

The Summer Meeting of the Nutrition Society hosted by the Scottish Section was held at Heriot-Watt University, Edinburgh on 28 June–1 July 2010

## Conference on ‘Nutrition and health: cell to community’

### Postgraduate Symposium

## 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 (20:5*n*-3) and DHA (22:6*n*-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.

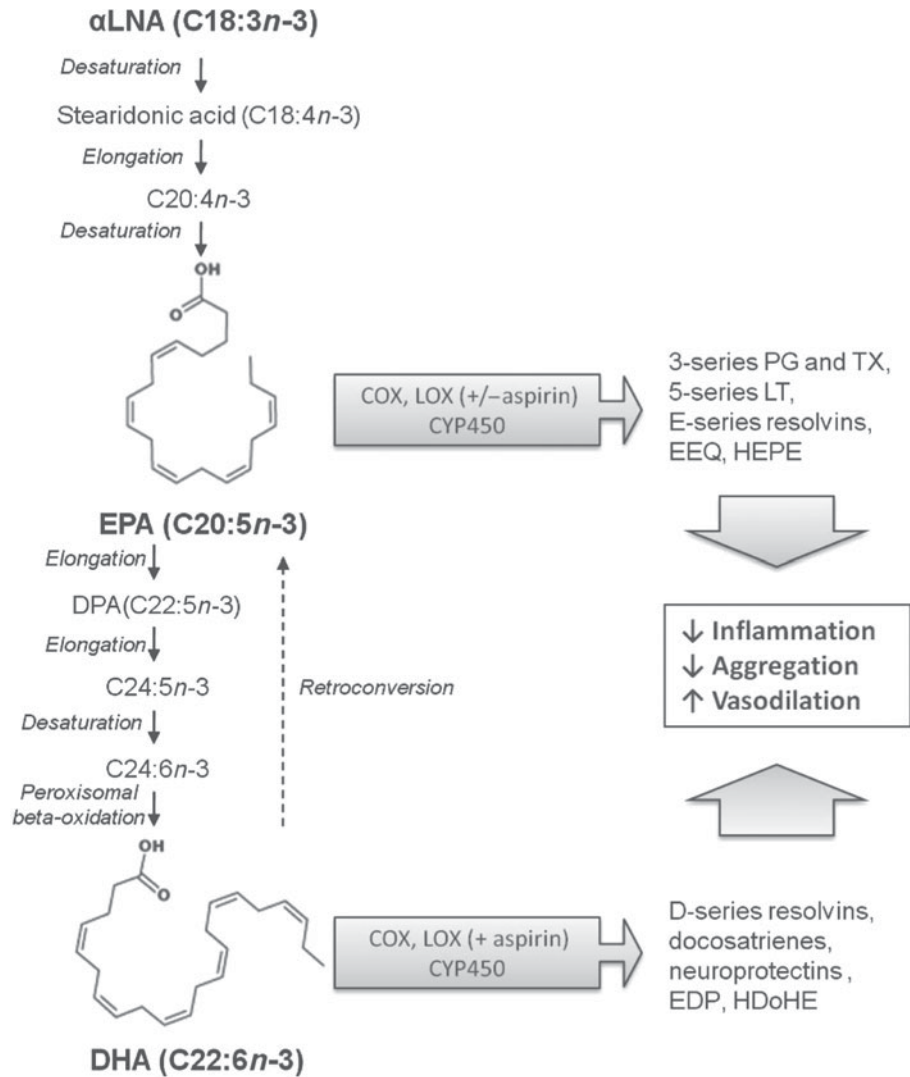
#### EPA: DHA: *n*-3 fatty acids: Cardiovascular risk: Vascular function

In the late 1970s, Dyerberg and Bang<sup>(1)</sup> 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<sup>(2,3)</sup> such as fatal CHD<sup>(4,5)</sup> and stroke<sup>(5,6)</sup>. 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<sup>(7,8)</sup>. *n*-3 LCP from fish oils include EPA

(20:5*n*-3) and DHA (22:6*n*-3), and have been developed commercially as dietary supplements. Recent evidence from randomised controlled trials has produced equivocal results<sup>(9–11)</sup>. 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;  $\alpha$ -LNA,  $\alpha$ -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



**Fig. 1.** Outline of the formation of EPA and DHA and their metabolites.  $\alpha$ -LNA,  $\alpha$ -linolenic acid; DPA, docosapentaenoic acid; COX, cyclooxygenase; LOX, lipoxygenase; CYP450, cytochrome P450 enzymes; TX, thromboxanes; LT, leukotriene; EEQ, epoxyeicosatetraenoic acid; HEPE, hydroxyeicosapentaenoic acid; EDP, epoxydocosapentaenoic acid; HDoHE, hydroxydocosahexaenoic acid.

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,  $\alpha$ -linolenic acid ( $\alpha$ -LNA; 18:3n-3) (Fig. 1), which is found in common vegetable oils, such as linseed or walnut oils.  $\alpha$ -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  $\alpha$ -LNA has cardioprotective effects, but evidence is not as robust as for EPA and DHA and there are insufficient data to encourage increasing  $\alpha$ -LNA consumption<sup>(12)</sup> in order to reduce cardiovascular risk. Human

subjects can only convert  $\alpha$ -LNA to longer-chain  $n$ -3 LCP at a very low rate, especially DHA<sup>(13,14)</sup>, and the reduced potency or absence of effect of dietary  $\alpha$ -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  $\alpha$ -LNA are mainly attributed to competition for  $\Delta$ 6-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<sup>(13)</sup>.

**Table 1.** Differential effect of EPA and DHA supplementation on plasma fasting TAG levels in human subjects.

Duration (weeks)	Design and sample size	Population	Fatty acid	Form	Dose (g/d)	Effect on TAG levels	Control	Reference
4	Parallel n 74	Normolipidaemic human subjects	EPA	Spread	2.2	↓ (15%)	None	(25)
			DHA		2.3	↓ (31%)		(28)
6	Parallel n 42	Normolipidaemic human subjects	EPA	EE	4.8	↓ (15%, NS)	Olive oil	(26)
			DHA		4.9	↓ (22%)		(24)
7	Parallel n 56	Hyperlipidaemic human subjects	EPA	EE	3.84	↓ (18%)	Olive oil	(30)
			DHA		3.68	↓ (20%)		(29)
4	Parallel n 224	Normolipidaemic human subjects	EPA	EE	3.8	↓ (21%)	Corn oil	(31)
			DHA		3.6	↓ (26%)		(32)
4	Crossover n 38	Normolipidaemic human subjects	EPA	TAG	3.3	↓ (28%)	Palmolein soyabean oil mix	(33)
			DHA		3.7	↓ (19%)		(34)
6	Parallel n 38	T2D human subjects with treated hypertension	EPA	EE	3.84	↓ (15%)	Olive oil	(35)
			DHA		3.68	↓ (19%)		(36)
7	Parallel n 50	Dyslipidaemic human subjects	EPA	EE	3.04	↓ (23%)	Olive oil	(37)
			DHA		2.84	↓ (32%)		(38)
4	Parallel n 33	Normolipidaemic human subjects	EPA	EE	3.8	=	Safflower oil	(39)
			DHA		3.8	=		(40)

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 vasodilation<sup>(15)</sup>. 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<sup>(16)</sup>, 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<sup>(17)</sup>, NF-κB<sup>(18)</sup> or sterol regulatory element binding protein (SREBP)<sup>(19)</sup>, 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 hypertriglyceridemia, hypercholesterolemia 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<sup>(20,21)</sup>. There is strong evidence from epidemiological and intervention studies that EPA+DHA consumption decreases TAG levels<sup>(22)</sup>, thus improving cardiovascular health, and this appears to be dose-dependent<sup>(23)</sup>. When administered individually for 6 weeks or more, both EPA and DHA

decrease TAG levels in normolipidaemic<sup>(24,25)</sup> and hyperlipidaemic subjects<sup>(26)</sup> 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<sup>(27)</sup>. More recently, Buckley *et al.*<sup>(28)</sup> 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<sup>(29)</sup>. However, when given for a sufficient period, EPA and DHA seem to reduce triglyceridaemia with no apparent differential effect<sup>(24–26,28–32)</sup> (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 009) and HDL (n15 106) cholesterol levels, but these were clinically insignificant<sup>(22)</sup>. 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<sup>(33–37)</sup>. 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<sup>(24,25)</sup>. However, DHA but not EPA (3.7 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<sup>(26)</sup>. Our recent research observed that neither EPA nor DHA (3 g/d, 6 weeks) affected TAG, HDL

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<sup>(38)</sup>. Larger HDL (HDL-2) carry more cholesterol and are more protective than their counterpart (HDL-3)<sup>(39)</sup>. In general, dietary fish oil increases HDL-2 levels<sup>(40,41)</sup>, sometimes without a significant change of HDL level<sup>(42)</sup>, and also decrease small dense LDL levels<sup>(41,43)</sup>. When given individually, DHA but not EPA increased both LDL and HDL particle size in hyperlipidaemic and healthy human subjects<sup>(26,27)</sup>, although EPA alone also increased HDL-2:HDL-3 in hypercholesterolaemic subjects<sup>(44)</sup>. High doses of DHA alone (3 g/d, 45 d) also increased LDL particle size in hypertriglyceridaemic men<sup>(35)</sup>, whereas low doses of DHA alone (0.7 g/d) increased LDL by 7% and LDL:apoB ratio by 3.1% in middle-aged women and men<sup>(45)</sup>, 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<sup>(46)</sup>, as well as altering VLDL concentration and particle size<sup>(47)</sup>. The potential molecular mechanisms have been comprehensively reviewed by Harris *et al.*<sup>(46)</sup> and notably involves the modulation of transcription factors activity, including SREBP and PPAR. SREBP-1c controls enzymes responsible for fatty acid and TAG synthesis, while SREBP-2 modulates enzymes involved in cholesterol synthesis. In animal and *in vitro* models, both EPA and DHA were reported to down-regulate SREBP-1c activity, and this was associated with a decrease in lipogenic enzymes expression<sup>(19,48,49)</sup>. In addition, both EPA and DHA are PPAR ligands and EPA and DHA stimulate  $\beta$ -oxidation of fatty acids through PPAR $\alpha$ -dependent mechanisms in rats<sup>(50)</sup>, thus contributing to the decrease in TAG release by the liver. Lipoprotein lipase, located in capillary endothelium, hydrolyses circulating TAG in TAG-rich lipoprotein, generating NEFA. EPA and DHA (4 g/d, 4 weeks) were equally as effective in accelerating chylomicron TAG clearance by stimulating lipoprotein lipase activity in healthy human subjects<sup>(29)</sup>; possibly via PPAR $\gamma$ -dependent mechanisms<sup>(51)</sup>.

