The differential effects of EPA and DHA on cardiovascular risk factors

S. C. Cottin*, T. A. Sanders and W. L. Hall
Diabetes and Nutritional Sciences Division, School of Medicine, King’s College London, 150 Stamford Street, London SE1 9NH, UK

Compelling evidence exists for the cardioprotective benefits resulting from consumption of fatty acids from fish oils, EPA (20:5n-3) and DHA (22:6n-3). EPA and DHA alter membrane fluidity, interact with transcription factors such as PPAR and sterol regulatory element binding protein, and are substrates for enzymes including cyclooxygenase, lipoxygenase and cytochrome P450. As a result, fish oils may improve cardiovascular health by altering lipid metabolism, inducing haemodynamic changes, decreasing arrhythmias, modulating platelet function, improving endothelial function and inhibiting inflammatory pathways. The independent effects of EPA and DHA are poorly understood. While both EPA and DHA decrease TAG levels, only DHA appears to increase HDL and LDL particle size. Evidence to date suggests that DHA is more efficient in decreasing blood pressure, heart rate and platelet aggregation compared to EPA. Fish oil consumption appears to improve arterial compliance and endothelial function; it is not yet clear as to whether differences exist between EPA and DHA in their vascular effects. In contrast, the beneficial effect of fish oils on inflammation and insulin sensitivity observed in vitro and in animal studies has not been confirmed in human subjects. Further investigation to clarify the relative effects of consuming EPA and DHA at a range of doses would enable elaboration of current understanding regarding cardioprotective effects of consuming oily fish and algal sources of long chain n-3 PUFA, and provide clearer evidence for the clinical therapeutic potential of consuming either EPA or DHA-rich oils.

EPA: DHA: n-3 fatty acids: Cardiovascular risk: Vascular function

In the late 1970s, Dyerberg and Bang(1) were the first to highlight the cardioprotective effect of dietary long chain n-3 PUFA (n-3 LCP) present in oily fish in the Inuit population. It is now widely accepted that habitual oily fish and fish oil intake decreases the risk of CVD(2,3) such as fatal CHD(4,5) and stroke(5,6). Over the past 30 years, the mechanisms by which fish oils improve cardiovascular health have been extensively investigated, showing anti-inflammatory, anti-arrhythmic and anti-aggregatory effects, as well as an improvement in endothelial function (EF). Responding to the abundance of evidence, national and international organisations encourage an increased fish oil consumption. n-3 LCP from fish oils include EPA (20:5n-3) and DHA (22:6n-3), and have been developed commercially as dietary supplements. Recent evidence from randomised controlled trials has produced equivocal results(9–11). Heterogeneity of the studies in terms of dosage, duration, population target, sample size, as well as the relative amount of EPA and DHA used in supplements could account for the variability of the results. Since the appearance of purified forms of DHA on the market in the 1990s, researchers have started to investigate the differential effects of EPA and DHA on cardiovascular health. However, the number of human studies is still limited in this field and the independent effects of EPA and DHA on various cardiovascular outcomes are yet to be firmly

Abbreviations: AA, arachidonic acid; α-LNA, α-linolenic acid; BP, blood pressure; COX, cyclooxygenase; CRP, C-reactive protein; CYP450, cytochrome P450; EF, endothelial function; FMD, flow-mediated dilation; HMG, 3-hydroxy-3-methylglutaryl; HR, heart rate; HRV, HR variability; n-3 LCP, long chain n-3 PUFA; LOX, lipoxygenase; LT, leukotriene; NOS, NO synthase; Rv, resolvin; SREBP, sterol regulatory element binding protein; TX, thromboxane.

*Corresponding author: Sarah Cottin, fax + 44 2078484171, email sarah.cottin@kcl.ac.uk
established. Further understanding in this field is needed to define optimal doses of EPA and/or DHA in order to target different metabolic disorders, and to assess the relative efficiency of algal DHA, which could be used as a source of n-3 LCP in vegetarians.

Structure, formation and metabolism of EPA and DHA

EPA and DHA are derived from another n-3 PUFA, α-linolenic acid (α-LNA; 18:3n-3) (Fig. 1), which is found in common vegetable oils, such as linseed or walnut oils. α-LNA is an essential fatty acid, i.e. it has to be provided in the diet as human subjects are unable to synthesise it. Some studies suggested that α-LNA has cardioprotective effects, but evidence is not as robust as for EPA and DHA and there are insufficient data to encourage increasing α-LNA consumption in order to reduce cardiovascular risk. Human subjects can only convert α-LNA to longer-chain n-3 LCP at a very low rate, especially DHA, and the reduced potency or absence of effect of dietary α-LNA in improving cardiovascular risk factors suggests that dietary intake of n-3 fatty acids in the form of oily fish or supplements is desirable for optimal health. Cardioprotective benefits of α-LNA are mainly attributed to competition for Δ6-desaturase with linoleic acid (C18:2n-6), found in abundance in vegetable oils, seeds and nuts, and a precursor for arachidonic acid (AA; C20:4n-6), also directly obtained from animal sources including meat, eggs and dairy products, leading to production of more EPA and less AA. EPA competes with AA through the cyclooxygenase (COX) and lipoxygenase (LOX) pathways, leading to a set of lipid mediators that improve vasodilation and decrease inflammation, as well as aggregation. Upon the action of aspirin, EPA and DHA can be converted by the COX and LOX pathways into similar families of resolvins, E and D series, respectively.

