studies in human subjects.

***n*-3 Fatty acids: Fish oils: Fish: Cardiovascular risk factors: Cardiometabolic risk factors:
Human studies**

There is considerable evidence from clinical, experimental and epidemiological studies that polyunsaturated *n*-3 fatty acids, particularly EPA (20:5) and DHA (22:6) the two main *n*-3 fatty acids from fish and fish oils, are protective against atherosclerotic heart disease and sudden coronary death[1–4]. *n*-3 Fatty acids have multiple effects benefiting a number of cardiometabolic risk factors including blood pressure[5–8] and cardiac function[4,7], arterial compliance[9,10], vascular reactivity[11,12], lipid metabolism[13,14], reduced leucocyte-derived cytokine formation[15], anti-platelet[16], anti-inflammatory[17,18] and pro-resolving[19] effects, and antioxidative actions[20]. There is also evidence from studies in human subjects that EPA and DHA have differential effects on blood pressure, heart rate, lipids and vascular reactivity[21]. The aim of the present paper is to review the evidence for beneficial effects of *n*-3 fatty acids on cardiometabolic risk factors, concentrating particularly on randomised controlled studies in human subjects.

Population studies

A number of population studies have demonstrated an inverse association between *n*-3 fatty acid consumption and CVD (Table 1). In meta-analyses, Wang *et al.*[22] showed that increased consumption of *n*-3 fatty acids from fish or fish oil supplements reduces rates of all-cause mortality, cardiac and sudden death, and Bucher *et al.*[23], He *et al.*[24] and Whelton *et al.*[25] showed an inverse association between *n*-3 fatty acids and CHD. An inverse relationship has also been shown between *n*-3 fatty acids and heart failure[26], particularly with consumption of tuna or other broiled or baked fish, but not fried fish[27]. Several meta-analyses have shown an inverse association between increased intake of *n*-3

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Table 1. Studies examining the effect of *n*-3 fatty acids as fish or fish oils on CHD, stroke and total mortality

Meta-analyses of population studies		
Wang *et al.*[22]	↓	all-cause mortality; cardiac and sudden death
Bucher *et al.*[23]	↓	total mortality; cardiac and sudden death
He *et al.*[24]	↓	CHD mortality
Whelton *et al.*[25]	↓	fatal and total CHD
He *et al.*[28]	↓	stroke, particularly ischaemic stroke
Xun *et al.*[29]	↓	stroke, particularly ischaemic stroke
Randomised controlled trials		
DART[30]	↓	all-cause mortality in post-MI patients
GISSI[31]	↓	total and cardiovascular mortality; sudden death in post-MI patients
GISSI-HF[32]	↓	total mortality and hospital admission for CVD in heart failure patients
JELIS[33]	↓	coronary events in patients with hypercholesterolaemia
ORIGIN[34]	↔	cardiovascular events in patients at high risk of CVD and impaired glucose or diabetes
OPERA[35]	↔	post-operative atrial fibrillation in patients undergoing cardiac surgery
Risk and prevention[36]	↔	cardiovascular mortality and morbidity in patients with multiple cardiovascular risk factors

MI, myocardial infarction.

fatty acids and risk of stroke, particularly ischaemic stroke[28,29].

Randomised controlled trials

Several randomised controlled trials have shown the beneficial effects of *n*-3 fatty acids, especially in secondary prevention of CHD (Table 1). The Diet and Reinfarction Trial study showed that *n*-3 fatty acids given either as oily fish or fish oil capsules, reduced all-cause mortality by 29 % in 2033 men with recent myocardial infarction[30]. The Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto (GISSI) study randomised 11 323 post-myocardial infarction patients to one daily capsule containing 850 mg *n*-3 fatty acids *v.* usual care[31]. After 1 year, patients taking *n*-3 fatty acids had a 21, 30 and 45 % reduction in total and cardiovascular mortality, and sudden cardiac death, respectively. In a follow-up study in approximately 7000 patients with class II to IV heart failure (GISSI-HF), the same investigators showed that the same dose of *n*-3 fatty acids had a significant effect in reducing total mortality by 9 % and total mortality or hospitalisation for cardiovascular diseases by 8 %[32]. The Japan EPA Lipid Intervention Study trial, randomised 18 645 patients with hypercholesterolaemia to statin alone or statin plus 1800 mg/d highly purified EPA[33]. At the end of the 5-year study, there was a 19 % reduction in major cardiovascular events in those patients randomised to EPA.