3-Hydroxy-3-methylglutaryl (HMG)-CoA reductase is a key enzyme in cholesterol synthesis and is inhibited by both EPA and DHA in hepatocytes, probably through SREBP-2 dependent mechanisms<sup>(52,53)</sup>. Although HMG-CoA reductase inhibitors (statins) are well known for their hypocholesterolemic effect, this has not been a consistent outcome of fish oils consumption. However, a common mechanism for statins and EPA/DHA may be related to increasing LDL and HDL particle size<sup>(54–56)</sup>; in fact *n*-3 LCP and statins may exert a synergistic beneficial effect on lipid levels<sup>(57)</sup>, and it can be postulated that EPA and DHA modulate particle size by a mechanism analogous to that of HMG-CoA reductase inhibitors<sup>(58)</sup>.

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.

## 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<sup>(59,60)</sup>. Numerous epidemiological and intervention studies have demonstrated a hypotensive role of fish oils<sup>(61)</sup>. 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<sup>(62)</sup>. 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<sup>(63)</sup>. 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<sup>(31,32,64)</sup>. However, low doses of DHA alone (from algal sources) were shown to decrease diastolic BP in healthy subjects<sup>(65)</sup>. 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<sup>(66)</sup>, is more sensitive than the conventional office BP in predicting cardiovascular events<sup>(67,68)</sup>. 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<sup>(69)</sup>.

### 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<sup>(70)</sup>. 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<sup>(71)</sup>. This effect appears to be mediated by DHA rather than EPA: DHA alone (2.8 g/d) decreased HR by 7% in postmenopausal women<sup>(72)</sup>, and DHA but not EPA decreased HR by 3.5 beats per minute (bpm) and 2.2 bpm, in hyperlipidaemic males<sup>(69)</sup> and healthy males<sup>(64)</sup>, respectively. In contrast, Woodman and co-workers showed no



significant effect of neither EPA nor DHA on HR in healthy males for similar dosage and treatment duration<sup>(31)</sup>.

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<sup>(73)</sup>, 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<sup>(74–76)</sup>. 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<sup>(77)</sup>, and failed to increase HRV calculated from 10 min recordings<sup>(78)</sup> or 24 h Holter recordings<sup>(79)</sup> 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<sup>(79)</sup>. 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<sup>(80)</sup>.

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 trans-membrane 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<sup>(81–83)</sup>. These effects, observed in a concentration-dependent manner, are thought to be mediated by the effect of EPA and DHA on membrane fluidity<sup>(83)</sup>, although other mechanisms, such as direct binding of *n*-3 LCP to the channel could be involved<sup>(84)</sup>. Furthermore, there is growing evidence from animal studies that DHA, compared to EPA, is preferentially incorporated into the myocardial cell membrane<sup>(73)</sup>. Collectively, these findings help to explain the anti-arrhythmic and HR-lowering effects observed with DHA but not EPA in human subjects<sup>(69)</sup>. In addition, incorporation of DHA into the membrane of cardiomyocytes influences the beta adrenergic system to a greater extent than EPA<sup>(85)</sup>, 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<sup>(86)</sup>. The induction of NO release, together with the decrease in noradrenaline levels, is likely to be responsible for the BP-lowering effect of DHA<sup>(86)</sup>.

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 prostacyclin 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<sup>(87)</sup> and digital volume pulse<sup>(88)</sup>.

#### *Endothelial function*

Animal studies demonstrated that EF could be modulated by feeding EPA and DHA<sup>(89–91)</sup>. 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<sup>(92)</sup>. 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  $\geq 0.5$  g/d<sup>(93–107)</sup>.

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<sup>(108)</sup>, whereas DHA alone (1.2 g/d; 6 weeks) improved endothelium-dependent FMD in hyperlipidaemic children receiving nutritional counselling<sup>(109)</sup>. 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<sup>(65)</sup>. 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<sup>(97)</sup>. However, DHA (but not EPA) also increased vasodilation in response to the co-infusion of acetylcholine and *N*<sub>G</sub>-monomethyl-L-arginine citrate (an NOS inhibitor), as well as sodium nitroprusside (a NO donor), suggesting that the vasodilatory effects of DHA were mainly mediated through endothelium-independent mechanisms<sup>(97)</sup>. In healthy volunteers, fish oil concentrate, but not EPA alone, increased urinary excretion of NO metabolites (nitrates/nitrites), 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.

Duration  (weeks)	Design	Population	Dosage	Measurement	Effect	Function assessed	References
Fish oils							
6	Cross-over	T2D patients <i>n</i> 34	2 g/d	Fasting FMD Fasting RH (Doppler) Postprandial FMD Postprandial RH	= = ↑ ↑	EF (fasting) EF (fasting) EF (postprandial) EF (postprandial)	(106)
12	Parallel	Elderly  <i>n</i> 46	2.5 g/d	RH (mercury strain gauge plethysmography)  NOx vWF E-selectin Endothelin	↑  ↑ = = = =	EF  ED/EA ED/EA ED/EA ED/EA	(104)
12	Parallel	Offsprings of T2D <i>n</i> 50	2 g/d	FMD VCAM ICAM E-selectin	↑ = = =	EF ED/EA ED/EA ED/EA	(102)
12	Parallel	PAD patients <i>n</i> 32	2 g/d	FMD sTM	↑ ↓	EF ED/EA	(103)
24	Parallel	Lupus erythematosus patients <i>n</i> 60	3 g/d	FMD	↑	EF	(107)
2	Parallel	Healthy subjects <i>n</i> 26	1 g/d	FMD FMD + GTN	↑ ↑	EF Endothelium independent vasodilation	(98)
6	Crossover	Chronic heart failure patients (>65-year-old) <i>n</i> 20	3 g/d	FBF + Ach  FBF + SNP	↑  =	EF  Endothelium independent vasodilation	(105)
6	Parallel	Healthy subjects <i>n</i> 13	5 g/d	FBF + AT-II FBF + L-NAME BA diameter (post contraction) BA conductance (post contraction) BA blood flow (post contraction)	= = ↑ ↑ ↑	Vasoconstriction Vasoconstriction EF EF EF	(101)
32	Parallel	Healthy subjects <i>n</i> 173	1.1–1.2 g/d	Laser Doppler + Ach Laser Doppler + SNP	↑ =	EF Endothelium independent vasodilation	(95)
16	Parallel	Hypercholesterolemic subjects <i>n</i> 30	4 g/d	FMD FMD + GTN	↑ =	EF Endothelium independent vasodilation	(94)
4	Parallel	Healthy subjects <i>n</i> 29	1.5–5.9 g/d	FBF + NAd + AT-II	↓ ↓	Vasoconstriction Vasoconstriction	(93)
6	Crossover	T2D patients	3 g/d	FBF + Ach	↑	EF	(96)

Table 2 (Continued)

Duration  (weeks)	Design	Population	Dosage	Measurement	Effect	Function assessed	References
EPA 12	Parallel	<i>n</i> 23 Untreated hypertriglyceridaemic subjects <i>n</i> 15	1.8 g/d	+ GTN FBF + Ach FBF + SNP	= ↑ =	Endothelium independent vasodilation EF Endothelium independent vasodilation	(108)
DHA 6	Crossover	Hyperlipidaemic children <i>n</i> 20	1.2 g/d	FMD ADMA	↑ =	EF ED/EA	(109)
12	Crossover	Healthy subjects <i>n</i> 38	0.7 g/d	sTM vWF E-selectin	= = =	ED/EA ED/EA ED/EA	(65)
EPA v. DHA 6	Parallel	Overweight mildly hyperlipidaemic subjects    <i>n</i> 56	4 g/d	EPA FBF + NAd  FBF + Ach FBF + Ach + L-NMMA  FBF + SNP  DHA FBF + NAd FBF + Ach FBF + Ach + L-NMMA  FBF + SNP	=  = = =  ↓ ↑ ↑  ↑	Vasoconstriction  EF Endothelium independent vasodilation Endothelium independent vasodilation Vasoconstriction EF Endothelium independent vasodilation Endothelium independent vasodilation	(97)

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-NAME, nitro-L-arginine-methyl ester (NOS inhibitor); GTN, glyceryl trinitrate (NO donor); AT-II, angiotensin II; NAd, noradrenaline (norepinephrine); ADMA, asymmetrical dimethylarginine; L-NMMA, *N*<sub>G</sub>-monomethyl-L-arginine.  
 ↑, increase; ↓, decrease; =, unchanged.

