Highlight Article

Very long chain omega-3 (n-3) fatty acids and human health[*]

Philip C. Calder[1, 2, 3]

[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

[3] Department of Biological Sciences, Faculty of Science, King Abdulaziz University, Jeddah, Saudi Arabia

Omega-3 (n-3) fatty acids are a family of polyunsaturated fatty acids that contribute to human health and well-being. Functionally the most important n-3 fatty acids appear to be eicosapentaenoic acid (EPA) and docosahexaenoioc acid (DHA), but roles for n-3 docosapentaenoic acid (DPA) are now emerging. Intakes of EPA and DHA are usually low, typically below recommended intakes. Increased intakes are reflected in greater incorporation into blood lipid, cell and tissue pools. Increased content of EPA and DHA modifies the structure of cell membranes and the function of membrane proteins involved as receptors, signaling proteins, transporters, and enzymes. EPA and DHA modify the production of lipid mediators and through effects on cell signaling can alter patterns of gene expression. Through these actions EPA and DHA alter cell and tissue responsiveness in a manner that seems to result in more optimal conditions for growth, development, and maintenance of health. The effects of n-3 fatty acids are evident right through the life course, meaning that there is a need for all sectors of the population to have a sufficient intake of these important nutrients. EPA and DHA have a wide range of physiological roles which are linked to certain health or clinical benefits.

Practical application: Very long chain omega-3 (n-3) fatty acids are found in seafood, especially fatty fish, and in supplements. They exert a range of health benefits as a result of their molecular, cellular and physiological actions. Consequently, very long chain n-3 fatty acids play important roles in growth, development, optimal function, and maintenance of health and well-being right across the life course. Therefore, all sectors of the population need to ensure sufficient intake of these important nutrients. This can be achieved through eating fatty fish or, failing that, use of good quality supplements.

Keywords: Brain / Cancer / Cardiovascular disease / Development / Diet / Docosahexaenoic acid / Eicosanoids / Eicosapentaenoic acid / Fish oil / Inflammation

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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
E-mail: mailto:pcc@soton.ac.uk
Fax: +44 (0)2381 204221

Abbreviations: CI, confidence interval; **CVD,** cardiovascular disease; **DHA,** docosahexaenoic acid; **DPA,** docosapentaenoic acid; **EPA,** eicosapentaenoic acid; **GPR,** G-protein coupled receptor; **IBD,** inflammatory bowel disease; **MI,** myocardial infarction; **RA,** rheumatoid arthritis; **RCT,** randomized controlled trial

1 Very long chain 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 that are characterized by the position of the double bond closest to the methyl terminus of the hydrocarbon chain being on carbon number three (counting the methyl carbon as number one). Functionally the most important n-3 fatty acids appear to be eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoioc acid (DHA; 22:6n-3) [1], although roles

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for docosapentaenoic acid (DPA; 22:5n-3) are now also emerging [2]. Because of their long hydrocarbon chain EPA, DPA, and DHA are termed very long chain n-3 fatty acids. EPA, DPA, and DHA are found in fairly high amounts in seafood, especially fatty fish (sometimes called "oily fish"), in supplements like fish oils, cod liver oil, and krill oil, in some algal oils, and in a limited number of pharmaceutical grade preparations. EPA, DPA, and DHA are metabolically related to one another, and there is a pathway by which EPA can be synthesized from simpler, plant-derived n-3 fatty acids (Fig. 1). The initial substrate for this pathway is α-linolenic acid (18:3n-3), an essential fatty acid in animals. The pathway of conversion of α-linolenic acid to EPA involves three steps, catalyzed in turn by delta-6 desaturase, elongase, and delta-5 desaturase (Fig. 1). These enzymes are shared with the analogous omega-6 (n-6) fatty acid biosynthetic pathway of
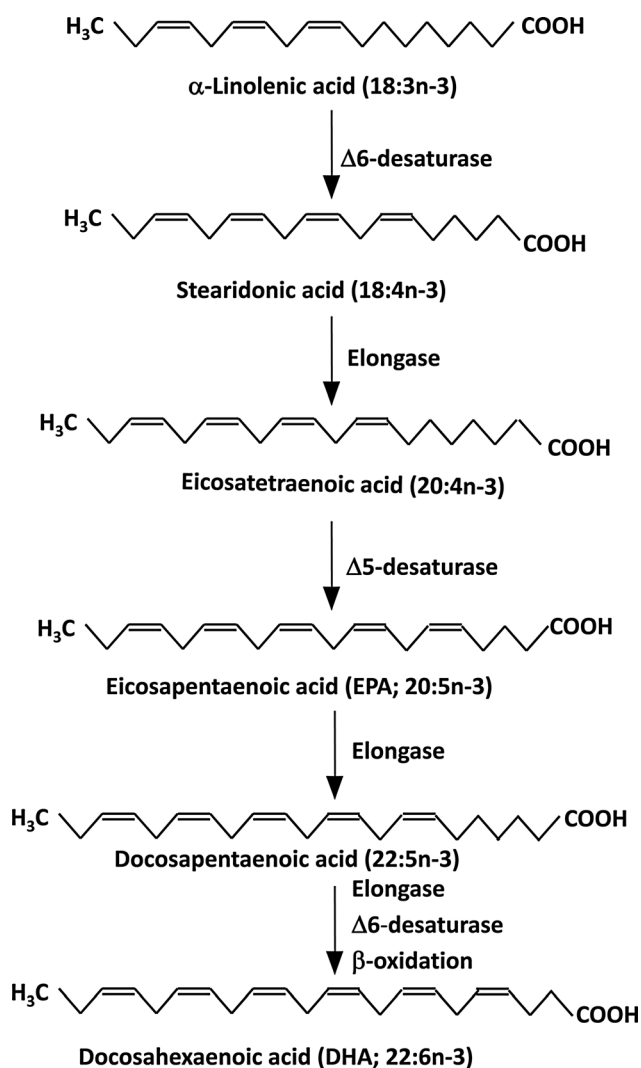

Figure 1. The metabolic pathway of biosynthesis of eiocosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid.

conversion of linoleic acid (18:2n-6) to arachidonic acid (20:4n-6). The high intake of linoleic acid relative to α-linolenic acid in many Western diets [3] favors linoleic acid conversion over that of α-linolenic acid; this may be one of the reasons why conversion of α-linolenic acid along this pathway is considered to occur at a low rate [4], although this rate may be affected by hormones [5], sex [6], genetics [7], age [8], and disease. Where α-linolenic acid has been demonstrated to have biological effects, these seem to be related to its conversion to EPA (see [4]); hence the limited conversion of α-linolenic acid to EPA and further to DHA, limits the functionality and health impact of α-linolenic acid (discussed in [4]). One-step β-oxidation of DHA can be used to produce EPA, a process sometimes referred to as retroconversion [9].

