The Role of Docosahexaenoic Acid in the First 1,000 Days

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Natalia Wagemans, MD
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Omega-3 (n-3) long-chain polyunsaturated fatty acids (LCPUFAs), including eicosahexaenoic acid (EPA) and docosahexaenoic acid (DHA), are dietary fats linked with health benefits along the life span of an individual. These include a role in anti-inflammatory processes, viscosity of cell membranes, fetal development, and healthy aging. Since DHA is a key component of all cell membranes and is abundant in the brain and retina, much of the work has been focused on the perinatal period and the first 1,000 days of life. Literature exists underscoring the critical significance of healthy growth and development in the first 1,000 days of life in relation to morbidity and mortality. Towards this end, this period of growth may also be critical to the development of the brain and neurocognitive outcome. For women and children, optimal intake of DHA and a proper healthy diet could improve overall human health as well as decrease morbidity and mortality from a variety of neonatal diseases. The sources of DHA in a human diet, its presence in human breast milk, as well as the high concentrations in the human eye and brain are outlined. Further, the importance of maintaining an appropriate concentration of DHA throughout pregnancy, lactation, and the first 1,000 days is discussed since insufficient supply of DHA can impact mental and visual development and performance.

In the first article, Philip Calder elegantly discusses the rapid accumulation of DHA and arachidonic (AA) acids during the last trimester of pregnancy which corresponds to the period of rapid growth and brain development. DHA comprises as much as 30–50% of neuronal plasma membranes by weight. He also points out that DHA accumulation in organs other than the brain as well as the accumulation of the other LCPUFAs is important to consider when determining optimal requirements. Premature infants, although having the capacity to convert α-linoleic acid to DHA and EPA, cannot fulfill the requirement based on fetal accretion rates, and current nutritional management leads to an early DHA deficit. In turn, DHA depletion may lead to reduced visual function and cognitive performance which may be improved by DHA and AA supplementation. Dr. Calder also goes on to discuss the mechanisms of the actions of DHA and that DHA is a substrate for biosynthesis of bioactive mediators. Since a lower DHA content in the brain and eye is linked to poorer cognitive development and visual function, it is vital that pregnant and lactating women as well as infants consume sufficient preformed DHA to support brain and eye development and function. DHA affects cell and tissue physiology; the effects include alterations in membrane structure and function, cell signaling, and...
lipid mediator production. In addition, DHA reduces inflammation, improves immune function, and through these effects may have positive effects including reducing the risk of insulin resistance, metabolic syndrome, hyperlipidemia, and cardiovascular disease.

Makrides and Best present the available evidence and discuss the relationship between prenatal n-3 LCPUFA supplementation during pregnancy and the incidence of preterm birth. The importance of this is the fact that preterm births account for almost 85% of all perinatal complications including death and that approximately 50% of all preterm births do not have clear causes and there are no effective primary prevention strategies to prevent preterm birth. They discuss epidemiological data and evidence from randomized clinical trials to support an effect of increased n-3 LCPUFA intake during pregnancy on the length of gestation. The authors again stress the fact that the prenatal period is a time for increased risk of n-3 LCPUFA deficiency and that the WHO recommends an intake of 300 mg/day of these fatty acids during pregnancy. However, women in many low-, middle-, and high-income countries do not achieve this amount with the exception of those living in coastal countries. While marine foods are rich sources of n-3 LCPUFA, warnings against consumption of certain marine species may have reduced overall fish intake and, therefore, LCPUFA intake. This may be associated with the increasing rate of preterm birth. The evidence to date consistently demonstrates that n-3 PUFA supplementation during pregnancy increases the mean duration of gestation and produces a significant reduction in preterm birth. The inconsistencies between epidemiological observations and randomized controlled trials may not be surprising since observational studies are likely based on lifetime exposure compared to a defined period of supplementation in randomized controlled trials.

Meldrum and Simmer underscore the placental transfer of these fatty acids during gestation and point out that diets being insufficient, particularly in Western nations during pregnancy and lactation, may be unable to meet the high demands of the fetus and that countries, which experience typically low levels of DHA within breast milk include the USA (0.2%), Canada (0.14%), and Australia (0.25%). They go on to discuss the associations between maternal DHA and neurodevelopment of the infant, maternal supplementation with LCPUFA and neurodevelopment of the infant, and supplementation during lactation and neurodevelopment. Lastly, supplementation of term infants either through infant formulas or direct supplementation and their effects on neurodevelopment are discussed. The authors conclude that there is no definite evidence that DHA supplementation in pregnancy, lactation, or infancy improves the neurodevelopment of healthy term infants and stress the need for larger trials to identify maternal factors, infant gender, and the optimal dose for supplementation to provide certainty for future recommendations.