Fig. 1. Outline of the formation of EPA and DHA and their metabolites. α-LNA, α-linolenic acid; DPA, docosapentaenoic acid; COX, cyclooxygenase; LOX, lipoxygenase; CYP450, cytochrome P450 enzymes; TX, thromboxanes; LT, leukotriene; EEQ, epoxyeicosatetraenoic acid; HEPE, hydroxyeicosapentaenoic acid; EDP, epoxycosapentaenoic acid; HDHE, hydroxydocosahexaenoic acid.
Table 1. Differential effect of EPA and DHA supplementation on plasma fasting TAG levels in human subjects.

<table>
<thead>
<tr>
<th>Duration (weeks)</th>
<th>Design and sample size</th>
<th>Population</th>
<th>Fatty acid</th>
<th>Form</th>
<th>Dose (g/d)</th>
<th>Effect on TAG levels</th>
<th>Control</th>
<th>Reference</th>
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<td>n 74</td>
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<td>EPA</td>
<td>Spread</td>
<td>2-2</td>
<td>↓ (15%)</td>
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<td>(25)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>DHA</td>
<td></td>
<td>2-3</td>
<td>↓ (31%)</td>
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<td>n 42</td>
<td>Normolipidaemic human subjects</td>
<td>EPA</td>
<td>EE</td>
<td>4-8</td>
<td>↓ (15%, NS)</td>
<td>Olive oil</td>
<td>(28)</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>4-9</td>
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<td>EE</td>
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<td>(26)</td>
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<tr>
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<td></td>
<td></td>
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</tr>
<tr>
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<td>n 224</td>
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<td>EE</td>
<td>3-8</td>
<td>↓ (21%)</td>
<td>Corn oil</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td>3-6</td>
<td>↓ (26%)</td>
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<tr>
<td>Crossover</td>
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<td>TAG</td>
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<td>Palmolein soyabean oil mix</td>
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<td></td>
<td></td>
<td></td>
<td>DHA</td>
<td></td>
<td>3-7</td>
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<td>(31)</td>
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<td>n 50</td>
<td>T2D human subjects with treated hypertension</td>
<td>DHA</td>
<td>EE</td>
<td>3-68</td>
<td>↓ (19%)</td>
<td>Olive oil</td>
<td>(32)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>EPA</td>
<td>EE</td>
<td>3-84</td>
<td>↓ (15%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parallel</td>
<td>n 38</td>
<td>Dyslipidaemic human subjects</td>
<td>EPA</td>
<td>EE</td>
<td>3-04</td>
<td>↓ (23%)</td>
<td>Olive oil</td>
<td>(52)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DHA</td>
<td>EE</td>
<td>2-84</td>
<td>↓ (32%)</td>
<td></td>
<td></td>
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<tr>
<td>Parallel</td>
<td>n 33</td>
<td>Normolipidaemic human subjects</td>
<td>EPA</td>
<td>EE</td>
<td>3-8</td>
<td>=</td>
<td>Safflower oil</td>
<td>(29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>DHA</td>
<td>EE</td>
<td>3-8</td>
<td>=</td>
<td></td>
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</tr>
</tbody>
</table>

EE, ethyl ester; NS, non-significant; T2D: type 2 diabetes; ↓, decrease; =, no change.

In addition, both EPA and DHA compete with AA for the cytochrome P450 (CYP450) enzymes, leading to the formation of important mediators of vasodilatation (18). These EPA- and DHA-derived eicosanoids are likely to exert varying effects within the cardiovascular system.

DHA possesses a longer carbon chain and one more double bond than EPA, which is thought to be the reason for the greater influence of DHA on membrane fluidity and cholesterol content (16), and thus on the activity of membrane protein or ion channels. EPA and DHA, as well as their broad range of derivatives, may also have a differential effect on transcription factors such as PPAR (17), NF-κB (18) or sterol regulatory element binding protein (SREBP) (19), with subsequent differences in lipid metabolism, insulin sensitivity and inflammation. This review will explore the differential effects of EPA and DHA in human subjects and relate it to possible molecular mechanisms.

Effects of EPA and DHA on plasma lipid and lipoprotein metabolism

Dyslipidaemia, specifically hypertriglyceridaemia, hypercholesterolaemia and/or a low HDL cholesterol level, is a major risk factor for development of atherosclerosis and CVD. The cardioprotective effects of fish oils are partially attributed to their TAG-lowering action, while their effect on cholesterol levels appears weak or inexistent.

Effect of EPA and DHA on TAG levels

Raised fasting and postprandial TAG concentrations are now widely recognised as markers of cardiovascular risk (20,21). There is strong evidence from epidemiological and intervention studies that EPA-DHA consumption decreases TAG levels (22), thus improving cardiovascular health, and this appears to be dose-dependent (23). When administered individually for 6 weeks or more, both EPA and DHA decrease TAG levels in normolipidaemic (24,25) and hyperlipidaemic subjects (26) from 15 to 30%. Interventions of ≤4 weeks are less consistent. One study showed that 3 weeks of supplementation with EPA or fish oil, but not DHA reduced TAG levels in healthy human subjects (27). More recently, Buckley et al. (28) showed that 4 weeks of supplementation with DHA significantly reduced TAG levels in normolipidaemic human subjects by 22%, while EPA decreased TAG levels by 15% without reaching significance. In another 4-week intervention in healthy human subjects, both EPA and DHA reduced postprandial TAG without affecting fasting TAG levels (29). However, when given for a sufficient period, EPA and DHA seem to reduce triglyceridaemia with no apparent differential effect (24–26,28–32) (Table 1).

