ANNUAL LECTURE

Omega-3: The good oil

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List of abbreviations: ALA, α-linolenic acid; CHD, coronary heart disease; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; GISSI, Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto; RR, relative risk.

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Omega-3 fatty acids: structure, naming, sources, intakes and recommendations for intake

Omega-3 (*n*-3) fatty acids are a family of polyunsaturated fatty acids. They are characterised by the position of the double bond closest to the methyl terminus of the hydrocarbon (acyl) chain being on carbon number three when counting the methyl carbon as number one. Functionally the most important omega-3 fatty acids are eicosapentaenoic acid (EPA; 20:5*n*-3) and docosahexaenoic acid (DHA; 22:6*n*-3) (Calder 2014, 2016), although roles for docosapentaenoic acid (DPA; 22:5*n*-3) are now also emerging (Kaur *et al*. 2011). EPA and DHA (and also DPA) are found in fairly high amounts in seafood, especially 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. Table 1 shows typical values for the EPA, DPA and DHA content of selected fish, while Table 2 shows typical values for the EPA and DHA content of different types of omega-3 supplements. EPA, DPA and DHA are related metabolically to one another, and there is a pathway by which EPA can be synthesised from simpler, plant-derived omega-3 fatty acids (Figure 1). The initial substrate for this pathway is the essential fatty acid α-linolenic acid (ALA; 18:3*n*-3). The pathway for conversion of ALA to EPA involves three steps, catalysed 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 catalysed by elongase 5, a second chain elongation catalysed 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 omega-3 fatty acid interconversion are shared with the analogous omega-6 (*n*-6) fatty acid biosynthetic pathway of conversion of linoleic acid (18:2*n*-6) to arachidonic acid (20:4*n*-6). The high intake of linoleic acid relative to ALA in many Western diets (Blasbalg *et al*. 2011) 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 (Arterburn *et al*. 2006; Baker *et al*. 2016), although this rate can be influenced by several factors including stage of the life course, age, sex, hormones, genetics and disease (Baker *et al*. 2016).

Because fatty fish are the richest dietary source of EPA and DHA, intake of those fatty acids is influenced strongly by fish consumption. In most Western populations the

distribution of fatty fish consumption is bimodal, with a smaller proportion of the population being regular fatty fish consumers. For example, in the UK it is estimated that only 25% of the adult population regularly consume fatty fish (Scientific Advisory Committee on Nutrition/Committee on Toxicity 2004). The other 75% of the population consume fatty fish rarely or never. Mean intakes of EPA+DHA among adults in many Western populations are considered to be around 0.1 to 0.2 g/day. However, it is difficult to be precise about this figure for several reasons, as discussed elsewhere (Calder 2014). Australian data suggest mean intake of EPA+DPA+DHA in adults to be around 0.2 g/day but with a skewed distribution such that the median intake is around 0.1 g/day or even less (Meyer *et al*. 2003; Howe *et al*. 2006). Australian children and adolescents aged 2- 16 years consumed a mean of about 0.08 g/day of EPA+DPA+DHA, with a median intake of about 0.03 g/day (Rahmawaty *et al*. 2013).

The intakes described for omega-3 fatty acids may be compared with current recommendations. In the UK, the Scientific Advisory Committee on Nutrition based its recommendation for all adults on the consumption of a minimum of one lean and one fatty fish portion per week and equated this to an EPA+DHA intake of at least 0.45 g per day (Scientific Advisory Committee on Nutrition/Committee on Toxicity 2004). The Food and Agriculture Organisation of the United Nations recommends a minimum intake of 0.25 g/day EPA+DHA for adult males and for non-pregnant or non-lactating adult females (Food and Agricultural Organisation of the United Nations 2010). For pregnant or lactating females the minimum daily intake is recommended to be 0.3 g EPA+DHA of which at least 0.2 g should be DHA (Food and Agricultural Organisation of the United Nations 2010). For children, the recommendations for EPA+DHA intake (g/day) are 0.1-0.15 for those aged 2-4 years, 0.15-0.2 for those aged 4-6 years, and 0.2-0.25 for those aged 6-10 years (Food and Agricultural Organisation of the United Nations 2010). The European Food Safety Authority recommends an intake of 0.25 g/day of EPA+DHA as adequate for adult males and non-pregnant females, with an additional 0.1-0.2 g/day of DHA being needed in pregnancy (European Food Safety Authority 2010). For infants and children aged 6 months-2 years the recommendation is 0.1 g/day DHA, while for children aged 2-18 years the recommendation ‘should be consistent with that for adults’ (European Food Safety Authority 2010). An international consensus group has recommended an intake of at least 0.2 g/day DHA for pregnant women (Koletzko *et al*. 2007). It is evident that for the majority of the population in Western countries, intakes of EPA+DHA are much lower than recommended (Sioen *et al*. 2017). It is also evident that