Finally, Lapillonne and Moltu discuss trials, which demonstrate that larger amounts of DHA than currently and routinely provided are associated with better neurological outcomes. Studies in preterm infants indicate possible benefits for retinal and cognitive development as suggested by greater retinal sensitivity to photic stimulation, more mature visual acuity, and short-term global developmental outcomes at 6–18 months after DHA supplementation in preterm infants. The studies are confounded by the variability in study designs and the amounts of DHA supplementation, where in some studies the amounts were chosen to produce the same concentrations of DHA and AA as in term human milk, which would be lower than the in utero accretion rate. The early advantage in neurological outcome up to 2 years of life does not appear to persist into childhood. However, as these authors caution, ‘this does not mean that supplementing with adequate amounts of LCPUFA during the perinatal period is not necessary’. They also cite studies demonstrating the growing evidence that besides the effect on growth, n-3 PUFA supplementation may attenuate immune and anti-inflammatory responses, reducing many of the comorbidities observed in preterm infants.

In summary, the n-3 LCPUFAs and their role in health and disease continue to evolve. While existing evidence for their role in the entire spectrum of the first 1,000 days is conflicting, the importance of a healthy diet including optimal amounts of these fatty acids cannot be overemphasized. The varying amounts of supplemental LCPUFAs in clinical studies, varying clinical designs, and selection of subjects results in meta-analyses producing conflicting results. Observational studies, which may also underscore lifelong healthy habits, demonstrate positive benefits of LCPUFA. Future trials need to be targeted to define subgroups of populations which may incur the most benefits while we also seek epigenetic data demonstrating the multiple benefits of LCPUFA in the human diet to improve pregnancy outcomes, infant neurodevelopment, and long-term health consequences.

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Jatinder Bhatia and Maria Makrides
The DHA content of a cell membrane can have a significant influence on cellular behaviour and responsiveness to signals

**Key insights**

Docosahexaenoic acid (DHA) is a long-chain omega-3 (n-3) polyunsaturated fatty acid that is a critical component of lipid structures. DHA plays important roles throughout the body and is essential for maintaining the structure and function of the brain and eye. Fetal development and infancy are key windows during which sufficient DHA levels are necessary for optimal mental and visual development and performance in later life.

**Current knowledge**

Humans have a limited ability to synthesise DHA from essential fatty acids; the main source of DHA is from the diet. Transported in the blood as a component of lipoproteins, DHA can be stored in adipose tissues. Compared to other bodily tissues, the eye and brain contain a high proportion of DHA. Due to its highly unsaturated composition, DHA adopts a three-dimensional shape that is different from that of other fatty acids in cell membranes. In the rod cells of retinal photoreceptors for example, DHA within the membrane facilitates the conformational change triggered by a light signal. In addition to its effects in the eye and brain, DHA also reduces inflammation, improves immune function, and optimises cellular metabolism.

**Practical implications**

Maternal plasma phospholipids are an important source of DHA for the fetus. Indeed, DHA is highly concentrated in the fetal circulation and in fetal tissues through the process of bio-magnification. DHA is naturally found in breast milk. The DHA content of breast milk can be increased by maternal consumption of DHA-rich foods such as fish, eggs, fish oil, or DHA-rich oil. Higher consumption of DHA by lactating women results in increased DHA in breast milk, ultimately raising the infant’s DHA status. Therefore, ensuring sufficient dietary DHA in pregnant women is key for optimal fetal development.

**Recommended reading**

The Role of DHA in the First 1,000 Days

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Docosahexaenoic Acid

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However, this pathway does not appear to be very efficient in many individuals, although the conversion of ALA to DHA is much better in young women than in young men. Furthermore, young infants may be more efficient converters of ALA to DHA than many adults, although the conversion rate is variable among infants. Many factors have been identified that affect the rate of conversion. The implication of poor conversion is that preformed DHA needs to be consumed. DHA is found in fairly high amounts in seafood, especially fatty fish, and in various forms of n-3 supplements. The amount of DHA in seafood and in supplements varies. Breast milk contains DHA. DHA is found esterified into complex lipids within the bloodstream, in adipose stores and in cell membranes. Its concentration in different compartments varies greatly. The brain and eye have high DHA contents compared to other organs. DHA is especially concentrated in the grey matter of the brain and in the rod outer segments of the retina. In the brain DHA is involved in neuronal signalling, while in the eye it is involved in the quality of vision. DHA is accumulated in the brain and eye late in pregnancy and in early infancy. A lower DHA content is linked to poorer cognitive development and visual function. DHA affects cell and tissue physiology and function through numerous mechanisms, including alterations in membrane structure and function, in membrane protein function, in cellular signalling and in lipid mediator production.