Studies demonstrating the benefits of *n*-3 fatty acids on cardiovascular outcomes need to be considered against several randomised controlled large-scale interventions where *n*-3 fatty acids at a dose of 1 g daily have not provided benefit. In the ORIGIN trial[34] in 12 536 patients with or at high risk for diabetes, *n*-3 fatty acids did not reduce the rate of cardiovascular events. Similarly, the OPERA study, comprising 1516 patients undergoing cardiac surgery, showed that *n*-3 fatty acids did not reduce the risk of post-operative atrial fibrillation relative to placebo[35]. A trial examining 12 513 patients with multiple cardiovascular risk factors or atherosclerotic vascular disease but not myocardial infarction, found that *n*-3 fatty acids did not reduce cardiovascular mortality and morbidity[36]. There are a number of possibilities that may account for differences between these latter studies and others showing the benefit of *n*-3 fatty acids. Perhaps *n*-3 fatty acids provide greater benefit to patients with recent myocardial infarction or heart failure due to their antiarrhythmic effects, or possibly studies have had limited power to detect a reduction in sudden deaths from cardiac causes or arrhythmic events. Other factors include the presence of confounding comorbidities, the effects of concomitant medications and the likelihood that patients are receiving optimal clinical care, studies using doses of *n*-3 fatty acids that are lower than the 800–900 mg/d previously shown to have an effect, and dietary background particularly in relation to *n*-3 fatty acid intake.

Effects on blood pressure, endothelial function and cardiac function

Meta-analyses

Randomised controlled intervention trials providing fish meals or fish oils unequivocally demonstrate that *n*-3 fatty acids lower blood pressure. Although some studies have reported no effect on blood pressure this is probably due to study design, relatively small sample sizes leading to a lack of statistical power and/or an insufficiently high dose of *n*-3 fatty acids. Three meta-analyses have examined the effect of *n*-3 fatty acids on blood pressure. Morris *et al.*[8] analysed thirty-one placebo controlled trials involving 1536 subjects and showed an overall reduction of –3·0/−1·5 mmHg with an average dose of 4·8 g/d. The hypotensive effect was strongest in treated and untreated hypertensives (−3·4/−2·0 mmHg) although healthy subjects showed no significant change (−0·4/−0·7 mmHg). Significant dose–response effects were observed with doses of greater than 6 g/d *n*-3 fatty acids. It was predicted that each 1 g/d increase in *n*-3 fatty acids decreased blood pressure −0·66/−0·35

mmHg. The dose–response effect was also greater for DHA than for EPA. Appel *et al.*[5] estimated that blood pressure fell −5·5/−3·5 mmHg in untreated hypertensives (from six trials) and −1·0/−0·5 mmHg in normotensives (from eleven trials) with an average intake of >3 g/d *n*-3 fatty acids. The authors did not show a dose–response effect. Geleijnse *et al.*[6] examined thirty-six trials in which 50 % of the participants were hypertensive (systolic blood pressure>140 mmHg and/or diastolic blood pressure>90 mmHg), the mean trial duration was 11·7 years and the median dose of the *n*-3 fatty acids was 3·7 g/d. Overall, *n*-3 fatty acids reduced blood pressure by −2·1/−1·6 mmHg, with the greatest effects in older (>45 years; −3·5/−2·4) and hypertensive (≥140/90 mmHg; −4·0/−2·5) individuals.