As indicated above, EPA, DPA, and DHA are found in fairly high amounts in seafood and products derived from seafood. Table 1 reports typical values for the content of these fatty acids in various seafoods [10]. It is evident that there is at least a ten-fold range in content of these fatty acids per portion (i.e., per serving) of seafood, with fatty fish able to provide 1–3 g of EPA + DPA + DHA per portion. Examples of fatty fish are mackerel, salmon, trout, herring, tuna, and sardines. In comparison lean fish like cod, haddock, and plaice, typically provide 0.1–0.4 g per portion. Although tuna is a fatty fish, canned tuna has had the oil removed during processing and so is low in content of very long chain n-3 fatty acids (Table 1). Meat and blubber of sea mammals like seals and whales is also rich in very long chain n-3 fatty acids, although these are not usually eaten by most humans. Likewise, some offal products like brain are rich in very long chain n-3 fatty acids but again these are rarely eaten in most populations. EPA, DPA, and DHA are found in modest amounts in animal-derived foods like eggs and meat (Table 1). Among foods, including fatty fish, the relative distribution of EPA, DPA, and DHA differs such that some foods are richer in EPA than DHA while others are richer in DHA than EPA (Table 1).

Because fatty fish are the richest dietary source of very long chain n-3 fatty acids, intake of those fatty acids is heavily influenced by fish consumption. In most Western populations the distribution of fatty fish consumption is bimodal, with a relatively small proportion of the population being regular fatty fish consumption; in the UK it is estimated that only 25% of the adult population regularly consume fatty fish [11]. The other 75% of the population consume fatty fish rarely or never. Mean intakes of EPA + DPA + DHA among adults in many Western populations are considered to be around 0.1–0.3 g/day [11]. However it is difficult to be precise about this figure for several reasons: (i) as indicated above, the distribution of fatty fish consumption is bimodal (and so mean intake is not very informative); (ii) the infrequent or irregular pattern of fatty fish consumption means that robust information about this may not be captured by tools that assess dietary information like recall, food frequency questionnaires, and food diaries; (iii) databases of nutrient composition of foods may have inadequate or incorrect data

Table 1. Typical content of EPA, DPA, and DHA in seafood and meat. Data are taken from ref. [10]. Note that both very long chain n-3 fatty acid content and portion size may vary

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
Canned sardines	0.89	0.10	0.68	100	1.67
Salmon	0.5	0.4	1.3	100	2.2
Trout	0.23	0.09	0.83	230	2.65
Herring	0.51	0.11	0.69	120	1.56
Cod	0.08	0.01	0.16	120	0.30
Haddock	0.05	0.01	0.10	120	0.19
Plaice	0.16	0.04	0.10	130	0.39
Canned tuna	0.02	0.02	0.14	45	0.08
Crab	0.47	0.08	0.45	85	0.85
Prawns	0.06	< 0.01	0.04	60	0.06
Mussels	0.41	0.02	0.16	40	0.24
Beef	0.02	0.02	0	90	0.04
Lamb	0.03	0.04	0.02	90	0.08
Pork	0.01	0.02	0.01	90	0.04
Chicken	0.01	0.02	0.03	100	0.06
Venison	0.04	0.09	< 0.01	120	0.16

on EPA, DPA, and DHA content of foods including fish and non-seafood sources; and (iv) the very long chain n-3 fatty acid content varies among fatty fish, even those of the same species, according to location caught, season, diet, water temperature, stage in the life cycle, and whether the fish was wild or farmed [12–14].

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 [15], consistent with the oft-quoted "average" intake of these fatty acids among Western adults [11]. However, median intakes of the three very long chain n-3 fatty acids were found to be only 8, 6, and 15 mg/day [15], the large differences between mean and median intakes reflecting the non-normal 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, giving a total very long chain n-3 fatty acid intake of 246 mg/day [16]. 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 very long chain n-3 fatty acids daily. An interesting observation from these studies is that meat (including poultry) provides 40–45% of the very long chain n-3 fatty acids consumed by Australian adults, with fish and seafood products providing 45–50% [16]. Australian children aged 2–16 years consumed a mean of 79 mg/day EPA + DPA + DHA, with a median intake of 29 mg/day [17]. Intakes increased with age and were much higher in fish eaters than non-fish eaters [17].

EPA, DPA, and DHA are present in oils, supplements, and pharmaceutical grade preparations. Most of these are available "over the counter" in supermarkets, pharmacies, health food shops and so on and on the internet, while some are available on prescription only. These vary according to very long chain n-3 fatty acid content. For example, cod liver oil and standard fish oil supplements contain about 30% of their fatty acids as EPA + DHA (i.e., about 300 mg/g oil), while concentrated n-3 supplements contain 45–60% of their fatty acids as EPA + DHA and the pharmaceutical preparation Omacor® (also known as Lovaza®) contains almost 90% EPA + DHA (in the ethyl ester form). Figure 2 illustrates the influence of daily consumption of a 1 g standard fish oil capsule, a 1 g "concentrated" supplement, one teaspoon of cod liver oil, one meal of salmon, and one or four capsules of the pharmaceutical grade preparation Omacor® on daily intake of EPA + DHA. It is clear 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.

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

Like all fatty acids, EPA and DHA are transported in the bloodstream esterified into triacylglycerols, phospholipids, and cholesteryl esters as components of lipoproteins and
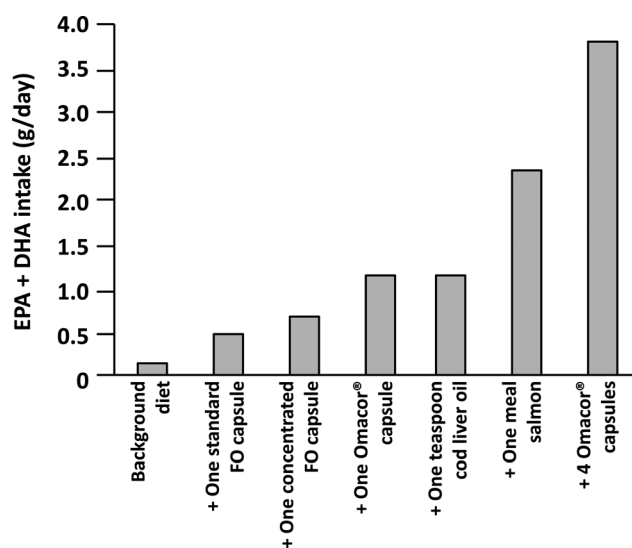
Figure 2. Typical intake of EPA + DHA from the background diet in an adult not regularly consuming fatty fish and what would be achieved by also consuming a 1 g standard fish oil (FO) capsule, a 1 g "concentrated" supplement, one teaspoon of cod liver oil, one meal of salmon, or one or four capsules of the pharmaceutical grade preparation Omacor®.

