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REVIEW

Long-chain n-3 polyunsaturated fatty acids: new insights into mechanisms relating to inflammation and coronary heart disease

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Evidence from observational studies, prospective cohort studies and randomized clinical intervention studies indicate that moderate doses of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) significantly decrease risk of fatal coronary heart disease (CHD). Higher doses and longer duration of intervention may also protect from non-fatal CHD events. The exact mechanisms through which LC n-3 PUFA has an effect on CHD are not well established but may include a decrease in fasting and postprandial triacylglycerol levels, a decrease in arrhythmias, modulation of platelet aggregation and decreased synthesis of pro-inflammatory agents. The mechanistic relation between LC n-3 PUFA and inflammation has attracted great interest, and *in vitro* studies have revealed that these fatty acids decrease endothelial activation, affect eicosanoid metabolism (including epoxygenation pathways) and induce inflammatory resolution. However, the effects of LC n-3 PUFA on established biomarkers of inflammation and endothelial activation *in vivo* are not strong. Consequently we need new and more sensitive and systemic biomarkers to reveal the effects of LC n-3 PUFA on localized inflammatory processes.
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Keywords: fish oil; LC n-3 PUFA; mechanisms; coronary heart disease; inflammation

Abbreviations: 5-HETE, 5-hydroxyeicosatetraenoic acid; 17,18-EEQ, 17(R),18(S)-epoxyeicosaquatraenoic acid; AA, arachidonic acid; ADP, adenosine diphosphate; apoE$^{-/-}$, apolipoprotein E knockout; AUDA, 12-(3-adamantan-1-yl-ureido)-dodecanoic acid; CD-62P, P-selectin; CHD, coronary heart disease; COX, cyclooxygenase; DART, diet and reinfarction trial; DHA, docosahexaenoic acid; DHET, dihydroxyeicosatrienoic acids; EET, epoxyeicosatrienoic acids; EPA, eicosapentaenoic acid; HDL, high-density lipoprotein; hsCRP, (high-sensitivity) C-reactive protein; ICAM-1, intercellular adhesion molecule 1; IL, interleukin; LC-MS/MS, liquid chromatography-tandem mass spectrometry; LC n-3 PUFA, long-chain n-3 polyunsaturated fatty acids; LDL, low-density lipoprotein; LDLR$^{-/-}$, low-density lipoprotein receptor knockout; LOX, lipoxygenase; LTB_4, leukotriene B_4; MALDI-MS, matrix-assisted laser desorption/ionization-mass spectrometry; NF-κB, nuclear factor-κB; PGH_2, prostaglandin H_2; PPAR, peroxisome proliferator-activated receptor; sEH, soluble epoxide hydrolase; SREBP-1c, sterol receptor element binding protein-1c; TNF-α, tumour necrosis factor-α; TxA_2, thromboxane A_2; VCAM-1, vascular cell adhesion molecule-1; VLDL, very low-density lipoprotein

Introduction

n-3 polyunsaturated fatty acids (n-3 PUFA or ω-3 fatty acids) are polyunsaturated fatty acids that have their first double bond at the third position when counted from the methyl end of the molecule. The simplest n-3 PUFA is α-linolenic acid (Figure 1). This fatty acid is an 18-carbon n-3 PUFA with three

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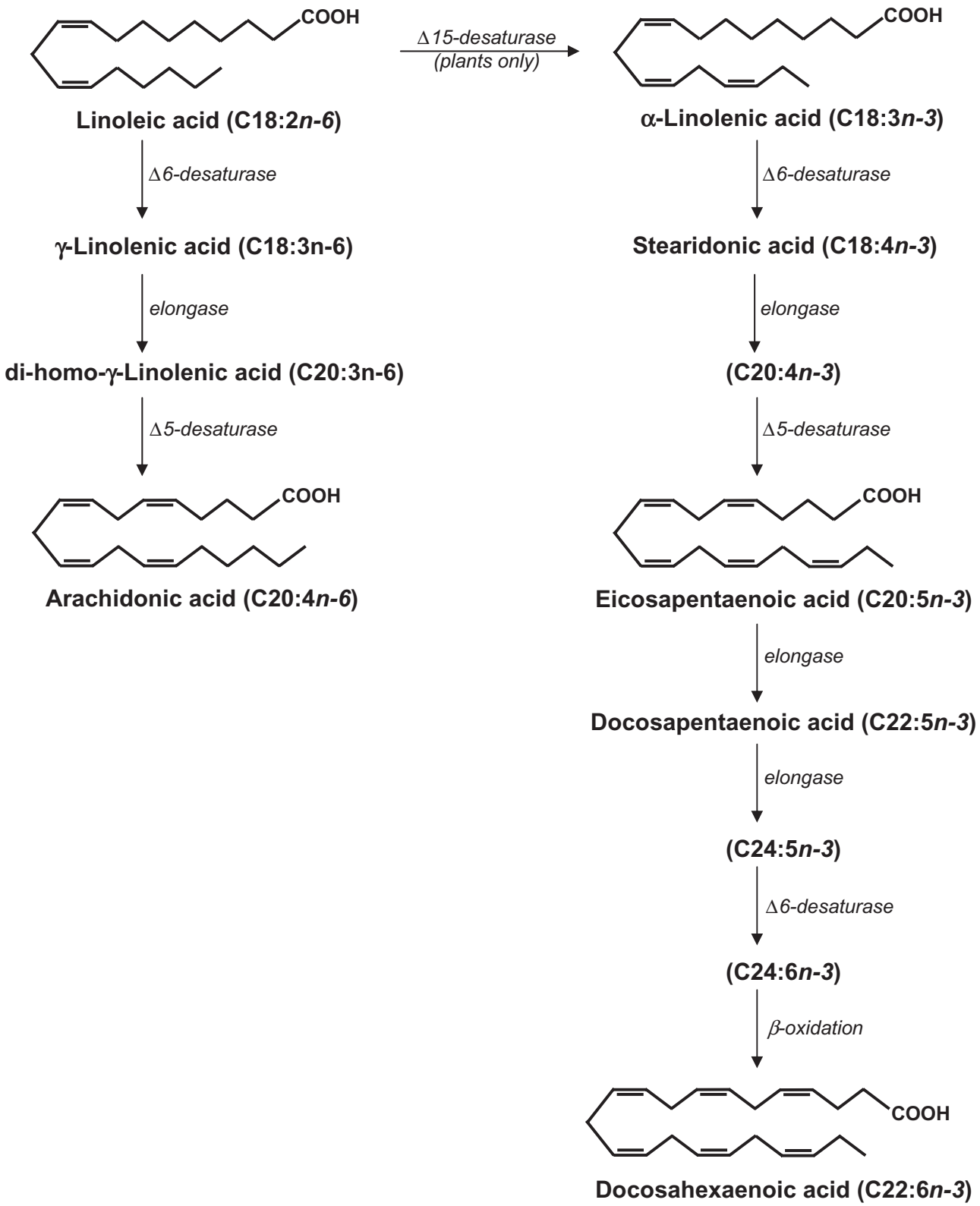

Figure 1 Biosynthesis pathways of n-6 and n-3 polyunsaturated fatty acids.

double bonds (18:3n-3) and its synthesis, as well as the synthesis of other n-3 PUFAs from n-6 fatty acids, requires the presence of the enzyme Δ15-desaturase. However, humans lack this enzyme and need to ensure adequate intake of n-3 PUFA by dietary means for example, through consumption of vegetables and nuts. α-Linolenic acid can however be converted to the longer-chain (LC) n-3 PUFA in the human body. The extent of this conversion is not precisely known and at best very limited (Goyens *et al*., 2005; 2006). Thus most LC n-3 PUFA will also have to be obtained from the diet. These

fatty acids, which predominantly are at least 20-carbon atoms long with five or more double bonds, primarily occur in fish and other seafoods. The main LC n-3 PUFA from these products are eicosapentaenoic acid (EPA; C20:5n-3) and docosahexaenoic acid (DHA: C22:6n-3) (Figure 1). The metabolism of n-3 and n-6 fatty acids is linked as their metabolic pathways compete for the same enzymes (desaturases and elongases). Thus downstream metabolism of linoleic and α-linolenic acid, producing LC derivatives like arachidonic acid (AA; 20:4n-6), EPA (20:5n-3) and DHA (22:6n-3), will influence each other (Figure 1).

