Very long chain omega-3 (n-3) fatty acids and human health: fact, fiction and the future

Philip C. Calder[1,2]

[1]Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, Southampton, United Kingdom;

[2]NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton, United Kingdom;

Address for correspondence: Philip C. Calder, Human Development and Health Academic Unit, Faculty of Medicine, University of Southampton, MP887 Southampton General Hospital, Tremona Road, Southampton SO16 6YD, United Kingdom

Email: pcc@soton.ac.uk

Phone: +44(0)2381205250

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Abbreviations: ADHD, attention deficit hyperactivity disorder; ALA, α-linolenic acid; ARA, arachidonic acid; CHD, coronary heart disease; CVD, cardiovascular disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; IBD, inflammatory bowel disease; MI, myocardial infarction; PUFA, polyunsaturated fatty acid; RA, rheumatoid arthritis; RCT, randomized controlled trial; SDA, stearidonic acid; TAG, triacylglycerol.

Abstract

Eicosapentaenoic acid (EPA) and docosahexaenoioc acid (DHA) appear to be the most important omega-3 (n-3) fatty acids, but roles for n-3 docosapentaenoic acid (DPA) are now also emerging. Intakes of EPA and DHA are usually low, typically below those recommended. Increased intakes result in higher concentrations of EPA and DHA in blood lipids, cells and tissues. Increased content of EPA and DHA modifies the structure of cell membranes and the function of membrane proteins. EPA and DHA modulate the production of lipid mediators and through effects on cell signaling can alter the patterns of gene expression. Through these mechanisms, EPA and DHA alter cell and tissue responsiveness in a way that often results in more optimal conditions for growth, development and maintenance of health. DHA has vital roles in brain and eye development and function. EPA and DHA have a wide range of physiological roles which are linked to certain health or clinical benefits, particularly related to cardiovascular disease, cancer, inflammation, and neurocognitive function. The benefits of EPA and DHA are evident throughout the life course. Future research will include better identification of the determinants of variation of responses to increased intake of EPA and DHA; more in-depth dose response studies of the effects of EPA and DHA; clearer identification of the specific roles of EPA, DPA and DHA; testing strategies to enhance delivery of n-3 fatty acids to the bloodstream; and exploration of sustainable alternatives to fish-derived very long chain n-3 fatty acids.

Omega-3 (n-3) fatty acids – structure, metabolic interrelationships, dietary sources and intakes

Omega-3 (n-3) fatty acids are a family of polyunsaturated fatty acids (PUFAs)[1]. They are defined by the position of the double bond closest to the methyl terminus of the hydrocarbon (acyl) chain. This is on carbon number three when counting the methyl carbon as number one. Eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) appear to be the most important n-3 fatty acids[2,3], although roles for docosapentaenoic acid (DPA; 22:5n-3) are now also emerging[4,5]. Because of their long hydrocarbon chain, EPA, DPA and DHA are sometimes termed very long chain n-3 fatty acids, in order to differentiate them from the 18-carbon plant-derived n-3 fatty acids like α-linolenic acid (ALA; 18:3n-3) and stearidonic acid (SDA; 18:4n-3). In this article, the term "very long chain n-3 fatty acids" will be used to individually and collectively describe EPA, DPA and DHA.

EPA, DPA and DHA are metabolically related to one another, and there is a pathway by which EPA can be synthesized from the simpler plant-derived n-3 fatty acids (Figure 1). The conversion of ALA to EPA involves three steps catalyzed, in turn, by delta-6 desaturase, elongase 5 and delta-5 desaturase (Figure 1). Further conversion of EPA to DHA, via DPA, occurs by a complex pathway (Figure 1) involving chain elongation catalyzed by elongase 5, a second chain elongation catalyzed by elongase 2 or 5, desaturation by delta 6-desaturase, and then removal of two carbon atoms by limited β-oxidation in peroxisomes. The enzymes of n-3 fatty acid interconversion are shared with the analogous omega-6 (n-6) fatty acid biosynthetic pathway of conversion of linoleic acid (18:2n-6) to arachidonic acid (ARA; 20:4n-6) and beyond. The high intake of linoleic acid relative to ALA in many Western diets[6] favours linoleic acid conversion over that of ALA. This may be one explanation for

the frequently reported low rate of conversion of ALA along this pathway[7,8], although this rate can be influenced by several other factors including age[9], sex[10,11,12], hormones[13], and genetics[14].

EPA and DHA are found in fairly high amounts in most seafood, especially in fatty fish (sometimes called "oily fish"), in the blubber and tissues of sea mammals like whales and seals, in supplements like fish oils, cod liver oil, and krill oil, in some algal oils, and in a limited number of pharmaceutical grade preparations[15,16]. There is at least a 10-fold range in content of these fatty acids per serving of seafood, with fatty fish (e.g. mackerel, salmon, trout, herring, tuna and sardines) being the richest source (Table 1). Hence, intake of very long chain n-3 fatty acids is strongly influenced by fish consumption. In most Western countries only a relatively small proportion of the population regularly consume fatty fish. For example, in the UK it is estimated that only 25% of the adult population are regular fatty fish consumers[17]. Consequently intakes of very long chain n-3 fatty acids are low in much of the population and mean intakes of EPA+DHA among adults in many Western populations are considered to be around 0.1 to 0.3 g/day[17]. However, it is difficult to be precise about this figure for several reasons, as discussed elsewhere[3]. A series of Australian studies in adults and children provide probably the most accurate data on intake of very long chain n-3 fatty acids. Data from over 10,000 Australian adults identified mean daily intakes of EPA, DPA and DHA as 56, 26 and 106 mg, respectively, to give a total very long chain n-3 fatty acid intake of 189 mg/day[18], consistent with the oft-quoted "average" intake of these fatty acids among Western adults. However, median intakes of EPA, DPA and DHA were found to be only 8, 6 and 15 mg/day, respectively[18]. The large differences between mean and median intakes reflect the skewed distribution of the intake data. A more recent study using an

updated nutrient composition database produced mean daily intake data for EPA, DPA and

DHA of 75, 71 and 100 mg, respectively, in Australian adults, giving a mean total very long

chain n-3 fatty acid intake of 246 mg/day[19]. Again, median intakes were lower, being about

50% of the mean. The data suggest that 50% of Australian adults consume less than about 120

mg of very long chain n-3 fatty acids daily. There is no reason to think that average intakes

would be higher than this in other Western countries, and in many they may be lower.

Australian children and adolescents aged 2 to 16 years consumed a mean of 79 mg/day

EPA+DPA+DHA, with a median intake of 29 mg/day[20]. Intakes increased with age and were

much higher in fish eaters than non-fish eaters[20].

The oil used in many n-3 fatty acid supplements is sourced from fish; as such this may

be referred to as "fish oil". Other, non-fish, sources of oil such as krill and algae are also used

in supplements. These different oils differ according to their very long chain n-3 fatty acid

content and the relative contributions of EPA and DHA (Table 2). Figure 2 illustrates the

estimated influence of daily consumption of a one g standard fish oil capsule, a one g

"concentrated" supplement, one teaspoon of cod liver oil, one meal of salmon, and one or four

capsules of a pharmaceutical grade preparation on daily intake of EPA+DHA. This shows that

a person who does not consume fatty fish or n-3 fatty acid supplements can markedly increase

their EPA+DHA intake through dietary change or through use of supplements.

