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Chronic and degenerative diseases Similarities and differences between the effects of EPA and DHA on markers of atherosclerosis in human subjects*

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We have reviewed effects of long chain (LC) *n*-3 PUFA on markers of atherosclerosis in human subjects with a focus on individual effects of EPA and DHA. Initial results from epidemiological studies suggested that LC *n*-3 PUFA from fish oils (FO) reduced incidence of CVD; those results have been confirmed in interventional studies. Dietary intervention with *n*-3 PUFA decreased fasting and postprandial TAG, number of remnant-like chylomicron particles, large VLDL, and total and small dense LDL particles. It increased mean size of LDL particles by increasing number of large and decreasing those of small dense particles. With some exceptions, *n*-3 PUFA decreased blood pressure (BP) and heart rate (HR), flow-mediated dilation (FMD) and plasma concentrations of inflammatory markers. *n*-3 PUFA also decreased circulating adhesion molecules and intima-media thickness (IMT) in some but not other studies. For IMT, results varied with the sex and artery being examined. EPA effects on FMD are endothelial cell dependent, while those of DHA seem to be endothelial cell independent. Individually, both EPA and DHA decreased TAG and inflammatory markers, but only DHA decreased HR, BP and number of small dense LDL particles. Results varied because of dose and duration of *n*-3 PUFA, EPA:DHA, health status of subjects and other reasons. Future studies are needed to determine optimal doses of EPA and DHA individually, their synergistic, additive or antagonistic effects, and to understand underlying mechanisms. In conclusion, *n*-3 PUFA decreased several risk factors for atherosclerosis without any serious adverse effects.

TAG: LDL size and numbers: Intima-media thickness: Blood pressure

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Atherosclerosis comes from the Greek words athero (meaning gruel or paste) and sclerosis (hardness). It is a common disorder of the arteries in which fat and other substances build up in and on the walls of arteries and form hard structures called plaques[1]. The plaques can make the artery narrow and less flexible, making it harder for blood to flow. If the coronary arteries become narrow, blood flow to the heart can slow down or stop. This can cause chest pain (stable angina), shortness of breath, heart attack and other symptoms. Pieces of plaque can break off and move through the bloodstream (embolisation). This is a common cause of heart attack and stroke. Blood clots can also form around a tear (fissure) in the plaque and block blood flow. If the clot moves into an artery in the heart, lungs or brain, it can cause a stroke, heart attack or pulmonary embolism[2]. Eighty million American adults (approximately one in three) have CVD[3]. CVD is the number one cause of deaths in the US accounting for 34 % of all deaths or 2400 deaths each day. It is a major public health problem with an annual economic loss of $400 billion[3].

Abbreviations: ALA, α-linolenic acid; BP, blood pressure; CAD, coronary artery disease; CRP, C-reactive protein; FMD, flow-mediated dilation; FO, fish oil; HDL-C, HDL cholesterol; HR, heart rate; ICAM, intracellular adhesion molecule; IMT, intima-media thickness; LC, long chain; LDL-C, LDL cholesterol; VCAM, vascular adhesion molecule.

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The arterial wall comprises three layers. Adventitia, the outermost layer carries blood and nerve supply to the artery itself. Media, the middle layer, comprises smooth muscle cells and controls vascular tone. Intima, the innermost or basement membrane, is covered by a single layer of endothelial cells which have adhesion molecules (intracellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1)) on their surface to which the immune cells can stick. It is the site of atherosclerosis and it regulates haemostasis, thrombosis, vascular tone and permeability. Atherosclerosis process starts within the walls of the artery and not in the lumen[4]. It is a heterogeneous disease, and does not uniformly affect all blood vessels[5].

Oxidation of LDL by free radicals, particularly reactive oxygen species initiates atherosclerosis. When oxidised LDL comes in contact with an artery wall, it alters the permeability of the arterial wall and penetrates under the endothelial cells. The body's immune system responds by sending specialised leucocytes (monocytes and T-lymphocytes) to repair the damage to the arterial wall by attaching to the adherence molecules expressed on the surface of endothelial cells. Next these cells pass under the endothelial cell layer where monocytes absorb the oxidised-LDL and are transformed into specialised foam cells. Unfortunately, these leucocytes are not able to process the oxidised-LDL, and ultimately grow then rupture, depositing a greater amount of oxidised cholesterol into the arterial wall. This triggers more leucocytes, and the cycle continues. Eventually, the artery becomes inflamed. The growth factors released from monocytes and T-cells cause the smooth muscle cells to enlarge and form a hard cover over the affected area. This hard cover is what causes a narrowing of the artery, reduces the blood flow and increases blood pressure (BP).