LC n-3 PUFA and heart disease: evidence from human studies

In the seventies, Bang and Dyerberg noted that Inuits from Greenland had very low levels of coronary heart disease (CHD). As the intake of fish and LC n-3 PUFA from sea animals in the Inuit population is very high, they suggested that high intake of LC n-3 PUFA may be associated with low prevalence of CHD (Bang *et al.*, 1971; 1976; Dyerberg *et al.*, 1978; Bang and Dyerberg, 1980). Since then many studies have been performed to investigate this association. The Zutphen study was the first prospective cohort study that showed that higher intake of fish was associated with less fatal CHD (Kromhout *et al.*, 1985). Subsequently many observational studies found that higher intakes of fish or fish fatty acids were associated with a lower risk of CHD (Dolecek and Granditis, 1991; Rodriguez *et al.*, 1996; Daviglus *et al.*, 1997; Albert *et al.*, 1998; Yuan *et al.*, 2001; Hu and Willett, 2002; Hu *et al.*, 2002). However, other studies reported no association or even a slightly increased risk of CHD in people with a higher fish intake (Ascherio *et al.*, 1995; Salonen *et al.*, 1995; Pietinen *et al.*, 1997; Osler *et al.*, 2003). High mercury concentrations in fish might explain the higher rates of fatal CHD in subjects with a high fish intake in Finland (Pietinen *et al.*, 1997). A meta-analysis of 13 prospective observational studies indicated that people who ate at least one fish meal per week had approximately a 15% lower risk of dying of CHD compared with people who ate a fish meal less than once a month, and consumption of every additional 20 g of fish per day decreased the risk of fatal CHD by 7% (Whelton *et al.*, 2004).

Studies suggest that LC n-3 PUFA in blood are the component responsible for the suspected protection against fatal heart disease. In three studies, a higher percentage of fish fatty acids in blood was associated with a lower risk of fatal heart disease, sudden death or cardiac arrest (Siscovick *et al.*, 1995; 2000; Albert *et al.*, 1998; 2002). In the Cardiovascular Health Study, higher levels of LC n-3 PUFA in plasma phospholipids were associated with a lower risk of fatal CHD, but not with a lower risk of non-fatal myocardial infarction (Lemaitre *et al.*, 2003). There was also no association between fish intake and risk of non-fatal CHD in the Physicians' Health Study (Ascherio *et al.*, 1995). Thus observational studies suggest that a modest intake of fish is associated with a lower risk of fatal CHD, but not with non-fatal heart disease. However, as with all observational studies, other lifestyle factors may have confounded such associations. Furthermore, the beneficial effects of fish consumption on heart disease may depend on the type of fish consumed. Consumption of tuna or other broiled or baked fish two times per week or more was associated with lower risk of fatal ischaemic heart disease compared with consumption of less than once per month. However, intake of fried fish or fish sandwiches was not associated with a lower risk, but with trends towards higher risks (Mozaffarian *et al.*, 2003).

By 2008 five randomized clinical intervention trials have been published on the direct effects of ω-3 fatty acid consumption on fatal CHD. The first GISSI-Prevenzione trial, a randomized open label study in 11 324 Italian patients with a recent myocardial infarction, demonstrated that patients had a 15% lower combined risk of mortality, non-fatal myocardial infarction and stroke upon supplementation for 3.5 years with 850 mg·day^{-1} of LC n-3 PUFA. The relative risk of cardiovascular death was also decreased by 30% and of sudden death by 45%. These effects were already evident just months after randomization. Additionally, the beneficial effects were seen in a population already protected with recommended secondary prevention that included anti-platelet therapy with acetylsalicylic acid (aspirin) and consumption of a 'Mediterranean diet'. The GISSI-Prevenzione trial demonstrated that a significant protective effect could be obtained with doses much lower than those previously considered necessary for significant beneficial effects (GISSI Investigators, 1999). The second GISSI-HF study, a randomized double-blind placebo-controlled trial in 6975 Italian patients with chronic heart failure, revealed a moderate decrease in both all-cause mortality and admissions to hospital for cardiovascular reasons upon supplementation for on average 3.9 years with 1 g of LC n-3 PUFA daily. Again, the beneficial effects were seen in a population already treated with recommended therapies (Gissi-Hf, 2008). The diet and reinfarction trial 1 (DART) was carried out in 2033 English men with a recent myocardial infarction. Men who received advice to increase their fish intake to at least two meals per week, compared with no advice, had a 29% lower mortality rate during the 2 year follow-up (Burr *et al.*, 1989). In contrast, the second DART trial of Burr *et al.* (Burr *et al.*, 2003), in 3114 Welsh patients with stable angina without a myocardial infarction, showed no beneficial effect of n-3 PUFA intake during 9 year follow-up. In this trial, advice to eat fatty fish did not lower mortality, and intake of fish oil supplements was associated with a higher risk of cardiac and sudden death (Burr *et al.*, 2003). However, methodological problems may have affected the outcome as compliance to advice was only shown for a subsample of patients. Unfortunately, both participants and providers were not masked, which implies that the intake of fish oil may have modified the behaviour of both patients and physicians towards intake of medication or diet and lifestyle. Furthermore, recruitment for DART-2 was interrupted for 1 year because of funding problems (Burr *et al.*, 2005). The JELIS trial was performed in 18 645 Japanese men and women with hypercholesterolaemia treated with statins. Supplementation with 1.8 g EPA per day decreased major coronary events by 19% over 4.6 years. Non-fatal coronary events, rather than CHD death, were decreased (Yokoyama *et al.*, 2007). This is consistent with the very low death rates from CHD in Japan because of a high background seafood consumption

(Mozaffarian, 2007; 2008). In summary, a modest intake of LC n-3 PUFA significantly decreases risk of fatal CHD risk. However, higher doses and longer duration of intervention, as reported for the JELIS study, has the potential to protect from non-fatal CHD events (Mozaffarian, 2008).