the recommendations can be met through regular consumption of fatty fish or, failing that, use of supplements providing EPA and DHA.

Omega-3 fatty acid concentrations in different body compartments are affected by intake

In common with all long chain fatty acids, EPA and DHA are transported in the bloodstream esterified into triacylglycerols, phospholipids and cholesteryl esters as components of lipoproteins and 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, the manner in which membranes change in response to external stimuli and the functional activities of membrane-bound proteins. The contribution of EPA and DHA to the total fatty acids present within any of the transport, storage or functional pools differs according to the pool (Table 3). 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 (Table 3). 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 (Table 3).

Increased intakes of EPA and DHA from fish or from supplements are reflected in increased concentrations (and proportions) of both fatty acids in blood lipid, blood cell and many tissue pools. This has been reported many times for total plasma and serum lipids and for the complex lipid components of plasma and serum (*i.e.* triacylglycerols, phospholipids and cholesteryl esters) and is also well described for erythrocytes (red blood cells), platelets and leukocytes (white blood cells) (see Calder 2014 for references). There are also descriptions of increased proportions of EPA and DHA in human tissues, including skeletal muscle, heart, gut mucosa and adipose tissue, when their intake is increased (see Calder 2014 for references). These locations all show a dose- and time-dependent incorporation of both EPA and DHA (Figure 2), but the precise pattern depends upon the specific location (Katan *et al*. 1997; Browning *et al*. 2012). 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 do blood cells (Katan *et al*. 1997; Browning *et*

al. 2012), whilst amongst blood cells, platelets and 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.

Increased EPA and DHA content in cell membranes alters cellular responses via a variety of mechanisms

Many, though not all, of the functional effects of EPA and DHA rely upon their incorporation into cell membrane phospholipids (Figure 3). Once they are incorporated, EPA and DHA alter the physical properties of membranes (because of their highly unsaturated nature), and in doing so they create a specific environment for membrane proteins such as receptors, transporters, ion channels and signaling enzymes that influences the activity of those proteins (see Calder 2014 for references). 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 signalling pathways. Studies in a variety of cell types including neurones, immune cells and cancer cells have shown that EPA and DHA modify raft formation in a manner which modifies intracellular signalling pathways (see Calder 2014 for references). As a result of their effects on membrane-generated intracellular signals, EPA and DHA can modulate transcription factor activation and, subsequently, gene expression patterns (Figure 3). Transcription factors shown to be affected by EPA and DHA include nuclear factor κ B, peroxisome proliferator activated receptor-α and -γ, and the sterol regulatory element binding proteins (see Calder 2014 for references). Effects of omega-3 fatty acids on transcription factor activation and gene expression are central to their role in controlling fatty acid and triacylglycerol metabolism, inflammation and adipocyte differentiation (Calder 2012)

A second consequence of increased abundance of EPA and DHA in cell membrane phospholipids, and the associated decreased abundance of the omega-6 fatty acid arachidonic acid, is that the availability of substrates for synthesis of bioactive lipid mediators is altered. Arachidonic acid is 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. Excess or inappropriate production of eicosanoids from arachidonic acid is associated with many disease processes. Increasing the omega-3 fatty acid content of cell membranes results in decreased production of eicosanoids from arachidonic

acid (see Calder 2015 for references), 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, and frequently the EPA-derived mediator has weaker biological activity than the arachidonic acid-derived mediator (see Calder 2015). 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) (Bannenberg and Serhan 2010). It has recently been discovered that DPA gives rise to bioactive mediators (Dalli *et al*. 2013). The major role of resolvins, protectins and maresins appears to be in the resolution of inflammation and modulation of immune function (Bannenberg and Serhan 2010).