Atherosclerosis starts at very early ages and progresses with age. There are six stages in the development of atherosclerosis[4]. The first four stages (foam cells, fatty streak, extracellular fatty streak and lipid core) are asymptomatic for decades and are compensated by artery enlargement. The last two stages (lipid core embedded in fibrosis, plaque rupture leading to thrombosis and haemorrhage) cause angina, heart attack and stroke. The atheromatous plaques, though long compensated for by artery enlargement, eventually lead to plaque ruptures and clot formation inside the artery lumen at the site of ruptures. The clots heal and usually shrink but leave behind stenosis (narrowing) or complete closure of the artery which leads to an insufficient blood supply to the tissues and organs it feeds. If the compensating artery enlargement process is excessive, it results in aneurysm.

Multiple factors including dyslipidaemia, increased oxidative stress, inflammation, endothelial dysfunction, plaque rupture, age, sex and smoking contribute to the pathogenesis of atherosclerosis[2]. Increased LDL cholesterol (LDL-C), TAG and the number of small dense LDL and chylomicron particles and low HDL cholesterol (HDL-C) are atherogenic. Endothelial dysfunction induced by LDL peroxidation is an initial step in atherosclerosis. Increased oxidative stress increases lipid peroxidation and inflammation. Macrophages and endothelial cells that have been modified by the oxidised LDL release a variety of inflammatory substances, cytokines and growth factors. Plasma concentration of markers of systemic inflammation are directly associated with the risk of atherosclerosis[6,7].

n-3 PUFA and CVD

First indications suggesting the improvement of cardiovascular health by *n*-3 PUFA came from epidemiological studies. Sinclair noted low incidence of CHD mortality rate in Greenland Eskimos, who consumed a high-fat diet, but rich in *n*-3 PUFA[8]. Similar conclusions were drawn based on the comparisons of CVD between the Greenland Inuits and Danish population or the Japanese and North Americans populations[9–12]. Other studies have reported an inverse association between dietary intake of *n*-3 PUFA or whole blood *n*-3 PUFA or adipose tissue PUFA and CVD [13–17].

n-3 PUFA can regulate atherosclerosis by modulating plasma concentrations of blood lipids, inflammation and adhesion molecules, lipid peroxidation, plaque formation and stability, platelet aggregation, thrombosis, BP and heart rate (HR). There have been a number of published reports regarding the effects of fish oils (FO) on risk factors for atherosclerosis; these studies have been the topics of a number of recent reviews[18–21]. We therefore will only summarise the findings with FO and discuss studies with EPA (20:5 *n*-3) and DHA (22:6 *n*-3) in greater detail; studies with FO will be discussed only for risk factors where there are no or limited number of studies with EPA and DHA individually. α-Linolenic acid (18:3 *n*-3, ALA) from flaxseed oil and walnuts has been reported to mimic several effects of EPA and DHA, but has been determined to be less potent than the long chain (LC) *n*-3 PUFA; therefore, the effects of ALA on atherosclerosis will not be discussed here.

DHA and not EPA is the major LC *n*-3 PUFA in human tissues. DHA concentration in brain, retina and reproductive organs is greater than 20 wt% of total fatty acids; its concentration in human heart is approximately 5·1 wt% and EPA is 0·5 wt%[22]. Mice heart contains about 13 wt% DHA, while EPA is not detectable[23]. Human erythrocyte membrane phospholipids contain 3 wt% DHA and less than 0·5 wt% EPA when not supplemented with FO[24]. Thus, most human tissues contain several fold more DHA than EPA. When an animal is put on an *n*-3 PUFA-free diet, the body specifically retains DHA not EPA. Human beings can readily retroconvert DHA to EPA, but the elongation of EPA to DHA is minimal[25]. In men and post-menopausal women, conversion of ALA to EPA is less than 5% and only negligent to DHA[26]. While both EPA and DHA have important roles, the earlier facts indicate that adequate supply of DHA may be able to meet the needs for both EPA and DHA, while EPA cannot meet the need for DHA.