Effect of EPA and DHA on lipoprotein profiles

Fish oils generally have no effect on total cholesterol but their influence on LDL and HDL cholesterol is variable, depending on the dose, form and population. Meta-analysis of EPA + DHA supplementation studies showed a very slight increase in LDL (n14:09) and HDL (n15:06) cholesterol levels, but these were clinically insignificant (22). The majority of studies investigating the effect of algal DHA (that also contains docosapentaenoic acid, 22:5n-3) reported a moderate but significant increase in both HDL and LDL levels (33–37). Few studies have reported the differential effect of purified EPA and DHA from fish oils on plasma LDL and HDL cholesterol. Relatively high doses of DHA (2–4 g/d; 6–7 weeks) increased HDL levels by 4–13% in normolipidaemics, whereas similar doses of EPA had no effect (24,25). However, DHA but not EPA (3–7 g/d and 3–8 g/d, respectively, 6 weeks) increased total LDL by 8% in hyperlipidaemic subjects, while no significant effect was observed on total HDL levels (26). Our recent research observed that neither EPA nor DHA (3 g/d, 6 weeks) affected TAG, HDL

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or LDL cholesterol levels in normolipidaemic young men (SC Cottin, TAB Sanders and WL Hall, unpublished results).

Beyond cholesterol levels, LDL and HDL subfractions have emerged as candidate markers of cardiovascular risk. LDL particle size correlates negatively with TAG levels and positively with HDL levels. Larger HDL (HDL-2) carry more cholesterol and are more protective than their counterpart (HDL-3). In general, dietary fish oil increases HDL-2 levels, sometimes without a significant change of HDL level, and also decrease small dense LDL levels. When given individually, DHA but not EPA increased both LDL and HDL particle size in hyperlipidaemic and healthy human subjects, although EPA alone also increased HDL-2:HDL-3 in hypercholesterolaemic subjects. High doses of DHA alone increased HDL particle size in hypertriglyceridaemic men, whereas low doses of DHA alone increased LDL by 7% and LDL:apoB ratio by 3·1% in middle-aged women and men, suggesting an increase in LDL size.

Hypertriglyceridaemia is a result of overproduction and/or decreased catabolism of TAG-rich lipoproteins, including VLDL and chylomicrons. There is growing evidence that EPA and DHA exert their TAG lowering effects by reducing VLDL TAG release from the liver and by increasing TAG clearance from chylomicrons and VLDL particles, as well as altering VLDL concentration and particle size. The potential molecular mechanisms have been comprehensively reviewed by Harris and positively with HDL levels. Larger HDL (HDL-2) correlate negatively with TAG levels and lipid transfer between lipoprotein.

Effects of EPA and DHA on haemodynamics

Blood pressure

Hypertension is a strong predictor of cardiovascular risk, and there is convincing evidence that reducing blood pressure (BP) decreases the risk of total mortality, cardiovascular mortality and stroke. Numerous epidemiological and intervention studies have demonstrated a hypotensive role of fish oils. In a meta-analysis of thirty-one placebo-controlled trials, Morris and co-workers showed that fish oils reduced BP with a dose-dependent effect (systolic BP/diastolic BP: −0·66/−0·35 mm Hg/g n-3 fatty acids), and so is of potential benefit to patients with hypertension, atherosclerosis or hypercholesterolaemia. A more recent meta-analysis of thirty-six intervention trials confirmed the hypotensive role of fish oils on both systolic BP and diastolic BP, especially in elderly and hypertensive patients, although the clinical effect of doses lower than 0·5 g/d, equivalent to one portion of oily fish a week, could not be established. Few human studies have investigated the separate effects of EPA and DHA on BP; these have generally been assessed by seated office measurements, with no significant lowering effects in hypertensive, dyslipidaemic and healthy human subjects. However, low doses of DHA alone (from algal sources) were shown to decrease diastolic BP in healthy subjects. Ambulatory BP, where monitors are worn and take readings at regular intervals over 24 h, considered to be an estimate of the true mean BP level, is more sensitive than the conventional office BP in predicting cardiovascular events. Mori and co-workers investigated the effect of 6-weeks supplementation with EPA or DHA (4 g/d) on ambulatory BP and showed that DHA but not EPA decreased both 24 h and daytime systolic and diastolic ambulatory BP in mildly hyperlipidaemic males.

Heart rate

A high heart rate (HR) has been long associated with cardiovascular morbidity and mortality in epidemiological studies. It is positively correlated with hypertension and has only recently emerged as an independent cardiovascular risk factor to be targeted to reduce cardiovascular events, especially in high-risk populations. A meta-analysis including thirty randomised controlled trials showed that fish oil intake reduces HR, especially in populations with a high-baseline HR and when consumed for a longer intervention period. This effect appears to be mediated by DHA rather than EPA: DHA alone (2·8 g/d) decreased HR by 7% in postmenopausal women, and DHA but not EPA decreased HR by 3·5 beats per minute (bpm) and 2·2 bpm, in hyperlipidaemic males and healthy males, respectively. In contrast, Woodman and co-workers showed no

In summary, both DHA and EPA reduce fasting plasma TAG concentrations with no apparent differential effect, probably by inhibiting VLDL-TAG release and increasing TAG clearance. DHA appears to increase HDL and LDL particle size through the regulation of cholesterol synthesis and lipid transfer between lipoprotein.
significant effect of neither EPA nor DHA on HR in healthy males for similar dosage and treatment duration(31).