Increased intake of EPA and DHA leads to increased content of EPA and DHA in blood, cells and tissues

As for all long chain and very long chain fatty acids, EPA and DHA are transported in the

bloodstream esterified into triacylglycerols (TAGs), phospholipids and cholesteryl esters as

components of lipoproteins. They also occur in the bloodstream in the non-esterified form non-covalently bound to albumin. EPA and DHA are stored in adipose tissue esterified into TAGs and they are found in all cell membranes esterified into phospholipids and related complex lipids. Cell membrane phospholipids and their fatty acid composition are important in determining the physical characteristics of cell membranes[21], the manner in which membranes change in response to external stimuli[22], and the functional activities of membrane-bound proteins[23]. EPA and DHA circulating in the bloodstream, stored in adipose tissue, and present in cell membranes may be regarded as transport, storage and functional pools, respectively. The contribution of EPA or DHA to the total fatty acids present within any of the transport, storage or functional pools differs according to the pool (Table 3)[24-36]. DHA is most often present in a greater concentration than EPA (Table 3). This is especially true in specific regions of the eye and brain where DHA makes a significant contribution to the fatty acid complement and EPA is virtually absent[27,28,37]. Within cell membranes, EPA and DHA are distributed differently among the different phospholipid components and in the brain and eye specific phospholipids are especially rich in DHA[37,38].

Increased intakes of EPA and DHA from fish or from supplements are reflected in increased concentrations (and proportions) of both fatty acids in blood lipids, blood cells and many tissues. This has been reported many times for total plasma and serum lipids and for the complex lipid components of plasma and serum (i.e. TAGs, phospholipids and cholesteryl esters)[24,39-47]. It is also well described for erythrocytes[24,42,43,47], platelets [24,39,48] and leukocytes[24,26,44-46,49]. When their intake is increased, the content of EPA and DHA increases in several human tissues, including skeletal muscle[31], heart[29], gut mucosa[35,50] and adipose tissue[24,43]. There is a dose- and time-dependent pattern of incorporation of both EPA and

DHA[24,26,39,43-46]. Although EPA is incorporated more quickly than DHA[24,43], the precise pattern of EPA and DHA incorporation that occurs depends upon the specific nature of the lipid pool[24,43]. Pools that are turning over rapidly show faster incorporation of EPA and DHA than slower turning over pools. For example, plasma lipids incorporate EPA and DHA more quickly than blood cells[24,43], and amongst blood cells, leukocytes have been usually shown to incorporate EPA and DHA more quickly than erythrocytes. However, dose- and time-dependence of EPA and DHA incorporation into tissues is not well investigated in humans, apart from for adipose tissue[24,43]. Modification of human brain fatty acid composition is more difficult than for other tissues, especially beyond childhood.

The higher concentration of EPA and DHA achieved in various lipid pools through increased intake of EPA and DHA is maintained so long as the higher intake of EPA and DHA is maintained. If, after a period of increased intake of EPA and DHA, intake returns to the earlier lower level, then EPA and DHA concentrations decline, eventually returning to earlier levels. This is well described for blood lipids[39,43,44], platelets[39], leukocytes[44] and erythrocytes[43,47]. Just as the incorporation of EPA into different pools is faster than the incorporation of DHA, the loss of EPA is faster than the loss of DHA[39,43,44,47]. The better retention of DHA than EPA may be because DHA is structurally and/or functionally preferred over EPA and that metabolic mechanisms have evolved to preserve it.

Molecular and cellular effects of increased EPA and DHA content

Many, though not all, of the functional effects of EPA and DHA are considered to require their incorporation into cell membrane phospholipids[51-53] (Figure 3). EPA and DHA are highly unsaturated and as a consequence they have been shown in some studies to decrease

membrane order (i.e. increase membrane fluidity)[21,54], although cells have mechanisms such as modifying membrane cholesterol content, to limit this effect. Through modulation of the physical properties of membranes, EPA and DHA provide a specific environment for membrane proteins like receptors, transporters, ion channels, and signaling enzymes to function[22.23,55]. As a result, EPA and DHA can modulate cell responses that are dependent upon membrane protein function. This has been shown to be especially important in the eye where the presence of DHA enables optimal activity of the photoreceptor protein rhodopsin[56]. Cell membranes contain microdomains called rafts[57]. These have specific lipid and fatty acid compositions and act as platforms for receptor action and for the initiation of intracellular signaling pathways[57-59]. EPA and DHA can modify raft formation in a variety of cell types including neurons, immune cells and cancer cells[59-61], so affecting intracellular signaling pathways[59-62]. As a result of their effects on membrane-generated intracellular signals, EPA and DHA can modulate transcription factor activation and, subsequently, gene expression patterns in a variety of cell types[51,55,63]. Nuclear factor κ B [64], the peroxisome proliferator activated receptors[65,66], and the sterol regulatory element binding proteins[67-71] are amongst the transcription factors shown to be affected by EPA and DHA. The effects of EPA and DHA on transcription factor activation and modulation of gene expression are central to their role in controlling fatty acid and TAG metabolism, inflammation, and adipocyte differentiation[51-53,55,63].

Increased abundance of EPA and DHA in cell membrane phospholipids is associated with decreased abundance of the n-6 PUFA ARA[26,39,44,45,72]. This alters the availability of substrates for synthesis of bioactive lipid mediators (Figure 4). ARA is quantitatively the major substrate for the biosynthesis of various prostaglandins, thromboxanes and leukotrienes,

together termed eicosanoids, which have well-established roles in regulation of inflammation, immunity, platelet aggregation, smooth muscle contraction and renal function. Eicosanoids are oxidized derivatives of 20-carbon PUFAs and are produced via the cyclooxygenase (prostaglandins, thromboxanes), lipoxygenase (leukotrienes and other products) and cytochrome P450 pathways. Although they have a number of obvious important physiological roles, excess or inappropriate production of eicosanoids from ARA is associated with many disease processes[73,74]. Increasing the EPA and DHA content of cell membranes results in decreased production of eicosanoids from ARA[39,45], resulting in an impact of EPA and DHA on inflammation, immune function, blood clotting, vasoconstriction, and bone turnover amongst other processes. In addition to decreasing production of eicosanoids from ARA, EPA and DHA are themselves substrates for the synthesis of lipid mediators (Figure 4). Some of these are simply analogues of those produced from ARA. For example, prostaglandin E_3 produced from EPA is an analogue of prostaglandin E_2 produced from ARA. Frequently, though not always, the EPA-derived mediator has weaker biological activity than the mediator derived from ARA[75]. For example, thromboxane A_3 produced from EPA is a much weaker platelet aggregator than thromboxane A_2 produced from ARA[76]. EPA and DHA are also substrates for more complex biosynthetic pathways that result in generation of mediators known as resolvins (E-series formed from EPA and D-series formed from DHA), protectins/neuroprotectins (formed from DHA) and maresins (formed from DHA)[77-79] (Figure 4). It has recently been discovered that DPA gives rise to a similar family of mediators[5,80]. The major role of resolvins, protectins and maresins appears to be in the resolution of inflammation and modulation of immune function[77-79]. It seems likely that

many of the anti-inflammatory and immune modulating actions of EPA and DHA that are described in the literature [52,53,72,81] are mediated through resolvins, protectins and maresins.