Long chain *n*-3 PUFA and blood lipids

Effects of DHA on blood lipids and other risk factors for CVD have recently been reviewed[27,28]. Dyslipidaemia,

specifically hypertriglyceridaemia, increase in the number of small dense LDL particles and a low HDL-C level have been viewed as major contributors to atherogenic phenotype. In addition to these three factors, increase in remnant cholesterol particles and non-HDL-C (VLDL, intermediate-DL and LDL) also contributes to the atherogenicity of lipoproteins. The cardioprotective effects of FO are partially attributed to their TAG-lowering and the lipoprotein particle size altering actions; their effects on total cholesterol levels are weak.

There are more than fifty studies regarding the effects of FO on fasting TAG and cholesterol, about twenty-four studies with EPA and DHA individually and eight studies compared the effects of EPA and DHA esters[27,28]. With some exceptions, overall results from these studies indicate that FO, EPA and DHA decreased fasting and postprandial TAG 15–30% depending upon the dose and duration of *n*-3 PUFA supplementation and the level of hypertriglycerdaemia. One recent study compared the effects of low (0·85 g/d) and high (3·4 g/d) doses of EPA + DHA on blood lipids in healthy moderately hypertriglyceridaemic subjects; TAG concentration was decreased by 27% by the high dose, and only 10% by the lower dose that did not attain statistical significance[29]. Both EPA and DHA seem to have similar potency in lowering fasting TAG, with some exceptions in which DHA was reported to be more effective than EPA[30,31] or vice versa[32]. Limited number of studies examined the effect of *n*-3 PUFA on postprandial TAG, but again the results were consistent in showing similar decreases, except one study in which EPA (4 g/d) caused only 19% decrease in the postprandial lipaemia, while an equivalent amount of DHA caused a 49% decrease[33].

For most studies, FO did not alter total cholesterol, but increased HDL-C by 5–10% and LDL-C by 8–17%[34]. This increase in LDL-C and HDL-C seems to result from a change in the distribution of the LDL-C and HDL-C among different sub-fractions. FO increased the numbers of large HDL and/or LDL particles, and decreased the number of small dense LDL particles in several studies[35–39]. Limited number of studies directly compared the effects of DHA and EPA on plasma concentrations of HDL-C and LDL-C. Results from these studies suggest that DHA and not EPA may be responsible for raising both the HDL-C and LDL-C[30–40]. These effects of DHA and EPA can be supported by their effects on the distribution of HDL and LDL particles among different subfractions and the increase in mean particle sizes. Seven studies investigated the effects of DHA supplementation on HDL, LDL and VLDL particle sizes and all reported an increase in LDL or HDL and mean particle size or a decrease in VLDL particle size[31,32,41–45]. Out of the four studies with EPA, none reported an increase in LDL particle size but one reported an increase in HDL particle size[31,32,41,46]. Thus, it seems that DHA and not EPA increases LDL particle sizes.

Plasma remnants such as particle-C or remnant lipoprotein-C are considered a novel risk factor for CVD. They are produced from VLDL and chylomicron and are the major atherogenic lipoproteins that can be taken up by macrophages without oxidative modification to form foam cells[47]. They increase atherosclerosis through several mechanisms, including formation of foam cells, increasing inflammation, prothrombotic effects, impairing endothelial cell functions and causing endothelial precursor cell senescence. Both EPA and DHA decreased remnant-like chylomicron particles-C in separate studies[48,49]. Overall, both EPA and DHA decreased fasting as well as postprandial TAG and remnant-like chylomicron particles-C, but did not affect total cholesterol. Only DHA increased HDL-C and LDL-C, and the number of large LDL and HDL particles. DHA also decreased the number of small dense LDL particles that are atherogenic. Both large LDL and HDL particles are cardioprotective. These findings suggest that lipid profile in subjects taking DHA supplements may be healthier than those taking EPA supplements.

n-3 PUFA and endothelial cell functions

A disturbance in endothelial cell functions is a key event in the development of atherosclerosis. Endothelial cell functions can be monitored by measuring: (1) regulation of endothelial dependent blood flow in response to changes in tissue or organ perfusion requirements; when blood flow increases through a vessel, the vessel dilates and this phenomenon has been called flow-mediated dilation (FMD); (2) morphological and mechanical characteristics of vascular wall (intima-media thickness (IMT), vessel diameter, compliance and distensibility) and (3) plasma concentrations of soluble endothelial markers (intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, E and P selectins, NO and others)[50].