HR variability (HRV) is also a strong predictor of CVD, including sudden cardiac death, arrhythmic CHD and atrial fibrillation. Fish oils have shown anti-arrhythmic properties in animal studies(73) and several clinical and epidemiological studies have reported an association between an increase (improvement) of HRV and n-3 LCP blood cell levels and/or fish oil consumption(74–76). However, fish oils fail to improve HRV in several other human interventions. For example, n-3 LCP supplementation did not increase HRV in haemodialysis patients(77), and failed to increase HRV calculated from 10 min recordings(78) or 24 h Holter recordings(79) in healthy men. Nevertheless, the authors of the latter study noted that subjects presented with a particularly high baseline HRV, and subanalysis showed a significant improvement of HRV for subjects with lower baseline values(79). Inconsistency in the results of intervention trials might be due to variability of design, treatment duration, sample size or duration of HRV measurement; a prospective observational study (n 4263) reported that fish oil consumption, recorded over a year, correlated with an increase of HRV calculated from 10 min recordings(78) or 24 h Holter recordings(79) in healthy men. Nevertheless, the authors of the latter study noted that subjects presented with a particularly high baseline HRV, and subanalysis showed a significant improvement of HRV for subjects with lower baseline values(79). Inconsistency in the results of intervention trials might be due to variability of design, treatment duration, sample size or duration of HRV measurement; a prospective observational study (n 4263) reported that fish oil consumption, recorded over a year, correlated with an improvement of HRV, especially in older people(80).

As previously mentioned, the incorporation of EPA and DHA into the cell membrane influences its organisation, fluidity and permeability, as well as the activity of transmembrane proteins, including receptors, enzymes and ion channels. Both EPA and DHA were shown to modulate K, Na and Ca channel activities in myocardial cells, regulating myocyte electrical excitability and contractility(81–85). These effects, observed in a concentration-dependent manner, are thought to be mediated by the effect of EPA and DHA on membrane fluidity(83), although other mechanisms, such as direct binding of n-3 LCP to the channel could be involved(84). Furthermore, there is growing evidence from animal studies that DHA, compared to EPA, is preferentially incorporated into the myocardial cell membrane(73). Collectively, these findings help to explain the anti-arrhythmic and HR-lowering effects observed with DHA but not EPA in human subjects(89). In addition, incorporation of DHA into the membrane of cardiomyocytes influences the beta adrenergic system to a greater extent than EPA(85), potentially an important mechanism in the hypotensive and anti-arrhythmic effects of DHA. DHA incorporation into the membrane of endothelial cells stimulates ATP release from the endothelium, increasing vasodilation by stimulating nitric oxide (NO) release(86). The induction of NO release, together with the decrease in noradrenaline levels, is likely to be responsible for the BP-lowering effect of DHA(86).

DHA, but not EPA, seems to have lowering effects on BP and HR, very probably mediated by the increased fluidity in the membrane cardiomyocytes, potentially improving channel activity and beta adrenergic signalling. More studies are necessary to confirm this differential effect and understand the mechanisms involved.

**Effects of EPA and DHA on endothelial function and arterial compliance**

Endothelial dysfunction is a key early event in the development of atherosclerosis and is characterised by an imbalance between molecules produced by the endothelium, impairing vasodilation, inflammatory status and haemostasis. In human subjects, EF can be assessed by measuring plasma markers of EF, including NO and pros-tacyclin metabolites (the two main vasodilators) or markers of endothelial damage and/or activation, such as soluble thrombomodulin, von Willebrand factor or E-Selectin. EF can also be assessed by non-invasive techniques such as plethysmography and flow-mediated dilation (FMD) or invasive techniques like forearm blood flow, with FMD being more commonly used. These techniques can also be used to measure endothelium-independent vascular response (using NO donors, or NO synthase (NOS) inhibitors) or vasoconstrictive response. Endothelium-derived mediators influence vascular tone and structure, thus influencing arterial stiffness and microvascular function. Non-invasive techniques have been developed to measure arterial stiffness/compliance in order to assess vascular function, which include pulse wave analysis, pulse wave velocity(87) and digital volume pulse(88).

**Endothelial function**

Animal studies demonstrated that EF could be modulated by feeding EPA and DHA(89–91). An observational study reported that plasma and erythrocyte DHA levels were positively associated with FMD in young smokers and young adults at greater metabolic risk(92). Recent findings suggest that fish oil consumption can improve EF in human subjects, particularly in those with a high risk of CVD (Table 2). Supplementation with n-3 LCP for periods ranging from 2 weeks up to 8 months improved endothelium-dependent vasodilation, prevented vasoconstriction or augmented exercise-induced blood flow at doses ≥0.5 g/d(93–107).

The comparative effects of EPA and DHA on EF have been seldom investigated in human subjects (Table 2). Supplementation with EPA alone (1.8 g/d; 3 months) increased endothelium-dependent forearm blood flow response in untreated hypertriglyceridaemic males(108), whereas DHA alone (1.2 g/d; 6 weeks) improved endothelium-dependent FMD in hyperlipidaemic children receiving nutritional counselling(109). Supplementation with low doses of algal DHA did not affect salbutamol-induced changes in digital volume pulse reflection index (a measure of endothelium-dependent vasodilation), but more extensively validated techniques such as FMD are required to confirm this(65). When the vasodilatory effects of high doses of EPA and DHA (4 g/d, 6 weeks) were compared in overweight mildly hyperlipidaemic males, DHA, but not EPA, decreased vasoconstrictive responses to noradrenaline and increased vasodilatory responses to acetylcholine(97). However, DHA (but not EPA) also increased vasodilation in response to the co-infusion of acetylcholine and NO(105). Sodium nitroprusside (an NO donor), as well as sodium nitroprusside (a NO donor), suggesting that the vasodilatory effects of DHA were mainly mediated through endothelium-independent mechanisms(97). In healthy volunteers, fish oil concentrate, but not EPA alone, increased urinary excretion of NO metabolites (nitrates/nitrates), suggesting that EPA is unlikely to be responsible for the enhancement of
Table 2. Effect of fish oils on endothelial function in human randomised controlled trials.