Higher omega-3 fatty acid intake and status are both associated with improved health and lowered disease risk

Through the mechanisms outlined in Figure 3, EPA and DHA can influence metabolism, inflammation, immune function, oxidative stress, platelet reactivity, blood coagulation, the vascular endothelium, organ (liver, heart, muscle, brain *etc*.) function and wound healing, amongst many other physiological and pathophysiological responses. It is through these effects that EPA and DHA act to improve health and well-being, to lower disease risk and, in some situations, to improve patient outcome. The most researched area of the health benefits of omega-3 fatty acids is in cardiovascular disease. A role for omega-3 fatty acids in protecting against coronary heart disease (CHD) mortality was identified in studies conducted among Greenland Inuit populations, and later extended to other Arctic native populations and to the Japanese. The effects seen on mortality were ascribed to the high intake of EPA and DHA from sea mammals and from fatty fish, and EPA and DHA were demonstrated to beneficially improve a number of risk factors for development of atherosclerosis including blood lipids, blood pressure and inflammation (see Calder 2004; Saravanan *et al*. 2010). A number of ecological, case-control and prospective cohort studies reported beneficial associations between omega-3 fatty acid intake from the diet or omega-3 fatty acid concentrations in a specific body pool (*e.g.* in blood plasma or serum or in red blood cells) and clinical markers of disease risk or a disease manifestation such as incident CHD,

myocardial infarction, stroke or mortality. These studies have been summarised and discussed in detail elsewhere (see Calder 2004). A fairly recent systematic review and meta-analysis brought together prospective studies examining the association of dietary or circulating fatty acids, including omega-3 fatty acids, with risk of coronary outcomes (Chowdhury *et al*. 2014). Outcomes included fatal or nonfatal myocardial infarction, CHD, coronary insufficiency, coronary death, angina or angiographic coronary stenosis. The aggregation of data from 16 studies involving over 422 000 individuals showed a relative risk (RR) of 0.87 for those in the top third of dietary EPA+DHA intake compared with those in the lower third of intake. The aggregation of data from 13 studies involving over 20 000 individuals showed RR of 0.78, 0.79 and 0.75 for those in the top third of circulating EPA, DHA and EPA plus DHA, respectively, compared with those in the lower third (Chowdhury *et al*. 2014). A smaller number of studies in fewer individuals gave a RR of 0.64 for circulating DPA (Chowdhury *et al*. 2014). A recent study presented data obtained by pooling 19 studies involving over 45 000 individuals (Del Gobbo *et al*. 2016). After adjustment for multiple variables, EPA, DPA and DHA concentrations (assessed in various pools and expressed as % of total fatty acids) were each associated with a lower risk of fatal CHD, with a RR of 0.91 for EPA, of 0.90 for DPA and of 0.90 for DHA. Thus, the accumulated data suggested that EPA, DPA and DHA all lower the risk of developing adverse coronary outcomes (Chowdhury *et al*. 2014; Del Gobbo *et al*. 2016). These data support a clear role for EPA, DPA and DHA in primary prevention of CHD and, perhaps more widely, of cardiovascular disease.