n-3 PUFA and flow-mediated dilation

FMD is a non-invasive surrogate for endothelial cell function, which is in part, dependent on endothelial production and release of NO. It is a useful tool to evaluate early atherosclerotic disease; larger FMD predicts decreased risk for CVD events. A mixture of *n*-3 PUFA (>0·5 g/d for a duration of 2 weeks to 8 months) from FO improved FMD in more than a dozen studies[34]. There are only a limited number of studies which examined the effects of EPA and DHA individually or compared the effects of these two fatty acids on FMD. In one study, DHA supplementation (1·2 g/d, 6 weeks) increased endothelial mediated FMD of the brachial artery after occlusion was increased in hyperlipidaemic children[51]. In another study DHA (0·7 g/d, 3 months) did not alter FMD or arterial stiffness in healthy subjects[52]. A study with hyperlipidaemic men compared the effects of EPA and DHA (4 g/d, 6 weeks) on endothelial dependent and independent blood flow[31]. DHA, but not EPA increased forearm blood flow in response to acetyl choline that induces endogenous NO release; however, it also increased forearm blood flow when choline was injected with an inhibitor of endothelial NO synthase, or with nitroprusside as donor of exogenous NO, suggesting that increase in forearm blood flow caused by DHA was independent of endothelial function. Similarly DHA but not EPA

decreased vasoconstriction in response to norepinephrine, which is again endothelial cell independent. In contrast to the results from this study, EPA (1·8 g/d, 12 weeks) increased forearm blood flow in response to acetyl choline (endothelial dependent) but not nitroprusside (endothelial independent) in hypertriglyceridaemic subjects[53]. Discrepancy regarding the effects of EPA between the two studies may be due to differences in the duration of the study or the decrease in TAG following EPA supplementation. Overall, results from these limited number of studies suggest that EPA effects on FMD are endothelial cell dependent, and those of DHA are endothelial cell independent.

n-3 PUFA and intima-media thickness

Carotid IMT has been used as a surrogate endpoint for evaluating the regression and/or progression of atherosclerotic CVD. Many studies have documented the relation between the carotid IMT and the presence and severity of atherosclerosis. In general, wall thickening may be in the intimal layer or in the muscular medial layer. A number of epidemiological, and observational human studies have indicated an inverse association between *n*-3 PUFA intake and atherosclerosis; however, results from human interventional studies with FO have been quite variable. One study compared the IMT and coronary artery calcification among the Japanese men (300, aged 40–49 years) from Kusatsu, Shiga and 306 US white men from Allegheny County Pennsylvania[54]. The Japanese men had twice the plasma concentrations of *n*-3 PUFA and significantly less IMT and coronary artery calcification than the US men. Once they corrected for the serum *n*-3 PUFA, the differences between the Japanese and American men became smaller, though still significant. Part of these differences between subject groups was due to *n*-3 PUFA and the remainder may be due to genetic, environmental and lifestyle factors. In the Harvard Atherosclerosis Reversibility Study, fifty-nine patients with coronary artery disease (CAD), took supplements of FO (12 g/d; *n*-3 PUFA 6 g/d) or olive oil as placebo for 2 years. Changes in diameter of atherosclerotic coronary arteries as determined by angiography did not differ between the FO and placebo groups[55]. In the Prevention of Atherosclerosis by Intervention with Marine Omega-3 fatty acids (SCIMO) study, 223 patients with angiographically proven CAD took either a supplement of FO (1·65 g EPA + DHA, 3 months and then 3 g/d for 21 months) or a placebo resembling the typical European fatty acid mixture. Although there were fewer cardiovascular events in the FO group, the loss in luminal diameter between the two groups was not significantly different[56]. Also, the progression of atherosclerosis in the carotid artery and the increase in mean IMT did not differ between the FO and placebo groups[57]. In the subjects without known CAD (Multi-ethnic Study of Atherosclerosis), men with the highest quartile of plasma *n*-3 PUFA had the lowest diameter of brachial artery, while in women *n*-3 PUFA had no association with brachial artery diameter; women in the highest quartile of plasma *n*-3 PUFA had the lowest change in percent FMD, while men had no association between *n*-3 PUFA and change in percent FMD[58]. In the same study, the intake of *n*-3 PUFA was associated with lower prevalence of subclinical atherosclerosis when classified by common carotid artery IMT, but not by internal carotid IMT, or coronary artery calcification or ankle brachial arterial pressure index[59]. It seems that the associations between *n*-3 PUFA intake and the risk of atherosclerosis varied by sex and the artery examined. Standardised and more sensitive techniques are needed to determine the role of IMT in the determination of atherosclerosis.