<table>
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<th>Duration (weeks)</th>
<th>Design</th>
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<th>Measurement</th>
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<th>Function assessed</th>
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<td>↑</td>
<td>EF (postprandial)</td>
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<td>↑</td>
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<td>(107)</td>
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<td>↑</td>
<td>EF</td>
<td>(96)</td>
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<tr>
<td></td>
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### Table 2 (Continued)

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<tr>
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<td>=</td>
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<td>FBF + SNP</td>
<td>↑</td>
<td>Endothelium independent vasodilation</td>
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T2D, type 2 diabetes; PAD, peripheral arterial disease; CAD, coronary artery disease; FMD, flow-mediated dilation; RH, reactive hyperaemia; BA, brachial artery; EF, endothelial function; ED/EA, endothelial dysfunction/endothelial damage; NOx, nitrates/nitrites; vWF, von Willebrand factor; VCAM, vascular cell adhesion molecule; ICAM, intercellular adhesion molecule; sTM, soluble thrombomodulin; FBF, forearm blood flow; Ach, acetylcholine; SNP, sodium nitroprusside (NO donor); L-NMMA, nitro-L-arginine-methyl ester (NOS inhibitor); GTN, glyceryl trinitrate (NO donor); AT-II, angiotensin II; NAd, noradrenaline (norepinephrine); ADMA, asymmetrical dimethylarginine; L-NMMA, N2-monomethyl-L-arginine.

↑, increase; ↓, decrease; =, unchanged.
NO production\textsuperscript{(110)}. In summary, there are too few data to conclude whether EPA and DHA have differing effects on endothelium-dependent vasodilation, but early indications are that DHA might be more effective in improving EF.

Arterial compliance

Little is known about the influence of dietary n-3 LCP on arterial stiffness, although it has been observed that Japanese populations with higher intakes of n-3 LCP have reduced arterial stiffness\textsuperscript{(111)}, and the results of two randomised controlled trials indicate a beneficial effect\textsuperscript{(112,113)}. Even less is known about the individual effects of either EPA or DHA. Three-month supplementation with low doses of DHA (0.7 g/d) in healthy subjects had no effect on indices of arterial stiffness using digital volume pulse, suggesting that either larger doses are necessary for measurable changes to occur during that time period, that fish oil is more effective in subjects at greater cardiovascular risk (our group also found no effects of 3 g/d EPA or DHA for 6 weeks in young healthy males (SC Cottin, TAB Sanders and WL Hall, unpublished results)) or lastly, that EPA rather than DHA is the active constituent of fish oil in relation to arterial stiffness\textsuperscript{(65)}. In support of this, EPA supplementation (1.8 g/d; 3 months) improved pulse wave velocity and cardio-ankle vascular index in obese Japanese subjects, the latter measure being a novel index of arterial stiffness that is less influenced by BP than pulse wave velocity\textsuperscript{(114)}. The same dose of EPA consumed for 12 months by hyperlipidaemic patients also prevented the increase of pulse wave velocity due to ageing, even after adjustment for gender, age and BP change\textsuperscript{(115)}. However, not all evidence supports the theory that EPA is the sole active constituent of fish oil in relation to arterial stiffness; the only trial to compare the individual effects of EPA and DHA showed that 7 weeks supplementation with EPA increased systemic arterial compliance in dyslipidaemics by 36\% and DHA increased it by 27\%, with no significant differences in the size of the effect between the two groups\textsuperscript{(32)}.

Microvascular dysfunction, as observed in hypertensive and insulin-resistant states, is characterised by capillary rarefaction in skin and muscle\textsuperscript{(116,117)}. Fish oil supplementation increased capillary density in ventricles\textsuperscript{(118)} and skin (cheek pouch)\textsuperscript{(119)} in hypertensive rats and hamsters, respectively, suggesting there might be beneficial effects on human microvasculature. Videomicroscopic techniques (capilloscopy) have been developed and used in human subjects in order to look at microcirculation in the skin and oral mucosa (tongue), which are readily accessible for microscopic measurements (see comprehensive review\textsuperscript{(120)}). Capillaries appear either parallel (loops) or perpendicular (dot or comma) shaped to the skin, and can be analysed in terms of shape (tortuosity and diameter) and number (density). Capilloscopy also gives the possibility to assess the velocity of the erythrocytes by video or laser Doppler. However, to date, the effect of fish oil on this outcome has not been investigated. In our recent trial, neither EPA nor DHA changed finger capillary density (Capiscope; KK Technologies) in healthy young men (SC Cottin, TAB Sanders and WL Hall, unpublished results), but further research is needed in sub-populations with impaired microvascular function to ascertain whether fish oils or individual n-3 LCP have protective effects.