In 2006, two systematic reviews were published, assessing all available evidence from fish oil intervention studies. Hooper *et al*. (2006), based their review on 48 randomized clinical trials and 41 cohort studies, concluded that long-chain and shorter-chain ω-3 fats did not clearly affect total mortality, combined cardiovascular events or cancer. However, two main points of criticism on their approach were: (i) the pooling of results of studies addressing α-linolenic acid with reports on LC n-3 PUFA from fish and epidemiological evidence of protective effects from α-linolenic acid is not very convincing; and (ii) combination of fatal and non-fatal cardiovascular events (Geleijnse *et al*., 2006). In addition, their overall conclusion on LC n-3 PUFA from fish was quite heavily influenced by the results from the DART-2 study, which was troubled by methodological problems (Burr *et al*., 2003). Removal of DART-2 from the meta-analysis resulted in a protective effect for LC n-3 PUFA (Hooper *et al*., 2006). Indeed, several earlier meta-analyses have shown a protective effect of fish intake on stroke and on fatal CHD (Bucher *et al*., 2002; He *et al*., 2004; Whelton *et al*., 2004). The second review by Wang *et al*. (2006), based on 14 randomized clinical trials, 25 prospective cohort studies and 7 case–control studies, concluded that increased consumption of LC n-3 PUFA from fish or fish oil supplements, but not of α-linolenic acid, decreases rates of all-cause mortality, cardiac and sudden death, and possibly stroke. In addition, the benefits of fish oil were stronger in secondary compared with primary prevention settings, and adverse effects appeared to be minor (Wang *et al*., 2006).

Mechanisms of LC n-3 PUFA

The exact mechanisms through which LC n-3 PUFA influence CHD are not well established, but appear to include a decrease in fasting and postprandial triacylglycerol, decreased atherosclerosis, a decrease in arrhythmias, modulation of platelet aggregation and decreased synthesis of pro-inflammatory agents (de Roos *et al*., 2005b) (Table 1). These favourable

Table 1 Overview of pathways and mechanisms through which long-chain n-3 polyunsaturated fatty acids could affect cardiovascular outcome

Pathways	*Mechanisms*	
↓ Plasma triacylglycerol	↓ Triacylglycerol production	↓ SREBP-1c activity
		↓ VLDL assembly and secretion
		↓ Activation of PPAR-α
		↓ β-Oxidation in mitochondria and peroxisomes
↓ Arrhythmias	Arrhythmogenesis	Action potential shortening
	No effect on ventricular tachyarrhythmia	
	No effect on spontaneous atrial fibrillation	
	Prevention of post-operative atrial fibrillation	
	↓ Heart rate	Better ventricular efficiency
↑ Platelet function	↓ Production of the pro-aggregatory TxA_2	
	Antagonists of the TxA_2/PGH_2 receptor *in vitro*	
↓ Inflammation	↓ Endothelial activation	↓ Expression of ICAM-1, VCAM-1 *in vitro only*
		↓ IL-6, IL-8 *in vitro only*
		↓ E-selectin *in vitro only*
		↓ NF-κB *in vitro only*
		↓↑ Production of TNF-α, IL-1 or IL-6 by activated mononuclear cells
	Changes in eicosanoid metabolism	↓ Production of PGE_2 by inflammatory cells *ex vivo*
		↓ TxB_2 by inflammatory cells *ex vivo*
		↓ LTB_4 by inflammatory cells *ex vivo*
		↓ 5-HETE by inflammatory cells *ex vivo*
		↓ LTE_4 by inflammatory cells *ex vivo*
		↓ COX-2 expression and activity
	Changes in epoxygenation pathways	↓ Protein levels of soluble epoxide hydrolase
		↑ Production of epoxy-EPA and DHA derivatives
↑ Inflammatory resolution	↓ Production of pro-inflammatory eicosanoids	
	↑ Production of lipoxins and aspirin-triggered lipoxins from AA	
	↑ Production of E-series resolvins from EPA	Protects against pro-inflammatory gene expression
		Blocks transendothelial migration of polymorphonuclear leukocytes
		↓ Leukocyte rolling
		↓ Platelet aggregation
		↓ Neutrophil infiltration
	↑ Production of D-series resolvins from DHA	↓ Neutrophil infiltration
	↑ Production of protectins from DHA	↓ Transmigration of neutrophils

AA, arachidonic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HETE, hydroxyeicosatetraenoic acid; ICAM, intercellular adhesion molecule; IL, interleukin; LT, leukotriene; NF-κB, nuclear factor-κB; PG, prostaglandin; PPAR-α: peroxisome proliferator-activated receptor-α; SREBP-1c, sterol receptor element binding protein-1c; TNF-α, tumour necrosis factor-α; Tx, thromboxane; VCAM, vascular cell adhesion molecule; VLDL, very low-density lipoprotein.

effects have been primarily attributed to EPA, which is present in large amounts in fish oil. However, controlled studies in humans now demonstrate that DHA has equally important anti-arrhythmic and anti-atherogenic effects, although it is often present in lower amounts in oily fish and fish oil supplements (Mori and Woodman, 2006).

Effects on triacylglycerol levels

Triacylglycerol-lowering properties are among the best-established *in vivo* actions of ω-3 fatty acids. LC n-3 PUFA decrease very low-density lipoprotein (VLDL) assembly and secretion, resulting in diminished triacylglycerol production, through a decreased sterol receptor element binding protein-1c activity (Jump and Clarke 1999; Davidson, 2006; Jump, 2008). Pharmacokinetic data obtained from obese male subjects with dyslipidaemia show that 4 g of fish oil per day decrease the production of VLDL apolipoprotein B, without a change in the pool size of low-density lipoprotein (LDL) apolipoprotein B, and also with no change in the fractional catabolic rate of VLDL apolipoprotein B (Chan *et al.*, 2002). Although kinetic studies fail to show an effect of LC n-3 PUFA on VLDL clearance, VLDL particles enriched in LC n-3 PUFA are more susceptible to *in vitro* lipase-mediated conversion to LDL than controls (Lu *et al.*, 1999; Park *et al.*, 2004). Indeed, a rise in LDL seen upon fish oil supplementation results directly from the conversion of VLDL into LDL particles in obese male subjects (Chan *et al.*, 2002). In addition, LC n-3 PUFA increase β-oxidation of other fatty acids in mitochondria and peroxisomes (Jump and Clarke 1999; Harris and Bulchandani, 2006; Jump, 2008), possibly through activation of peroxisome proliferator-activated receptor (PPAR)-α (Nakamura *et al.*, 2004; Davidson, 2006). This occurs despite the evidence that LC n-3 PUFA are weak PPAR agonists (Krey *et al.*, 1997; Jump, 2008).

Clinical trials clearly suggest beneficial effects of LC n-3 PUFA consumption on plasma triacylglycerol levels. A review of placebo-controlled human studies concludes that an average intake of 3–4 $g \cdot day^{-1}$ of LC n-3 PUFA decreases serum triacylglycerol concentrations by 25–30% in a dose-dependent manner. The same intake does not affect total cholesterol, but increases LDL concentrations by 5–10% and high-density lipoprotein (HDL) by 1–3% (Harris, 1997). The increase in LDL is mainly through a rise in amounts of the larger, more buoyant and potentially less atherogenic LDL particles, whereas the smaller, more dense and potentially more atherogenic LDL particles decrease (Calabresi *et al.*, 2000; Stalenhoef *et al.*, 2000; Durrington *et al.*, 2001). Based on the triacylglycerol-lowering effects of LC n-3 PUFA, the American Food and Drug Administration has approved a prescription form of LC n-3 PUFA fatty acids. Lovaza™ (former Omacor) is prescribed as an adjunct to appropriate diet for the treatment of very high triacylglycerol levels ($\geq$5.65 $mmol \cdot L^{-1}$ or $\geq$500 $mg \cdot dL^{-1}$) in adults. Each 1 g capsule of Lovaza™ contains approximately 465 mg EPA ethyl esters and 375 mg DHA ethyl esters. Clinical trials have shown that administration of 4 $g \cdot day^{-1}$ of Lovaza™ results in a decrease in triacylglycerol levels of 30–50% (Harris *et al.*, 1997; Pownall *et al.*, 1999; Stalenhoef *et al.*, 2000; Bays, 2006). In addition, administration of Lovaza™ does not affect the efficacy of statins (McKenney *et al.*, 2006). In patients with combined hyperlipidaemia, co-administration of Lovaza™ with statins was a safe and effective means of lowering serum triacylglycerol, despite the persistent high triacylglycerol levels when the patients received statins alone (Durrington *et al.*, 2001; Davidson *et al.*, 2007). Currently, also many types of non-prescription dietary supplements of LC n-3 PUFA are available; however, their efficacy, quality and safety are uncertain as they are not regulated by the same standards as pharmaceutical agents.