EPA and DHA: separate cardiovascular effects

NO production<sup>(110)</sup>. 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<sup>(111)</sup>, and the results of two randomised controlled trials indicate a beneficial effect<sup>(112,113)</sup>. 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<sup>(65)</sup>. 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<sup>(114)</sup>. 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<sup>(115)</sup>. 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<sup>(32)</sup>.

Microvascular dysfunction, as observed in hypertensive and insulin-resistant states, is characterised by capillary rarefaction in skin and muscle<sup>(116,117)</sup>. Fish oil supplementation increased capillary density in ventricles<sup>(118)</sup> and skin (cheek pouch)<sup>(119)</sup> in hypertensive rats and hamsters, respectively, suggesting there might be beneficial effects on human microvasculature. Videomicroscopic techniques (capillaroscopy) 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<sup>(120)</sup>). 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). Capillaroscopy 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 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<sup>(121)</sup>. 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<sup>(122,123)</sup>. 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<sup>(124)</sup>. In addition, EPA was shown to increase acetylcholine-induced endothelium-dependent hyperpolarising factor-mediated vasodilation in diabetic rats<sup>(125)</sup>. Endothelium-independent vasodilatory effects are mediated by the modulation of Ca<sup>2+</sup> signalling in smooth muscle cells, but the mechanisms, especially with respect to the type of Ca channels involved, remain uncertain<sup>(126–129)</sup>.

AA, EPA and DHA are also substrates for the CYP450 enzymes that act as monooxygenases, 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<sup>(130)</sup>. Interestingly, different CYP450 isoforms have a different affinity, regioselectivity and stereospecificity for EPA and DHA (see comprehensive review<sup>(15)</sup>), 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<sup>(131)</sup>. 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<sup>(132–137)</sup>. 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- $\kappa$ B<sup>(136)</sup>, 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<sup>(138,139)</sup>. Another hypothesis for the cardioprotective effects of fish oil supplementation is the inhibition of cytokine production, 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<sup>(102,103,140–150)</sup>. In their recent meta-analysis including twenty-one trials, Balk *et al.*<sup>(151)</sup> 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- $\alpha$  remained unchanged in hypertensive type 2 diabetics<sup>(152)</sup>. 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<sup>(153)</sup>, while EPA (1.8 g/d, 8 weeks) decreased CRP levels in obese subjects<sup>(114)</sup>. Lower doses of DHA, representative of levels of intake obtained from dietary sources, failed to affect plasma CRP levels in healthy subjects<sup>(65)</sup>. 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) B<sub>4</sub> and PGE<sub>2</sub> and increasing EPA-derived mediators such as LTB<sub>5</sub> and PGE<sub>3</sub> (see review<sup>(154)</sup> for further information).

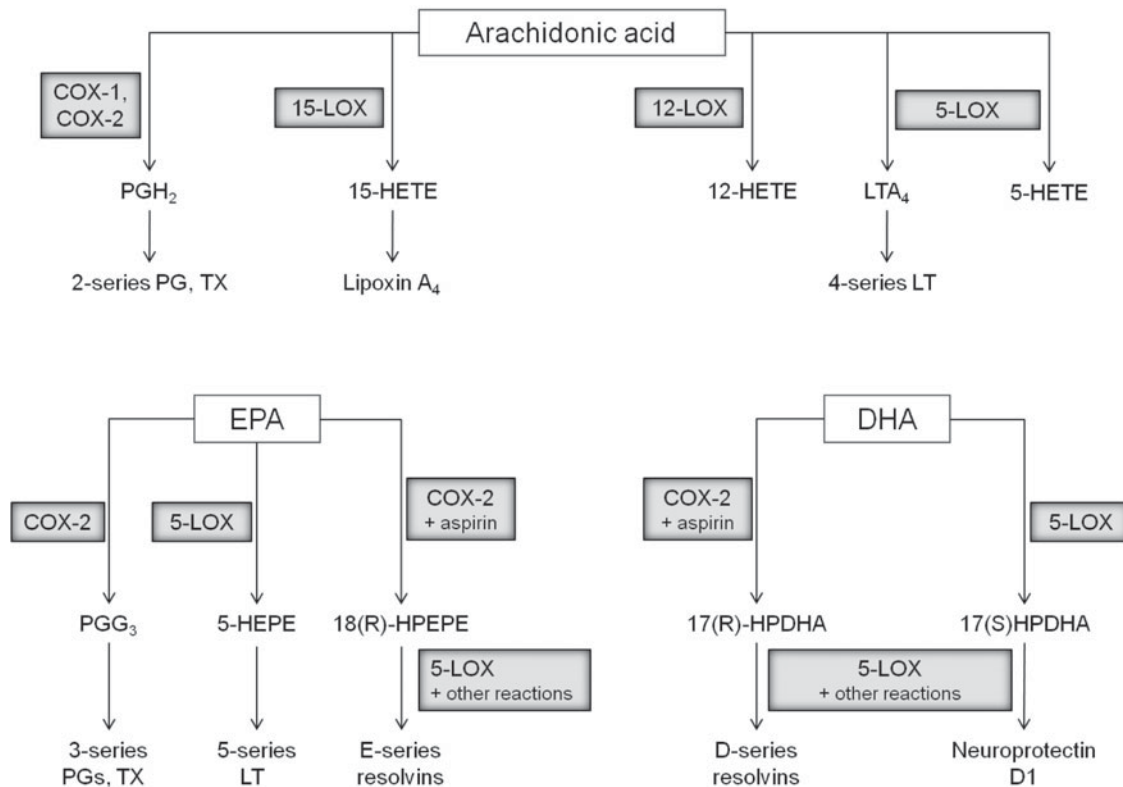
As indicated earlier, EPA and DHA supplementation lowers the cell membrane *n*-6:*n*-3 ratio. This reduces AA availability for the production of lipid mediators through the COX and LOX pathways, including 4-series LT, 2-series PG and thromboxane (TX), while increasing the production of 5-series LT, and 3-series PG and TX<sup>(154)</sup>. 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<sup>(154)</sup> 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 LTB<sub>4</sub> and PGD<sub>2</sub> production in macrophages to a greater extent than DHA<sup>(155)</sup>. The slight but significant effect of DHA might be due to its partial reversion to EPA<sup>(156)</sup>.

In addition, both EPA and DHA undergo a series of reactions involving COX-2 in the presence of aspirin and 5-LOX<sup>(2)</sup>, 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-1 $\beta$  and TNF- $\alpha$  by endothelial cells and reduced leucocyte transmigration through the endothelium<sup>(157)</sup>. However, the DHA-derived compound RvD1, but not the EPA-derived RvE1, decreased PGE<sub>2</sub> production in endothelial cells<sup>(157)</sup>.

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<sup>(1)</sup>. 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<sup>(158,159)</sup>, while lower doses (<2 g/d) seem to have no significant effect<sup>(160,161)</sup>. 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<sup>(162)</sup>. In general, fish oils seem to reduce platelet aggregation and TX A<sub>2</sub> production in response to ADP and collagen in healthy people<sup>(163,164)</sup> and in subjects with mildly raised BP and cholesterol levels<sup>(165)</sup>. However, platelet



**Fig. 2.** Outline of the pathways of eicosanoid and lipid mediators synthesis from arachidonic acid (AA), EPA and DHA. Through cyclooxygenases (COX) and lipoxygenases (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.

aggregation is usually measured in the laboratory in response to various stimuli and there is uncertainty regarding the correlation between platelet activity *ex vivo* and *in vivo*. The effect of fish oils on *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<sup>(166)</sup>.

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<sup>(167)</sup>. Interestingly, EPA but not DHA was also able to reduce mean platelet volume, a simple marker of platelet activation in healthy subjects<sup>(168)</sup>. Four-week supplementation with 3.6 g EPA alone, daily, was also shown to decrease platelet aggregation and TX production in response to collagen in healthy males<sup>(169)</sup>. However, in agreement with animal studies<sup>(170–172)</sup>, human studies suggest that DHA is a more potent anti-aggregatory

agent than EPA at high doses<sup>(173,174)</sup>. 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<sup>(175)</sup>.

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<sup>(151,176)</sup>. However, studies generally show no significant effect of *n*-3 LCP on haemostatic factors levels or activities in healthy subjects<sup>(177–179)</sup>, with similar findings for algal DHA<sup>(180)</sup>. In type 2 diabetics, fish oil supplementation decreased fibrinogen levels by 10%<sup>(162)</sup>, and increased factor VII by 25%<sup>(181)</sup>, 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<sub>2</sub> production, leading to the formation of TXA<sub>3</sub>, a less

potent vasoconstrictor and pro-aggregatory mediator. Accordingly, both EPA and DHA decrease AA-induced TXA<sub>2</sub> production by platelets, while only EPA increases TXA<sub>3</sub> production, showing that EPA, but not DHA is a direct substrate for the COX/TX synthase complex<sup>(182)</sup>. 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<sup>(124)</sup>. In addition, both EPA and DHA inhibit platelet-activating factor synthesis<sup>(183)</sup> and stimulate endothelial NOS activity<sup>(122,123)</sup> 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<sup>(170)</sup>. 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 diabetics<sup>(184–186)</sup>. There is also growing evidence from animal studies that fish oil intake increases insulin sensitivity and adiponectin levels in insulin resistant rats and mice<sup>(187,188)</sup>. 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) and a slight but non-significant increase in fasting blood sugar (seventeen trials, 1427 participants)<sup>(151)</sup>. This was more recently confirmed in a meta-analysis including 1075 type 2 diabetics, where the authors showed no effect of EPA and DHA on HbA1c, fasting glucose, fasting insulin or body weight<sup>(189)</sup>.