The expression of endothelial NOS is vital to EF and therefore a major factor in atherogenesis. Cell membranes are organised in microdomains, called lipid rafts, that co-localise transmembrane proteins involved in intracellular signalling pathways. When incorporated into the membrane, EPA and DHA can alter this organisation, thus modulating signalling in various types of cells\textsuperscript{(121)}. In endothelial cells, both EPA and DHA were shown to alter the organisation of caveolae, a particular subset of lipid rafts, and displace endothelial NOS from caveolae, a necessary step in the activation of endothelial NOS\textsuperscript{(122,123)}. This could potentially lead to an increase in NO release by the endothelium and explain the putative beneficial effects of EPA and DHA on vasodilation observed to date (Table 2). EPA and DHA probably also influence the production of the two other main vasodilators produced by the endothelium: prostacyclin and endothelium-dependent hyperpolarising factor. EPA, as a direct substrate for COX, may be converted to 3-series prostacyclin, analogue to the 2-series prostacyclin derived from AA. Both EPA and DHA increased 3-series prostacyclin production by endothelial cells to the same extent without affecting 2-series prostacyclin levels, suggesting a retroconversion from DHA to EPA\textsuperscript{(124)}. In addition, EPA was shown to increase acetylcholine-induced endothelium-dependent hyperpolarising factor-mediated vasodilation in diabetic rats\textsuperscript{(125)}. Endothelium-independent vasodilatory effects are mediated by the modulation of Ca\textsuperscript{2+} signalling in smooth muscle cells, but the mechanisms, especially with respect to the type of Ca channels involved, remain uncertain\textsuperscript{(126–129)}.

AA, EPA and DHA are also substrates for the CYP450 enzymes that act as monoxygenases, catalysing hydroxylation, epoxidation or allylic oxidation. CYP450-dependent derivatives include epoxyeicosatrienoic acids and hydroxyeicosatetraenoic acids, potentially important factors in the regulation of vasodilation and vasoconstriction, and modulate renal, vascular and cardiac function. CYP450 enzymes are highly regioselective and stereospecific, and several isoforms prefer n-3 LCP as substrates rather than n-6 LCP. Investigation into the physiological roles of CYP450-dependent EPA and DHA metabolites is at an early stage, but recent data suggest that CYP450-dependent mediators derived from EPA and DHA contribute to the vasodilatory and cardioprotective effects of fish oils\textsuperscript{(130)}. Interestingly, different CYP450 isoforms have a different affinity, regioselectivity and stereospecificity for EPA and DHA (see comprehensive review\textsuperscript{(125)}), leading to various sets of mediators that will exert varying effects on the vasculature.

In addition, EPA and DHA modulate EF through anti-inflammatory effects. When endothelial cells undergo inflammatory activation, they increase the expression of adhesion molecules, allowing the migration of leucocytes through the endothelium, an important process in the pathophysiology of atherosclerosis\textsuperscript{(131)}. General patterns that emerge from in vitro experimental literature indicate that DHA has a greater effect than EPA in reducing endothelial inflammation. DHA tends to inhibit markers of EF, such as inflammatory cell adhesion molecules and...
monocyte chemoattractant protein-1 gene and protein expression, and the adhesion of leucocytes to the endothelium, whereas EPA either up-regulated gene expression of monocyte chemoattractant protein-1 or was a weaker inhibitor of cell adhesion molecules than DHA(132–137). The effect of DHA on vascular cell adhesion molecule-1 is likely to be mediated by the inhibition of the mobilisation of the nuclear transcription factor, NF-κB(136), which regulates the expression of numerous cytokines and other adhesion molecules.

Fish oils generally improve EF and arterial compliance in subjects at high cardiovascular risk. However, the effects of fish oils in healthy human subjects and the mechanisms (endothelium dependent and independent) by which EPA and/or DHA improve vascular function are yet to be fully established.

**Effects of EPA and DHA on inflammation**

Inflammation is an important process in the development of CVD; and chronic inflammation, characterised by elevated plasma levels of inflammatory markers, is commonly found in subjects at high cardiovascular risk, including type 2 diabetics and patients with CHD.

Epidemiological studies strongly suggest that fish oils have anti-inflammatory properties, and levels of n-3 LCP in plasma, as well as in erythrocyte membrane, negatively correlate with plasma pro-inflammatory markers, including C-reactive protein (CRP) and IL-6(138,139). Another hypothesis for the cardioprotective effects of fish oil supplementation, as measured directly in plasma or ex vivo, and studies have been published that support and challenge the hypothesis that n-3 LCP inhibit cytokine and CRP production(102,103,140–150). In their recent meta-analysis including twenty-one trials, Balk et al.(151) concluded that the effect of n-3 LCP, including EPA and DHA, on CRP levels in human subjects was unconvincing. In human subjects, only one study investigated the differential role of EPA and DHA (4 g/d; 6 weeks), reporting that plasma CRP, IL-6 and TNF-α remained unchanged in hypertensive type 2 diabetics(152). When investigated separately, DHA (3 g/d, 3 months) reduced CRP at 6 weeks and IL-6 at 12 weeks of intervention in hypertriglyceridaemic subjects(153), while EPA (1.8 g/d, 8 weeks) decreased CRP levels in obese subjects(115). Lower doses of DHA, representative of levels of intake obtained from dietary sources, failed to affect plasma CRP levels in healthy subjects(65). Complete understanding of this topic requires intervention studies on the anti-inflammatory effects of long-term combined EPA and DHA intakes at low doses (<1.5 g/d), relevant to dietary guidelines for optimal health, and also shorter-term higher doses EPA and DHA (1.5–5 g/d), potentially important in developing therapies for at-risk patients.