Table 2. EPA and DHA content of different transport, storage and functional pools in healthy humans with low fatty fish intake. Data are expressed as % of total fatty acids present, are mean from 168 individuals (∼50% men; aged 20–80 (mean 50) years) and are taken from ref. [21]

Pool	EPA	DHA
Plasma phosphatidylcholine	1.2	3.6
Plasma cholesteryl ester	1.0	0.6
Plasma triacylglycerol	0.3	0.8
Plasma non-esterified fatty acid	0.4	1.6
Blood mononuclear cells	0.8	1.9
Erythrocytes	2.3	5.2
Platelets	1.1	2.0
Cheek buccal cells	1.5	0.8
Subcutaneous adipose tissue	0.2	0.2

non-covalently bound to albumin in the non-esterified form. They are stored in adipose tissue esterified into triacylglycerols 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 [18], the manner in which membranes change in response to external stimuli [19], and the functional activities of membrane-bound proteins [20]. The proportional 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 2) [21]. Most often DHA is present in a greater proportion than EPA. 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. For example, DHA was reported to contribute an average of 18% of fatty acids to adult human brain gray matter [22], while Makrides et al. [23] reported average DHA contents of about 8% and 12% of fatty acids for human infant cerebral cortex and retina, respectively. In the latter study the contributions of EPA were <0.05% and 0.1%, respectively [24]. 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. For example, DHA was reported to contribute an average of 36% of fatty acids in mammalian brain gray matter phosphatidylserine [24] and an average of 22% of fatty acids in retina phosphatidylcholine [25].

Increased intakes of EPA and DHA from fish or from supplements are reflected in increased proportions of both fatty acids in blood lipid, blood cell, and many tissue pools. This has been reported many times for total plasma or serum lipids or for the complex lipid components of plasma or serum (i.e., triacylglycerols, phospholipids, and cholesteryl esters) [21, 26–34] and is also well described for erythrocytes [21, 29, 34], platelets [21, 26, 35], and leukocytes [21, 30, 32, 36, 37]. There are also descriptions of increased proportions of EPA and DHA in human tissues, including skeletal muscle [38], heart [39], gut mucosa [40, 41], and adipose tissue [21, 30] when their intake is increased. These locations all show a dose- and time-dependent incorporation of both EPA and DHA [21, 26, 30–32, 36], but the precise pattern depends upon the specific location. Pools that are turning over rapidly show faster incorporation of EPA and DHA than slower turning over pools. Thus, plasma lipids incorporate EPA and DHA more quickly than blood cells do [2], whilst amongst blood cells, leukocytes have been usually shown to incorporate EPA and DHA more quickly than erythrocytes. Modification of human brain fatty acid composition is more difficult than for other tissues, especially beyond childhood. A recent study examined in detail the dose- and time-dependent appearance of EPA and DHA in different transport, functional, and storage pools in humans [21]. Healthy human volunteers consumed one of three doses of EPA + DHA (providing 3.27, 6.54, or 13.08 g/wk; the ratio of EPA to DHA was 1:1.1) in capsules or placebo capsules daily for 12 months. Blood was collected at the start of the study and at several intervals up to 12 months; in addition adipose tissue biopsies were collected at study entry and after 6 and 12 months. The study clearly demonstrated that all pools show time- and dose-dependence of incorporation of both EPA and DHA and that, in time, pools reach a new steady state content of EPA and DHA that is determined by intake, but that the exact nature of these changes varies among

pools. Figure 3 shows the incorporation of EPA and DHA into plasma phosphatidylcholine and into platelets. EPA appears to be incorporated more quickly than DHA into both pools, the incremental increase in both fatty acids is greater in plasma phosphatidylcholine than in platelets, and the new steady state level of EPA and DHA that is reached is precisely related to the dose of EPA or DHA being consumed. Table 3 summarises the time to reach a new steady state and the approximate increment in EPA and DHA content (as a proportion of total fatty acids) that the new steady state represents.

The higher status of EPA and DHA achieved 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 levels then EPA and DHA status decline, eventually returning to earlier levels. This is well described for blood lipids [26, 30, 31], platelets [26], leukocytes [31], and erythrocytes [34]. However, just as the incorporation of EPA into different pools is faster than the incorporation of DHA (Fig. 3; Table 3), the loss of EPA is faster than the loss of DHA; this is shown in Fig. 4 for human blood mononuclear cells [31] but is also described for other pools [26, 34]. One interpretation of this preferential retention of DHA is that DHA is structurally and/or functionally

preferred over EPA and that metabolic mechanisms have evolved to preserve it.

Since EPA and DHA content in lipid pools is frequently described as a percentage or proportion, the increase in content of these fatty acids is accompanied by a decrease in content (proportion) of other, usually unsaturated, fatty acids. Depending upon the pool, since once again there are differences among pools, these other fatty acids include oleic (18:1n-9), linoleic (18:2n-6), dihomo-γ-linolenic (20:3n-6), and arachidonic (20:4n-6) acids. There are good demonstrations of dose- and time-dependent decreases in the proportion of arachidonic acid in leukocytes and platelets [26, 31, 32, 36], which might be functionally important (see later).

3 Molecular and cellular effects of increased EPA and DHA status

Many, though not all, of the functional effects of EPA and DHA rely upon their incorporation into cell membrane phospholipids [42–44]. Because of their highly unsaturated nature, EPA and DHA have been shown in some studies to decrease membrane order (i.e., increase membrane fluidity) [18, 45], although cells have mechanisms such as modifying membrane cholesterol content, to limit this effect, and they create a specific
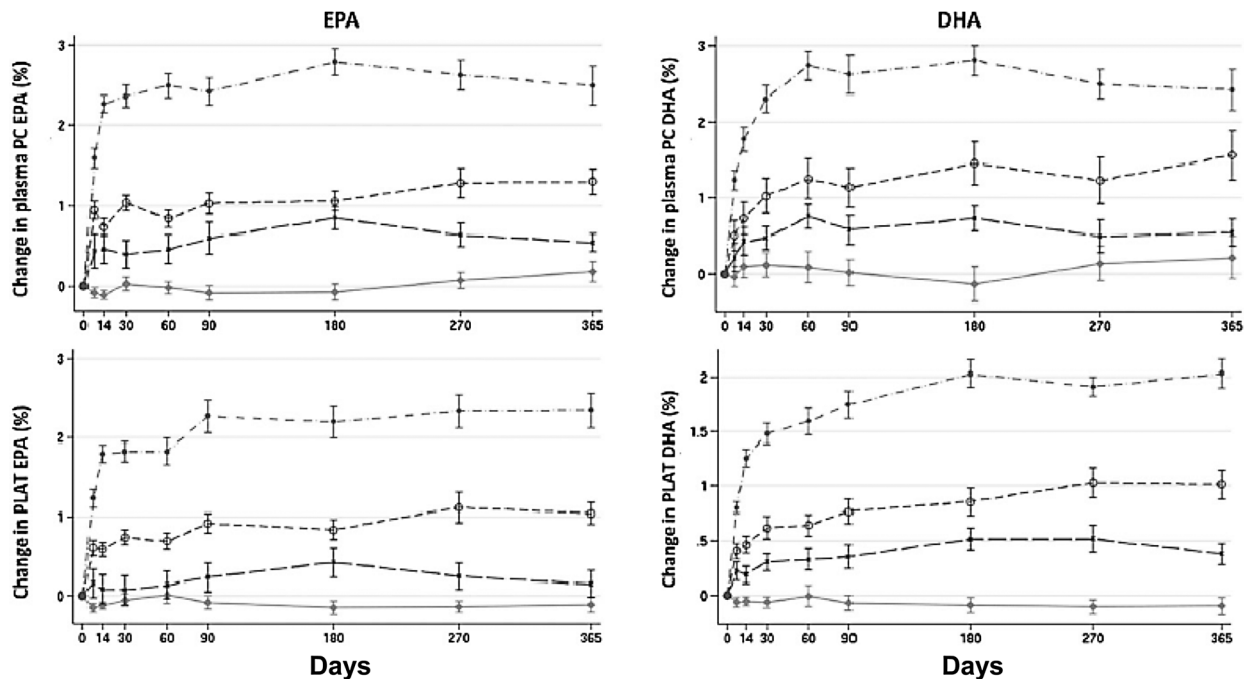