Translational research with omega-3 fatty acids: Atherosclerotic plaque stabilisation

There has been significant interest in the effect of EPA and DHA in people with existing cardiovascular disease, for example in survivors of myocardial infarction or in patients with heart failure. A number of controlled intervention trials, usually with omega-3 supplements, have been conducted and the primary outcome measure in these studies has most often been the occurrence of a major cardiovascular event (*e.g.* myocardial infarction), including one that is fatal. Several such studies published between 1989 and 2008 reported lower rates of death in patients receiving EPA and DHA at doses of 0.5- 1.8 g/day for periods of 1 - 5 years. The most well-known of these studies is the the *Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto* (*GISSI*)-*Prevenzione* study (Anonymous, 1999). The *GISSI-Prevenzione* study enrolled just over 11 000 patients, who were within 3 months of having a

myocardial infarction, and studied the effects of EPA+DHA (0.885 g/day) provided as pharmaceutical grade ethyl esters and/or vitamin E with up to 3.5 years of follow-up. EPA+DHA significantly lowered (by 15%) the risk of the combined primary outcome of death and non-fatal cardiovascular events. There was a significant reduction in risk of mortality (by 20%), cardiovascular mortality (by 30%) and sudden death (by 45%). Three key mechanisms have been suggested to contribute to this therapeutic effect of EPA and DHA. These are a) altered cardiac electrophysiology seen as lower heart rate, increased heart rate variability and fewer arrhythmias; b) an anti-thrombotic action resulting from the altered pattern of production of eicosanoid mediators that control platelet aggregation; c) an anti-inflammatory effect which would act to stabilise atherosclerotic plaques preventing their rupture.

Plaque rupture is the acute occurrence that exposes the plaque contents to the highly prothrombotic environment of the vessel lumen, so initiating thrombosis that may lead to myocardial infarction or other vascular events. Plaque rupture is essentially an inflammatory event and the characteristics of an atherosclerotic plaque that make it vulnerable to rupture are a thin fibrous cap and increased numbers of inflammatory cells, such as macrophages (Stary *et al*. 1995; Plutzky 1999). A randomised, double blind, placebo controlled intervention study conducted in patients awaiting the common surgical procedure, carotid endarterectomy (removal of advanced plaque from the carotid artery), showed that EPA and DHA from fish oil supplements (providing EPA+DHA 1.4 g /day) are incorporated into advanced atherosclerotic (carotid) plaques and that this incorporation is associated with structural changes consistent with increased plaque stability (Thies *et al*. 2003). A follow-up study, using EPA+DHA (1.8 g /day) provided as pharmaceutical grade ethyl esters, confirmed the higher EPA content of carotid plaque phospholipids in patients receiving EPA+DHA and the association between a higher EPA content of the plaque and lower plaque inflammation and instability (Cawood *et al*. 2010). Furthermore, messenger RNA levels for matrix metalloproteinase-7, metalloproteinase-9 and metalloproteinase-12 were lower in plaques from patients who had received EPA+DHA. The significance of this is that these proteases are released from inflammatory macrophages and are linked to plaque instability as a result of degradation of proteins that make up the plaque's fibrous cap. These two intervention studies demonstrate that incorporation of omega-3 fatty acids into advanced atherosclerotic plaques is linked to a reduction in inflammation and to an increase in stability, which might explain the findings of the *GISSI-Prevenzione* trial.