The above-mentioned mechanisms of action of EPA and DHA rely upon incorporation of those fatty acids into cell membrane phospholipids (Figure 3). It is now recognized that EPA and DHA (in their non-esterified form) can also act directly via membrane G-protein coupled receptors that exhibit some specificity for very long chain n-3 fatty acids over other fatty acids as ligands[82]. In particular, free fatty acid receptor 4 (also known as GPR120) which is highly expressed on inflammatory macrophages and on adipocytes, was shown in cell culture experiments to play a central role in mediating the anti-inflammatory effects of DHA on macrophages and the insulin-sensitising effects of DHA on adipocytes[82].

Roles of EPA and DHA in supporting optimal cell and tissue function and promoting health

Cardiovascular disease

Native populations in Greenland, Northern Canada and Alaska consuming their traditional diet were found to have much lower rates of death from cardiovascular disease (CVD) than predicted, despite their high dietary fat intake[83-86]. The protective component of the diet was suggested to be the very long chain n-3 fatty acids consumed in very high amounts as a result of the regular intake of seal and whale meat, whale blubber and fatty fish[87]. Low cardiovascular mortality is also seen in Japanese consuming a traditional diet[88] and this diet is rich in seafood including fatty fish and sometimes marine mammals, which contain significant amounts of EPA and DHA. Much evidence has now accumulated from prospective and case-control studies indicating that higher intake of EPA and DHA is associated with

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reduced risk of CVD outcomes in Western populations, although not all studies agree. These studies have been summarised and discussed in detail elsewhere[89-93] and they have been subject to systematic review and meta-analysis. For example, Chowdhury et al.[94] brought together prospective studies examining the association of dietary or circulating fatty acids, including very long chain n-3 fatty acids, with risk of coronary outcomes. Data from 16 studies involving over 422,000 individuals showed a relative risk of 0.87 for those in the top third of dietary intake of very long chain n-3 fatty acids compared with those in the lower third of intake. Data from 13 studies involving over 20,000 individuals showed relative risks of 0.78, 0.79 and 0.75 for those in the top third of circulating EPA, DHA and EPA+DHA, respectively, compared with those in the lower third[94]. A smaller number of studies in fewer individuals gave a relative risk of 0.64 for circulating DPA[94]. A more recent analysis pooled data from 19 studies that investigated the association between EPA or DHA concentration in a body compartment like plasma, serum, red blood cells or adipose tissue and risk of future coronary heart disease (CHD) in adults who were healthy at study entry[95]. EPA and DHA were each associated with a lower risk of fatal CHD, with relative risks of 0.91 and 0.90, respectively[95]. These analyses[94,95] support a clear role for EPA and DHA in primary prevention of CHD, and perhaps, more widely, of CVD, as discussed elsewhere[96].

Beneficial modification of a broad range of risk factors probably explains the protective effect of very long chain n-3 fatty acids towards CHD. These risk factors include plasma/serum TAG concentrations, blood pressure, thrombosis, cardiac function, vascular function and inflammation which are all improved by very long chain n-3 fatty acids[89,93,97-101]. A recent meta-analysis of randomized controlled trials (RCTs) evaluated the effects of EPA+DHA on a range of risk factors for CVD[102]; the findings are summarized in Table 4.

Significant effects of EPA+DHA were identified for plasma/serum TAG and high-density lipoprotein cholesterol concentrations, systolic and diastolic blood pressure, heart rate, and C-reactive protein concentration, a marker of inflammation (Table 4). There was also a significant elevation of low-density lipoprotein concentration (Table 4), but this apparently deleterious effect may be offset by an increased size of low-density lipoprotein particles[103], rendering them less atherogenic. The improvement in the risk factor profile with very long chain n-3 fatty acids would account for the lowered risk of coronary disease reported in many previous epidemiological studies and identified in the recent meta-analysis[94] and pooled analysis[95] of cohort studies.

A role in prevention of CVD is an obvious important health benefit of EPA+DHA. There has also been great interest in the ability of very long chain n-3 fatty acids to treat people with existing CVD. The outcome in studies in this area has most often been the occurrence of a major cardiovascular event (e.g. MI), fatal MI, or death. Several large studies published between 1989 and 2008 reported lower rates of death in patients receiving very long chain n-3 fatty acids[104-108]. Doses of very long chain n-3 fatty acids used in these studies were 500 mg to 1.6 g per day and durations of intervention were one to five years. As a result of these positive findings, meta-analyses published in period 2002 to 2009 supported that very long chain n-3 fatty acids lower mortality in patients with existing CVD[109-111]. For example, a meta-analysis including 11 studies involving almost 16,000 patients reported that, compared with control, very long chain n-3 fatty acids lower the risk of fatal myocardial infarction (MI), sudden death and all-cause mortality (relative risks 0.7, 0.7 and 0.8, respectively)[109]. A second meta-analysis including 14 studies involving over 20,000 patients reported that, compared with control, very long chain n-3 fatty acids lower the risk of cardiac mortality and

all-cause mortality (relative risks 0.68 and 0.77, respectively)[110]. It is likely that the mechanisms that reduce the likelihood of cardiovascular events and mortality in patients with established disease are different from the mechanisms that act to slow the development of atherosclerosis. Three key mechanisms have been suggested to contribute to this therapeutic effect of very long chain n-3 fatty acids. The first is altered cardiac electrophysiology seen as lower heart rate[112], increased heart rate variability[113] and fewer arrhythmias[114]. These effects make the heart more able to respond robustly to stress. The second is an anti-thrombotic action resulting from the altered pattern of production of eicosanoid mediators from ARA and from EPA that control platelet aggregation[39]. This effect would lower the likelihood of clot formation or would result in weaker clots less able to stop blood flow to affected organs. The third mechanism is the well-documented anti-inflammatory effect of very long chain n-3 fatty acids which would serve to stabilise atherosclerotic plaques preventing their rupture[115,116]. This effect would reduce the likelihood of a cardiovascular event (MI, stroke) from happening[117]. Thus, there is a biological plausibility to EPA+DHA having a benefit in secondary prevention of cardiovascular events and mortality.

Despite the positive findings with very long chain n-3 fatty acids, supported by meta-analyses and biologically plausible candidate mechanisms, more recent studies in patients with existing CVD have failed to replicate the earlier findings[118-122]. This has influenced some of the most recent meta-analyses which have concluded that there is little protective effect of very long chain n-3 fatty acids on cardiovascular mortality[123-125]. Nevertheless, the meta-analysis by Rizos et al.[123] which included the recent studies, but excluded the landmark GISSI Prevenzione trial[105], did identify a reduction in cardiac death with very long chain n-3 fatty acids (relative risk 0.91) and trends towards reductions in sudden death and MI (relative

risks 0.91 and 0.89, respectively). It is important to recognize that the most recent studies of very long chain n-3 fatty acids and cardiovascular mortality have been criticised for various reasons related to small sample size, the low dose of EPA+DHA used, the too short duration of follow up, and, in some studies, the low rate of events[126]. Despite this, meta-analyses that include the GISSI-Prevenzione study and some of the more recent neutral studies, still report benefits from very long chain n-3 fatty acids[127,128]. For example, Casula et al.[127] identified reductions in cardiac death, sudden death and MI (relative risks 0.68, 0.67 and 0.75, respectively) with very long chain n-3 fatty acids and a trend towards lower all-cause mortality (relative risk 0.89). Likewise, Wen et al.[128] identified reductions in cardiac death, sudden death, MI and all-cause mortality (relative risks 0.88, 0.86, 0.86 and 0.92, respectively) with very long chain n-3 fatty acids.