A meta-analysis by Dickinson *et al.*[37] examined the efficacy of dietary nutrients and lifestyle in patients with raised blood pressure in 105 trials randomising 6805 participants with a mean baseline blood pressure of 147/92 mmHg and a mean age of 50 years. They showed that 0·1–1·7 g/d *n*-3 fatty acids reduced blood pressure by −2·3/−2·2 mmHg. These effects were modest in comparison with the estimated benefits of improved diet, salt restriction, aerobic exercise and alcohol restriction. Dokholyan *et al.*[38] showed that low doses of *n*-3 fatty acids were ineffective in reducing blood pressure in patients with high–normal diastolic blood pressure or stage 1 hypertension, and concluded that relatively high doses of *n*-3 fatty acids (>3 g/d) are required for blood pressure reduction.

Clinical trials

The blood pressure-lowering effects of *n*-3 fatty acids were potentiated by concomitant sodium restriction in healthy elderly volunteers[39] and by β-adrenergic receptor blockade in mild-to-moderate hypertensives[40]. Lungershausen *et al.*[41] also showed that *n*-3 fatty acids potentiated the antihypertensive effects of β-blockers or diuretics in treated hypertensives.

Bao *et al.*[42] reported that *n*-3 fatty acids were additive to the blood pressure-lowering effects of weight reduction. Sixty-three overweight treated hypertensives were randomised to an energy-restricted weight loss programme, a daily fish meal providing approximately 3·65 g/d *n*-3 fatty acids, the two regimens combined or a control diet, for 4 months. Weight fell on average 5·6 kg in the two weight-loss groups. Relative to the control group, daytime blood pressures fell −6·0/−3·0 mmHg in the fish group, −5·5/−2·2 in the weight loss group and −13·0/−9·3 with the combined regimens. Awake heart rate was reduced by 1·8, 4·3 and 6·1 bpm in the weight loss, fish and combined weight loss+fish groups, respectively, relative to controls, suggesting an autonomic/cardiac component to the blood pressure reduction.

Independent effects of EPA and DHA

Blood pressure and heart rate are differentially affected by EPA and DHA. Mori *et al.*[43] showed in overweight, mildly hypercholesterolaemic patients, that 4 g/d highly purified DHA, but not EPA, supplemented for 6 weeks, significantly reduced 24 h (−5·8/−3·3 mmHg) and awake (−3·5/−2·0 mmHg) blood pressure, relative to olive oil. DHA, but not EPA, also significantly reduced 24 h, awake and asleep heart rate by −3·5, −3·7 and −2·8 bpm, respectively[43]. Of note, EPA resulted in a small, but non-significant rise in heart rate. These differential effects of EPA and DHA on heart rate responses in human subjects were supported by Grimsgaard *et al.*[44]. The blood pressure changes with DHA[43] were accompanied by significant improvements in endothelial and smooth muscle function as well as reduced vasoconstrictor responses, in the forearm microcirculation[12]. DHA, but not EPA, improved vasodilator responses to endogenous and exogenous nitric oxide donors and attenuated vasoconstrictor response to noradrenaline in the forearm microcirculation. The mechanisms were predominantly endothelium-independent, based on the fact that co-infusion of acetylcholine with NG-mono-methyl-L-arginine and infusion of nitroprusside, both of which are endothelium-independent, resulted in enhanced vasodilatory responses. However, the data do not preclude an endothelial component in the dilatory responses associated with DHA. These findings contrast with those reported by Woodman *et al.*[45] who showed that using the same study design neither EPA nor DHA decreased blood pressure in treated hypertensive type 2 diabetic patients. The lack of effect in the latter trial could be related to concomitant use of pharmacological agents, the presence of glycaemia and increased blood pressure variability in diabetic patients.