Life course research with omega-3 fatty acids: The Salmon in Pregnancy Study

A supply of omega-3 fatty acids, especially DHA, to the growing fetus and the newborn infant is vital to support optimal growth and development, especially of the neural and visual systems (Calder 2016). Early life exposures can have long-term consequences on physiology, health and disease risk (British Nutrition Foundation 2013). There is a hypothesis that excessive early exposure to the omega-6 fatty acid arachidonic acid programmes the immune system to a pro-allergic state and that this can be prevented by ensuring an adequate early supply of omega-3 fatty acids (Calder *et al*. 2010). A systematic review identified that intake of fish, fatty fish and omega-3 fatty acids in pregnancy is associated with reduced risk of allergic disease in the offspring infants (Kremmyda *et al*. 2011). Findings with fish were more robust than with omega-3 fatty acids, possibly because fish provides beneficial nutrients in addition to EPA and DHA. In common with its advice to other adults, the UK government advises that pregnant women should consume two portions of fish per week, at least one of which should be fatty (Scientific Advisory Committee on Nutrition/Committee on Toxicity, 2004). The *Salmon in Pregnancy Study* was a randomised, controlled dietary intervention study testing this advice in the context of offspring allergic disease. Pregnant women who were low consumers of fatty fish and at risk of giving birth to an infant who would become allergic were recruited (Miles *et al*. 2011). They maintained their habitual diet or consumed salmon twice per week from week 19 of pregnancy until giving birth. Women in the salmon group showed much greater dietary intake of EPA and DHA: intake of EPA+DHA from the diet was equivalent to 0.03 g/day in the control group and 0.4 g/day in the salmon group (Miles *et al*. 2011). Women in the control group showed a decline in the percentage of both EPA and DHA in plasma phosphatidylcholine from week 19 to week 38 pregnancy (Miles *et al*. 2011); this decline is consistent with other reports (Al *et al*. 1995; Otto *et al*. 1997). However, in the salmon group this decline did not occur and EPA and DHA actually increased in plasma phosphatidylcholine over the course of pregnancy (Miles *et al*. 2011). Furthermore, both EPA and DHA were significantly higher in umbilical cord plasma phosphatidylcholine in the salmon group compared with the control group (Miles *et al*. 2011). Thus, by consuming salmon twice per week mothers were providing more EPA and DHA to their growing fetus. There were also some differences in umbilical cord blood immune cell responses between the two groups, including a lower production of pro-allergic prostaglandin E_2 by cord blood mononuclear cells in response to inflammatory stimuli in the salmon group

(Noakes *et al*. 2012). Breast milk DHA was higher from women in the salmon group at days 1, 5, 14 and 28 after birth, even though women ceased consuming salmon at birth (Urwin *et al*. 2012). Thus, women in the salmon group were most likely able to provide a greater amount of DHA to their newborn infant during the early weeks of lactation. Despite, these important findings, at 6 months of age there was no significant difference between the two groups in the number of infants with atopic dermatitis or in atopic dermatitis severity, in the number of infants sensitised to common allergens or in various allergic manifestations (Noakes *et al*. 2012). However, the numbers of infants affected was low in both groups. At follow-up at age 2.5 - 3 years, children whose mothers were in the salmon group were less likely to have doctor diagnosed asthma than those whose mothers were in the control group (unpublished data). Thus, there may be an important clinical effect of maternal consumption of fatty fish during pregnancy but there may be a delay in when this effect is seen. It is interesting to note that a recently published trial of fish oil supplementation in pregnant women (EPA+DHA 2.4 g/day from week 24 of pregnancy until 1 week after delivery) reported a significant reduction in persistent wheeze or asthma in the offspring during follow-up between age 3 and 5 years (Bisgaard *et al*., 2016).

Summary and conclusions

Omega-3 fatty acids are a family of polyunsaturated fatty acids that contribute to human health and well-being. Functionally the most important omega-3 fatty acids appear to be EPA and DHA found in fatty fish and in omega-3 supplements. Intakes of EPA and DHA are typically low and much below what is recommended. Increased intakes of EPA and DHA are reflected in greater incorporation of these fatty acids 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, signalling proteins, transporters and enzymes. EPA and DHA also modify the production of lipid mediators and through effects on cell signalling can alter patterns of gene expression. As a result of 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. This has been well explored in the context of cardiovascular disease, where new actions around stabilisation of advanced plaques have been identified. The effects of EPA and DHA are evident right through the life course meaning that there is a need for all sectors of the population to have an adequate intake of these important nutrients. Fatty fish are an ideal source of EPA and DHA but in

those not willing or able to increase their intake of fatty fish, omega-3 supplements are an alternative source.

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Conflicts of interest

The author serves on scientific advisory boards of Pronova BioPharma, Smartfish and DSM and acts as a consultant to Cargill and Fresenius-Kabi.