Thus, there is a significant literature gathered over more than 45 years from association studies, from RCTs investigating the impact on risk factors, and from RCTs investigating the effect on hard clinical outcomes like mortality that very long chain n-3 fatty acids lower the risk of developing CVD, especially CHD, and can be used to treat people with CVD. Although the most recent RCTs in patients with CVD have produced findings that do not agree with the previously accumulated literature, it is too early to discard the earlier evidence. Instead reasons to explain the different findings need to be identified, in order to better understand the actions of EPA and DHA. The conclusion that very long chain n-3 fatty acids have a role in reducing risk of CVD, especially CHD, remains well supported, for example by the American Heart Association[89,129].

Cancer

EPA and DHA have a number of biological activities that may influence tumour cell viability and proliferation. For example, DHA can promote tumour cell apoptosis[130,131], possibly through inducing oxidative stress. The replacement of ARA in cell membranes by EPA and DHA is also an important anti-cancer mechanism of action of n-3 PUFAs, since ARA-derived eicosanoids like prostaglandin E_2 and leukotriene B_4 promote tumour cell proliferation, survival, migration and invasion[131-133]. Through these effects, EPA and DHA can directly influence cancer cells and the tumour environment, and they can also influence the host response to tumour bearing. Recent reviews provide a detailed description of the mechanisms by which very long chain n-3 fatty acids affect tumour cell proliferation, survival, invasion and metastasis[130,131]; the ability of very long chain n-3 fatty acids to enhance the effectiveness of anti-cancer treatments[131,133]; and the current evidence of the efficacy of very long chain n-3 fatty acids in humans in the context of cancer and its treatment[131,133].

Some prospective and case-control studies show that very long chain n-3 fatty acids are associated with lower risk of colorectal and breast cancers, but there is inconsistency in the findings from such studies[134]. Recent systematic reviews conclude that very long chain n-3 fatty acids are protective against colorectal[135] and breast[136] cancers, while a fairly recent prospective cohort study among post-menopausal women found that higher dietary intake of either EPA or DHA was associated with lower risk of developing breast cancer[137]. Whether very long chain n-3 fatty acids increase or decrease risk of prostate cancer is currently under debate[138,139].

In addition to effects that lower the risk of developing some forms of cancer, there seems to be a role for very long chain n-3 fatty acids in patients who already have cancer. For example, quality of life and physical functioning can be improved in cancer patients with oral

provision of very long chain n-3 fatty acids. A systematic review[140] concluded that lung cancer patients receiving supplements containing EPA and DHA had improved appetite, energy intake, body weight and quality of life. Breast cancer patients with a higher concentration of very long chain n-3 fatty acids in their bloodstream had lower inflammation and less physical fatigue than seen in patients with a lower concentration of very long chain n-3 fatty acids[141]. Lung cancer patients given 1.8 g EPA+DHA per day had improved appetite and less fatigue than controls[142]. Van der Meij et al.[143] reported improved quality of life, physical function, cognitive function and health status in patients with non-small cell lung cancer receiving 2.9 g EPA+DHA per day. The patients receiving very long chain n-3 fatty acids also tended to have greater physical activity compared to the control group[143].

EPA and DHA sensitise cultured tumour cells to chemotherapeutic agents, increasing the efficacy of those agents[144]. The mechanism by which this occurs is not clear, but it might involve increased EPA and DHA content of tumour cell membranes resulting in increased lipid peroxidation in those membranes in the presence of the cancer therapeutic. If this occurred *in vivo* it would result in improved efficacy of the therapy and perhaps reduced side effects. Murphy et al.[145] conducted a trial in patients with non-small cell lung cancer and showed that 2.5 g EPA+DHA per day caused a two-fold increase in response rate to the chemotherapy being used and prolonged the period over which patients could receive the chemotherapy. They also reported a trend towards improved survival with very long chain n-3 fatty acids. Bougnoux et al.[146] reported improved chemotherapy outcomes in breast cancer patients receiving 1.8 g DHA per day.

Cancer cachexia (loss of lean and fat tissue) is a complication that occurs in patients with advanced solid tumors and greatly increases risk of mortality. Weed et al.[147] reported

that patients with squamous cell cancer of the head and neck taking 3.08 g EPA+DHA per day had increased lean body mass. Murphy *et al.*[148] showed that 2.2 g EPA+DHA per day was able to maintain body weight and muscle mass during chemotherapy in patients with non-small cell lung cancer. In other studies in patients with cancer, very long chain n-3 fatty acids increased body weight[149,150].

Thus, there is increasing evidence from studies in humans that very long chain n-3 fatty acids have a role in reducing risk of developing some cancers, particularly colorectal and breast. Furthermore, a number of intervention trials demonstrate that very long chain n-3 fatty acids have a range of benefits in patients with various types of cancer. Most intervention studies have used approximately 2 g/day EPA+DHA. From their review of the literature Vaughan *et al.*[133] concluded "There is now sufficient literature to suggest that the use of supplements containing EPA and DHA may have potential use as an effective adjuvant to chemotherapy treatment and may help ameliorate some of the secondary complications associated with cancer…… our investigations indicate that supplementation with fish oil or EPA/DHA (> 1 g EPA and > 0.8 g DHA per day) is associated with positive clinical outcomes."

Inflammation

Inflammation is an essential component of normal host defence mechanisms, initiating the immune response and later playing a role in tissue repair. The inflammatory response is normally self-limiting (i.e. resolving) in order to protect the host from damage. However, the loss of the normal mechanisms inducing tolerance (e.g. to self, to endogenous commensal micro-organisms, or to environmental components such as foods) or loss of resolving factors

can allow inflammation to become chronic and in this state the damage done to host tissues may become pathological[151,152]. As such, inflammation is the central adverse response seen in a range of conditions including rheumatoid arthritis (RA), inflammatory bowel disease (IBD), multiple sclerosis, asthma, psoriasis, and atopic dermatitis. Furthermore, chronic low-grade inflammation is now recognised to be a contributor to CVD[153,154] and to play a role in cardiometabolic diseases like obesity, type-2 diabetes and non-alcoholic fatty liver disease and in cognitive decline[155]. Cancer also has a permissive inflammatory component[156].

Eicosanoids and related lipid mediators provide a direct link between fatty acids and inflammatory processes. As described earlier, the n-6 PUFA ARA is the precursor for the production of prostaglandins and leukotrienes that are directly involved in inflammatory processes[73,74]. In contrast to the effects of ARA, EPA and DHA give rise to mediators which are less pro-inflammatory, anti-inflammatory or inflammation resolving[52,53,72,77-79,81]. In addition to their effects on lipid mediators (prostaglandins, leukotrienes, resolvins, protectins, maresins), EPA and DHA modulate many other aspects of inflammatory processes including leukocyte migration and production of inflammatory cytokines[52,53,72,81]. These effects on inflammation relate to the modulation of cell signaling, transcription factor activation and gene expression by EPA and DHA. There is good evidence that EPA and DHA given in combination at sufficiently high doses are anti-inflammatory and have a therapeutic role in inflammatory diseases. This has been most widely studied in RA[157], IBD[158] and asthma[159]. Evidence of efficacy is strongest in RA, although high doses (several g/day of EPA+DHA) are typically used[157]. Recent systematic reviews and meta-analyses have highlighted the benefits of EPA+DHA on arthritic pain[160,161].