There is growing evidence from animal and *in vitro* studies that both EPA and DHA, taken individually, exert an insulin-sensitising action<sup>(187,190–193)</sup>. However, the relative effect of EPA and DHA on insulin sensitivity in human subjects has been poorly investigated. EPA alone decreased insulin reactivity and increased adiponectin levels in obese Japanese, without affecting leptin levels<sup>(114)</sup>. In type 2 diabetics, EPA had no effect on

adiponectin levels but an additive positive effect when combined with statin treatment<sup>(194)</sup>. To date, only three studies investigated the independent effects of EPA and DHA on insulin sensitivity in human subjects. In hyperlipidaemic subjects, both EPA and DHA (6 weeks, 4 g/d) decreased fasting insulin levels, and fasting glucose tended to increase in the EPA group, remaining unchanged following DHA supplementation<sup>(26)</sup>. In treated hypertensive type 2 diabetics, neither EPA nor DHA influenced insulin levels, secretion or sensitivity, but both increased fasting glucose<sup>(31)</sup>. More recently, Egert and co-workers confirmed that neither EPA nor DHA had an effect on HbA1c, insulin level or sensitivity in healthy subjects, although EPA showed a minor increase in glucose levels while DHA had no effect<sup>(195)</sup>.

### Conclusion

Numerous studies have proven the cardioprotective effects of fish oils in human subjects, showing their hypotriglyceridaemic, hypotensive, anti-arrhythmic and anti-thrombotic properties. Recent data suggest that fish oils also improve arterial stiffness and EF, and increase HDL and LDL particle size. Most studies have investigated the effect of oily fish or fish oil supplements containing mixtures of EPA and DHA, and current UK dietary guidelines recommend the consumption of one portion of oily fish a week to maintain general good health. However, over the past 20 years, there has been growing evidence that EPA and DHA exert a heterogeneous effect on various cardiovascular outcomes, which is of considerable relevance for primary and secondary cardiovascular prevention. While both EPA and DHA are able to reduce TAG levels, DHA appears responsible for the BP and HR-lowering effect of fish oils. DHA also seems to be beneficial for EF and platelet function, although an active role for EPA has not been ruled out. 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 inflammation or 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 *Cryptocodinium 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.

### Acknowledgements

The authors declare no conflict of interest. The authors' research was supported by funding from King's College London and we thank Croda Chemicals Europe Ltd, Goole, UK, who donated the EPA-enriched oil (Incromega EPA 500TG SR) and DHA-enriched oil (Incromega DHA 500TG SR) used in the authors' study. S.C. drafted this paper and W.L.H. and T.A.S. discussed and modified it.

### References

- Dyerberg J & Bang HO (1979) Lipid metabolism, atherogenesis, and haemostasis in Eskimos: The role of the prostaglandin-3 family. *Haemostasis* **8**, 227–233.
- Serhan CN & Chiang N (2008) Endogenous pro-resolving and anti-inflammatory lipid mediators: A new pharmacologic genus. *Br J Pharmacol* **153**, S200–S215.
- von Schacky C & Harris WS (2007) Cardiovascular benefits of omega-3 fatty acids. *Cardiovasc Res* **73**, 310–315.
- Hu FB & Willett WC (2002) Optimal diets for prevention of coronary heart disease. *JAMA* **288**, 2569–2578.
- He K, Song Y, Davi GL *et al.* (2004) Fish consumption and incidence of stroke: A meta-analysis of cohort studies. *Stroke* **35**, 1538–1542.
- Keli SO, Feskens EJ & Kromhout D (1994) Fish consumption and risk of stroke. The Zutphen Study. *Stroke* **25**, 328–332.
- Lichtenstein AH, Appel LJ, Brands M *et al.* (2006) Diet and lifestyle recommendations revision 2006: A scientific statement from the American Heart Association Nutrition Committee. *Circulation* **114**, 82–96.
- SACN. (2004) *Scientific Advisory Committee on Nutrition – Advice on Fish Consumption: Benefits and Risks*. London: FSA.
- Hooper L, Thompson RL, Harrison RA *et al.* (2006) Risks and benefits of omega 3 fats for mortality, cardiovascular disease, and cancer: Systematic review. *BMJ* **332**, 752–760.
- Kromhout D, Giltay EJ & Geleijnse JM (2010) *n*-3 Fatty acids and cardiovascular events after myocardial infarction. *N Engl J Med* **363**, 2015–2026.
- Filion K, El Khoury F, Bielinski M *et al.* (2010) Omega-3 fatty acids in high-risk cardiovascular patients: A meta-analysis of randomized controlled trials. *BMC Cardiovasc Disord* **10**, 24.
- Wendland E, Farmer A, Glasziou P *et al.* (2006) Effect of alpha linolenic acid on cardiovascular risk markers: A systematic review. *Heart* **92**, 166–169.
- Burdge GC & Calder PC (2005) Conversion of alpha-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod Nutr Dev* **45**, 581–597.
- Gerster H (1998) Can adults adequately convert alpha-linolenic acid (18:3 $n$ -3) to eicosapentaenoic acid (20:5 $n$ -3) and docosahexaenoic acid (22:6 $n$ -3)? *Int J Vitam Nutr Res* **68**, 159–173.
- Konkel A & Schunck W-H (2011) Role of cytochrome P450 enzymes in the bioactivation of polyunsaturated fatty acids. *Biochim Biophys Acta* **1814**, 210–222.
- Hashimoto M, Hossain S, Yamasaki H *et al.* (1999) Effects of eicosapentaenoic acid and docosahexaenoic acid on plasma membrane fluidity of aortic endothelial cells. *Lipids* **34**, 1297–1304.
- Krey G, Braissant O, L'Horsset F *et al.* (1997) Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay. *Mol Endocrinol* **11**, 779–791.
- Weldon SM, Mullen AC, Loscher CE *et al.* (2007) Docosahexaenoic acid induces an anti-inflammatory profile in lipopolysaccharide-stimulated human THP-1 macrophages more effectively than eicosapentaenoic acid. *J Nutr Biochem* **18**, 250–258.
- Caputo M, Zirpoli H, Torino G *et al.* (2011) Selective regulation of UGT1A1 and SREBP-1c mRNA expression by docosahexaenoic, eicosapentaenoic and arachidonic acids. *J Cell Physiol* **226**, 187–193.
- Cullen P (2000) Evidence that triglycerides are an independent coronary heart disease risk factor. *Am J Cardiol* **86**, 943–949.
- Stalenhoef AF & de Graaf J (2008) Association of fasting and nonfasting serum triglycerides with cardiovascular disease and the role of remnant-like lipoproteins and small dense LDL. *Curr Opin Lipidol* **19**, 355–361.
- Eslick GD, Howe PR, Smith C *et al.* (2009) Benefits of fish oil supplementation in hyperlipidemia: A systematic review and meta-analysis. *Int J Cardiol* **136**, 4–16.
- Mattar M & Obeid O (2009) Fish oil and the management of hypertriglyceridemia. *Nutr Health* **20**, 41–49.
- Grimsgaard S, Bonna KH, Hansen JB *et al.* (1997) Highly purified eicosapentaenoic acid and docosahexaenoic acid in humans have similar triacylglycerol-lowering effects but divergent effects on serum fatty acids. *Am. J. Clin. Nutr.* **66**, 649–659.
- Egert S, Kannenberg F, Somoza V *et al.* (2009) Dietary alpha-linolenic acid, EPA, and DHA have differential effects on LDL fatty acid composition but similar effects on serum lipid profiles in normolipidemic humans. *J Nutr* **139**, 861–868.
- Mori TA, Burke V, Puddey IB *et al.* (2000) Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *Am J Clin Nutr* **71**, 1085–1094.
- Rambjor GS, Walen AI, Windsor SL *et al.* (1996) Eicosapentaenoic acid is primarily responsible for hypotriglyceridemic effect of fish oil in humans. *Lipids* **31**, Suppl, S45–S49.