Figure 3. Time course of changes in EPA and DHA content of human plasma phosphatidylcholine and platelets in subjects consuming placebo oil or one of three doses of fish oil. Healthy subjects supplemented their diet with capsules providing 0 (solid line), 3.27 (− − −), 6.54 (- - -), or 13.08 (- - -) g EPA + DHA per week for a period of 12 months; the ratio of EPA to DHA was 1:1.1. Plasma phosphatidylcholine and platelets were isolated at 0, 1, 2, 4, 8, 12, 24, 36, and 52 weeks and their fatty acid composition determined by gas chromatography. Data are mean ± standard error from at least 30 subjects per group. Taken from ref. [21] with permission from the American Society of Nutrition.

Table 3. Estimated time to peak incorporation and maximum incorporation of EPA and DHA in different transport, storage and functional pools in healthy humans with low fatty fish intake. Data are median from 33 individuals who consumed 13.08 g EPA + DHA per week for 12 months. The ratio of EPA to DHA was 1:1.1 and the very long chain n-3 fatty acids were taken over 4 days of each week. Data are taken from ref. [21]

Pool	EPA			DHA		
	Time to peak (days)	Maximum incorporation (% of fatty acids)	Increase from baseline (% of fatty acids)	Time to peak (days)	Maximum incorporation (% of fatty acids)	Increase from baseline (% of fatty acids)
Plasma phosphatidylcholine	18	3.5	2.5	23	5.9	2.5
Plasma cholesteryl ester	24	3.2	2.2	ND	1.0	0.4
Plasma triacylglycerol	16	1.7	2.0	20	2.5	2.0
Plasma non-esterified fatty acid	38	1.1	0.5	32	3.1	2.0
Blood mononuclear cells	249	2.3	1.6	207	3.3	1.5
Erythrocytes	55	3.7	2.0	136	7.7	3.0
Platelets	25	3.1	2.2	32	3.6	2.0
Subcutaneous adipose tissue	ND	0.3	0.1	ND	0.35	0.15

ND, not determined.

environment for membrane proteins like receptors, transporters, ion channels, and signaling enzymes that influences the activity of those proteins [19, 20, 46]. Through these actions EPA and DHA can modulate cell responses that are dependent upon membrane proteins. Cell membranes contain microdomains called rafts; rafts have specific lipid and fatty acid compositions and act as platforms for receptor action and for the initiation of intracellular signaling pathways [47–49].
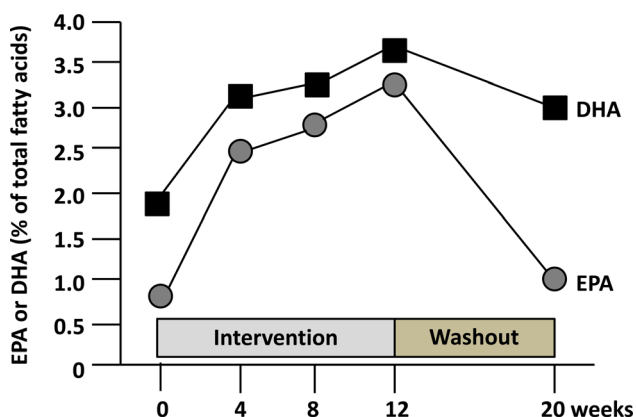

Figure 4. Time course of changes in EPA and DHA content of human blood mononuclear cells in subjects consuming fish oil. Healthy subjects supplemented their diet with fish oil capsules providing 2.1 g EPA plus 1.1 g DHA per day for a period of 12 weeks (indicated by the box "intervention"). During the period between 12 and 20 weeks subjects did not use fish oil (indicated by the box "washout"). Blood mononuclear cell phospholipids were isolated at 0, 4, 8, 12, and 20 weeks and their fatty acid composition determined by gas chromatography. Data are mean from eight subjects and are from ref. [31]. EPA is shown as gray circles and DHA as black squares. Error bars are omitted for clarity.

Studies in a variety of cell types including neurones, immune cells, and cancer cells have shown that EPA and DHA modify raft formation [49, 50], although the exact effect varies according to cell type, in a manner which modifies intracellular signaling pathways [49–51]. As a result of their effects on membrane-generated intracellular signals, EPA and DHA can modulate transcription factor activation and, subsequently, gene expression patterns [42, 46, 52]. Transcription factors shown to be affected by EPA and DHA include nuclear factor κB [53], peroxisome proliferator activated receptor-α and γ [54, 55], and the sterol regulatory element binding proteins [56–60]. These effects of n-3 fatty acids on transcription factor activation and gene expression are central to their role in controlling inflammation, fatty acid and triacylglycerol metabolism, and adipocyte differentiation [42–44, 46, 52].

A second consequence of increased abundance of EPA and DHA in cell membrane phospholipids, and the associated decreased abundance of arachidonic acid, is that the availability of substrates for synthesis of bioactive lipid mediators is altered. Arachidonic acid 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 polyunsaturated fatty acids and are produced via the cyclooxygenase (prostaglandins, thromboxanes), lipoxygenase (leukotrienes and other products) and/or cytochrome P450 pathways. Excess or inappropriate production of eicosanoids from arachidonic acid is associated with many disease processes [61, 62]. It is well described that increasing the very long chain n-3 fatty acid content of cell membranes results in decreased production of eicosanoids from arachidonic acid [26, 31–33, 36], 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 arachidonic acid, EPA and DHA are themselves substrates for the synthesis of lipid mediators. Some of these are simply analogues of those produced from arachidonic acid. For example, prostaglandin E_3 produced from EPA is an analogue of prostaglandin E_2 produced from arachidonic acid. Frequently, though not always, the EPA-derived mediator has weaker biological activity than the arachidonic acid-derived mediator [63]. For example, thromboxane A_3 from EPA is a much weaker platelet aggregator than thromboxane A_2 from arachidonic acid [64]. EPA and DHA are also substrates for more complex biosynthetic pathways that result in generation of a large number 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) [65–67]. It has recently been discovered that DPA gives rise to similar mediators [68]. The major role of resolvins, protectins, and maresins appears to be in the resolution of inflammation and modulation of immune function [65–67]. It seems likely that many of the anti-inflammatory actions of very long chain n-3 fatty acids that are described in the literature are mediated through resolvins, protectins, and maresins [43, 44].