Possible mechanisms

The antihypertensive effects of *n*-3 fatty acids are likely to be multifactorial involving improvements in endothelial function and arterial compliance, along with a cardiac effect mediated by a decrease in heart rate[7]. Possible mechanisms include suppression of vasoconstrictor prostanoids, enhanced production and/or release of nitric oxide, reduced plasma noradrenaline, changes in calcium flux, increased membrane fluidity, antioxidative actions of *n*-3 fatty acids or an increase in HDL cholesterol[7].

n-3 Fatty acids reduce heart rate[7,42,43,46] suggesting a significant cardiac component associated with the antihypertensive effects possibly mediated by effects on cardiac myocytes, autonomic nerve function or β-adrenoreceptor activity. In a meta-analysis of thirty studies Mozaffarian *et al.*[46] showed that *n*-3 fatty acids reduce heart rate overall by −1·6 bpm, with a greater reduction in trials with baseline heart rate greater than 69 bpm (−2·5 bpm) and those of longer than 12 weeks duration (−2·5 bpm).

It is well recognised that blood pressure is strongly affected by arterial compliance, which in turn is influenced by endothelial function. McVeigh *et al.*[9] showed that in individuals with type 2 diabetes compliance in the large arteries and more peripheral vasculature improved significantly after 6 weeks of fish oil compared with olive oil. EPA and DHA supplementation also improved arterial compliance by 35 and 27 %, respectively, in patients with dyslipidaemia[10]. Pase *et al.*[47]

showed in a meta-analysis that included ten randomised controlled trials, four using pulse wave velocity and six using arterial compliance measured as capacitance compliance or systemic arterial compliance, that *n*-3 fatty acids significantly improved both pulse wave velocity and arterial compliance.

Human studies strongly suggest that *n*-3 fatty acids increase heart rate variability in patients at high risk of sudden cardiac death and in healthy individuals[48,49]. A meta-analysis that included fifteen randomised controlled trials showed that short-term *n*-3 fatty acid supplementation favourably affects the frequency domain of heart rate variability as indicated by enhancement of vagal tone, which may be an important mechanism underlying the antiarrhythmic effect of *n*-3 fatty acids[50].

Mechanisms through which the *n*-3 fatty acids affect heart rate probably relate to their incorporation into myocardial cells and altering electrophysiological function in a manner that reduces the vulnerability to ventricular fibrillation[51]. The anti-arrhythmic effects of *n*-3 fatty acids are due to their ability to inhibit the fast, voltage-dependent sodium current and the L-type calcium currents, and also to modulate potassium channels[51].

Effects on plasma lipids and lipoproteins

n-3 Fatty acids reduce plasma TAG by approximately 20–30 %, but have very little effect on total cholesterol, HDL cholesterol and LDL cholesterol[13,14]. In overweight, treated hypertensive patients[52] and in dyslipidaemic men[53], *n*-3 fatty acids increased HDL cholesterol due primarily to an increase in HDL$_2$-cholesterol subfraction. A reduction in hepatic very-low density lipoprotein cholesterol synthesis probably contributes to the fall in plasma TAG. Mechanisms for this effect include a reduction in fatty acid availability for TAG synthesis as a result of decreased *de novo* lipogenesis, increased fatty acid β-oxidation, a reduction in the delivery of NEFA to the liver, altered enzymatic activity for TAG assembly in the liver and increased hepatic synthesis of phospholipids instead of TAG[13,14,54]. Using proton magnetic resonance spectroscopy, Cussons *et al.*[55] showed that *n*-3 fatty acids significantly reduced liver fat by 18 % in women with hepatic steatosis.

Mozaffarian *et al.*[4] have shown that TAG lowering is linearly dose-dependent across a wide range of *n*-3 fatty acid consumption. Overall plasma TAG are reduced by 0·33 mM for a 1 g/d increase in EPA plus DHA. In trials supplementing highly purified EPA or DHA, Mori *et al.*[45,53] showed the TAG-lowering actions of *n*-3 fatty acids were attributable to both EPA and DHA.

A small, albeit significant increase in LDL cholesterol observed in some studies supplementing *n*-3 fatty acids, is accompanied by an increase in LDL particle size. Mori *et al.*[53,56] showed that DHA and not EPA supplementation increased LDL particle size, suggesting that the lipid-regulating effects of DHA are at least as important as those of EPA. Reduced LDL particle size is an important cardiovascular risk factor[57] and correlates with sub-clinical atherosclerosis as measured by intima-media thickening[58].