28. Buckley R, Shewring B, Turner R *et al.* (2004) Circulating triacylglycerol and apoE levels in response to EPA and docosahexaenoic acid supplementation in adult human subjects. *Br J Nutr* **92**, 477–483.
29. Park Y & Harris WS (2003) Omega-3 fatty acid supplementation accelerates chylomicron triglyceride clearance. *J Lipid Res* **44**, 455–463.
30. Olano-Martin E, Anil E, Caslake MJ *et al.* (2010) Contribution of apolipoprotein E genotype and docosahexaenoic acid to the LDL-cholesterol response to fish oil. *Atherosclerosis* **209**, 104–110.
31. Woodman RJ, Mori TA, Burke V *et al.* (2002) Effects of purified eicosapentaenoic and docosahexaenoic acids on glycemic control, blood pressure, and serum lipids in type 2 diabetic patients with treated hypertension. *Am J Clin Nutr* **76**, 1007–1015.
32. Nestel P, Shige H, Pomeroy S *et al.* (2002) The *n-3* fatty acids eicosapentaenoic acid and docosahexaenoic acid increase systemic arterial compliance in humans. *Am J Clin Nutr* **76**, 326–330.
33. Holub BJ (2009) Docosahexaenoic acid (DHA) and cardiovascular disease risk factors. *Prostaglandins Leukot Essent Fatty Acids* **81**, 199–204.
34. Theobald HE, Chowienczyk PJ, Whittall R *et al.* (2004) LDL cholesterol-raising effect of low-dose docosahexaenoic acid in middle-aged men and women. *Am J Clin Nutr* **79**, 558–563.
35. Kelley DS, Siegel D, Vemuri M *et al.* (2007) Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men. *Am J Clin Nutr* **86**, 324–333.
36. Geppert J, Kraft V, Demmelmair H *et al.* (2006) Microalgal docosahexaenoic acid decreases plasma triacylglycerol in normolipidaemic vegetarians: A randomised trial. *Br J Nutr* **95**, 779–786.
37. Nelson G, Schmidt P, Bartolini G *et al.* (1997) The effect of dietary docosahexaenoic acid on plasma lipoproteins and tissue fatty acid composition in humans. *Lipids* **32**, 1137–1146.
38. Kondo A, Muranaka Y, Ohta I *et al.* (2001) Relationship between triglyceride concentrations and LDL size evaluated by malondialdehyde-modified LDL. *Clin Chem* **47**, 893–900.
39. Morgan J, Carey C, Lincoff A *et al.* (2004) High-density lipoprotein subfractions and risk of coronary artery disease. *Curr Atheroscler Rep* **6**, 359–365.
40. Chan DC, Watts GF, Nguyen MN *et al.* (2006) Factorial study of the effect of *n-3* fatty acid supplementation and atorvastatin on the kinetics of HDL apolipoproteins A-I and A-II in men with abdominal obesity. *Am J Clin Nutr* **84**, 37–43.
41. Wilkinson P, Leach C, Ah-Sing EE *et al.* (2005) Influence of alpha-linolenic acid and fish-oil on markers of cardiovascular risk in subjects with an atherogenic lipoprotein phenotype. *Atherosclerosis* **181**, 115–124.
42. Agren JJ, Hanninen O, Julkunen A *et al.* (1996) Fish diet, fish oil and docosahexaenoic acid rich oil lower fasting and postprandial plasma lipid levels. *Eur J Clin Nutr* **50**, 765–771.
43. Griffin MD, Sanders TA, Davies IG *et al.* (2006) Effects of altering the ratio of dietary *n-6* to *n-3* fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45–70 y: The OPTILIP Study. *Am J Clin Nutr* **84**, 1290–1298.
44. Nozaki S, Matsuzawa Y, Hirano K *et al.* (1992) Effects of purified eicosapentaenoic acid ethyl ester on plasma lipoproteins in primary hypercholesterolemia. *Int J Vitam Nutr Res* **62**, 256–260.
45. Liao FH, Liou TH, Shieh MJ *et al.* (2010) Effects of different ratios of monounsaturated and polyunsaturated fatty acids to saturated fatty acids on regulating body fat deposition in hamsters. *Nutrition* **26**, 811–817.
46. Harris WS, Miller M, Tighe AP *et al.* (2008) Omega-3 fatty acids and coronary heart disease risk: Clinical and mechanistic perspectives. *Atherosclerosis* **197**, 12–24.
47. Burdge GC, Powell J, Dadd T *et al.* (2009) Acute consumption of fish oil improves postprandial VLDL profiles in healthy men aged 50–65 years. *Br J Nutr* **102**, 160–165.
48. Howell Iii G, Deng X, Yellaturu C *et al.* (2009) *N-3* polyunsaturated fatty acids suppress insulin-induced SREBP-1c transcription via reduced trans-activating capacity of LXR[alpha]. *Biochim Biophys Acta* **1791**, 1190–1196.
49. Kajikawa S, Harada T, Kawashima A *et al.* (2009) Highly purified eicosapentaenoic acid prevents the progression of hepatic steatosis by repressing monounsaturated fatty acid synthesis in high-fat/high-sucrose diet-fed mice. *Prostaglandins Leukot Essent Fatty Acids* **80**, 229–238.
50. Harris WS & Bulchandani D (2006) Why do omega-3 fatty acids lower serum triglycerides? *Curr Opin Lipidol* **17**, 387–393.
51. Chambrier C, Bastard JP, Rieusset J *et al.* (2002) Eicosapentaenoic acid induces mRNA expression of peroxisome proliferator-activated receptor gamma. *Obes Res* **10**, 518–525.
52. Arai T, Kim HJ, Chiba H *et al.* (2009) Interaction of fenofibrate and fish oil in relation to lipid metabolism in mice. *J Atheroscler Thromb* **16**, 283–291.
53. Le Jossic-Corcous C, Gonthier C, Zaghini I *et al.* (2005) Hepatic farnesyl diphosphate synthase expression is suppressed by polyunsaturated fatty acids. *Biochem J* **385**, 787–794.
54. Sone H, Takahashi A, Shimano H *et al.* (2002) HMG-CoA reductase inhibitor decreases small dense low-density lipoprotein and remnant-like particle cholesterol in patients with type-2 diabetes. *Life Sci* **71**, 2403–2412.
55. Pontrelli L, Parris W, Adeli K *et al.* (2002) Atorvastatin treatment beneficially alters the lipoprotein profile and increases low-density lipoprotein particle diameter in patients with combined dyslipidemia and impaired fasting glucose/type 2 diabetes. *Metabolism* **51**, 334–342.
56. Ikewaki K, Terao Y, Ozasa H *et al.* (2009) Effects of atorvastatin on nuclear magnetic resonance-defined lipoprotein subclasses and inflammatory markers in patients with hypercholesterolemia. *J Atheroscler Thromb* **16**, 51–56.
57. Nordoy A (2002) Statins and omega-3 fatty acids in the treatment of dyslipidemia and coronary heart disease. *Minerva Med* **93**, 357–363.
58. Das UN (2008) Essential fatty acids and their metabolites could function as endogenous HMG-CoA reductase and ACE enzyme inhibitors, anti-arrhythmic, anti-hypertensive, anti-atherosclerotic, anti-inflammatory, cytoprotective, and cardioprotective molecules. *Lipids Health Dis* **7**, 37.
59. Turnbull F, Neal B, Ninomiya T *et al.* (2008) Effects of different regimens to lower blood pressure on major cardiovascular events in older and younger adults: Meta-analysis of randomised trials. *BMJ* **336**, 1121–1123.
60. Law MR, Morris JK & Wald NJ (2009) Use of blood pressure lowering drugs in the prevention of cardiovascular disease: Meta-analysis of 147 randomised trials in the context of expectations from prospective epidemiological studies. *BMJ* **338**, b1665.