The above-mentioned mechanisms of action of EPA and DHA rely upon incorporation of those fatty acids into cell membrane phospholipids. It is now recognized that EPA and DHA (in their unesterified form) can also act directly via G-protein coupled receptors (GPR) that exhibit some specificity for very long chain n-3 fatty acids over other fatty acids as ligands [69]. In particular, 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-sensitizing effects of DHA on adipocytes [69].

4 Human health benefits of EPA and DHA

4.1 Introduction

In addition to studies in model systems like cell culture and laboratory animals, evidence for a role of EPA and DHA in human health comes from two different experimental approaches. The first of these are studies of the association between very long chain n-3 fatty acid intake from the diet or very long chain n-3 fatty acid concentration in a specific body pool (e.g., in blood plasma or serum or in erythrocytes) and biomarkers or clinical markers of disease risk or a disease manifestation. Such studies can involve comparisons between populations or sub-populations (called ecological studies; e.g., comparison between populations in Greenland and Denmark or between Japanese living in Japan and in the

USA), comparisons between individuals with disease and those without (called case-control studies), or the tracking of a group of individuals over time to identify the likelihood of emergence of disease (called prospective cohort studies). Such studies typically involve long term exposure to very long chain n-3 fatty acids and often include large numbers of individuals. The second experimental approach is the clinical trial, where individuals consume an increased amount of EPA and DHA for a period of time and the effect on disease risk factors, disease manifestations, or disease occurrence is monitored. Such studies are typically relatively short and often involve relatively small numbers of individuals. A clinical trial is more robust if there is a control (placebo) group, if allocation to the control or the very long chain n-3 fatty acid group is random, and if the participants and the researchers are unaware ("blind") to the group which each participant is allocated. This design is termed a randomized, controlled trial (RCT) and this is considered the highest level of experimental evidence. Experimental results from several association studies or RCTs can be aggregated in meta-analyses which consequently include large numbers of participants and have great statistical power to identify effects. Consequently, meta-analyses are regarded as a high level of evidence. It is beyond the scope of this article to describe all of the health benefits of very long chain n-3 fatty acids; such wide-ranging coverage may be found elsewhere [70, 71].

4.2 Very long chain n-3 fatty acids and cardiovascular disease

Cardiovascular disease (CVD) includes heart disease, cerebrovascular disease, and peripheral vascular disease. The major causes of death as a result of CVD are myocardial infarction (MI, i.e., heart attack) and stroke. Native populations in Greenland, Northern Canada, and Alaska consuming their traditional diet had much lower rates of death from CVD than predicted, despite their high dietary fat intake [72–75]. Typically the rate of mortality was less than 10% of that predicted. The protective component 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 oily fish [76]. Japanese consuming a traditional diet also exhibit a low cardiovascular mortality [77] and this diet is rich in seafood including oily fish and sometimes marine mammals, which contain significant amounts of EPA and DHA. Substantial evidence from prospective and case-control studies has now accumulated indicating that consumption of EPA and DHA reduces the risk of CVD outcomes in Western populations, although not all studies agree. These studies have been summarized and discussed in detail elsewhere [78–82]. A recent systematic review and meta-analysis 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 [83]. The aggregation of data from 16 studies involving over 422,000 individuals showed a relative risk of 0.87 (95% confidence interval (CI) 0.78–0.97) for those in the top third of dietary very long chain n-3 fatty acid intake compared with those in the lower third of intake. The aggregation of data from 13 studies involving over 20,000 individuals showed relative risks of 0.78 (95% CI 0.65–0.94), 0.79 (95% CI 0.67–0.93), and 0.75 (95% CI 0.62–0.89) for those in the top third of circulating EPA, DHA, and EPA + DHA, respectively, compared with those in the lower third [83]. A smaller number of studies in fewer individuals gave a relative risk of 0.64 (95% CI 0.47–0.89) for DPA [83].

The protective effect of very long chain n-3 fatty acids towards CVD development most likely relates to beneficial modification of a broad range of risk factors. These include plasma triacylglycerol concentrations, blood pressure, and inflammation which are all lowered by very long chain n-3 fatty acids [78, 82, 84–86] (Table 4). The healthier risk factor profile would result in improved blood flow and reduced build-up of fatty deposits (atherosclerotic plaques) within the blood vessel wall. Thus the improvement of the risk factor profile caused by very long chain n-3 fatty acids lowers the risk of developing CVD.

There has also been significant interest in the effect of very long chain n-3 fatty acids in people with existing CVD. The outcome in these studies has most often been the occurrence of a major cardiovascular event (e.g., MI), including one that is fatal. Several studies published between 1989 and 2008 reported lower rates of death in patients receiving very long chain n-3 fatty acids [87–91]. Doses used in these studies were 500–900 mg EPA + DHA/day for 2 years [87], 885 mg EPA + DHA/day for 1 [89], 3.5 [88] or 3.9 [91] years, and 1.8 g EPA/day for 5 years [90]. As a result of these positive findings several meta-analyses supported that very long chain

Table 4. Factors involved in cardiovascular risk that are influenced by very long chain n-3 fatty acids

Factor	Effect of very long chain n-3 fatty acids
Plasma triacylglycerol concentration (fasting and post-prandial)	↓
Production of chemoattractants	↓
Production of growth factors	↓
Cell surface expression of adhesion molecules	↓
Production of inflammatory eicosanoids and cytokines	↓
Blood pressure	↓
Endothelial relaxation	↑
Thrombosis	↓
Cardiac arrhythmias	−/↓
Heart rate variability	↑
Atherosclerotic plaque stability	↑

n-3 fatty acids lower mortality in patients with existing CVD [92–94]. In a meta-analysis including 11 studies involving almost 16,000 patients, Bucher et al. [92] reported that compared with control, very long chain n-3 fatty acids lower the risk of fatal MI (relative risk 0.7; 95% CI 0.6–0.8), sudden death (relative risk 0.7; 95% CI 0.6–0.9) and all-cause mortality (relative risk 0.8; 95% CI 0.7–0.9). In a meta-analysis including 14 studies involving over 20,000 patients, Studer et al. [93] reported that compared with control, very long chain n-3 fatty acids lower the risk of cardiac mortality (relative risk 0.68; 95% CI 0.52–0.90) and all-cause mortality (relative risk 0.77; 95% CI 0.63–0.94).

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 the therapeutic effect of very long chain n-3 fatty acids. The first is altered cardiac electrophysiology seen as lower heart rate [95], increased heart rate variability [96], and fewer arrhythmias [97]. 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 that control platelet aggregation from arachidonic acid and from EPA [26]. 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 stabilize atherosclerotic plaques preventing their rupture [98, 99]. This effect would reduce the likelihood of a cardiovascular event (MI, stroke) from happening [100].