As the dose of LC n-3 PUFA required to achieve a lowering in triacylglycerol concentrations is higher than the doses required for a decrease in mortality from CHD, it is questionable whether the triacylglycerol-lowering effects of LC n-3 PUFA could affect CHD mortality. Thus, the major mechanisms underlying the beneficial effects of LC n-3 PUFA appear to be different, or in addition to, the effects on lowering plasma triacylglycerol concentrations (Deckelbaum *et al.*, 2008). Indeed, the GISSI and JELIS trial discussed above showed only minor changes in plasma triacylglycerol levels (GISSI Investigators, 1999; Yokoyama *et al.*, 2007).

Effects on atherosclerosis

Dietary intake of LC n-3 PUFA may have anti-atherosclerotic potential as supplementation with fish oil concentrate (6 $g \cdot day^{-1}$ for 3 months and then 3 $g \cdot day^{-1}$ for 21 months) in 223 patients with angiographically proven coronary artery disease modestly mitigated the course of coronary atherosclerosis (von Schacky *et al.*, 1999). However, 12 randomized trials on the effect of fish oil on restenosis of carotid arteries after percutaneous transluminal coronary angioplasty or coronary artery bypass graft showed equivocal effects (Balk *et al.*, 2006). Additionally, the only randomized intervention study on the effects of fish oil versus placebo on intima media thickness and the media of the carotid artery indicated worsening rather than improvement upon fish oil supplementation (Angerer *et al.*, 2002). In animals, effects of physiological doses of LC n-3 PUFA on atherosclerosis development are again not convincing. One study assessing the effects of a low-fat high-cholesterol diet containing 1% ($w \cdot w^{-1}$) fish oil for 14 weeks in male apolipoprotein E knockout ($apoE^{-/-}$) mice did not find an effect on atherogenesis, but this study lacked an appropriate control group (Xu *et al.*, 2007). In a second study, 1% ($w \cdot w^{-1}$) dietary fish oil, compared with 1% ($w \cdot w^{-1}$) corn oil or an un-supplemented diet for 20 weeks, did not affect the size of atherosclerotic plaque in female $apoE^{-/-}$ mice, nor did it affect plasma lipids (Zampolli *et al.*, 2006). However, the background diet in this study was a low-fat diet without added cholesterol, whereas a high-fat high-cholesterol diet may be required to exacerbate lesions and reveal any inhibitory effects of dietary compounds on atherogenesis. This is especially the case in the low-density lipoprotein receptor knockout ($LDLR^{-/-}$) mouse model of diet-dependent diseases, characterized by high plasma cholesterol levels when fed a high-fat high-cholesterol diet (Breslow, 1996). Indeed, a high-fat high-cholesterol diet supplemented with low doses of fish oil (1% $w \cdot w^{-1}$) did inhibit atherosclerosis development in a study with $LDLR^{-/-}$ mice after 20 weeks of intervention (Zampolli *et al.*, 2006). Much higher doses of dietary fish oil are causing a more consistent decrease in atherosclerosis

development in both animal models. Wang *et al*. (Wang *et al*., 2004) found that a high-fat diet containing 20% ($w \cdot w^{-1}$) dietary fish oil, but no added cholesterol, after 10 weeks of intervention decreased atherosclerotic plaque size development in male $apoE^{-/-}$ mice compared with a high-fat diet containing 20% corn oil. Similarly, Casos *et al*. (2008) found that a low-fat high-cholesterol diet containing 5% ($w \cdot w^{-1}$) fish oil, compared with a diet containing 5% ($w \cdot w^{-1}$) corn oil, decreased atherosclerotic plaque development after 8, 12 and 20 weeks of intervention in male $apoE^{-/-}$ mice. In female $LDLR^{-/-}$ mice, a high-fat high-cholesterol diet containing 5% ($w \cdot w^{-1}$) menhaden oil, compared with 5% ($w \cdot w^{-1}$) olive oil, also decreased atherosclerotic lesion area (Saraswathi *et al*., 2007). Furthermore, a high-fat high-cholesterol diet containing 5% ($w \cdot w^{-1}$) EPA ethyl ester, compared with no EPA, for 12 weeks decreased plaque size in both male $apoE^{-/-}$ mice and $LDLR^{-/-}$ mice (Matsumoto *et al*., 2008). However, such high doses of fish oil or EPA intake would not be feasible, or indeed advisable, in humans. It should be noted that both models of murine atherosclerosis are far more aggressive than human disease, as disease develops in a few months rather than over a few decades. Thus any ability of dietary component to ameliorate disease would indicate an impressive anti-atherogenic capacity.

Effects on arrhythmias

Long-chain n-3 PUFA could decrease sudden death (Siscovick *et al*., 1995; GISSI Investigators, 1999; Albert *et al*., 1998; 2002;) through prohibiting cardiac arrhythmia. Animal models show that n-3 PUFA can reduce susceptibility to arrhythmia (McLennan *et al*., 1988; Billman *et al*., 1994; Leaf and Kang, 1996; Billman *et al*., 1999). Studies in dogs with ligated coronary arteries showed that intravenous infusion of n-3 PUFA prevented ventricular arrhythmia after an exercise programme (Billman *et al*., 1997; Billman *et al*., 1999). Dietary intake of fish oil also prevented ventricular fibrillation after electrophysiological stimulation in rats and marmoset monkeys (McLennan *et al*., 1988; 1993; McLennan, 1993). Furthermore, in cultured cardiomyocytes n-3 PUFA changed the conductance of ion channels in the membrane and thereby prevent occurrence of arrhythmia (Leaf *et al*., 1999). Thus, evidence from both *in vivo* and *in vitro* studies suggests that LC n-3 PUFA may decrease arrhythmias (Brouwer *et al*., 2006a; von Schacky, 2008). The majority of acute sudden deaths are caused by ventricular tachyarrhythmia (Huikuri *et al*., 2001), and three trials have used patients with implantable cardioverter defibrillators to test whether LC n-3 PUFA from fish oil can prevent ventricular tachycardia and ventricular fibrillation. These trials, however, did not show a strong protective effect of fish oil on life-threatening ventricular arrhythmia (Leaf *et al*., 2005; Raitt *et al*., 2005; Brouwer *et al*., 2006c). Similar to the lack of effects of LC n-3 PUFA on ventricular arrhythmias, three cross-sectional studies (Mozaffarian *et al*., 2004; Frost and Vestergaard, 2005; Brouwer *et al*., 2006b) did also not detect a consistent association between intake of fish fatty acids and spontaneous atrial fibrillation, the most common form of sustained arrhythmias. However, one experimental study (Calo *et al*., 2005) suggests that fish oil could prevent post-operative atrial fibrillation.