61. Mori TA (2006) Omega-3 fatty acids and hypertension in humans. *Clin Exp Pharmacol Physiol* **33**, 842–846.
62. Morris MC, Sacks F & Rosner B (1993) Does fish oil lower blood pressure? A meta-analysis of controlled trials. *Circulation* **88**, 523–533.
63. Geleijnse JM, Giltay EJ, Grobbee DE *et al.* (2002) Blood pressure response to fish oil supplementation: Metaregression analysis of randomized trials. *J Hypertens* **20**, 1493–1499.
64. Grimsgaard S, Bonna K, Hansen J *et al.* (1998) Effects of highly purified eicosapentaenoic acid and docosahexaenoic acid on hemodynamics in humans. *Am J Clin Nutr* **68**, 52–59.
65. Theobald HE, Goodall AH, Sattar N *et al.* (2007) Low-dose docosahexaenoic acid lowers diastolic blood pressure in middle-aged men and women. *J Nutr* **137**, 973–978.
66. Pickering TG, Shimbo D & Haas D (2006) Ambulatory blood-pressure monitoring. *N Engl J Med* **354**, 2368–2374.
67. Ohkubo T, Imai Y, Tsuji I *et al.* (1997) Prediction of mortality by ambulatory blood pressure monitoring versus screening blood pressure measurements: A pilot study in Ohasama. *J Hypertens* **15**, 357–364.
68. Staessen JA, Thijs L, Fagard R *et al.* (1999) Predicting cardiovascular risk using conventional vs ambulatory blood pressure in older patients with systolic hypertension. Systolic hypertension in Europe trial investigators. *JAMA* **282**, 539–546.
69. Mori TA, Bao DQ, Burke V *et al.* (1999) Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. *Hypertension* **34**, 253–260.
70. Perret-Guillaume C, Joly L & Benetos A (2009) Heart rate as a risk factor for cardiovascular disease. *Prog Cardiovasc Dis* **52**, 6–10.
71. Mozaffarian D, Geelen A, Brouwer *et al.* (2005) Effect of fish oil on heart rate in humans: A meta-analysis of randomized controlled trials. *Circulation* **112**, 1945–1952.
72. Stark KD & Holub BJ (2004) Differential eicosapentaenoic acid elevations and altered cardiovascular disease risk factor responses after supplementation with docosahexaenoic acid in postmenopausal women receiving and not receiving hormone replacement therapy. *Am J Clin Nutr* **79**, 765–773.
73. McLennan PL (2001) Myocardial membrane fatty acids and the antiarrhythmic actions of dietary fish oil in animal models. *Lipids* **36**, Suppl, S111–S114.
74. Christensen JH, Skou HA, Fog L *et al.* (2001) Marine *n*-3 fatty acids, wine intake, and heart rate variability in patients referred for coronary angiography. *Circulation* **103**, 651–657.
75. Holguin F, Tellez-Rojo MM, Lazo M *et al.* (2005) Cardiac autonomic changes associated with fish oil vs soy oil supplementation in the elderly. *Chest* **127**, 1102–1107.
76. Christensen JH, Christensen MS, Dyerberg J *et al.* (1999) Heart rate variability and fatty acid content of blood cell membranes: A dose-response study with *n*-3 fatty acids. *Am J Clin Nutr* **70**, 331–337.
77. Svensson M, Schmidt EB, Jorgensen KA *et al.* (2007) The effect of *n*-3 fatty acids on heart rate variability in patients treated with chronic hemodialysis. *J Ren Nutr* **17**, 243–249.
78. Geelen A, Zock PL, Swenne CA *et al.* (2003) Effect of *n*-3 fatty acids on heart rate variability and baroreflex sensitivity in middle-aged subjects. *Am Heart J* **146**, E4.
79. Dyerberg J, Eskesen DC, Andersen PW *et al.* (2004) Effects of trans- and *n*-3 unsaturated fatty acids on cardiovascular risk markers in healthy males. An 8 weeks dietary intervention study. *Eur J Clin Nutr* **58**, 1062–1070.
80. Mozaffarian D, Stein PK, Prineas RJ *et al.* (2008) Dietary fish and omega-3 fatty acid consumption and heart rate variability in US adults. *Circulation* **117**, 1130–1137.
81. Xiao YF, Gomez AM, Morgan JP *et al.* (1997) Suppression of voltage-gated L-type Ca<sup>2+</sup> currents by polyunsaturated fatty acids in adult and neonatal rat ventricular myocytes. *Proc Natl Acad Sci USA* **94**, 4182–4187.
82. Li GR, Sun HY, Zhang XH *et al.* (2009) Omega-3 polyunsaturated fatty acids inhibit transient outward and ultra-rapid delayed rectifier K<sup>+</sup> currents and Na<sup>+</sup> current in human atrial myocytes. *Cardiovasc Res* **81**, 286–293.
83. Xiao YF, Sigg DC & Leaf A (2005) The antiarrhythmic effect of *n*-3 polyunsaturated fatty acids: Modulation of cardiac ion channels as a potential mechanism. *J Membr Biol* **206**, 141–154.
84. Kang JX & Leaf A (1996) Evidence that free polyunsaturated fatty acids modify Na<sup>+</sup> channels by directly binding to the channel proteins. *Proc Natl Acad Sci USA* **93**, 3542–3546.
85. Grynberg A, Fournier A, Sergiel JP *et al.* (1995) Effect of docosahexaenoic acid and eicosapentaenoic acid in the phospholipids of rat heart muscle cells on adrenoceptor responsiveness and mechanism. *J Mol Cell Cardiol* **27**, 2507–2520.
86. Hashimoto M, Shinozuka K, Gamoh S *et al.* (1999) The hypotensive effect of docosahexaenoic acid is associated with the enhanced release of ATP from the caudal artery of aged rats. *J Nutr* **129**, 70–76.
87. Nelson MR, Stepanek J, Cevette M *et al.* (2010) Non-invasive measurement of central vascular pressures with arterial tonometry: Clinical revival of the pulse pressure waveform? *Mayo Clin Proc* **85**, 460–472.
88. Millasseau SC, Ritter JM, Takazawa K *et al.* (2006) Contour analysis of the photoplethysmographic pulse measured at the finger. *J Hypertens* **24**, 1449–1456.
89. Mark G & Sanders TA (1994) The influence of different amounts of *n*-3 polyunsaturated fatty acids on bleeding time and in vivo vascular reactivity. *Br J Nutr* **71**, 43–52.
90. Shimokawa H, Aarhus LL & Vanhoutte PM (1988) Dietary omega 3 polyunsaturated fatty acids augment endothelium-dependent relaxation to bradykinin in coronary microvessels of the pig. *Br J Pharmacol* **95**, 1191–1196.
91. Shimokawa H & Vanhoutte PM (1988) Dietary cod-liver oil improves endothelium-dependent responses in hypercholesterolemic and atherosclerotic porcine coronary arteries. *Circulation* **78**, 1421–1430.
92. Leeson CPM, Mann A, Kattenhorn M *et al.* (2002) Relationship between circulating *n*-3 fatty acid concentrations and endothelial function in early adulthood. *Eur Heart J* **23**, 216–222.
93. Chin JP, Gust AP, Nestel PJ *et al.* (1993) Marine oils dose-dependently inhibit vasoconstriction of forearm resistance vessels in humans. *Hypertension* **21**, 22–28.
94. Goodfellow J, Bellamy MF, Ramsey MW *et al.* (2000) Dietary supplementation with marine omega-3 fatty acids improve systemic large artery endothelial function in subjects with hypercholesterolemia. *J Am Coll Cardiol* **35**, 265–270.
95. Khan F, Elherik K, Bolton-Smith C *et al.* (2003) The effects of dietary fatty acid supplementation on endothelial function and vascular tone in healthy subjects. *Cardiovasc Res* **59**, 955–962.
96. McVeigh GE, Brennan GM, Johnston GD *et al.* (1993) Dietary fish oil augments nitric oxide production or release

- in patients with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* **36**, 33–38.
97. Mori TA, Watts GF, Burke V *et al.* (2000) Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. *Circulation* **102**, 1264–1269.
  98. Shah AP, Ichiuji AM, Han JK *et al.* (2007) Cardiovascular and endothelial effects of fish oil supplementation in healthy volunteers. *J Cardiovasc Pharmacol Ther* **12**, 213–219.
  99. Tagawa H, Shimokawa H, Tagawa T *et al.* (1999) Long-term treatment with eicosapentaenoic acid augments both nitric oxide-mediated and non-nitric oxide-mediated endothelium-dependent forearm vasodilatation in patients with coronary artery disease. *J Cardiovasc Pharmacol* **33**, 633–640.
  100. Tagawa T, Hirooka Y, Shimokawa H *et al.* (2002) Long-term treatment with eicosapentaenoic acid improves exercise-induced vasodilation in patients with coronary artery disease. *Hypertens Res* **25**, 823–829.
  101. Walser B, Giordano RM & Stebbins CL (2006) Supplementation with omega-3 polyunsaturated fatty acids augments brachial artery dilation and blood flow during forearm contraction. *Eur J Appl Physiol* **97**, 347–354.
  102. Rizza S, Tesaro M, Cardillo C *et al.* (2009) Fish oil supplementation improves endothelial function in normoglycemic offspring of patients with type 2 diabetes. *Atherosclerosis* **206**, 569–574.
  103. Schiano V, Laurenzano E, Brevetti G *et al.* (2008) Omega-3 polyunsaturated fatty acid in peripheral arterial disease: Effect on lipid pattern, disease severity, inflammation profile, and endothelial function. *Clin Nutr* **27**, 241–247.
  104. De Berrazueta JR, Gómez de Berrazueta JM, Amado Señarís JA *et al.* (2009) A diet enriched with mackerel (*Scomber scombrus*)-derived products improves the endothelial function in a senior population (Prevención de las Enfermedades Cardiovasculares: Estudio Santofña – PECES project). *Eur J Clin Invest* **39**, 165–173.
  105. Morgan DR, Dixon LJ, Hanratty CG *et al.* (2006) Effects of dietary omega-3 fatty acid supplementation on endothelium-dependent vasodilation in patients with chronic heart failure. *Am J Cardiol* **97**, 547–551.
  106. Stirban A, Nandreaan S, Gotting C *et al.* (2010) Effects of n-3 fatty acids on macro- and microvascular function in subjects with type 2 diabetes mellitus. *Am J Clin Nutr* **91**, 808–813.
  107. Wright SA, O'Prey FM, McHenry MT *et al.* (2008) A randomised interventional trial of omega-3-polyunsaturated fatty acids on endothelial function and disease activity in systemic lupus erythematosus. *Ann Rheum Dis* **67**, 841–848.
  108. Okumura T, Fujioka Y, Morimoto S *et al.* (2002) Eicosapentaenoic acid improves endothelial function in hypertriglyceridemic subjects despite increased lipid oxidizability. *Am J Med Sci* **324**, 247–253.
  109. Engler MM, Engler MB, Malloy M *et al.* (2004) Docosahexaenoic acid restores endothelial function in children with hyperlipidemia: Results from the EARLY study. *Int J Clin Pharmacol Ther* **42**, 672–679.
  110. Harris WS, Rambjor GS, Windsor SL *et al.* (1997) n-3 fatty acids and urinary excretion of nitric oxide metabolites in humans. *Am J Clin Nutr* **65**, 459–464.
  111. Hamazaki T, Urakaze M, Sawazaki S *et al.* (1988) Comparison of pulse wave velocity of the aorta between inhabitants of fishing and farming villages in Japan. *Atherosclerosis* **73**, 157–160.
  112. McVeigh GE, Brennan GM, Cohn JN *et al.* (1994) Fish oil improves arterial compliance in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb* **14**, 1425–1429.
  113. Wang S, Ma AQ, Song SW *et al.* (2008) Fish oil supplementation improves large arterial elasticity in overweight hypertensive patients. *Eur J Clin Nutr* **62**, 1426–1431.
  114. Satoh N, Shimatsu A, Kotani K *et al.* (2009) Highly purified eicosapentaenoic acid reduces cardio-ankle vascular index in association with decreased serum amyloid A-LDL in metabolic syndrome. *Hypertens Res* **32**, 1004–1008.
  115. Tomiyama H, Takazawa K, Osa S *et al.* (2005) Do eicosapentaenoic acid supplements attenuate age-related increases in arterial stiffness in patients with dyslipidemia? A preliminary study. *Hypertens Res* **28**, 651–655.
  116. Jonk AM, Houben AJHM, de Jongh RT *et al.* (2007) Microvascular dysfunction in obesity: A potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension. *Physiology* **22**, 252–260.
  117. Antonios TF, Singer DR, Markandu ND *et al.* (1999) Structural skin capillary rarefaction in essential hypertension. *Hypertension* **33**, 998–1001.
  118. Mitasikova M, Smidova S, Macsaliiova A *et al.* (2008) Aged male and female spontaneously hypertensive rats benefit from n-3 polyunsaturated fatty acids supplementation. *Physiol Res* **57**, Suppl 2, S39–S48.
  119. Conde CMS, Cyrino FZGA, Bottino DA *et al.* (2007) Longchain n-3 polyunsaturated fatty acids and microvascular reactivity: Observation in the hamster cheek pouch. *Microvasc Res* **73**, 237–247.
  120. Awan ZA, Wester T & Kvernebo K (2010) Human microvascular imaging: A review of skin and tongue videomicroscopy techniques and analysing variables. *Clin Physiol Funct Imaging* **30**, 79–88.
  121. Yaqoob P (2009) The nutritional significance of lipid rafts. *Annu Rev Nutr* **29**, 257–282.
  122. Li Q, Zhang Q, Wang M *et al.* (2007) Docosahexaenoic acid affects endothelial nitric oxide synthase in caveolae. *Arch Biochem Biophys* **466**, 250–259.
  123. Li Q, Zhang Q, Wang M *et al.* (2007) Eicosapentaenoic acid modifies lipid composition in caveolae and induces translocation of endothelial nitric oxide synthase. *Biochimie* **89**, 169–177.
  124. Hishinuma T, Yamazaki T & Mizugaki M (1999) Effects of long-term supplementation of eicosapentaenoic and docosahexaenoic acid on the 2-, 3-series prostacyclin production by endothelial cells. *Prostaglandins Other Lipid Mediat* **57**, 333–340.
  125. Matsumoto T, Nakayama N, Ishida K *et al.* (2009) Eicosapentaenoic acid improves imbalance between vasodilator and vasoconstrictor actions of endothelium-derived factors in mesenteric arteries from rats at chronic stage of type 2 diabetes. *J Pharmacol Exp Ther* **329**, 324–334.
  126. Engler MB & Engler MM (2000) Docosahexaenoic acid-induced vasorelaxation in hypertensive rats: Mechanisms of action. *Biol Res Nurs* **2**, 85–95.
  127. Singh TU, Kathirvel K, Choudhury S *et al.* (2010) Eicosapentaenoic acid-induced endothelium-dependent and -independent relaxation of sheep pulmonary artery. *Eur J Pharmacol* **636**, 108–113.
  128. Engler MM, Engler MB, Pierson DM *et al.* (2003) Effects of docosahexaenoic acid on vascular pathology and reactivity in hypertension. *Exp Biol Med (Maywood)* **228**, 299–307.
  129. Engler MB, Ma YH & Engler MM (1999) Calcium-mediated mechanisms of eicosapentaenoic acid-induced