Allergic disease begins in infancy and the immune imbalances that predispose to allergy may be influenced by the relative exposure to n-6 and n-3 PUFAs[162]. Therefore, there has been significant interest in whether increased intake of very long chain n-3 fatty acids by pregnant and breast feeding women will reduce the risk of allergic disease in their babies. There is some evidence that increased intake of EPA and DHA during human pregnancy has an effect on the immune system of the baby[163-165] and that this is linked to reduced allergic symptoms later in childhood[163,166,167]. Best *et al.*[168] reported a meta-analysis of offspring clinical outcomes from trials of increased maternal intake of very long chain n-3 fatty acids in pregnancy. They identified lower risk of atopic eczema, and less likelihood of having a positive skin prick test to any allergen tested, to hens' egg, or to any food extract, all in the first 12 months of life. A recent study reported that fish oil consumption by pregnant women decreased risk of persistent wheeze and asthma in the offspring at ages 3 to 5 years[169]. Supplementing the diets of very young infants has also now been shown to have immune effects consistent with reduced likelihood of allergy [170]. This area of research has been reviewed recently[171-173].

Thus, the anti-inflammatory actions of EPA and DHA are extensively demonstrated and the underlying mechanisms are increasingly understood. High doses of very long chain n-3 fatty acids can be used to treat frank inflammatory conditions like RA, while lower doses likely have a role in protecting against development of childhood allergic disease and low-grade inflammatory conditions in adulthood.

Neurocognitive health

More than 50% of the dry weight of the brain is lipid, particularly structural and functional lipid (i.e. phospholipids). The human brain and retina contain an especially high proportion of DHA relative to other tissues but little EPA (Table 3). Grey matter phosphatidylethanolamine contains 24% of its fatty acids as DHA, whereas grey matter phosphatidylserine contains 37% of its fatty acids as DHA[174]. DHA contributes 50 to 70% of the fatty acids present in the rod outer segments of the retina[38]. These rod outer segments contain the eye's photoreceptors and DHA has been clearly shown to be essential for optimal visual functioning[56]. DHA is important for neurotransmission, neuronal membrane stability, neuroplasticity and signal transduction[174-176]. An adequate supply of very long chain n-3 fatty acids, especially DHA, seems essential for optimal visual, neural and behavioural development of the infant/child. The need for DHA so early in life was demonstrated in studies conducted in the 1980s and 1990s with pre-term infants, where formulas that included DHA (and often also ARA) were shown to improve visual development[177-182]. However, a recent meta-analysis of 17 trials of inclusion of DHA (and ARA) in infant feeds involving 2,260 preterm infants found little evidence to support improved visual acuity or neurodevelopment, although three out of seven studies of neurodevelopment did report some benefit of the PUFAs[183]. Studies of DHA in preterm infants are discussed in detail elsewhere[184]. The literature on the effect of DHA on visual and cognitive outcomes in term infants is mixed with some studies reporting benefits[185,186] and others not[187-190]. One reason for this might be that an early beneficial effect of DHA is lost with time so that early assessments show benefit and later assessments do not; at least one study has shown this[185,191]. A recent meta-analysis of 15 trials of inclusion of DHA (and ARA) in infant feeds involving 1,889 term infants found little evidence to support improved visual or neurodevelopment, although four out of nine studies

of visual acuity and two out of eleven studies of cognitive development reported benefit[192].

Despite the inconsistencies in the literature, it still seems important that pregnant and breastfeeding women and infants consuming formula instead of breast milk have adequate intakes of very long chain n-3 fatty acids, especially DHA. A systematic review and meta-analysis of eleven RCTs of very long chain n-3 fatty acids in pregnancy involving over 5,000 women could not conclusively support or refute that very long chain n-3 fatty acids in pregnancy improve infant visual or cognitive development[193].

Very long chain n-3 fatty acids are likely to have important roles in the brain beyond infancy and are probably important for brain function throughout the life course. A number of studies have reported lower levels of EPA and DHA in the bloodstream of children with attention deficit hyperactivity disorder (ADHD) or autistic spectrum disorders than in control children[194]. It is possible that these and other developmental disorders might be related to some sort of fatty acid deficiency state. If this is the case, then normalisation of fatty acid levels should lead to clinical benefit in these conditions. This has been examined in a number of trials in children and adolescents with attention, learning, or behavioural disorders, some showing some improvements[195-205] and others finding no effect[206-214]. These trials have been reviewed many times, with differing conclusions. The different findings may relate to the dose of n-3 fatty acids used, the duration of supplementation, the precise outcome(s) measured and differences in the children studied. Indeed, one review concluded that studies using higher doses of very long chain n-3 fatty acids or of longer duration or in children/adolescents with low socioeconomic status were more likely to find effects[215]. A recent meta-analysis of five RCTs involving 189 children with autism spectrum disorder identified benefit of very long chain n-3 fatty acids on some outcomes, but concluded that the

limited number of studies and small sample sizes restrict the ability to make firm conclusions [216]. Another recent meta-analysis used data from 15 case-control studies involving 1193 individuals with autism to show lower blood EPA, DHA and ARA and higher ratio of n-6 to n-3 PUFAs in autism[217]. Data from four RCTs involving 107 individuals with autism showed improvements in some outcomes with very long chain n-3 fatty acids, but the authors suggested the need for larger and longer studies in order to be clear about the effect[217]. A meta-analysis of 10 RCTs of very long chain n-3 fatty acids in children with ADHD showed no improvements in measures of emotional lability, oppositional behaviour, conduct problems or aggression[218]. However, subgroup analyses of higher quality studies and those meeting strict inclusion criteria found a significant reduction in emotional lability and oppositional behaviour[218]. The authors concluded that a number of treatment effects may have failed to reach statistical significance due to small sample sizes and within and between study heterogeneity in terms of design and study participants[218]. In seven RCTs involving 534 adolescents with ADHD, very long chain n-3 fatty acids improved ADHD clinical symptom scores, while in three RCTs involving 214 adolescents, very long chain n-3 fatty acids improved cognitive measures associated with attention[219]. A very recent systematic review was supportive of use of n-3 fatty acid supplements in children with ADHD[220].