Increased heart rate is another independent risk factor for sudden death (Jouven *et al*., 2001), and LC n-3 PUFA from fish decrease heart rate (Geelen *et al*., 2005; Mozaffarian *et al*., 2005). In a cohort of 5073 men and women consumption of fish rich in LC n-3 PUFA was not only associated with lower heart rate, but also with lower systemic vascular resistance and a greater stroke volume. The reduction in heart rate may have been the result of better ventricular efficiency (Mozaffarian *et al*., 2006). Nevertheless the underlying mechanisms of the effects of LC n-3 PUFA on heart rate and sudden death are largely unknown. Animal and cell studies, as well as human studies, suggest that the underlying disease might determine whether or not fish oil can protect against fatal heart disease (Verkerk *et al*., 2006; Coronel *et al*., 2007; den Ruijter *et al*., 2007). Fish oil may be harmful in patients vulnerable to life-threatening arrhythmia based on re-entry, whereas it is probably protective in patients with a prior myocardial infarction. In patients with prior myocardial infarction and heart failure, arrhythmias may have been based on triggered activity and prolonged action potentials (den Ruijter *et al*., 2007; 2008). These are the type of arrhythmias that are likely to be prevented by fish oil (Baartscheer *et al*., 2003; den Ruijter *et al*., 2007).

Effects on platelet function

Long-chain n-3 PUFA may decrease the risk of atherothrombosis by affecting platelet aggregation and haemostasis. The anti-thrombotic properties of EPA and DHA have been attributed to the incorporation into platelet phospholipids at the expense of the n-6 PUFA, such as AA (Smith, 2005). An important set of pathways clearly influenced by changes in the n-3/n-6 ratio are those for synthesis of eicosanoids. These include the cyclooxygenase (COX), lipoxygenase (LOX) and P450 epoxygenase pathways (Smith, 2005), for which EPA and DHA compete with AA as a substrate, inhibiting the production of the pro-aggregatory thromboxane A_2 (TxA_2) originating from AA (Figure 2). Indeed, the production of TxA_2 from platelets stimulated by a variety of agonists decreased by between 60% and 80% after fatty acid supplementation both *in vitro* and *in vivo* (Siess *et al*., 1980; Goodnight, 1986; Kristensen *et al*., 1989; Weber, 1989; Christensen *et al*., 1997). Different biological effects of EPA and DHA are supported by experimental evidence. For example, incorporation of DHA into human platelets produced greater inhibition of platelet aggregation than either EPA alone or a combination of EPA and DHA (Croset and Lagarde, 1986). DHA was also more potent than EPA in inhibiting platelet TxA_2 synthesis (Kramer *et al*., 1996). In addition DHA, and to some extent EPA, act as antagonists of the TxA_2/prostaglandin H_2 receptor in human platelets, thereby blocking the activation of platelets through the AA pathway (Swann *et al*., 1989).

The effects of LC n-3 PUFA on haemostatic function and thrombogenesis *in vivo* are however unclear. Results of measurements of platelet aggregation after dietary LC n-3 PUFA intervention have yielded inconsistent findings, partly because of differences in study design, the source and quantity of these fatty acids given, and methodology (Mori *et al*., 1997; Hornstra, 2001). Relatively high doses of LC n-3 PUFA

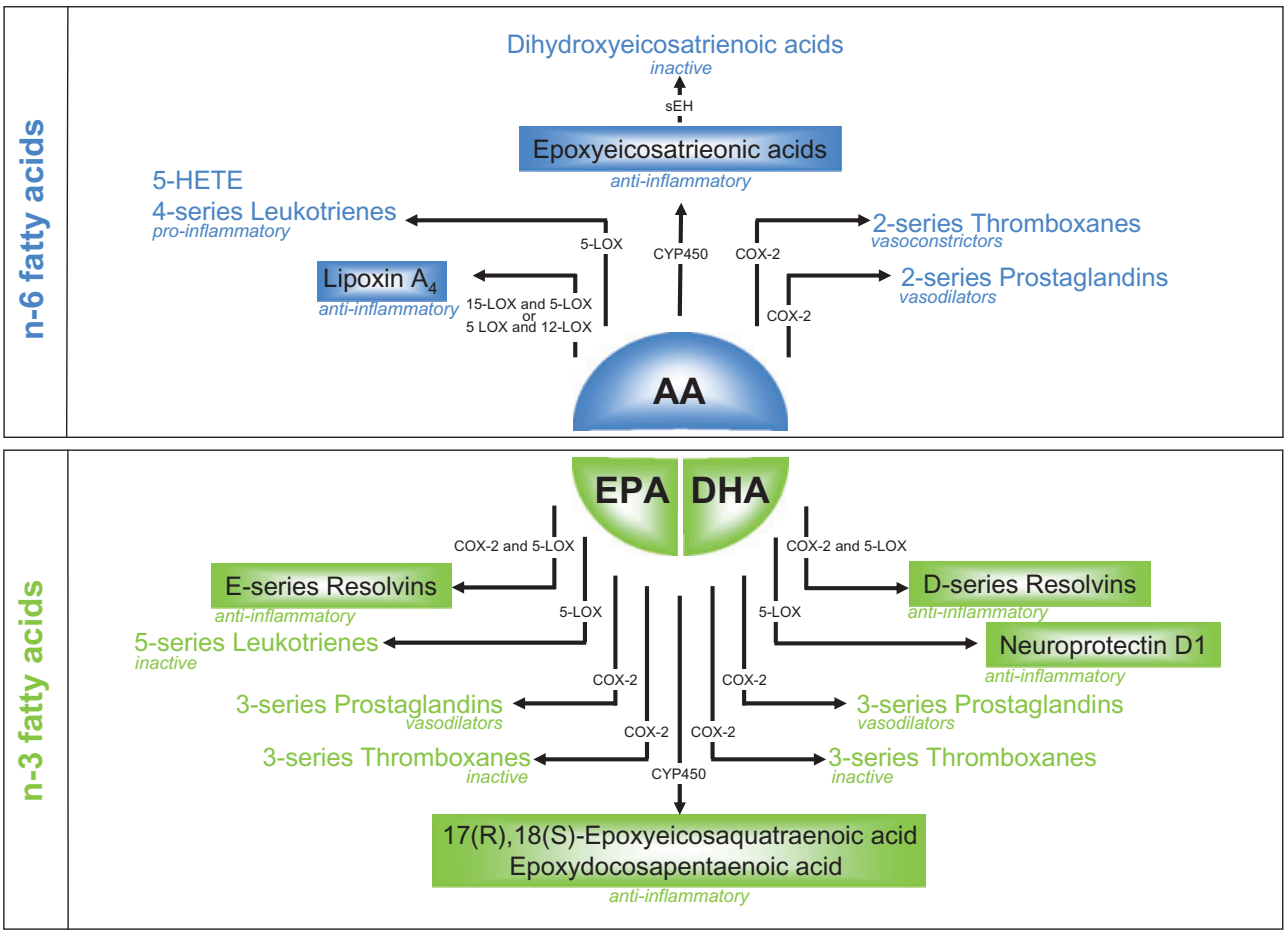

Figure 2 Pathways in eicosanoid metabolism leading to the generation of pro- and anti-inflammatory, or inactive eicosanoids, from arachidonic acid (an n-6 fatty acid – in blue) and eicosapentaenoic acid and docosahexaenoic acid (n-3 fatty acids – in green). 5-HETE, 5-hydroxyeicosatetraenoic acid; AA, arachidonic acid; COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LOX, lipoxygenase; sEH, soluble epoxide hydrolase.

inhibited both platelet aggregation and platelet thromboxane release in response to collagen and adenosine diphosphate (ADP) (Hirai *et al.*, 1980; Brox *et al.*, 1983; Kristensen *et al.*, 1989), and inhibited platelet aggregation by thrombin (Ahmed and Holub, 1984), and by adrenalin (Kristensen *et al.*, 1987), or platelet-activating factor (Codde *et al.*, 1987). In order to achieve significant reductions in platelet aggregation, doses of 3 g·day^{-1} appear to be necessary. At such high doses, lengthening of the bleeding time is also seen (Hornstra, 2001).