Chan *et al.*[59] also demonstrated in dyslipidaemic, viscerally obese men with insulin resistance, that *n*-3 fatty acid supplementation combined with statin therapy provided the optimal change in lipid profile, as reflected by decreased plasma TAG and increased HDL cholesterol.

Effects on glycaemic control, insulin sensitivity and secretion

Reports of disparate results on glycaemia, particularly from early studies examining the effects of *n*-3 fatty acids on glycaemic control in type 2 diabetic patients, are probably related to the dose of *n*-3 fatty acids provided, concomitant oral diabetic medication, presence of obesity and/or insulin resistance, presence of other comorbidities such as hypertension, not controlling subjects' diets during intervention and duration of intervention[60]. However, three independent meta-analyses of twenty-six, eighteen and twenty-three randomised controlled studies, have shown no overall effect of the *n*-3 fatty acids in a dose range of 0·9 to 18 g/d on fasting glucose or glycated Hb in patients with type 2 diabetes[60–62].

Two placebo-controlled trials have examined the independent effects of purified EPA or DHA supplementation on plasma glucose and insulin in type 2 diabetic patients[45] and in mild dyslipidaemic men[53]. These studies showed the differential effects of EPA and DHA on plasma glucose and insulin levels depending on the background condition. Mori *et al.*[53] reported a borderline increase in fasting glucose with 4 g daily EPA but no change with DHA. Fasting serum insulin significantly increased relative to placebo after DHA but not after EPA[53]. Woodman *et al.*[45] reported that in type 2 diabetic patients fasting glucose increased following 4 g daily EPA or DHA, but insulin and C-peptide were not altered by either fatty acid[45]. Self-monitored blood glucose measured four times daily on 4 d of each week throughout the 6-week intervention, rose following EPA and DHA in the first 3 weeks, but had returned to baseline values by week 6[45]. Overall, HbA$_{1c}$, insulin secretion and insulin sensitivity were unaffected by EPA or DHA[45].

Effects on platelet function and thrombosis

A number of trials have studied the effect of *n*-3 fatty acids on platelet function, and measures of coagulation and fibrinolysis, in healthy human subjects and in patients at increased risk of CVD[16,63]. *n*-3 Fatty acids, particularly at large doses, reduced *ex vivo* platelet aggregation, an effect mediated, in part, via alterations in thromboxane formation[63]. In contrast, *n*-3 Fatty acids have shown inconsistent and minor effects on measures of fibrinolysis and coagulability[64]. To date, there is little

evidence that an increased intake of *n*-3 fatty acids will increase the risk of major bleeding in patients receiving anticoagulants or platelet inhibitors.

Effects on inflammation

The anti-inflammatory and immunomodulatory effects of *n*-3 fatty acids are most probably related to their attenuating actions on inflammatory eicosanoids including altered leucotriene formation, cytokines, oxidative stress, endothelial and cell–cell activation and immune cell function[17,18]. *n*-3 Fatty acids were shown to reduce *ex vivo* production of proinflammatory cytokines including TNFα, IL-1 and IL-6 following lipopolysaccharide-stimulation of monocytes/lymphocytes[15,17,18]. *In-vitro* studies also showed that DHA but not EPA decreased the expression of pro-inflammatory cytokines, cell-adhesion molecules and monocyte adhesion to endothelial cells[65]. *n*-3 Fatty acids attenuated the expression of adhesion molecules on the surface of cultured human endothelial cells, monocytes and lymphocytes[65]. Of note, DHA was more potent than EPA in inhibiting the expression of vascular cell adhesion molecule-1, intercellular adhesion molecule-1 and E-selectin, after stimulation. The EPA and DHA-induced reduction in adhesion molecule expression was accompanied by decreased binding of human lymphocytes and monocytes to cytokine-stimulated endothelial cells[65].