Related to the observations for cytokines, an increasing dietary intake of n-3 LCP also modifies the eicosanoid profile in blood, reducing production of AA-derived mediators by inflammatory cells, such as leukotriene (LT) B4 and PGD2 and increasing EPA-derived mediators such as LTB5 and PGE3 (see review(154) for further information). As indicated earlier, EPA and DHA supplementation lowers the cell membrane n-6:n-3 ratio. This reduces AA availability for the production of lipid mediators through the COX and LOX pathways, including 4-series LT, 2-series PG and thromboxane (TX), while increasing the production of 5-series LT, and 3-series PG and TX(154). 3-series EPA-derived eicosanoids, are thought to be less potent than AA-derived eicosanoids, thus contributing to the anti-inflammatory, but also the anti-aggregatory and vasodilatory effects of fish oils(154) previously described. As mentioned earlier, EPA, unlike DHA, is a direct substrate for COX and LOX for the synthesis of LT, PG and TX, which might explain why it reduces LTB4 and PGD2 production in macrophages to a greater extent than DHA(155). The slight but significant effect of DHA might be due to its partial reconversion to EPA(156).

In addition, both EPA and DHA undergo a series of reactions involving COX-2 in the presence of aspirin and 5-LOX(2), leading to a novel class of lipid mediators, known as E-series resolvins (Rv) from EPA and D-series Rv and neuroprotectin D1 from DHA, which are involved in the resolution of inflammation. Although EPA and DHA-derived compounds possess strong similarities, they exert different actions that could account for the differential effect of EPA and DHA on various processes in cardiovascular health and disease. For example, both RvE1 and RvD1 reduced the expression of vascular cell adhesion molecule-1, IL-8, macrophage inflammatory protein-1β and TNF-α by endothelial cells and reduced leucocyte transmigration through the endothelium(157). However, the DHA-derived compound RvD1, but not the EPA-derived RvE1, decreased PGE2 production in endothelial cells(157).

In summary, fish oils decrease inflammation, although efficacy in human studies depends on dose, population and inflammation marker chosen. Individually, DHA, and to a lesser extent EPA, have anti-inflammatory properties in vitro but there is insufficient information to determine whether one is more potent than the other.

**Effects of EPA and DHA on thrombosis and haemostasis**

While noting the cardioprotective effects of n-3 LCP from fish oils, Bang and Dyerberg reported that very high oily fish consumption was associated with lengthened bleeding time(1). The anti-thrombotic action of fish oils in both healthy human subjects and people at high cardiovascular risk have been extensively investigated during the ensuing decades. Several intervention studies later confirmed the effect on bleeding time in healthy, hyperlipidaemic and patients with heart disease at generally relatively high doses of fish oils(158,159), while lower doses (<2 g/d) seem to have no significant effect(160,161). A recent meta-analysis including twenty-four trials in type 2 diabetics (1533 subjects) concluded that fish oils reduced platelet aggregation to ADP and to collagen by 22 and 21%, respectively(162). In general, fish oils seem to reduce platelet aggregation and TX A2 production in response to ADP and collagen in healthy people(163,164) and in subjects with mildly raised BP and cholesterol levels(165). However, platelet
aggregation is usually measured in the laboratory in response to various stimuli and there is uncertainty regarding the correlation between platelet activity \textit{ex vivo} and \textit{in vivo}. The effect of fish oils on \textit{in vivo} platelet aggregation in healthy young males was recently investigated by Din and co-workers measuring platelet monocyte aggregates by flow cytometry; low doses of fish oils (1 g/d; 4 weeks) reduced platelet monocyte aggregate, while markers of platelet activation (soluble P-selectin, soluble CD40L) remained unchanged\textsuperscript{(166)}. Anti-thrombotic properties of fish oils were initially attributed to EPA due to its competition with AA in the COX and LOX pathways. Accordingly, 1.8 g EPA given daily to hyperlipidaemic diabetics for 4 weeks was shown to reduce platelet- and monocyte-derived particles, as well as the expression of CD62P, CD63 and PAC-1, all markers of platelet activation (soluble P-selectin, soluble CD40L) remained unchanged\textsuperscript{(166)}.

Fig. 2. Outline of the pathways of eicosanoid and lipid mediators synthesis from arachidonic acid (AA), EPA and DHA. Through cyclooxygenases (COX) and lipoxigenases (LOX), AA is converted into a set of lipid mediators including 2-series PG and thromboxanes (TX), 4-series leukotrienes (LT) and lipoxins. Competing with AA for COX and LOX enzymes, EPA is converted to 5-series LT, 3-series PG and TX, which are overall less inflammatory than the AA-derived eicosanoids. In the presence of aspirin, both EPA and DHA are substrates for COX-2, eventually leading to the formation of E- and D-series resolvins, respectively, involved in the resolution of inflammation. In addition, DHA may undergo lipoxygenation (through 5-LOX) and other reactions, producing the anti-inflammatory mediator neuroprotectin D1. HETE, hydroxyeicosatetraenoic; HPEPE, hydroperoxy-EPA; HPDHA, hydroperoxy-DHA.

Due to great variability in terms of design, dose of fish oils and population type, there is inconsistency regarding the effect of fish oils on haemostatic factors in human subjects\textsuperscript{(151,176)}. However, studies generally show no significant effect of \textit{n}-3 LCP on haemostatic factors levels or activities in healthy subjects\textsuperscript{(177–179)}, with similar findings for algal DHA\textsuperscript{(180)}. In type 2 diabetics, fish oil supplementation decreased fibrinogen levels by 10\%\textsuperscript{(162)}, and increased factor VII by 25\%\textsuperscript{(181)}, based on meta-analysis of three trials (159 participants) and two trials (116 participants), respectively. More studies are needed to clarify the independent effects of EPA and DHA on haemostatic factors.