- relaxation in hypertensive rat aorta. *Am J Hypertens* **12**, 1225–1235.
130. Conquer JA & Holub BJ (1996) Supplementation with an algae source of docosahexaenoic acid increases (*n*-3) fatty acid status and alters selected risk factors for heart disease in vegetarian subjects. *J Nutr* **126**, 3032–3039.
  131. Ross R (1993) The pathogenesis of atherosclerosis: A perspective for the 1990s. *Nature* **362**, 801–809.
  132. Shaw DI, Hall WL, Jeffs NR *et al.* (2007) Comparative effects of fatty acids on endothelial inflammatory gene expression. *Eur J Nutr* **46**, 321–328.
  133. De Caterina R & Massaro M (2005) Omega-3 fatty acids and the regulation of expression of endothelial pro-atherogenic and pro-inflammatory genes. *J Membr Biol* **206**, 103–116.
  134. De Caterina R, Cybulsky MI, Clinton SK *et al.* (1994) The omega-3 fatty acid docosahexaenoate reduces cytokine-induced expression of proatherogenic and proinflammatory proteins in human endothelial cells. *Arterioscler Thromb* **14**, 1829–1836.
  135. De Caterina R, Cybulsky MA, Clinton SK *et al.* (1995) Omega-3 fatty acids and endothelial leukocyte adhesion molecules. *Prostaglandins Leukot Essent Fatty Acids* **52**, 191–195.
  136. Weber C, Erl W, Pietsch A *et al.* (1995) Docosahexaenoic acid selectively attenuates induction of vascular cell adhesion molecule-1 and subsequent monocytic cell adhesion to human endothelial cells stimulated by tumor necrosis factor- $\alpha$ . *Arterioscler Thromb Vasc Biol* **15**, 622–628.
  137. Goua M, Mulgrew S, Frank J *et al.* (2008) Regulation of adhesion molecule expression in human endothelial and smooth muscle cells by omega-3 fatty acids and conjugated linoleic acids: Involvement of the transcription factor NF- $\kappa$ B? *Prostaglandins Leukot Essent Fatty Acids* **78**, 33–43.
  138. Farzaneh-Far R, Harris WS, Garg S *et al.* (2009) Inverse association of erythrocyte *n*-3 fatty acid levels with inflammatory biomarkers in patients with stable coronary artery disease: The Heart and Soul Study. *Atherosclerosis* **205**, 538–543.
  139. Ferrucci L, Cherubini A, Bandinelli S *et al.* (2006) Relationship of plasma polyunsaturated fatty acids to circulating inflammatory markers. *J Clin Endocrinol Metab* **91**, 439–446.
  140. Perunicic-Pekovic GB, Rasic ZR, Pljesa SI *et al.* (2007) Effect of *n*-3 fatty acids on nutritional status and inflammatory markers in haemodialysis patients. *Nephrology (Carlton)* **12**, 331–336.
  141. Rasic-Milutinovic Z, Perunicic G, Pljesa S *et al.* (2007) Effects of *n*-3 PUFAs supplementation on insulin resistance and inflammatory biomarkers in hemodialysis patients. *Ren Fail* **29**, 321–329.
  142. Damsgaard CT, Lauritzen L, Calder PC *et al.* (2009) Reduced ex vivo interleukin-6 production by dietary fish oil is not modified by linoleic acid intake in healthy men. *J Nutr* **139**, 1410–1414.
  143. Trebble T, Arden NK, Stroud MA *et al.* (2003) Inhibition of tumour necrosis factor- $\alpha$  and interleukin 6 production by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. *Br J Nutr* **90**, 405–412.
  144. Caughey G, Mantzioris E, Gibson R *et al.* (1996) The effect on human tumor necrosis factor alpha and interleukin 1 beta production of diets enriched in *n*-3 fatty acids from vegetable oil or fish oil. *Am J Clin Nutr* **63**, 116–122.
  145. Bowden RG, Wilson RL, Deike E *et al.* (2009) Fish oil supplementation lowers C-reactive protein levels independent of triglyceride reduction in patients with end-stage renal disease. *Nutr Clin Pract* **24**, 508–512.
  146. Bloomer RJ, Larson DE, Fisher-Wellman KH *et al.* (2009) Effect of eicosapentaenoic and docosahexaenoic acid on resting and exercise-induced inflammatory and oxidative stress biomarkers: A randomized, placebo controlled, crossover study. *Lipids Health Dis* **8**, 36.
  147. Yusof HM, Miles EA & Calder P (2008) Influence of very long-chain *n*-3 fatty acids on plasma markers of inflammation in middle-aged men. *Prostaglandins Leukot Essent Fatty Acids* **78**, 219–228.
  148. Fujioka S, Hamazaki K, Itomura M *et al.* (2006) The effects of eicosapentaenoic acid-fortified food on inflammatory markers in healthy subjects – a randomized, placebo-controlled, double-blind study. *J Nutr Sci Vitaminol (Tokyo)* **52**, 261–265.
  149. Burns T, Maciejewski SR, Hamilton WR *et al.* (2007) Effect of omega-3 fatty acid supplementation on the arachidonic acid:eicosapentaenoic acid ratio. *Pharmacotherapy* **27**, 633–638.
  150. Blok WL, Deslypere JP, Demacker PN *et al.* (1997) Pro- and anti-inflammatory cytokines in healthy volunteers fed various doses of fish oil for 1 year. *Eur J Clin Invest* **27**, 1003–1008.
  151. Balk EM, Lichtenstein AH, Chung M *et al.* (2006) Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: A systematic review. *Atherosclerosis* **189**, 19–30.
  152. Mori TA, Woodman RJ, Burke V *et al.* (2003) Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers in treated-hypertensive type 2 diabetic subjects. *Free Radic Biol Med* **35**, 772–781.
  153. Kelley DS, Siegel D, Fedor DM *et al.* (2009) DHA supplementation decreases serum C-reactive protein and other markers of inflammation in hypertriglyceridemic men. *J Nutr* **139**, 495–501.
  154. Calder PC (2008) Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseases. *Mol Nutr Food Res* **52**, 885–897.
  155. Mickleborough TD, Tecklenburg SL, Montgomery GS *et al.* (2009) Eicosapentaenoic acid is more effective than docosahexaenoic acid in inhibiting proinflammatory mediator production and transcription from LPS-induced human asthmatic alveolar macrophage cells. *Clin Nutr* **28**, 71–77.
  156. Gronn M, Christensen E, Hagve TA *et al.* (1991) Peroxisomal retroconversion of docosahexaenoic acid (22:6(*n*-3)) to eicosapentaenoic acid (20:5(*n*-3)) studied in isolated rat liver cells. *Biochim Biophys Acta* **1081**, 85–91.
  157. Tian H, Lu Y, Sherwood AM *et al.* (2009) Resolvins E1 and D1 in choroid-retinal endothelial cells and leukocytes: Biosynthesis and mechanisms of anti-inflammatory actions. *Invest Ophthalmol Visual Sci* **50**, 3613–3620.
  158. Saynor R, Verel D & Gillott T (1984) The long-term effect of dietary supplementation with fish lipid concentrate on serum lipids, bleeding time, platelets and angina. *Atherosclerosis* **50**, 3–10.
  159. Lorenz R, Spengler U, Fischer S *et al.* (1983) Platelet function, thromboxane formation and blood pressure control during supplementation of the Western diet with cod liver oil. *Circulation* **67**, 504–511.
  160. Saynor R & Verel D (1982) Eicosapentaenoic acid, bleeding time, and serum lipids. *Lancet* **320**, 272–272.
  161. Green D, Barreres L, Borensztajn J *et al.* (1985) A double-blind, placebo-controlled trial of fish oil concentrate (MaxEpa) in stroke patients. *Stroke* **16**, 706–709.