An emerging method to assess platelet function is to measure the change in markers of activation of the pro-coagulant processes after intervention. Large-scale studies are now increasingly turning to the technique to provide platelet-specific, consistent data, which are less susceptible to handling conditions than platelet aggregation techniques. The most widely used and validated markers are CD-62P (P-selectin) and the active conformation of the glycoprotein IIb/IIIa complex. In addition, future studies should not only examine 'functional indices' of platelet behaviour such as platelet aggregation, but also measures which are capable of showing the underlying changes induced in platelets after supplementation. For example, 3 g·day^{-1} of LC n-3 PUFA decreased the expression of platelet surface proteins important in supporting platelet–platelet and platelet–leukocyte interactions and tethering of the platelet to blood vessel walls (Vanschoonbeek *et al.*, 2004). In addition, our group recently found that consumption of a moderate amount of fish oil (1 g·day^{-1}) caused significant changes in levels of 65 platelet proteins (related to platelet structure, inflammation and thrombosis) in healthy volunteers after 3 weeks of intervention, without affecting platelet aggregation (B. de Roos, unpubl. results), indicating that changes in platelet function do indeed occur at lower dosage levels.

LC n-3 PUFA and inflammation: novel mechanisms *in vitro* and *in vivo*

The mechanistic connection between LC n-3 PUFA and inflammation has attracted great interest. This is mainly due to the multiple involvement of inflammatory processes, especially at the site of the vascular endothelium, in the development and progression of atherosclerosis (Ross, 1993; 1999). Inflammation is now recognized as a prominent process in

the development of atherosclerosis and CHD. Instigation of inflammation may well provide the link between hyperlipidaemia and atherogenesis (Libby, 2002). LC n-3 PUFA are believed to affect inflammatory processes mainly through two mechanistic pathways: endothelial activation and changes in eicosanoid production, or a combination of the two.

Endothelial activation

A number of inflammatory compounds are involved in vascular activation and atherogenesis. Various exogenous triggers for example, bacterial endotoxin (or lipopolysaccharide), can directly activate monocytes and macrophages. Activation results in the production and secretion of cytokines such as interleukin (IL)-1β and tumour necrosis factor-α (TNF-α) and other inflammatory mediators. This also results in the induction of adhesion molecule expression on the surface of endothelial cells and leukocytes. Adhesion molecules, such as vascular cell adhesion molecule-1 (VCAM-1), are involved in monocyte binding to the vascular endothelium (Libby, 2002). VCAM-1 is expressed by endothelial cells in response to for example cholesterol feeding, especially in areas of the vasculature prone to lesion formation (Cybulsky and Gimbrone, 1991). In addition to VCAM-1, P-selectin and E-selectin also contribute to leukocyte recruitment in atherosclerosis-susceptible mice (Dong *et al.*, 1998). Transcriptional activation of VCAM-1 is, in part, regulated by nuclear factor-κB (NF-κB), upon exposure to oxidized lipoproteins or to the pro-inflammatory cytokines in endothelial cells (Collins and Cybulsky, 2001). The action of inflammatory cytokines, which initiate a cascade of inflammatory mediators, thus amplifies the initial inflammatory signal (Calder, 2006).

In vitro studies have shown that LC n-3 PUFA, and especially DHA, beneficially affect endothelial activation by decreasing monocyte and leukocyte rolling and adhesion by inhibiting expression of intercellular adhesion molecule (ICAM)-1, VCAM-1, IL-6, IL-8 and E-selectin. These functions are partially attributed to the inhibitory effects of DHA and EPA on the transcription factor NF-κB (De Caterina *et al.*, 1994; 1998; 2000; Weber *et al.*, 1995; Khalfoun *et al.*, 1996). In humans, however, the effects of LC n–3 fatty acid consumption on biomarkers of inflammation are not convincing. A majority of intervention studies found no significant effect of fish oil supplementation or fish consumption on levels of plasma endothelial activation markers such as soluble adhesion molecules (s-ICAM, s-VCAM) and soluble P-selectin, pro-inflammatory cytokines (IL-1β, IL-6, IL-10), or the classic inflammation marker C-reactive protein (hsCRP), in healthy or diseased subjects (Thies *et al.*, 2001; Grundt *et al.*, 2003; Mori *et al.*, 2003; Geelen *et al.*, 2004; Jellema *et al.*, 2004; Vega-Lopez *et al.*, 2004; Seierstad *et al.*, 2005; Fujioka *et al.*, 2006; Lee *et al.*, 2006; Browning *et al.*, 2007; Kabir *et al.*, 2007; Schiano *et al.*, 2008; Yusof *et al.*, 2008). A few studies report lowered plasma levels of s-VCAM-1 and IL-6 (Seierstad *et al.*, 2005), TNF-α and IL-β1 (Accini *et al.*, 2006), or VCAM-1 and E-selectin (Thies *et al.*, 2001) upon consumption of LC n-3 PUFA. One study reports that ω-3 fatty acid supplementation in elderly men at risk of CHD increased VCAM (Berstad *et al.*, 2003). The same group also reported that the same amount of LC n-3 PUFA in elderly men with hyperlipidaemia decreased levels of plasma s-ICAM (Hjerkinn *et al.*, 2005). In addition, supplementation with LC n-3 PUFA decreased the production of TNF-α, IL-1 or IL-6 by activated mononuclear cells in some studies (Endres *et al.*, 1989; Meydani *et al.*, 1991; Abbate *et al.*, 1996; Caughey *et al.*, 1996; Kelley *et al.*, 1999; Mori *et al.*, 2003; Trebble *et al.*, 2003), but other work using a wide range of supplementation doses failed to show such anti-inflammatory effects (Molvig *et al.*, 1991; Cooper *et al.*, 1993; Cannon *et al.*, 1995; Schmidt *et al.*, 1996; Blok *et al.*, 1997; Yaqoob *et al.*, 2000; Thies *et al.*, 2001; Kew *et al.*, 2003; Wallace *et al.*, 2003; Kew *et al.*, 2004; Miles *et al.*, 2004). The discrepancy between the *in vivo* effects of LC n-3 PUFA are not clear, but may involve technical factors like treatment, dose, study design and choice of research subjects (Calder, 2001; de Roos *et al.*, 2005b). Supplementation dose is, however, not related consistently to differential outcomes (Calder, 2006).