Dietary EPA and DHA are readily incorporated into platelet membrane, leading to the formation of eicosanoids from the 3-series, less pro-thrombotic than the 2-series eicosanoids derived from AA (Fig. 2). This, in addition to the effect on platelet membrane fluidity, is likely to influence haemostatic and thrombotic processes. Competition of EPA with AA in the COX pathway (Fig. 2) reduces TXA\textsubscript{2} production, leading to the formation of TXA\textsubscript{3}, a less

agent than EPA at high doses\textsuperscript{(173,174)}. More recently, 8-week supplementation with DHA alone were shown to reduce platelet aggregation to collagen in healthy males for doses as low as 0.4 g/d\textsuperscript{(175)}.
potent vasoconstrictor and pro-aggregatory mediator. Accordingly, both EPA and DHA decrease AA-induced TXA2 production by platelets, while only EPA increases TXA2 production, showing that EPA, but not DHA is a direct substrate for the COX/TX synthase complex (182). Anti-thrombotic effects of EPA and DHA might also be endothelium dependent. 3-series prostacyclin is synthesised from EPA by endothelial cells, which adds on to the anti-aggregatory effect of 2-series prostacyclin (124). In addition, both EPA and DHA inhibit platelet-activating factor synthesis (183) and stimulate endothelial NOS activity (122,123) in endothelial cells. The decrease in platelet-activating factor levels, as well as the increase of NO, which has anti-aggregatory properties, may also contribute to the anti-thrombotic effects of fish oils.

Fish oils seem to exert their anti-thrombotic action in human subjects by influencing platelet activation and aggregation rather than haemostatic factors levels and/or activity. DHA is more potent than EPA in reducing platelet aggregation in animals, and possibly in human subjects, possibly as a result of its greater effect on membrane fluidity (170). In contrast to DHA, EPA is a direct substrate of COX for the synthesis of anti-inflammatory and anti-aggregatory mediators, a key factor in the inhibition of platelet activation. Further investigation is needed to specify the individual role of EPA and DHA in platelet function in human subjects, especially *in vivo*.

### Insulin sensitivity and glycaemic control

Insulin resistance is characteristic of type 2 diabetes and is associated with several disorders involved in the development of CVD, including chronic inflammation, dyslipidaemia, hypertension and endothelial dysfunction.

Plasma and erythrocyte n-3 LCP, n-3:n-6, and especially EPA:AA ratios correlate positively with insulin sensitivity in healthy subjects and type 2 diabetes (184–186). There is also growing evidence from animal studies that fish oil intake increases insulin sensitivity and adiponectin levels in insulin resistant rats and mice (187,188). In contrast, intervention studies generally show little or no effect of fish oils on insulin sensitivity and glycaemic control in human subjects. Balk’s meta-analysis considered healthy subjects, type 2 diabetics, hypertensives, dyslipidaemics or patients with CVD and concluded that fish oils induced no change in glycated Hb (HbA1c, eighteen trials, 578 participants) with CVD and concluded that fish oils induced no change in glycated Hb (HbA1c, eighteen trials, 578 participants) with CVD and concluded that fish oils induced no change in glycated Hb (HbA1c, eighteen trials, 578 participants) with CVD and concluded that fish oils induced no change in glycated Hb (HbA1c, eighteen trials, 578 participants). Although fish oils show anti-inflammatory and insulin-sensitising properties *in vitro* and in animal studies, human studies are often conflicting and efficacy remains uncertain; accordingly, neither EPA nor DHA alone showed an effect on insulin sensitivity in human subjects, despite indications for potency *in vitro*.

The apparent efficacy of DHA in improving a number of cardiovascular risk factors, and the remaining uncertainty surrounding the actions of EPA, suggest that there is a need for n-3 LCP oils that are a purified or enriched source of either EPA or DHA. An increasing number of studies are being published on the cardioprotective effects of DHA TAG from algal sources, either *Cryptophydomium cohnii* or *Schizochytrium* sp. (Martek Biosciences Corporation, Columbia, MD, USA). Supplements, infant formula, infant foods and certain other food categories (dairy, bakery, eggs and non-alcoholic beverages) fortified with algal DHA are now available to buy in many countries. The potential benefits of algal DHA supplements for subgroups that have low intakes, such as vegetarians, should be a high priority for investigation. EPA TAG-enriched oils and purified EPA ethyl ester oils are available but currently a large amount of effort is being directed by industry towards the development of non-fish oil-derived EPA. As more
DHA- and EPA-only products become available, partly as a result of concern over the sustainability of fish oil supplies and partly in response to consumer demand for non-fish sources, future research can be focused on establishing the most effective doses of DHA and EPA for improvement of cardiovascular risk factors. This will inform dietary advice on the optimal intake for life-long health, and should enable a decision to be made on the most effective supplement dose to be taken over short periods to reduce risk factors such as hypertriglyceridaemia or hypertension in various at-risk populations. It will be important to bear in mind that not all individuals will respond to DHA and/or EPA in the same way, and ongoing nutrigenetic and gender research will be crucial in defining future advice regarding dietary and supplementary EPA and DHA. The role of dietary n-3 LCP in cardiovascular health is an area of nutritional science/medicine that has undergone more investigation than most during the past 30 or more years, yet the gaps in our understanding of this field remain substantial.

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