Over 35 years ago Rudin suggested that mental disorders might result from a deficiency in very long chain n-3 fatty acids and might respond to provision of these fatty acids[221]. Data from nine countries demonstrated a significant correlation between high annual fish consumption and lower prevalence of major depression[222], an observation that is compatible with a proposed protective effect of very long chain n-3 fatty acids. A reduction in depressive symptoms was reported in a small study using a very high dose of EPA+DHA (9.6

g/day)[223], while this effect was not seen in a study using a lower dose of DHA alone (2 g/day)[224]. Intervention with 6.2 g/day EPA+DHA in patients with bipolar manic depression resulted in significant improvements in nearly all outcomes, especially with respect to depressive symptoms, after 4 months[225]. Likewise, 2 g/day EPA improved symptoms in patients with unipolar depressive disorder after 4 weeks[226]. A meta-analysis of 13 RCTs involving 1233 participants with major depressive disorder showed an overall beneficial effect of very long chain n-3 fatty acids on depressive symptoms[227]; interestingly higher EPA dose was one factor associated with better outcome for n-3 PUFA supplementation. This finding fits with an earlier analysis of 15 RCTs investigating the effects of EPA which concluded that supplements containing ≥ 60% EPA in doses ranging from 0.2 to 2.2 g/day EPA were effective against primary depression[228]. There is also evidence from meta-analysis that depressive symptoms seen in bipolar disorder may be improved by the adjunctive use of very long chain n-3 fatty acids[229].Another meta-analysis that included 25 studies involving 1373 participants identified a "small-to-modest benefit" for depressive symptomology with very long chain n-3 fatty acids[230] but the authors expressed doubts about the robustness of their finding.

Schizophrenic patients have lower levels of EPA and DHA in their erythrocytes than do controls[231-235]. The first trial of very long chain n-3 fatty acids in schizophrenia identified clinical improvement with EPA (2 g/day), but not with DHA[236], while subsequent trials also showed benefit with EPA[237,238], but not all studies have seen this[239]. A recent study reported significant benefits of very long chain n-3 fatty acids given for 26 weeks to patients with schizophrenia[240]. Although these findings are encouraging, a Cochrane review concluded that very long chain n-3 fatty acids should be regarded only as an experimental treatment for

schizophrenia[241]. A study reported significant benefit from 1 g/day EPA in borderline personality disorder[242], while a small number of studies report anti-aggressive effects of DHA[243,244].

Post-mortem studies showed that the brains of people with Alzheimer's disease contain less DHA than those without the disease[245-248] and some studies have linked low blood levels of very long chain n-3 fatty acids to dementia[249]. Sinn et al. reported that 1.8 g EPA+DHA/day for 6 months reduced depressive symptoms and improved cognition in adults with mild cognitive impairment[250]. Improved memory performance in subjects with mild Alzheimer's disease was reported with 1.5 g EPA+DHA/day for 6 months[251]. However, a number of other studies using various doses and ratios of EPA and DHA reported no effect on cognitive performance in people with Alzheimer's disease[252-257]. Meta-analyses provide a mixed view of the findings for n-3 fatty acid treatment in the area of cognitive impairment, reflecting the mixed findings from individual trials. A Cochrane review of RCTs studying the role of very long chain n-3 fatty acids in preventing cognitive decline in healthy older people showed no benefits[258], while two more recent meta-analyses differ in their findings. One meta-analysis of six RCTs of duration 3 to 40 months and using 0.l4 to 1.8 g EPA+DHA per day identified a slower rate of cognitive decline in those receiving very long chain n-3 fatty acids[259]. In contrast, a meta-analysis of three RCTs involving 632 participants with mild to moderate Alzheimer's disease and followed for 6, 12 or18 months found no evidence of a benefit from very long chain n-3 fatty acids on any outcome that was assessed[260]. A recent large trial found no effects of very long chain n-3 fatty acids (0.225 g EPA plus 0.8 g DHA daily) over three years on cognitive decline[261].

Thus, DHA is a key structural component of the brain and retina, where it plays particular, unique functional roles. A supply of DHA is very important early in life, especially during the fetal and early infant periods when the eye and central nervous system are developing. Since the supply must come from maternal sources (via the placenta and breast milk), maternal DHA status is likely to be important in determining eye and brain development early in life. Newly emerging areas of interest relate to the influence of very long chain n-3 fatty acids on childhood developmental disorders, adult psychiatric and psychological disorders, and neurodegenerative diseases of aging. These conditions appear to be associated with a lower status of very long chain n-3 fatty acids. Additionally, there is some epidemiological evidence for a lowered risk of psychiatric, psychological disorders, and neurodegenerative disorders with increased dietary intake of very long chain n-3 fatty acids. Intervention studies indicate that there may be some benefit from very long chain n-3 fatty acids in childhood developmental and adult psychiatric and psychological disorders, particularly in depression. Interestingly many of these studies are indicative that EPA is more important than DHA, which contrasts with the relative roles of these two fatty acids in very early eye and brain development. Although there may be a role for very long chain n-3 fatty acids in slowing cognitive decline[259], this has not been well demonstrated[258,260]. There is a clear need for larger, longer and higher quality human trials in this area of research.

Recommendations for intake of EPA and DHA

The demonstration of physiological actions of EPA and DHA that result in improved health outcomes and reduced risk of disease, along with the increased understanding of the molecular and cellular mechanisms of action involved, indicates a need to set

recommendations for the intake of these important fatty acids. However, the exact requirement for very long chain n-3 fatty acids in order to maintain health is not known. Furthermore, there has been a lack of clarity about the extent to which EPA and DHA can be synthesized in humans so long as there is sufficient intake of the precursor ALA[8]. Nevertheless, the recognition of the health benefits of EPA and DHA has resulted in several recommendations to increase the intake of fish and, more specifically, of EPA and DHA by various governmental, non-governmental and professional agencies[17,89,129,262-267]. These recommendations are summarised in Table 4.

The future of research in n-3 PUFAs

Very long chain n-3 fatty acids have been a focus of research since the late 1980s, although important pioneering work in the field was performed before then. A PubMed search conducted in late August 2017 identified over 25,500 publications using the search term "omega-3" and 28,500 publications using the search term "fish oil". The earliest publications identified using those terms were from 1922 and 1945, respectively, although the earliest of the almost 1,000 publications identified with the search term "cod liver oil" was from 1845. By 1990, there was a publication on the topic of n-3 fatty acids on average every day. By the mid-2010s this had risen to almost five publications every day. Thus, a tremendous effort has been, and is continuing to be, expended on research in the area of n-3 fatty acids. This research effort obviously falls into many categories, not all related to human health and well-being, although much of it does. Research aimed at better defining the roles that very long chain n-3 fatty acids play in human growth, development, function, health, well-being, and disease risk and better understanding the molecular and cellular mechanisms involved will

continue for the foreseeable future. Despite the vast amount of knowledge that is currently available about n-3 PUFAs, there remain significant gaps and inconsistencies in the literature that impair the ability to make robust recommendations for both the healthy population and specific patient groups. One reason for this is that it is not clear what the extent of the variation in response to very long chain n-3 fatty acids is within the population or amongst particular subgroups, or what the determinants of such variation might be, although these are likely to include other dietary components, sex, body composition, genetics, stage in the life course, physiological state, and the presence of disease. These determinants are likely to be more thoroughly explored in the coming years, with researchers taking advantage of new analytical and data handling technologies to study larger numbers of samples. Surprisingly, there is insufficient information currently available on dose responses of many physiological and pathological outcomes to very long chain n-3 fatty acid intervention. This hampers the ability to use the full potential of very long chain n-3 fatty acids in both public health and clinical settings. Most research on very long chain n-3 fatty acids to date has focused on EPA and DHA, usually studied in combination. This has resulted in many researchers thinking that EPA and DHA have the same biological effects. This seems not to be the case[268,269], but again this is an area of research that is likely to become better explored in the next period. Related to this, biological actions of DPA have emerged[4,5] but the effects of DPA and its mechanisms of action are poorly described at the moment. It seems likely that DPA will become a greater focus of research in this area. It is also likely that research into the molecular mechanisms of the action of each individual very long chain n-3 fatty acid will continue, with membrane structure-function relationships, early intracellular signaling pathways, transcription factors, novel membrane receptors, and novel lipid mediators all

being important candidates for future study. Whatever the mechanism involved, it is evident that EPA, DPA and DHA consumed orally need to be found at the site of their action in the body. As described earlier, it is also clear that the effects of these fatty acids on cell and tissue function are dose dependent[39,40,42,45]. Thus, it seems likely that strategies for more effective delivery of these bioactive fatty acids will be explored. These might include different chemical[270,271] or physical[272] formulations.