There are no published reports that have examined the effects of supplementing EPA or DHA individually on IMT or arterial diameter in human subjects; however, there is one observational study. Coronary artery diameters and stenosis were monitored at the start and end of a study in 228 postmenopausal women with CAD, who took oestrogen or placebo supplements for 3 years[60]. There was no effect of hormone therapy on either response tested. The subjects were then divided into groups with those below and above the median concentrations of ALA, EPA and DHA in plasma phospholipids. The differences between the initial and final readings for mean diameter and stenosis were not affected by the plasma phospholipids, ALA and EPA concentrations; both response variables were significantly lower in the subjects who had DHA concentrations above the median than those who had it below the median ($P = 0{\cdot}007$). The number of new lesions that appeared was also lower in the high than in the low DHA group. Authors concluded that plasma DHA significantly reduced progression of coronary atherosclerosis over the 3-year follow up in postmenopausal women with established CAD.

n-3 PUFA and adhesion molecules

Recruitment of circulating leucocytes at sites of atherosclerosis is mediated through a family of adhesion molecules. The function of circulating forms of these adhesion molecules remains unknown, but their levels may serve as molecular markers of subclinical CHD. Activated endothelium releases the soluble VCAM-1 and ICAM-1. In addition, endothelial cells also produce E and P selectins. Measurement of the circulating concentrations of these molecules can be used to quantify endothelial activation. There are only a few studies that examined the effects of purified EPA and/or DHA on the plasma concentrations adhesion molecules (VCAM-1, ICAM-1, and E and P selectins). In one study, Omacor (4 g/d; EPA-EE 465 + DHA-EE 375 mg/g) supplementation given to hypertriglyceridaemic subjects for 7 months significantly decreased the plasma concentrations of soluble cellular adhesion molecule 1 ($P = 0{\cdot}02$), and soluble E selectin ($P = 0{\cdot}0001$), but did not change soluble VCAM-1 concentration[61]. In another study with type 2 diabetic patients, EPA (1·8 g/d, 4 weeks) significantly decreased plasma concentrations of E selectin, and those of platelet and monocyte-derived microparticles that have pro-coagulant activities ($P = 0{\cdot}05$); concentration of P selectin was also decreased but did not attain significance[62]. In contrast to the results from earlier studies with hypertriglyceridaemic or diabetic subjects supplementing 2 or

6·6 g/d of a 1:1 mixture of EPA and DHA to healthy subjects for 12 weeks did not decrease the plasma concentrations of VCAM and ICAM; only the higher concentration of *n*-3 PUFA decreased that of E-selectin[63]. In another study with healthy subjects, DHA + EPA but not DHA alone decreased plasma concentrations of soluble VCAM-1 and E-selectin[64]. DHA supplementation (3 g/d, 90 d) in hypertriglyceridaemic men did not alter the circulating concentrations of soluble ICAM-1, soluble VCAM-1 and E-selectin[65]. Thus, the effects of *n*-3 PUFA on plasma concentrations of adhesion molecules have been variable. This limited number of studies suggests that EPA may be more effective than DHA in decreasing the circulating concentrations of adhesion molecules. However, this contradicts the results from *in vitro* findings in which DHA was more potent than EPA in inhibiting the expression of adhesion molecules on human endothelial cells, monocytes and lymphocytes[66]. Further studies are needed to determine the effects of individual *n*-3 PUFA on the plasma concentration of these molecules.