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Key Messages

- Docosahexaenoic acid (DHA) is a bioactive omega-3 polyunsaturated fatty acid that influences membrane structure and function, cell signalling and communication mechanisms, gene expression and lipid mediator production.
- DHA is found in high concentrations in the human brain and eye, where it is linked to better development and function.
- Maintenance of DHA concentration is important throughout the life course, but pregnancy, lactation and infancy are vulnerable periods, where insufficient DHA supply can impact mental and visual development and performance.

Key Words
Docosahexaenoic acid · Fish · Fish oil · Omega-3 · Pregnancy · Lactation · Life course

Abstract
Docosahexaenoic acid (DHA) is a long-chain, highly unsaturated omega-3 (n-3) fatty acid. It has a structure that gives it unique physical and functional properties. DHA is metabolically related to other n-3 fatty acids: it can be synthesised from the plant essential fatty acid α-linolenic acid (ALA).

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**Introduction**

Docosahexaenoic acid (DHA) is a long-chain, highly unsaturated omega-3 (n-3) fatty acid (see Appendix). It has 22 carbons in its acyl chain, which includes 6 double bonds. Chemically it can be described as all-cis-4,7,10,13,16,19-docosahexaenoic acid, with the numbers 4, 7, 10, 13, 16 and 19 referring to the carbon atoms in the acyl chain that bear double bonds when the carboxyl or α-carbon is counted as number 1 (fig. 1). DHA is shown in the common fatty acid nomenclature as 22:6ω-3 or 22:6n-3, with the ω-3 (or n-3) indicating the position of the first double bond in the acyl chain, in this case counting the methyl or ω-carbon as number 1 (fig. 1). The common name for DHA, which is rarely used, is cervonic acid. Fatty acyl chains with no double bonds, such as in saturated fatty acids, are straight and pack together tightly. Introduction of a *cis* double bond into an acyl chain introduces a ‘kink’ into the chain, making it less easy for such chains to pack together and lowering their melting point. As the acyl chain of DHA contains 6 *cis* double bonds, it becomes highly twisted (fig. 1), giving it unique physical properties and resulting in a very low melting point (–44 °C). DHA is metabolically related to other n-3 fatty acids. It can be synthesised from the plant-derived α-linolenic acid (ALA; 18:3n-3) or obtained directly from the diet. In common with other fatty acids, DHA is most often found linked via its carboxyl group into a more complex lipid structure such as a triglyceride, phospholipid or cholesteryl ester. Here, the pathway of DHA biosynthesis, dietary sources of DHA, the status (i.e., concentration) of DHA at different sites in the human body and the response of those sites to increased DHA intake, and selected actions of DHA at the molecular and cellular levels will be described. DHA plays vital roles in the structure and function of the brain and eye and an appropriate supply during fetal life and in infancy is essential to assure optimal development.

**Biosynthesis of DHA**

ALA is an essential fatty acid. It is synthesised in plants and in many lower organisms and is found in the human diet mainly as a component of green leaves, some nuts, seeds and vegetable oils, and foods made from or containing those ingredients. There is a metabolic pathway that links ALA to DHA (fig. 2). This pathway involves a series of enzyme-catalysed elongation and desaturation reactions. Elongation enzymes, called elongases, add pairs of carbon atoms to the growing acyl chain, in this case converting an 18-carbon fatty acid into a 22-carbon one, while desaturase enzymes insert double bonds into the acyl chain, in this case converting a fatty acid with 3 double bonds in its acyl chain into one with 6 double bonds. These reactions occur predominantly within the endoplasmic reticulum. The pathway is believed to mainly occur within the liver, but there is some evidence that other

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**Fig. 1.** Different depictions of the structure of DHA. DHA has 22 carbons and 6 *cis* double bonds in its hydrocarbon (acyl) chain. The α-carbon is the carbon of the terminal carboxyl group (COOH) and the ω-carbon is the carbon of the terminal methyl (CH₃) group.
tissues, including brain and testis, have high expression of the genes encoding the relevant enzymes.