Despite the positive findings with very long chain n-3 fatty acids, supported by meta-analyses and biologically plausible candidate mechanisms, a series of more recent studies have failed to replicate the earlier findings [101–105] and this has influenced 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 [106–108]. Nevertheless, the meta-analysis by Rizos et al. [108] which included the recent studies, but excluded the landmark GISSI Prevenzione trial [88], did identify a reduction in cardiac death with very long chain n-3 fatty acids (relative risk 0.91; 95% CI 0.85–0.98) and trends towards reductions in sudden death (relative risk 0.87; 95% CI 0.75–1.01), and MI (relative risk 0.89; 95% CI 0.76–1.04). It is important to recognize that the most recent studies of very long chain n-3 fatty acids and cardiovascular mortality have been criticized for various reasons related to small sample size (i.e., too few patients being studied), the low dose of very long chain n-3 fatty acids used, and the too short duration of follow up [109]. The most recent meta-analyses that include the GISSI-Prevenzione study and some of the more recent neutral studies, report benefits from very long chain n-3 fatty acids [110, 111]. For example, Casula et al. [110] identified

reductions in cardiac death (relative risk 0.68; 95% CI 0.56–0.83), sudden death (relative risk 0.67; 95% CI 0.52–0.87), and MI (relative risk 0.75; 95% CI 0.63–0.88) with very long chain n-3 fatty acids and a trend towards lower all-cause mortality (relative risk 0.89; 95% CI 0.78–1.02). Likewise, Wen et al. [111] identified reductions in cardiac death (relative risk 0.88; 95% CI 0.80–0.96), sudden death (relative risk 0.86; 95% CI 0.76–0.98), MI (relative risk 0.86; 95% CI 0.73–1.00), and all-cause mortality (relative risk 0.92; 95% CI 0.86–0.99) 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 cardiovascular disease and can be used successfully to treat people with cardiovascular disease. Although the most recent RCTs in patients with cardiovascular disease have produced findings that do not agree with the previously accumulated literature, it is too early to discard the earlier evidence. The conclusion that very long chain n-3 fatty acids have a role in reducing risk of cardiovascular disease remains well supported.

4.3 Very long chain n-3 fatty acids and cancer

EPA and DHA exert a range of biological activities that may influence tumor cell proliferation and viability; for example, DHA can promote tumor cell apoptosis [112, 113], possibly through inducing oxidative stress. EPA and DHA also replace the n-6 fatty acid arachidonic acid in cell membranes resulting in reduced production of mediators like prostaglandin E_2 that drive tumor cell proliferation and tumor growth [113–115]. Through these effects, EPA and DHA can directly influence cancer cells and the tumor environment and they can influence the host response to tumor bearing. Anti-inflammatory actions of very long chain n-3 fatty acids may also be important in preventing or slowing some steps in tumor initiation particularly in some cancers such as colorectal cancer. Recent reviews provide in-depth analysis of the mechanisms by which very long chain n-3 fatty acids affect tumor cell proliferation, invasion, and metastasis [112, 113]; the ability of very long chain n-3 fatty acids to enhance the effectiveness of anti-cancer treatments [113, 115]; 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 [113, 115].

The concentrations of very long chain n-3 fatty acids are reported to be lower in blood lipids and cells from some cancer patients than in controls, probably due both to dietary changes and to altered metabolism [116]. Some prospective and case-control studies suggest that very long chain n-3 fatty acids lower risk of colorectal, prostate, and breast cancers, but there is significant inconsistency in the findings from such studies [117]. A recent systematic review concluded that very long chain n-3 fatty acids are protective against breast cancer [118], while a 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 [119].

In addition to effects that lower the risk of developing cancer, there seems to a role for very long chain n-3 fatty acids in patients with existing 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 published by Elia et al. [120] concluded that lung cancer patients receiving supplements containing EPA and DHA had improved appetite, energy intake, body weight, and quality of life. Alfano et al. [121] reported lower inflammation and less physical fatigue in breast cancer patients with a higher concentration of very long chain n-3 fatty acids in their bloodstream than seen in patients with a lower concentration. Cerchietti et al. [122] reported that lung cancer patients given 1.8 g EPA + DHA per day had improved appetite and less fatigue than controls. Van der Meij et al. [123] reported that 2.9 g EPA + DHA per day improved quality of life, physical function, cognitive function, and health status in patients with non-small cell lung cancer. The patients receiving very long chain n-3 fatty acids also tended to have higher physical activity compared to the control group [123].

Both EPA and DHA sensitise cultured tumor cells to chemotherapeutic agents, increasing the efficacy of those agents [124]. The mechanism by which this occurs is not clear, but it might involve increased EPA and DHA content of tumor cell membranes resulting in increased lipid peroxidation in those membranes in the presence of the cancer therapeutic. This would result in improved efficacy of the therapy and perhaps reduced side effects. Murphy et al. [125] 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. [126] reported improved chemotherapy outcomes with 1.8 g DHA per day in breast cancer patients.

Cancer cachexia (loss of lean and fat tissue) is a complication that occurs in patients with advanced solid tumors; it greatly increases risk of mortality. Many of the factors involved in inducing and sustaining cachexia are targets for very long chain n-3 fatty acids. Weed et al. [127] 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. [128] conducted a trial in patients with non-small cell lung cancer and showed that 2.2 g EPA + DHA per day was able to maintain body weight and muscle mass during chemotherapy. In other studies very long chain n-3 fatty acids have been shown to increase body weight in cancer patients [129, 130].

Thus there is increasing recent evidence from studies in humans, including a number of intervention trials 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 in cancer patients to demonstrate benefits. From their review of the literature Vaughan et al. [115] 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. Although this review was not exhaustive, 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."

4.4 Very long chain n-3 fatty acids and inflammation

Inflammation is a key 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. Of the fatty acids studied, EPA and DHA appear to possess the most potent effects on the immune system and its inflammatory component [43, 44, 131]. When continuously exposed to an inflammatory trigger, the loss of the normal mechanisms inducing tolerance or loss of resolving factors can allow inflammation to become chronic and in this state the damage done to host tissues may become pathological [132, 133]. As such, inflammation is the central adverse response seen in a range of inflammatory conditions including rheumatoid arthritis (RA), inflammatory bowel diseases (IBD), asthma, psoriasis, and atopic dermatitis [132, 133]. Furthermore, chronic low-grade inflammation is now recognized to be a contributor to cardiovascular disease [134, 135] and to play a role in cardiometabolic diseases like obesity, type-2 diabetes, and non-alcoholic fatty liver disease [136]. The key link between fatty acids and inflammatory processes is that the n-6 fatty acid arachidonic acid is the precursor for the production of eicosanoids, which are intimately involved in inflammation [61, 62]. In contrast to the effects of arachidonic acid, EPA and DHA give rise to mediators which are less pro-inflammatory, anti-inflammatory or inflammation resolving [43, 44, 131]. In addition to their effects on lipid mediators (prostaglandins, leukotrienes, resolvins, protectins), EPA and DHA influence several other aspects of inflammatory processes including leukocyte migration and production of inflammatory cytokines [43, 44, 131]. These effects also seem to involve incorporation of EPA and DHA into the membranes of inflammatory cells from where they influence cell signaling, transcription factor activation, and gene expression [43, 44, 52, 131]. There is reasonably good evidence that EPA and DHA given in combination at sufficiently high doses are anti-inflammatory. As a result they are suggested to have a therapeutic role in