n-3 PUFA and plaque stability

Carotid endarterectomy is an operation during which a vascular surgeon removes the plaque from the carotid artery to restore blood flow. In a randomised study with patients who were waiting for carotid endarterectomy (7–189 d, median 42 d; *n* 52–57/group), they took a supplement of either FO or sunflower oil, or a placebo made of soyabean and palm oils (4 g/d, EPA + DHA 1·4 g/d)[64]. On the day of surgery carotid plaques were collected, rinsed, fixed and evaluated according to the American Heart Association criterion for the plaque stages and for cap thickness. The group receiving the FO had significantly higher number of stage 4 ($P = 0{\cdot}05$), and less of the stage 5 plaques ($P = 0{\cdot}03$) than those in the sunflower oil group (stage 5 is more advanced than 4). There were also fewer plaques with thinner caps in the FO group than those in sunflower oil group; thinner caps rupture easier than those with thicker caps. Since *n*-3 PUFA from FO had been incorporated into the caps, the authors concluded that *n*-3 PUFA may enhance the stability of the caps. The same authors repeated another study with the same dose of FO in another set of endarterectomy patients (7–102 d, median 21 d; *n* 47 or 53/group); in the second study, they did not find a difference in plaque stability between the placebo and FO groups, but fewer foam cells were found in the FO group[67]. Both EPA and DHA contents of the plaques were increased; only the increase in EPA was statistically significant ($P = 0{\cdot}02$). This may be because of the relatively much lower initial concentration of EPA than that of DHA. EPA content of the plaques was inversely associated with plaque instability, plaque inflammation and the number of T-cells in the plaques. Authors suggest that the lack of an effect of FO on plaque stability in the second study may be because of the shorter duration of FO supplementation. Based on these two studies it is difficult to predict if EPA is more effective than DHA in improving plaque stability, hence further studies are needed.

Effects of *n*-3 PUFA on blood pressure and heart rate

Initial indications for the BP lowering effects of *n*-3 PUFA came from the studies of Bang and Dyersburg with Greenland Inuits, when they found a negative association between plasma concentration of *n*-3 PUFA and diastolic BP[9]. Preliminary studies with normotensive and hypertensive subjects support the BP lowering effects of *n*-3 PUFA, although the results have been variable[27,28,68]. Two different meta-analyses concluded that the effect of *n*-3 PUFA on BP is dose dependent with a minimal efficacious dose of 3 g/d; a BP decrease of −0·66/−0·35 mmHg/g *n*-3 PUFA for systolic/diastolic BP[68,69].

The earlier conclusions were based on results from studies that used a mixture of EPA and DHA. More recent studies used EPA and DHA individually. In a study with mildly hypercholesterolaemic individuals, DHA but not EPA significantly reduced BP and HR[70]. However, neither EPA nor DHA reduced BP in another study by the same investigators in treated hypertensive type 2 diabetic patients[71]. This discrepancy may be because of hyperglycaemia or the use of pharmacologic treatments. DHA decreased both BP and HR in two studies with hypertriglyceridaemic subjects[44,72] and in another study with healthy middle-aged men and women[52]. The amount of DHA supplemented in those human studies showing reduction in BP ranged from 0·7 to 4·0 g/d for 6–13 weeks. In one of these studies, DHA supplementation (3 g/d) for 45 d decreased HR by 8 %, systolic BP by 6 % and diastolic BP by 4 %[44]. With the continued supplementation of DHA for 90 d, HR and BP were still reduced compared with the corresponding values prior to the start of DHA supplementation, but the decreases were not statistically significant. At the end of the study HR was decreased by only 5 %, systolic BP by 2·3 % and diastolic BP by 0·5 %. In two other studies with healthy normotensive subjects DHA did not decrease BP[73,74]. In another study with postmenopausal women DHA supplementation (2·8 g/d, 4 weeks) reduced HR by 7 %, but did not change BP[75]. Similarly, DHA but not EPA (4 g/d, 7 weeks) decreased HR but not BP in healthy subjects[76]. Results from this limited number of studies suggest that DHA may be more effective than EPA in lowering BP and HR. Further studies are needed to compare the effects of EPA and DHA on these variables.

Effects of DHA on the concentrations of inflammatory markers

Inflammation is the normal response of an organism's immune system to the damage caused to its cells and vascularised tissues by viruses, bacteria, injurious chemicals or physical insults. Blood flow to the infected site is increased so leucocytes can neutralise and remove the damage-causing agents. Although painful, inflammation is usually a healing response. Inadequate inflammatory response leads to immunodeficiency, cancer and infections; however, in some instances inflammation proceeds to a chronic state and is associated with debilitating diseases such as diabetes and CVD. Progression of inflammatory

diseases is associated with an increase in the plasma concentrations of one or more markers of inflammation[77,78].