Eicosanoid metabolism

Eicosanoid metabolism is likely to play a key role in the anti-inflammatory effects of LC n-3 PUFA. Eicosanoids include prostaglandins, thromboxanes and leukotrienes that are key mediators of inflammatory responses (Figure 2). AA is the substrate for production of series 2-prostaglandins and thromboxanes and series 4-leukotrienes. When AA is substituted by LC n-3 PUFA – mainly EPA, the resulting eicosanoids are series 3-prostaglandins and thromboxanes and series 5-leukotrienes (Capdevila *et al.*, 1981). EPA actually can, in contrast to DHA, function as a substrate for COX-1 and 5-LOX (Needleman *et al.*, 1979; Lee *et al.*, 1984; 1985). Due to the smaller size of the substrate binding site of COX-1 compared with COX-2, the 22-carbon DHA can however be metabolized by COX-2 (Serhan *et al.*, 2002; Massaro *et al.*, 2006). The products of n-3 PUFA may be beneficial as they do not induce the same level of inflammation as those derived from AA. In humans, supplementation with fish oil has resulted in decreased production of prostaglandin E_2 (PGE_2), thromboxane B_2, leukotriene B_4, 5-hydroxyeicosatetraenoic acid and leukotriene E_4 by inflammatory cells *ex vivo* (Lee *et al.*, 1985; Endres *et al.*, 1989; Meydani *et al.*, 1991; von Schacky *et al.*, 1993; Sperling *et al.*, 1993; Caughey *et al.*, 1996; Kelley *et al.*, 1999; Trebble *et al.*, 2003). *In vitro*, DHA decreased COX-2 activity and expression in human endothelial cells (Massaro *et al.*, 2006) at concentrations of free fatty acids compatible with those found in healthy volunteers before and after intervention with fish oil (1.5–20 $\mu mol \cdot L^{-1}$) (Sublette *et al.*, 2007; den Ruijter *et al.*, 2008). The importance of this finding lies in the implication of COX-2 activation in inflammation (Pairet and Engelhardt, 1996), atherosclerotic plaque growth (Burleigh *et al.*, 2002) and stability (Thies *et al.*, 2003). The decreased COX-2 activity and expression was regulated through a decreased IL-1α-mediated NF-κB activation and an inhibition of p65 NF-κB subunit nuclear translocation (Massaro *et al.*, 2006). However, the beneficial effects of LC n-3 fatty acids may not exclusively be mediated through eicosanoid production. For example, DHA exerts greater anti-inflammatory effects than EPA in the vascular endothelium. Being the direct precursor of eicosanoids EPA would be expected to have greater effects than DHA if eicosanoids played an important role. Also, these anti-inflammatory

effects of fish oils are not altered by a COX blocker, eliminating an exclusive role of for example prostaglandins (De Caterina *et al.*, 2000).

Long-chain n-3 PUFA could influence eicosanoid metabolism also via a different pathway. Preliminary data from our laboratory indicate that dietary fish oil directly affects hepatic levels of the enzyme soluble epoxide hydrolase (sEH), which regulates availability and metabolism of the cardioprotective and anti-inflammatory epoxyeicosatrienoic acids (EET) (de Roos *et al.*, 2005a; Spector and Norris, 2007). EET are synthesized by the oxygenation of AA by cytochrome P450 NADPH-dependent epoxygenases. There are four regio-isomers of EET: 5,6-, 8,9-, 11,12- and 14,15-EET. EET inhibit expression of VCAM-1, ICAM-1 and E-selectin through an NF-κB-related mechanism (Node *et al.*, 1999). sEH is responsible for the hydrolysis of the bioactive EETs to the less biologically active dihydroxyeicosatrienoic acids DHET (Spector *et al.*, 2004). sEH is a therapeutic target for acute inflammation, thus the development of potent and stable inhibitors of sEH has attracted significant interest in recent years. The conversion of epoxides to their corresponding diols is blocked by sEH inhibitors by which the cardioprotective effects of EETs are better maintained (Yu *et al.*, 2000; Imig *et al.*, 2002; Zhao *et al.*, 2004). A number of studies have provided evidence for the potential anti-inflammatory properties of sEH inhibitors. For example, the sEH inhibitor 12-(3-adamantan-1-yl-ureido)-dodecanoic acid (AUDA) decreased plasma levels of pro-inflammatory cytokines and nitric oxide in mice after endotoxin exposure. At the same time inflammatory resolution was enhanced through increased formation of lipotoxins (Schmelzer *et al.*, 2005). After treatment of endothelial cells with AUDA, EET increased PPAR-γ transcription activity, which suppresses NF-κB-mediated inflammatory responses (Liu *et al.*, 2005). Co-administration of AUDA and anti-inflammatory drugs decreased levels of the pro-inflammatory prostaglandin D_2 and PGE_2 to a greater extent than the sum of individual treatments. Furthermore, AUDA alone is more effective than anti-inflammatory drugs in decreasing levels of PGE_2 in mice (Schmelzer *et al.*, 2006). Therefore, regulation of sEH by fish oil could affect EET levels, thereby affecting inflammatory responses and eventually development of atherosclerosis. Such effects may be mediated through EPA, DHA or both. We recently found dietary fish oil (2% $w \cdot w^{-1}$), but not dietary DHA alone (2% $w \cdot w^{-1}$) decreased hepatic sEH protein levels over 10 weeks in $apoE^{-/-}$ mice, suggesting decreased degradation of anti-inflammatory EET (Y. Mavrommatis *et al.*, unpubl. data).

In addition to a likely direct effect on sEH protein levels, dietary fish oil may also indirectly affect the epoxygenation pathway through substitution of AA in the phospholipids membranes. Epoxy-EPA and epoxy-DHA derivatives have bioactive properties, where the main EPA epoxide is 17(R),18(S)-epoxyeicosaquatraenoic acid (17,18-EEQ) and the main DHA epoxide is epoxydocosapentaenoic acids. Both 17,18-EEQ and epoxydocosapentaenoic acids induce vasodilation and decrease blood pressure with greater potency than EET (Lauterbach *et al.*, 2002; Lu *et al.*, 2002; Ye *et al.*, 2002), and they may be responsible for some of the functional effects that n-3 fatty acids possess, either directly or by competing for CYP epoxygenases and decreasing availability of EET (Spector and Norris, 2007). Despite the important roles of EET analogs from EPA and DHA on blood pressure, their role in inflammation remains to be elucidated.