162. Hartweg J, Farmer AJ, Holman RR *et al.* (2009) Potential impact of omega-3 treatment on cardiovascular disease in type 2 diabetes. *Curr Opin Lipidol* **20**, 30–38.
163. Siess W, Roth P, Scherer B *et al.* (1980) Platelet-membrane fatty acids, platelet aggregation, and thromboxane formation during a mackerel diet. *Lancet* **1**, 441–444.
164. Mann N, Sinclair A, Pille M *et al.* (1997) The effect of short-term diets rich in fish, red meat, or white meat on thromboxane and prostacyclin synthesis in humans. *Lipids* **32**, 635–644.
165. Mori TA, Beilin LJ, Burke V *et al.* (1997) Interactions between dietary fat, fish, and fish oils and their effects on platelet function in men at risk of cardiovascular disease. *Arterioscler Thromb Vasc Biol* **17**, 279–286.
166. Din JN, Harding SA, Valerio CJ *et al.* (2008) Dietary intervention with oil rich fish reduces platelet-monocyte aggregation in man. *Atherosclerosis* **197**, 290–296.
167. Nomura S, Kanazawa S & Fukuhara S (2003) Effects of eicosapentaenoic acid on platelet activation markers and cell adhesion molecules in hyperlipidemic patients with Type 2 diabetes mellitus. *J Diabetes Complications* **17**, 153–159.
168. Park Y & Harris W (2002) EPA, but not DHA, decreases mean platelet volume in normal subjects. *Lipids* **37**, 941–946.
169. Wojenski CM, Silver MJ & Walker J (1991) Eicosapentaenoic acid ethyl ester as an antithrombotic agent: Comparison to an extract of fish oil. *Biochim Biophys Acta* **1081**, 33–38.
170. Hashimoto M, Hossain S & Shido O (2006) Docosahexaenoic acid but not eicosapentaenoic acid withstands dietary cholesterol-induced decreases in platelet membrane fluidity. *Mol Cell Biochem.* **293**, 1–8.
171. Adan Y, Shibata K, Sato M *et al.* (1999) Effects of docosahexaenoic and eicosapentaenoic acid on lipid metabolism, eicosanoid production, platelet aggregation and atherosclerosis in hypercholesterolemic rats. *Biosci Biotechnol Biochem* **63**, 111–119.
172. Yamada N, Shimizu J, Wada M *et al.* (1998) Changes in platelet aggregation and lipid metabolism in rats given dietary lipids containing different *n*-3 polyunsaturated fatty acids. *J Nutr Sci Vitaminal (Tokyo)* **44**, 279–289.
173. von Schacky C & Weber PC (1985) Metabolism and effects on platelet function of the purified eicosapentaenoic and docosahexaenoic acids in humans. *J Clin Invest* **76**, 2446–2450.
174. Woodman RJ, Mori TA, Burke V *et al.* (2003) Effects of purified eicosapentaenoic acid and docosahexaenoic acid on platelet, fibrinolytic and vascular function in hypertensive type 2 diabetic patients. *Atherosclerosis* **166**, 85–93.
175. Guillot N, Caillet E, Laville M *et al.* (2009) Increasing intakes of the long-chain omega-3 docosahexaenoic acid: Effects on platelet functions and redox status in healthy men. *FASEB J.* **23**, 2909–2916.
176. Robinson JG & Stone NJ (2006) Antiatherosclerotic and antithrombotic effects of omega-3 fatty acids. *Am J Cardiol* **98**, 39–49.
177. Damsgaard CT, Frokiaer H, Andersen AD *et al.* (2008) Fish oil in combination with high or low intakes of linoleic acid lowers plasma triacylglycerols but does not affect other cardiovascular risk markers in healthy men. *J Nutr* **138**, 1061–1066.
178. Sanders TA, Lewis F, Slaughter S *et al.* (2006) Effect of varying the ratio of *n*-6 to *n*-3 fatty acids by increasing the dietary intake of alpha-linolenic acid, eicosapentaenoic and docosahexaenoic acid, or both on fibrinogen and clotting factors VII and XII in persons aged 45–70 y: The OPTILIP study. *Am J Clin Nutr* **84**, 513–522.
179. Elvevoll EO, Barstad H, Breimo ES *et al.* (2006) Enhanced incorporation of *n*-3 fatty acids from fish compared with fish oils. *Lipids* **41**, 1109–1114.
180. Sanders TA, Gleason K, Griffin B *et al.* (2006) Influence of an algal triacylglycerol containing docosahexaenoic acid (22:6*n*-3) and docosapentaenoic acid (22:5*n*-6) on cardiovascular risk factors in healthy men and women. *Br J Nutr* **95**, 525–531.
181. Hartweg J, Farmer A, Holman R *et al.* (2007) Meta-analysis of the effects of *n*/*i*-3 polyunsaturated fatty acids on haematological and thrombogenic factors in type 2 diabetes. *Diabetologia* **50**, 250–258.
182. Krämer HJ, Stevens J, Grimminger F *et al.* (1996) Fish oil fatty acids and human platelets: Dose-dependent decrease in dienoic and increase in trienoic thromboxane generation. *Biochem Pharmacol* **52**, 1211–1217.
183. Mayer K, Merfelds M, Muhly-Reinholz M *et al.* (2002) Omega-3 fatty acids suppress monocyte adhesion to human endothelial cells: Role of endothelial PAF generation. *Am J Physiol Heart Circ Physiol* **283**, H811–818.
184. Yanagisawa N, Shimada K, Miyazaki T *et al.* (2010) Polyunsaturated fatty acid levels of serum and red blood cells in apparently healthy Japanese subjects living in an urban area. *J Atheroscler Thromb* **17**, 285–294.
185. Thorseng T, Witte DR, Vistisen D *et al.* (2009) The association between *n*-3 fatty acids in erythrocyte membranes and insulin resistance: The Inuit Health in Transition Study. *Int J Circumpolar Health* **68**, 327–336.
186. Nigam A, Frasare-Smith N, Lesperance F *et al.* (2009) Relationship between *n*-3 and *n*-6 plasma fatty acid levels and insulin resistance in coronary patients with and without metabolic syndrome. *Nutr Metab Cardiovasc Dis* **19**, 264–270.
187. Lombardo Y, Hein G & Chicco A (2007) Metabolic syndrome: Effects of *n*-3 PUFAs on a model of dyslipidemia, insulin resistance and adiposity. *Lipids* **42**, 427–437.
188. Arai T, Kim HJ, Chiba H *et al.* (2009) Anti-obesity effect of fish oil and fish oil-fenofibrate combination in female KK mice. *J Atheroscler Thromb* **16**, 674–683.
189. Hartweg J, Perera R, Montori V *et al.* (2008) Omega-3 polyunsaturated fatty acids (PUFA) for type 2 diabetes mellitus. *Cochrane Database Syst Rev* **23**, CD003205.
190. Murata M, Kaji H, Iida K *et al.* (2001) Dual action of eicosapentaenoic acid in hepatoma cells. *J Biol Chem* **276**, 31422–31428.
191. Li M, Pittman CU Jr & Li T (2009) Extraction of polyunsaturated fatty acid methyl esters by imidazolium-based ionic liquids containing silver tetrafluoroborate – extraction equilibrium studies. *Talanta* **78**, 1364–1370.
192. Andersen G, Harnack K, Erbersdobler HF *et al.* (2008) Dietary eicosapentaenoic acid and docosahexaenoic acid are more effective than alpha-linolenic acid in improving insulin sensitivity in rats. *Ann Nutr Metab* **52**, 250–256.
193. Shimura T, Miura T, Usami M *et al.* (1997) Docosahexaenoic acid (DHA) improved glucose and lipid metabolism in KK-Ay mice with genetic non-insulin-dependent diabetes mellitus (NIDDM). *Biol Pharm Bull* **20**, 507–510.
194. Nomura S, Inami N, Shouzu A *et al.* (2009) The effects of pitavastatin, eicosapentaenoic acid and combined therapy on platelet-derived microparticles and adiponectin in hyperlipidemic, diabetic patients. *Platelets* **20**, 16–22.
195. Egert S, Fobker M, Andersen G *et al.* (2008) Effects of dietary alpha-linolenic acid, eicosapentaenoic acid or docosahexaenoic acid on parameters of glucose metabolism in healthy volunteers. *Ann Nutr Metab* **53**, 182–187.