The anti-inflammatory effects of *n*-3 fatty acids are in part mediated by a novel family of local lipid mediators generated during self-limited resolution of inflammation. Serhan *et al.*[19] described the E-series resolvins derived from EPA, and D-series resolvins, protectins, neuroprotectins and maresins derived from DHA[66–69]. These mediators acting via G-coupled protein receptors[70] have potent anti-inflammatory and pro-resolving actions[71,72] and increase with time during the inflammatory process[19,73]. Two series of resolvins and protectins have been identified: those derived via lipoxygenase metabolism of EPA and DHA, and a second series derived from aspirin-triggered cyclooxygenase-2 or cytochrome P450 metabolism of EPA and DHA. Mas *et al.*[74] have shown that the resolvin and protectin pathway precursors 18R/S-HEPE and 17R/S-HDHA, as well as the resolvins 17S-RvD2, 17S-RvD1 and 17R-RvD1, were present in human plasma following *n*-3 fatty acid supplementation, at concentrations that are known to have potent anti-inflammatory effects.

The anti-inflammatory effects of *n*-3 fatty acids may play an important role in preventing plaque development and also in plaque stabilisation. Thies *et al.*[75] supplemented *n*-3 fatty acids to patients with symptomatic carotid atherosclerotic disease undergoing carotid endarterectomy. *n*-3 Fatty acids were readily incorporated into the atherosclerotic plaque and this associated with reduced macrophage infiltration into the plaque and a thickened fibrous cap, suggestive of increased plaque stability. These findings represent an important mechanism by which *n*-3 fatty acids could reduce ischaemic cardiovascular events.

Effects on oxidative stress

Despite any benefits of *n*-3 fatty acids, there remains concern that these fatty acids may increase lipid peroxidation and oxidative stress. In contrast, *n*-3 fatty acids given as fish meals[76] or purified EPA or DHA to patients with type 2 diabetes[77], and purified EPA or DHA supplemented to overweight, mildly dyslipidaemic men[78] decreased urinary F_2-isoprostane excretion. F_2-isoprostanes are lipid peroxidation products derived from the non-enzymatic free radical oxidation of arachidonic acid in membrane lipids and are considered the most reliable biomarkers of *in vivo* lipid peroxidative damage[79]. Barden *et al.*[80] also showed that cord plasma and urinary F_2-isoprostanes were reduced in infants whose mother received a daily *n*-3 fatty acid supplement during pregnancy. Mas *et al.*[20] showed that plasma F_2-isoprostanes were reduced by EPA and DHA in type 2 diabetic and overweight, dyslipidaemic men. Furthermore, the fall in plasma F_2-isoprostanes was not altered in analyses that corrected for changes in plasma arachidonic acid, EPA or DHA[20]. The mechanisms by which *n*-3 fatty acids reduce oxidative stress remain unresolved, but probably relate to decreased leucocyte activation and the immunomodulatory actions of these fatty acids. This hypothesis is supported by data from Mori *et al.*[77] who showed that changes in urinary F_2-isoprostanes were significantly positively associated with changes in TNFα.

Conclusions

Present data support the concept that *n*-3 fatty acids beneficially influence a number of cardiometabolic risk factors. Most, albeit not all, clinical trials demonstrate that *n*-3 fatty acids are protective particularly in the setting of secondary prevention. There are no clinically significant adverse effects of *n*-3 fatty acids at doses up to at least 4 g/d. *n*-3 Fatty acids also do not appear to have adverse interactions with medications such as statins and other lipid-lowering drugs or antihypertensive medications. Small doses of 1 g/d are achievable with consumption of two to three oily fish meals per week; otherwise numerous purified encapsulated preparations are now readily available. *n*-3 Fatty acids consumed as fish should be considered an important component of a healthy diet and as a potential therapeutic modality in patients with coronary artery disease and those at heightened risk of CVD[81].

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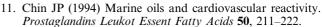Research Foundation of Royal Perth Hospital, Perth, Western Australia.

Conflicts of interest

None.

Authorship

The author is solely responsible for the research and writing of this review.

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