The richest dietary source of very long chain n-3 fatty acids is seafood, especially fatty fish (Table 1). Despite recommendations by many authorities to increase consumption of fish, the majority of the Western population are low consumers. An alternative to eating fish is to use n-3 supplements ("fish oil") which give an assured intake of EPA and DHA. A concern is that the supply of fish for human consumption and for the production of oil for use in n-3 supplements is not sustainable. Thus, alternative sources need to be sought and then proven to be beneficial to human health. Such alternatives include algal oils, some of which are already widely used in the infant formula industry, and seed oils from plants genetically modified to produce EPA and DHA[273]. Finally, although the focus of this article is the very long chain n-3 fatty acids EPA, DPA and DHA, plant n-3 fatty acids are widely available and are sustainable. Most research on plant n-3 fatty acids conducted to date has been on ALA as an alternative to very long chain n-3 fatty acids[8], but there has been some research on SDA[8,274]. From the research conducted to date, ALA and SDA need to be converted to very long chain n-3 fatty acids by the pathway shown in Figure 1 to be biologically effective[8]. However, the studies done so far show very poor conversion of ALA and SDA to DHA in humans, although SDA is a better precursor for EPA synthesis than ALA[275]. Future research will attempt to identify strategies to enhance endogenous biosynthesis of very long chain n-3 fatty acids from

their simpler plant precursors. These strategies might include modifying other aspects of the diet or taking advantage of genetic polymorphisms that favour conversion. Thus, there are many important questions yet to be answered and this assures continued research in the field of n-3 fatty acids and human health.

Summary and conclusions

N-3 fatty acids are a family of PUFAs that contribute to human development, health and well-being. Functionally the most important n-3 fatty acids are the very long chain EPA and DHA found in fatty fish and in supplements; roles for DPA are now emerging. Intakes of EPA and DHA are typically low and much below those that are recommended. Increased intakes of EPA and DHA, either from fish or from supplements, are reflected in greater incorporation into blood lipid, cell and tissue pools. Increased content of EPA and DHA can modify the structure of cell membranes and also the function of membrane proteins involved as receptors, signaling proteins, transporters and enzymes. EPA and DHA also modify the production of lipid mediators and through effects on cell signaling can alter patterns of gene expression. Through these actions EPA and DHA act to alter cellular responsiveness in a manner that seems to result in more optimal conditions for growth, development and maintenance of health. The effects of very long chain n-3 fatty acids are evident right through the life course meaning that there is a need for all sectors of the population to increase the intake of these important nutrients. EPA and DHA have a wide range of physiological roles which are linked to certain health or clinical benefits. A number of risk factors for CVD are modified in a beneficial way by increased intake of EPA and DHA: these include blood pressure, platelet reactivity and thrombosis, plasma TAG concentrations, vascular function, cardiac arrhythmias,

heart rate variability, and inflammation. Thus, there is a key role for these fatty acids in prevention and slowing progression of CVD. Furthermore, some supplementation studies with EPA and DHA have demonstrated reduced mortality in at risk patients, such as post-MI, indicating a therapeutic role, although this is currently disputed by some. A number of other, non-cardiovascular, actions of EPA and DHA have also been documented, suggesting that increased intake of these fatty acids could be of benefit in reducing the risk of (i.e. protecting from) or treating many conditions. For example, they have been used successfully in RA and, in some studies, in IBD, and may be useful in other inflammatory conditions like asthma and psoriasis. EPA and DHA may also have a role as part of cancer therapy; some recent studies show that they improve the effectiveness of chemotherapeutic agents. DHA has an important structural role in the eye and brain, and its supply early in life when these tissues are developing is known to be of importance in terms of optimising visual and neurological development. For this reason it is very important that pregnant and breast feeding women have adequate DHA intake. Recent studies have highlighted the potential for EPA and DHA to contribute to enhanced mental development and improved childhood learning and behaviour and to reduce the burden of psychiatric and depressive illnesses in adults, although these areas of possible action require more robust scientific support. There may also be a role for EPA and DHA in preventing neurodegenerative disease of ageing, but evidence for this is currently weak. The effects of EPA and DHA on health outcomes are likely to be dose-dependent, but clear dose response data have not been identified in most cases. Also in many cases it is not clear whether both EPA and DHA have the same effect or potency and therefore which one will be the most important for a particular indication. Thus, despite several decades of productive research on the health effects of very long chain n-3 fatty acids and the

mechanisms involved, important questions are currently unanswered and many areas remain to be explored in future research.

Conflict of interest

The author serves on the Scientific Advisory Boards of Pronova BioPharma (part of BASF), Smartfish, DSM, FrieslandCampina and Danone Nutricia Research.

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Figure legends

Figure 1. The metabolic pathway of biosynthesis of eiocosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid.

Figure 2. Typical intake of EPA+DHA from the background diet in an adult not regularly consuming fatty fish and what would be achieved by also consuming a one g standard fish oil (FO) capsule, a one g "concentrated" supplement, one teaspoon of cod liver oil, one meal of salmon, or one or four capsules of the pharmaceutical grade preparation Omacor®. Reproduced from Ref. 3 with permission from John Wiley and Sons.

Figure 3. General overview of the mechanisms by which very long chain n-3 fatty acids can influence the function of cells. Modified from European Journal of Pharmacology, Vol 668 (Supplement 1), P.C. Calder, Fatty acids and inflammation: the cutting edge between food and pharma, Pages S50-S58, Copyright (2011), with permission from Elsevier.

Figure 4. Overview of the bioactive lipid mediators produced from arachidonic, eicosapentaenoic and docosahexaenoic acids.

Table 1. Typical content of EPA, DPA and DHA in a selection of seafood and meat. Data are taken from Ref. 15. Note that both very long chain n-3 fatty acid content and portion size may vary. Modified from Ref. 3 with permission from John Wiley and Sons.

Food	EPA	DPA	DHA	Typical adult portion size	EPA+DPA+DHA
	g/100 g food			g	g/portion
Mackerel	0.71	0.12	1,10	160	3.09
Canned pilchards	1.17	0.23	1.20	110	2.86
Trout	0.23	0.09	0.83	230	2.65
Salmon	0.50	0.40	1.30	100	2.20
Canned sardines	0.89	0.10	0.68	100	1.67
Herring	0.51	0.11	0.69	120	1.56
Crab	0.47	0.08	0.45	85	0.85
Plaice	0.16	0.04	0.10	130	0.39
Cod	0.08	0.01	0.16	120	0.30
Mussels	0.41	0.02	0.16	40	0.24
Haddock	0.05	0.01	0.10	120	0.19
Venison	0.04	0.09	< 0.01	120	0.16
Canned tuna	0.02	0.02	0.14	45	0.08
Lamb	0.03	0.04	0.02	90	0.08
Prawns	0.06	< 0.01	0.04	60	0.06
Chicken	0.01	0.02	0.03	100	0.06
Beef	0.02	0.02	0	90	0.04
Pork	0.01	0.02	0.01	90	0.04

Table 2. Typical EPA and DHA contents of omega-3 supplements. Reproduced from Ref. 16 with permission from John Wiley and Sons.