Plasma markers of inflammation include an increase in the number of circulating leucocytes, acute phase proteins (C-reactive protein (CRP), serum amyloid A, fibrinogen), cytokines and their soluble receptors (TNFα, IL-1, IL-6, IL-7, IL-8 and IL-18, interferon γ), adhesion molecules (ICAM-1, VCAM-1, E and P selectin) and plasminogen activator inhibitor-1. An increase in the concentration of insulin and decrease in the concentrations of leptin and adiponectin are also associated with inflammation. Plasma concentrations of several of these markers are increased in a number of inflammatory diseases and are used to evaluate the disease status. Plasma CRP is one of the most commonly used markers of inflammation. There is a 4·4-fold increase in the Relative Risk for CVD comparing the highest and lowest quartiles of CRP, while this increase is only 2·4-fold comparing the quartiles of cholesterol[6,79]. CRP stimulates mononuclear cells to release tissue factors which are central to initiation of coagulation reactions, complement activation and neutralisation of platelet activating factor. Together these factors promote thrombotic response.

Several lines of evidence support the claim that *n*-3 PUFA have anti-inflammatory effects. First, epidemiological studies which showed lesser incidence of inflammatory diseases such as CVD and arthritis in populations consuming more fish than those populations consuming less or no fish[14,15,80,81]. Second, several studies indicated inverse associations between the estimated *n*-3 PUFA consumption and the plasma concentrations of inflammatory markers[82–84]. Third, other studies demonstrated an inverse association between tissue concentrations of *n*-3 PUFA and of inflammatory markers[85–89]. Fourth, a number of intervention studies with FO showed a decrease in the symptoms for inflammatory diseases and a decrease in the *in vivo* and *ex vivo* secretion of inflammatory markers. There have been dozens of studies regarding the effects of FO and of individual *n*-3 PUFA on the *ex vivo* production of inflammatory cytokines and eicosanoids. Those have been summarised in several recent reviews[77,78]. In general, the results indicate anti-inflammatory effects of FO, but there have been a number of inconsistencies due to health and age of the participants, amount, duration and fatty acid composition of the supplements, diets and the methods used to evaluate the inflammatory status.

Only a limited number of studies have investigated the anti-inflammatory effects of individual *n*-3 PUFA in human subjects and we found only six studies that examined the effects of DHA alone. Three of these studies found decrease in markers of inflammation following DHA supplementation[65,87,90] the other three did not[91–93]. In one study, healthy men were given a supplement of DHA (6 g/d for 90 d) producing a 10 % decrease in the number of circulating leucocytes which resulted from a 20 % reduction in the number of circulating neutrophils[90]. The absolute number of other types of leucocytes did not change, but the percentage of lymphocytes was increased because of a reduction in the number of granulocytes. The change in the number of circulating neutrophils was detectable within 56 d of DHA supplementation. In the same study, DHA supplementation caused a 60–75 % decrease in the *ex vivo* secretion of PGE_2 and leukotriene B_4 and a 30–40 % decrease in the secretion of IL-1β and TNFα within 12 weeks[94]. Production of both IL-1β and TNFα were decreased within 8 weeks of DHA supplementation, but those were not significant. None of these variables changed in the placebo group.

The second DHA study was of the same duration as the one discussed earlier (91 d), but it was conducted in hypertriglyceridaemic men and the DHA supplement was one-half (3 g/d) of the amount served in the first study[65]. In this study, the number of circulating neutrophils decreased by 11 % within 45 d of DHA supplementation and this reduction was maintained until the end of study[65]. This change in neutrophil numbers is about half of what was found in the first study, most likely due to the dose of DHA used. The reduction in the number of circulating neutrophils caused by DHA may be clinically important in conditions like acute respiratory distress syndrome which result from an increase in the activity and number of circulating neutrophils. The number of circulating neutrophils did not change in the placebo group. The concentration of other markers of inflammation did not significantly change within 45 d, but by 91 d, CRP decreased by 15 %, IL-6 by 23 % and granulocyte macrophage colony-stimulating factor by 21 % and the anti-inflammatory marker, matrix metalloproteinase-2, increased by 7 %. Plasma concentrations of other cytokines (IL-1β, IL-2, IL-8, IL-10 and TNFα) and adhesion molecules (ICAM-1, VCAM-1 and E-selectin) did not change in both the DHA and placebo groups.