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Figure captions

Figure 1. The pathway of biosynthesis of eicosapentaenoic, docosapentaenoic and docosahexaenoic acids from α-linolenic acid

Figure 2. Time course of changes in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) content of human plasma phosphatidylcholine (PC) 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 (dotted lines) g EPA+DHA per week for a period of 12 months; the ratio of EPA to DHA was 1:1.1. Plasma PC was isolated at 0, 1, 2, 4, 8, 12, 24, 36 and 52 weeks and the fatty acid composition determined by gas chromatography. Data are mean ± standard error from at least 30 subjects per group. Taken from Browning *et al*. (2012) with permission from the American Society of Nutrition

Figure 3. A schematic summary of the mechanisms by which eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) affect cell and tissue responses so impacting on health and disease risk

Table 1. Typical eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) contents of selected fish. Note that both omega-3 fatty acid content and portion size may vary

Fish	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	2.20
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

*Classified as fatty (oily) fish

Table 2. Typical eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents of omega-3 supplements

	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 eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) concentrations reported in different compartments in humans. Data are taken from the selected references and are expressed as % of total fatty acids

Population	Compartment	EPA	DHA	Reference
Generally healthy men and women aged 20 to 80 years; UK	Plasma triglycerides*	0.3	0.8	Browning *et al.* (2012)
Generally healthy men and women aged 20 to 80 years; UK	Plasma phospholipids*	1.2	3.6	Browning *et al.* (2012)
Healthy pregnant women aged 18 to 40 years; week 38 of pregnancy; UK	Plasma phospholipids*	0.4	3.8	Miles *et al*. (2011)
Newborn infants (umbilical cord); healthy pregnancies; UK	Plasma phospholipids	0.3	6.4	Miles *et al*. (2011)
Generally healthy men and women aged 20 to 80 years; UK	Plasma cholesteryl esters*	1.0	0.6	Browning *et al*. (2012)
Generally healthy men and women aged 20 to 80 years; UK	Plasma non-esterified fatty acids*	0.4	1.6	Browning *et al*. (2012)
Generally healthy men and women aged 20 to 80 years; UK	Red blood cells	2.3	5.2	Browning *et al*. (2012)
Generally healthy men and women aged 20 to 80 years; UK	Platelets	1.1	2.0	Browning *et al*. (2012)
Generally healthy men aged 18 to 40 years; UK	Blood neutrophils	0.6	1.6	Healy *et al*. (2000)
Generally healthy men and women aged 20 to 80 years; UK	Blood mononuclear cells (mainly lymphocytes)	0.8	1.9	Browning *et al*. (2012)
Men mean age 68 years with no evidence of dementia; UK	Brain grey matter	Not reported	18	Skinner *et al*. (1993)
Men mean age 68 years with no evidence of dementia; UK	Brain white matter	Not reported	4	Skinner *et al*. (1993)
Breast fed term infants who had died at mean age 4 months; Australia	Cerebral cortex	< 0.1	8	Makrides *et al*. (1994)
Breast fed term infants who had died at mean age 4 months; Australia	Retina	0.1	12	Makrides *et al*. (1994)
Men mean age 55 years	Cardiac muscle	0.2	1.5	Harris *et al*. (2004)

who had received a heart transplant; US				
Mainly men mean age 60 years undergoing cardiac surgery; Australia	Cardiac muscle phospholipids	0.5	4.8	Metcalf *et al*. (2007)
Healthy men mean age 21 years; UK	Skeletal muscle	0.6	1.5	McGlory *et al*. (2014)
Healthy men and women aged 25 to 45 years; US	Skeletal muscle phospholipids	0.7	1.9	Smith *et al*. (2011)
Men and women aged 38 to 41 years undergoing surgery; Chile	Liver	0.4	6.8	Araya *et al*. (2004)
Men and women aged 23 to 63 years undergoing surgery; Chile	Liver phospholipids	4.8	15.1	Elizondo *et al*. (2007)
Patients with inflammatory bowel disease; UK	Colonic mucosa	0.3	1.7	Hillier *et al*. (1991)
Generally healthy men and women aged 20 to 80 years; UK	Subcutaneous adipose tissue	0.2	0.2	Browning *et al*. (2012)
Generally healthy men mean age 34 years; Iran	Spermatozoa	0.6	9.6	Safarinejad (2011)

*Blood collected after an overnight fast