inflammatory diseases. This has been most widely studied in RA [137], IBD [138], and asthma [139]. Evidence of efficacy is strongest in RA, although high doses (several g/day of EPA + DHA) are typically used [137]. One area of significant current interest is 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 [140, 141]. During pregnancy very long chain n-3 fatty acids, especially DHA, are efficiently transferred from the mother to her fetus [142], with the amount transferred being directly related to the mother's intake [143]. There is some evidence that increased intake of EPA and DHA during human pregnancy has an effect on the immune system of the baby [144–146] and that this may reduce allergic symptoms later in life [140, 141, 145, 147, 148]. Supplementing the diets of very young infants has also now been shown to have immune effects consistent with reduced likelihood of allergy [149].

Thus, the anti-inflammatory actions of very long chain n-3 fatty acids are extensively demonstrated and the underlying mechanisms are increasingly understood [43, 44, 131]. High doses of very long chain n-3 fatty acids can be used to treat frank inflammatory conditions while lower doses likely have a role in protecting against low-grade inflammatory conditions.

4.5 Very long chain n-3 fatty acids and the brain

More than 50% of the dry weight of the brain is lipid, particularly structural lipid (i.e., phospholipids). The human brain and retina contain an especially high proportion of DHA relative to other tissues but little EPA (see earlier). DHA contributes 50–70% of the fatty acids present in the rod outer segments of the retina [25]. These rod outer segments contain the eyes' photoreceptors. DHA is important for neurotransmission, neuronal membrane stability, neuroplasticity, and signal transduction [150–152]. The human brain growth spurt occurs from approximately the beginning of the third trimester of pregnancy to 18 months after birth. The amount of DHA in the brain increases dramatically during the brain growth spurt. In humans, brain weight increases from about 100 g at 30 weeks of gestation to about 1100 g at 18 months of age; during this time there is three- to four-fold increase in DHA concentration in the brain and a 35-fold increase to total brain DHA. This DHA is provided by the mother across the placenta during pregnancy and in breast milk after birth. 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 with preterm infants, where feeds that included DHA (and often also arachidonic acid) were shown to improve visual development [153–158]. However, a recent meta-analysis of 17 trials of inclusion of DHA (and arachidonic acid) in preterm infant feeds found little evidence to support improved visual acuity or neurodevelopment, although three out of seven studies did report some benefit

of polyunsaturated fatty acids [159]. Studies of DHA in preterm infants are discussed in detail elsewhere [160]. The literature on the effect of DHA on visual and cognitive outcomes in term infants is mixed with some studies reporting benefits [161, 162] and others not [163–166]. 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. Agostoni et al. found that DHA-supplemented formula improved cognitive development of term infants assessed at 4 months of age [161], but that the effect was lost at 2 years of age [167]. A recent meta-analysis of 15 trials of inclusion of DHA (and arachidonic acid) in term infant feeds 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 [168]. 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 recent systematic review and meta-analysis of eleven RCTs of very long chain n-3 fatty acids in pregnancy involving over 5000 women could not conclusively support or refute that very long chain n-3 fatty acids in pregnancy improve infant visual or cognitive development [169].

It is now thought that very long chain n-3 fatty acids have important roles in the brain beyond infancy and indeed may be important for brain function throughout the life course. Children with attention deficit hyperactivity disorder or autistic spectrum disorders have been reported in some studies to have lower levels of EPA and DHA in their bloodstream than control children [170], leading to the suggestion that these and other developmental disorders such as dyslexia and dyspraxia are related to some sort of fatty acid deficiency state. Therefore, it has been considered that normalization of fatty acid levels might lead to clinical benefit in these conditions. This has been examined in a number of trials in children and adolescents with attention, learning, or behaviour disorders, some showing some improvements [171–179] and others finding no effect [180–186]. These trials have been reviewed recently, with the conclusion 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 [187].

Rudin [188] was the first to suggest 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. Schizophrenic patients have lower levels of EPA and DHA in their erythrocytes than do controls [189–192]. In a study of nine countries, Hibbeln [193] demonstrated a significant correlation between high annual fish consumption and lower prevalence of major depression, an observation that is compatible with a proposed protective effect of very long chain n-3 fatty acids. A small study using a very high dose of EPA + DHA (9.6 g/day) reported a reduction in depressive

symptoms [194], while a study using a lower dose of DHA alone (2 g/day) did not see this effect [195]. 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 [196]. Likewise, 2 g/day EPA improved symptoms in patients with unipolar depressive disorder after 4 wk [197]. 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 [198], while subsequent trials also showed benefit with EPA [199, 200], although not all studies have seen this [201]. 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 [202]. One study reported significant benefit from 1 g/day EPA in borderline personality disorder [203], while two studies report anti-aggressive effects of DHA [204, 205]. Many of these studies suggest that EPA is superior to DHA, which may account for discrepancies between study findings. Sublette et al. [206] analyzed the findings from 15 RCTs investigating the effects of EPA, concluding that supplements containing ≥60% EPA in doses ranging from 0.2 to 2.2 g/day EPA in excess of DHA were effective against primary depression. 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 [207].

Post-mortem studies showed that the brains of Alzheimer's disease sufferers contain less DHA than those without the disease [208–210]. Some studies have linked low blood levels of very long chain n-3 fatty acids to dementia [211] and cognitive impairment [212]. However, 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 [213]. Sinn et al. [214] reported that 1.8 g EPA + DHA/day for 6 months reduced depressive symptoms and improved cognition in adults with mild cognitive impairment. Although Scheltens et al. [215] reported that 1.5 g EPA + DHA/day for 6 months improved memory performance in subjects with mild Alzheimer's disease, a number of studies using various doses and ratios of EPA and DHA reported no effect on cognitive performance in people with Alzheimer's disease [216–220].

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. Thus maintenance of maternal DHA status is the key to optimizing DHA supply to the developing fetus and newborn infant. 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 lowered very long chain n-3 fatty acid status. 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. Interestingly many of these studies are indicative that EPA is more important than DHA, which contrasts with the relative roles of the 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, this has not yet been well demonstrated [213, 221].

5 Recommendations for intake of EPA and DHA

The demonstration of physiological actions of very long chain n-3 fatty acids that result in reduced risk of disease and improved health outcomes, 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 α-linolenic acid [4]. 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.