Another novel approach to the protective effects of LC n-3 PUFA against inflammatory pathways is based on the process of inflammatory resolution. At the site of inflammation, peripheral blood neutrophils initially release pro-inflammatory mediators, such as prostaglandins and leukotrienes, which activate and amply inflammation. But with time, sometimes within hours, PGE_2 and prostaglandin D_2 gradually cause a switch to resolution of inflammation through removal of leukocytes and cellular debris from an inflamed site, and production of the anti-inflammatory and pro-resolution lipoxins, resolvins and protectins (Serhan *et al.*, 2008). This allows re-establishment of homeostasis and prevents inflammation from spreading, becoming chronic and resulting in disease. The potent anti-inflammatory and pro-resolving lipoxins and aspirin-triggered lipoxins are derived from the n-6 fatty acid AA, emphasizing that n-6 fatty acids are not just a precursor of pro-inflammatory eicosanoids (Serhan and Chiang, 2008). Indeed, endogenous aspirin-triggered lipoxins may offer new and potentially important mechanisms that underlie the clinical benefits of aspirin (Serhan *et al.*, 2008). Resolvins are derived from EPA (E-series) and DHA (D-series) and are biologically active in low concentrations *in vitro* and *in vivo* (Serhan *et al.*, 2000; 2002; Hong *et al.*, 2003; Marcheselli *et al.*, 2003). Indeed, significant amounts of resolvin E1, resolvin D3 occurred in colonic tissue, in addition to a reduction in inflammation and tissue injury in colitis, in fat-1 mice that express the humanized *fat-1* gene from *Caenorhabditis elegans*. This gene allows them to endogenously produce n-3 fatty acids from the n-6 type (Hudert *et al.*, 2006). This contrasts with the biological activity of the oxygenated products from LC n-3 PUFA, like thromboxanes and prostaglandins, which are either devoid of bioactivity or far less potent than their AA counterparts (Figure 2). Resolvin E1 protects against excessive pro-inflammatory gene expression in humans and blocks transendothelial migration of polymorphonuclear leukocytes, an integral step of atherogenesis (Serhan *et al.*, 2000; Arita *et al.*, 2005). Additionally, resolving E1 decreases leukocyte rolling by approximately 40% and inhibits platelet aggregation induced by ADP or thromboxane receptor agonist U46619. These anti-aggregatory effects were not present in collagen stimulated platelets, or when stimulated with DHA lipid mediators, suggesting a fatty acid- and agonist-specific anti-platelet action (Dona *et al.*, 2008). Resolvin E2 also has potent anti-inflammatory action, mainly through decreased neutrophil infiltration (Tjonahen *et al.*, 2006). Similarly to series E resolvins, D-series resolvins originating from DHA exert important anti-inflammatory action mainly by blocking neutrophil infiltration (Serhan *et al.*, 2002). DHA also serves as substrate for the synthesis of protectins. Although there are a number of protectin isomers derived from DHA, protectin D1 is the most potent isomer and it is biosynthesized via a LOX-mediated pathway (Hong *et al.*, 2003). Its principal activity is to decrease transmigration of neutrophils and synthetic protectin D1 decreases polymorphonuclear leukocytes infiltration *in vivo* (Hong *et al.*, 2003), and at 10 $nmol \cdot L^{-1}$ it decreases human neutrophil transmigration by approximately 50% *in vitro* (Serhan *et al.*, 2002; 2006). Aspirin impinges on the endogenous production of lipoxins

and resolvins. Its well-known ability to acetylate COX-2 and thereby inhibit the production of pro-inflammatory prostaglandins does not affect the generation of monohydroxy fatty acid species. Aspirin therefore can trigger the production of aspirin triggered lipoxins, as well as the production of the 18R-hydroxyeicosapentenoic acid precursor of resolvin E1. Because resolvin E1 is rapidly inactivated to 18-oxo-resolvin E1, synthetic resolvin analogues have already been synthesized and licensed for clinical development, and these pro-resolving agonists of resolution may be a useful approach to treat many diseases characterized by uncontrolled inflammation (Serhan and Chiang, 2008). The assessment of the impact of resolvin E1 production on human health so far is hampered by the difficulty of measuring the compound in human plasma, although one paper states to have detected resolvin E1 in plasma from six donors plasma of individuals taking aspirin and EPA (Arita *et al.*, 2005). In addition, the generation of these agents is aspirin-dependent and is therefore unlikely to be physiologically relevant for dietary treatment only.

Conclusion

A large amount of new evidence from *in vitro* studies indicates that LC n-3 PUFA significantly affect mechanisms relating to inflammatory processes, such as endothelial activation, modification of eicosanoid metabolism – including epoxygenation pathways, and inflammatory resolution. Because inflammatory processes play such an important role in the development and progression of atherosclerosis, and thus myocardial infarction, it is not surprising to find that fish oil supplementation caused significant protective effects on mortality, non-fatal myocardial infarction and stroke, as well as cardiovascular and sudden death, in the GISSI-Prevenzione trial (GISSI Investigators, 1999). It may also explain why in the GISSI heart failure study the beneficial effects on mortality were much less pronounced (Gissi-Hf, 2008). Anti-inflammatory dietary compounds like fish oil may simply not be effective to any further extent when more permanent damage has been inflicted, like in heart failure patients.

However, it has so far been very difficult to provide evidence for the anti-inflammatory effects LC n-3 PUFA in humans *in vivo*. LC n-3 PUFA do not appear to affect the most extensively studied clinical marker of inflammation, CRP. There is, however, considerable debate regarding the usefulness of CRP as a risk biomarker of CHD and its potential causal role in atherogenesis. Various epidemiological studies have revealed a consistent, robust and significant association between increased serum or plasma CRP levels and the risk of future cardiovascular events (Scirica and Morrow, 2006). Nevertheless, the additional discriminative ability of elevated CRP beyond traditional risk predictors has been minimal (Danesh *et al.*, 2004), partly because it correlates well with known risk factors such as smoking, low levels of HDL cholesterol and obesity (Lowe, 2005; Miller *et al.*, 2005). Furthermore, CRP, an acute-phase reactant produced by the liver upon stimulation by IL-6, is a non-specific indicator of inflammation and thus may not directly participate in atherogenesis (Scirica and Morrow, 2006). Multiple *in vitro* and *in vivo* animal studies have identified several potential pro-inflammatory mechanisms by which CRP may promote atherosclerosis (Scirica and Morrow, 2006). However, in most of the initial studies, the recombinant CRP that was used was contaminated with bacterial lipopolysaccharide and the preservative azide, which are potent pro-inflammatory compounds themselves. Studies that used either local preparations of recombinant CRP or specific techniques to purify commercial CRP have not produced similar inflammatory reactions (Scirica and Morrow, 2006).

As well as CRP, LC n-3 PUFA also do not appear to affect plasma levels of soluble markers of endothelial activation and cytokines. On the other hand, such systemic markers may simply not enable the detection of a local inflammatory response, either in healthy or diseased subjects. Therefore, we may need to develop more specific and sensitive biomarkers to reveal similar or alternative pathways that are affected by dietary fatty acids. For that we will need to take into account that effects instigated by dietary intervention often produce relatively subtle effects (de Roos *et al.*, 2008a). In one of our recent dietary intervention trials we assessed the effects of daily fish oil supplements on the serum proteome, using 2D, matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Serum levels of apolipoprotein A_1, apolipoprotein L_1, zinc-α-2-glycoprotein, haptoglobin precursor, α-1-anti-trypsin precursor, anti-thrombin III-like protein, serum amyloid P component and haemopexin were all down-regulated by fish oil supplementation. In addition, the decrease in serum apolipoprotein A_1 was associated with a significant shift towards the larger, more cholesterol-rich, HDL_2 particles. The alterations in serum proteins and HDL size imply that fish oil activates anti-inflammatory and lipid-modulating mechanisms believed to impede the early onset of CHD. These proteins are potential diagnostic markers to assess the mechanisms whereby fish oils protect against CHD in humans (de Roos *et al.*, 2008b). Thus, determining changes in the plasma proteome upon dietary intervention offers the opportunity to systematically search for proteins that might be biomarkers of chronic diseases, and which may be altered by such treatments. Similarly, the lipidome could provide valuable insights into how fish oil consumption affects levels of bioactive lipid mediators that either are pro-inflammatory, or help to establish resolution of inflammation.

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Conflict of interest

None.

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