The third human study examined the effects of doubling the dose of DHA (200, 400, 800, 1600 mg/d, over 2-week intervals each) supplementation to healthy men on the *ex vivo* secretion of LTB_4 and LTB_5 by the cultured peripheral blood mononuclear cells stimulated with the Ca ionophore A23187[95]. The ratio of *ex vivo* secreted LTB_5 and LTB_4 was significantly increased ($P < 0{\cdot}001$) at DHA concentrations of 800 and 1600 mg/d, and they returned to normal within 2 weeks of discontinuation of DHA. These results show that DHA supplementation of 800 mg/d or higher reduced the inflammatory response.

The lack of an effect of DHA on the concentration of inflammatory markers in the other three studies was most likely due to the low dose of DHA (0·7 g/d, 12 weeks)[91] or short duration (4 or 6 weeks)[92,93] of supplementation, or a combination of the two. Results from two other studies supplementing DHA at 3 or 6 g/d demonstrated that the plasma concentrations and the *ex vivo* production of inflammatory cytokines were decreased at 90 d after supplementation, but not at 45 d[65,94]. Two studies directly compared the effects of EPA and DHA on inflammatory markers. In one study with healthy men and women, neither DHA nor EPA (4·7 g/d, 12 weeks) decreased plasma concentrations of inflammatory cytokines[92]. Similarly in another study with type 2 diabetes mellitus patients, neither fatty acid (4 g/d, 6 weeks) decreased plasma concentrations of CRP and IL-6, but both decreased concentrations of TNFα by 25 %[93]. Results from other studies comparing the effects of EPA and DHA varied; some suggested more potent effects of DHA,[93,96] while others suggested

greater effects of EPA[97]. Further studies are needed to determine the anti-inflammatory potencies of EPA and DHA individually.

Effect of *n*-3 PUFA on lipid peroxidation

Increased lipid peroxidation has been associated with the development and progression of a number of chronic human diseases including CVD and diabetes[98–101]. It damages biological membranes changing membrane fluidity and functions including receptor activity, and nutrient and ion transport. Peroxidation of LDL lipids renders the lipoprotein pro-atherogenic. Many lipid peroxidation products exert cytotoxic effects and alter cell signalling[102–105]. Thus, control of lipid peroxidation plays a critical role in health maintenance and disease prevention.

Lipid peroxidation in biological systems is believed to increase in proportion to an increase in the number of double bonds in the fatty acid chain (PUFA) and inversely to the antioxidant levels within the cells. LC PUFA, particularly of the *n*-3 type, reduces the risk of a number of chronic diseases, yet there remains a concern that they may also increase the risk for chronic diseases by increasing lipid peroxidation.

Human dietary studies with *n*-3 PUFA regarding their effects on lipid peroxidation have had variable results[35,74,106–110]. The effect of DHA ranged from protection to increased lipid peroxidation depending upon its dose[111]. These inconsistencies result from the different methods used (Cu^{2+} catalysed lipid peroxidation *ex vivo*, F-2 isoprostanes, malondialdehyde, oxygen-radical absorbance capacity, etc.) and their limitations. Future studies using more sensitive and reproducible methods are needed to establish if there is any risk of lipid peroxidation with increased consumption of *n*-3 PUFA and how to minimise it by increasing the intake of antioxidant nutrients.

Conclusions and future directions

Dietary intervention with LC *n*-3 PUFA decreased the risk factors for atherosclerosis in most human studies discussed in this review. It improved both fasting and postprandial lipid profile including the mean size and numbers of lipoprotein particles, decreased BP and HR and increased FMD. *n*-3 PUFA decreased blood vessel calcification, IMT, improved plaque stability and plasma concentrations of adhesion molecules in some, but not other studies. In general, both EPA and DHA provided health benefits, but some effects such as the reduction in BP, HR, the number of small dense LDL particles, and improved ratio between HDL-C and LDL-C seem to be limited to DHA. Future studies are needed to determine the optimal doses of EPA and DHA individually, their interaction and the mechanisms involved.

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