In the UK the recommendation is for an intake of at least two fish meals per week including at least one of oily fish [11]. This was translated into an EPA + DHA recommendation of "at least" 450 mg/day [11]. The International Society for the Study of Fatty Acids and Lipids suggested a target intake of 650 mg/day EPA + DHA [222], later modified to a minimum intake of 500 mg/day [223]. In France, an official recommendation for a target intake of 400–500 mg/day EPA + DHA with at least 100–120 mg/day DHA was made [224]. The target intake for Australia and New Zealand is 430–610 mg/day EPA + DHA [225]. The Food and Agriculture Organisation of the United Nations recommended a minimum intake of 250 mg/day EPA + DHA for adult males and for non-pregnant or non-lactating adult females [226]. For pregnant or lactating females the minimum daily intake was recommended to be 300 mg EPA + DHA of which at least 200 mg should be DHA [226]. They also made recommendations for DHA intake infants and for children. For children, the recommendations for EPA + DHA intake (mg/day) are 100–150 for those aged 2–4 years, 150–200 for those aged 4–6

years, and 200–250 for those aged 6–10 years [226]. The European Food Safety Authority recommended an intake of 250 mg/day of EPA + DHA as adequate, with an additional 100–200 mg/day of DHA being needed in pregnancy [227]. For infants and children aged 6 months to 2 years the recommendation is 100 mg/day DHA, while for children aged 2–18 years the recommendation "should be consistent with that for adults" [227]. An international consensus group recommended an intake of at least 200 mg/day DHA for pregnant women [228]

Recommendations for very long chain n-3 fatty acid intake have also been made for persons who have survived an MI or who have high blood triacylglycerol concentrations. For the secondary prevention of MI, 1 g/day EPA + DHA is recommended by the American Heart Association [78], the European Society for Cardiology and European Atherosclerosis Society [229], and a network of British Societies [230]. These recommendations are based upon the dose used in the GISSI-Prevenzione trial [88]. For triacylglycerol lowering, a very long chain n-3 fatty acid dose of 2–4 g/day is recommended [78].

6 Summary and conclusions

N-3 fatty acids are a family of polyunsaturated fatty acids that contribute to human health and well-being. Functionally the most important n-3 fatty acids appear to be the very long chain EPA and DHA found in oily fish and in supplements, but roles for DPA are emerging. Intakes of EPA and DHA are typically low and much below recommended intakes. Increased intakes 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 (Fig. 5). EPA
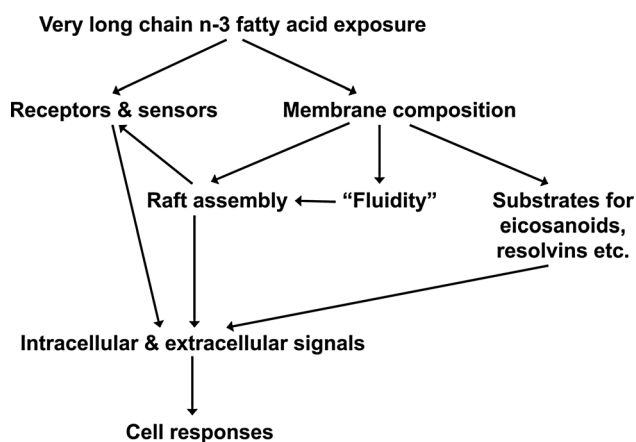
Figure 5. General overview of the mechanisms by which very long chain n-3 fatty acids can influence the function of cells. Modified from ref. [131] with permission from Elsevier.

and DHA also modify the production of lipid mediators and through effects on cell signaling can alter patterns of gene expression (Fig. 5). 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 (Table 5). A number of risk factors for cardiovascular disease are modified in a beneficial way by increased intake of EPA and DHA: these include blood pressure, platelet reactivity and thrombosis, plasma triacylglycerol concentrations, vascular function, cardiac arrhythmias, heart rate variability, and inflammation. As a result of these effects, increased EPA and DHA intake is associated with a reduced risk of cardiovascular morbidity and mortality. Thus, there is a key role for these fatty acids in prevention and slowing progression of cardiovascular disease. Furthermore, some supplementation studies with EPA and DHA have demonstrated reduced mortality in at risk patients, such as post-MI, indicating a therapeutic role. 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

Philip Calder is Professor of Nutritional Immunology within the Human Development and Health Academic Unit of the Faculty of Medicine at the University of Southampton in the UK. He has a PhD in Biochemistry from University of Auckland (New Zealand) and a DPhil in Biochemistry from University of Oxford (UK). He is a Registered Nutritionist and a Fellow of both the Society of Biology and the Association for Nutrition. For over 25 years he has conducted research on the metabolism and functionality of fatty acids with an emphasis on the roles of omega-3 fatty acids in immunity, inflammation and cardiometabolic disease.

as part of cancer therapy; some recent studies show that they improve the effectiveness of some 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 optimizing 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 illnesses in adults, although these areas of possible action require more robust scientific support.

Table 5. Summary of the physiological roles and potential clinical benefits of very long chain n-3 fatty acids

Physiological role	Potential clinical benefit	Target
Regulation of blood pressure	Decreased blood pressure	Hypertension; CVD
Regulation of platelet function	Decreased likelihood of thrombosis	Thrombosis; CVD
Regulation of blood coagulation	Decreased likelihood of thrombosis	Thrombosis; CVD
Regulation of plasma triacylglycerol concentrations	Decreased plasma triacylglycerol concentrations	Hypertriglyceridemia; CVD
Regulation of vascular function	Improved vascular reactivity	CVD
Regulation of cardiac rhythm	Decreased arrhythmias	CVD
Regulation of heart rate	Increased heart rate variability	CVD
Regulation of inflammation	Decreased inflammation	Inflammatory diseases (arthritis, inflammatory bowel disease, psoriasis, lupus, asthma, cystic fibrosis, dermatitis, neurodegeneration ….); CVD
Regulation of immune function	Improved immune function	Compromised immunity
Regulation of fatty acid and triacylglycerol metabolism	Decreased triacylglycerol synthesis and storage	Weight gain; Weight loss; Obesity; Fatty liver disease
Regulation of bone turnover	Maintained bone mass	Osteoporosis
Regulation of insulin sensitivity	Improved insulin sensitivity	Type-2 diabetes
Regulation of tumor cell growth	Decreased tumor cell growth & survival	Some cancers
Regulation of visual signaling (via rhodopsin)	Optimised visual signaling	Poor infant visual development (especially pre-term)
Structural component of brain and central nervous system	Optimised brain development – cognitive and learning processes	Poor infant and childhood cognitive processes and learning

There may also be a role for EPA and DHA in preventing neurodegenerative disease of ageing. 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 five decades of productive research on the health effects of very long chain n-3 fatty acids, many questions remain unanswered and many areas remain to be explored.

The author serves on the Scientific Advisory Boards of Pronova BioPharma, Aker Biomarine, DSM, Solutex, Sancilio and the Danone Research Centre for Specialised Nutrition; acts as a consultant to Smartfish, Mead Johnson Nutritionals, Vifor Pharma, Amarin Corporation and Enzymotec; and has recently received speaking honoraria from Pronova BioPharma, Smartfish, DSM, Fresenius Kabi, B. Braun and Vifor Pharma.

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