	EPA (mg/g oil)	DHA (mg/g oil)	EPA+DHA (mg/g/oil)
Cod liver oil	110	90	200
Krill oil	140	65	205
Standard fish oil	180	120	300
Typical 45% fish oil concentrate	270	180	450
Tuna oil	110	350	460
Algal oil used in infant formula	0	> 400	> 400
Typical 60% fish oil concentrate	360	240	600
Omacor®* (Omega-3 ethyl ester concentrate)	460	380	840
Flaxseed oil	0	0	0

*Also known as Lovaza®

Table 3. Typical EPA and DHA concentrations reported in different lipid pools in humans. Data are taken from the selected references and are expressed as % of total fatty acids. Reproduced from Ref. 16 with permission from John Wiley and Sons.

Population	Lipid pool	EPA	DHA	Reference
Generally healthy men and women aged 20 to 80 years; UK	Plasma triacylglycerols*	0.3	0.8	(24)
Generally healthy men and women aged 20 to 80 years; UK	Plasma phospholipids*	1.2	3.6	(24)
Healthy pregnant women aged 18 to 40 years; week 38 of pregnancy; UK	Plasma phospholipids*	0.4	3.8	(25)
Newborn infants (umbilical cord); healthy pregnancies; UK	Plasma phospholipids	0.3	6.4	(25)
Generally healthy men and women aged 20 to 80 years; UK	Plasma cholesteryl esters*	1.0	0.6	(24)
Generally healthy men and women aged 20 to 80 years; UK	Plasma non-esterified fatty acids*	0.4	1.6	(24)
Generally healthy men and women aged 20 to 80 years; UK	Red blood cells	2.3	5.2	(24)
Generally healthy men and women aged 20 to 80 years; UK	Platelets	1.1	2.0	(24)
Generally healthy men aged 18 to 40 years; UK	Blood neutrophils	0.6	1.6	(26)
Generally healthy men and women aged 20 to 80 years	Blood mononuclear cells (mainly lymphocytes)	0.8	1.9	(24)
Men mean age 68 years with no evidence of dementia	Brain grey matter	Not reported	18	(27)
Men mean age 68 years with no evidence of dementia	Brain white matter	Not reported	4	(27)
Breast-fed term infants who had died at mean age 4 months; Australia	Cerebral cortex	< 0.1	8	(28)
Breast-fed term infants who had died at mean age 4 months; Australia	Retina	0.1	12	(28)
Men mean age 55 years who had received a heart transplant; USA	Cardiac muscle	0.2	1.5	(29)
Mainly men mean age 60 years undergoing cardiac surgery; Australia	Cardiac muscle phospholipids	0.5	4.8	(30)
Healthy men mean age 21 years; UK	Skeletal muscle	0.6	1.5	(31)
Healthy men and women aged 25	Skeletal muscle	0.7	1.9	(32)

to 45 years; USA	phospholipids			
Men and women aged 38 to 41 years undergoing surgery; Chile	Liver	0.4	6.8	(33)
Men and women aged 23 to 63 years undergoing surgery; Chile	Liver phospholipids	4.8	15.1	(34)
Patients with inflammatory bowel disease; UK	Colonic mucosa	0.3	1.7	(35)
Generally healthy men and women aged 20 to 80 years; UK	Subcutaneous adipose tissue	0.2	0.2	(24)
Generally healthy men mean age 34 years; Iran	Spermatozoa	0.6	9.6	(36)

*Blood collected after an overnight fast

Table 4. Summary of the effects of very long chain n-3 fatty acids on risk factors for cardiovascular disease identified through the meta-analysis of AbuMweis *et al.*[(102)].

Risk factor	Number of studies included	Effect size (95% CI)	P
Plasma/serum triacylglycerol concentration (mmol/l)	110	-0.368 (-0.427, -0.309)	0.0001
Plasma/serum total cholesterol concentration (mmol/l)	108	-0.051 (-0.166, 0.064)	0.387
Plasma/serum LDL cholesterol concentration (mmol/l)	100	0.150 (0.058. 0.243)	0.001
Plasma/serum HDL cholesterol concentration (mmol/l)	110	0.039 (0.024, 0.054)	0.0001
Systolic blood pressure (mm Hg)	50	-2.195 (-3.172, -1.217)	0.0001
Diastolic blood pressure (mm Hg)	50	-1.08 (-1.716, -0.444)	0.0001
Heart rate (beats per minute)	26	-1.370 (-2.415, -0.325)	0.01
C-reactive protein (mg/l)	20	-0.343 (-0.454, -0.232)	0.0001
Flow-mediated dilatation (%)	6	1.460 (-0.475, 3.395)	0.139

Table 5. Some of the current recommendations for intake of very long chain n-3 fatty acids.

Recommending organisation	Population subgroup	Recommendation	Reference
(UK) Scientific Advisory Committee on Nutrition/Committee on Toxicity	Adults in the general population	At least two portions of fish per week, at least one of which is fatty fish; equated to 450 mg EPA+DHA per day	(17)
International Society for the Study of Fatty Acids and Lipids	Adults in the general population	Minimum of 500 mg EPA+DHA per day; Target of 650 mg EPA+DHA per day	(262,263)
French Agency for Food, Environmental and Occupational Health Safety	Adults in the general population	Target of 400-500 mg EPA+DHA per day with at least 100-120 mg DHA per day	(264)
(Australian) National Health and Medical Research Council	Adults in the general population	Target of 430-610 mg EPA+DHA per day	(265)
Food and Agricultural Organisation of the United Nations	Adult males and non-pregnant or non-lactating adult females	Minimum of 250 mg EPA+DHA per day	(266)
Food and Agricultural Organisation of the United Nations	Pregnant or lactating females	Minimum of 300 mg EPA+DHA per day of which at least 200 mg should be DHA	(266)
Food and Agricultural Organisation of the United Nations	Children aged 2-4 years	100-150 mg EPA+DHA per day	(266)
Food and Agricultural Organisation of the United	Children aged 4-6 years	150-200 mg EPA+DHA per day	(266)

Nations

Food and Agricultural Organisation of the United Nations	Children aged 6-10 years	200-250 mg EPA+DHA per day	(266)
European Food Safety Authority	Adult males and non-pregnant adult females	Adequate intake is 250 mg EPA+DHA per day	(267)
European Food Safety Authority	Pregnant females	An additional 100-200 mg DHA per day	(267)
European Food Safety Authority	Infants and children aged 6 months to 2 years	100 mg DHA per day	(267)
European Food Safety Authority	Children aged 2-18 years	"consistent with adults"	(267)
American Heart Association	Patients who have had a recent myocardial infarction	1000 mg EPA+DHA per day in concentrated form	(89,129)
American Heart Association	For patients with elevated blood triacylglycerol concentrations	2000 to 4000 mg EPA+DHA per day	(89)