The initial step in the pathway is the conversion of ALA to stearidonic acid (18:4n-3), catalysed by Δ-6-desaturase, which is generally considered to be the rate-limiting reaction in the pathway. Δ-6-desaturase is encoded by the gene fatty acid desaturase 2 (Fads2). Stearidonic acid is converted to 20:4n-3 by the addition of 2 carbons by the enzyme elongase-5, encoded by fatty acid elongase 5 (Elovl5). 20:4n-3 is then converted to eicosapentaenoic acid (EPA; 20:5n-3) by insertion of a double bond catalysed by Δ-5-desaturase, which is encoded by the gene fatty acid desaturase 1 (Fads1). EPA can be elongated by elongase 2 (encoded by Elovl2) to form n-3 docosapentaenoic acid (DPA; 22:5n-3) and then to 24:5n-3 followed by desaturation that again uses Δ-6-desaturase activity to form 24:6n-3. This desaturation seems to be catalysed by the same Δ-6-desaturase as in the first step of the pathway. 24:5n-3 is then translocated from the endoplasmic reticulum to the peroxisome where it undergoes one round of β-oxidation to form DHA.

It is important to note that the same enzymes are active in the metabolism of the n-6 fatty acid family, converting the essential n-6 fatty acid linoleic acid (18:2n-6) to arachidonic acid (20:4n-6) and on to n-6 DPA (22:5n-6). Thus, competition exists between the conversion of n-6 and n-3 fatty acids. The rate-limiting enzyme, Δ-6-desaturase, has a preference for ALA over linoleic acid. However, this may be more than offset by the greater abundance of linoleic acid than ALA in most human diets, meaning that the metabolism of the former is favoured.

In addition to the availability of the essential fatty acid substrates and the competition between them, a number of other factors have been demonstrated to regulate the pathway. These include the availability of several trace elements including zinc and iron, since the enzymes involved in the pathway require these as co-factors; sensitivity to insulin; female sex hormone status; polymorphisms in Fads genes which control gene expression and enzyme activity, and epigenetic modification of Fads and Elovl genes, which will affect their expression. Other nutrients, metabolites and hormones, and ageing may also affect the pathway. Measurements of EPA and DHA status reveal differences among some population subgroups, for example between men and women [1, 2] and among individuals with different Fads polymorphisms [3], that are likely to reflect different activities of the biosynthetic pathway. There has been much interest in the reported differences in EPA and DHA status between men and women. Studies using stable isotopes to trace metabolism of ALA have demonstrated that the conversion of ALA to both EPA and DHA is more efficient in young women than in young men [4, 5]. In men, conversion of ALA to EPA has been reported to be between 0.3 and 8%, and conversion to DHA <1%, whereas in women up to 21% conversion to EPA and up to 9% conversion to DHA have been reported. It has been suggested that the higher rate of conversion in women may be because of their greater requirement to produce DHA during pregnancy and lactation. Infants may be more effective at converting ALA to DHA than adults, and newborns appear to be better at synthesising DHA than older infants [6].

Fig. 2. The metabolic pathway of conversion of α-linolenic acid to DHA showing the enzymes involved.
Dietary Sources of DHA

Along with EPA and n-3 DPA, DHA is found in fairly high amounts in seafood and products derived from seafood. Table 1 reports typical values for the content of DHA in various seafoods [7]. It is evident that there is at least a 10-fold range in content of DHA per portion (i.e. per serving) of seafood, with fatty fish able to provide as much as 1–1.75 g of 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 about 0.1–0.2 g of DHA per portion. Although tuna is a fatty fish, canned tuna has had the oil removed during processing and so is low in content of n-3 fatty acids including DHA (table 1). Meat and blubber of sea mammals like seals and whales is also rich in n-3 fatty acids including DHA, although these are not usually eaten by most humans. Likewise, some organ meats like brain are rich in DHA but again these are rarely eaten in most populations. Humans and, as a result, DHA is found in modest amounts in animal-derived foods like eggs and meat (table 1). Human breast milk and other mammalian milks contain DHA. An analysis of data from 65 studies of over 2,400 women from around the globe gave a mean concentration of DHA in breast milk as 0.32% of total fatty acids by weight with a range of 0.06–1.4% [8]. The highest breast milk DHA concentrations were found in coastal populations and were associated with marine food consumption [8].

Because fatty fish are the richest dietary source of DHA, DHA intake 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 (e.g. 15–25%) being regular fatty fish consumers. Thus, typical intakes of DHA across a population are likely to be low. Data from over 10,000 Australian adults identified a mean daily DHA intake of 106 mg, with DHA contributing about 60% of n-3 long-chain polyunsaturated fatty acid (LCPUFA) intake [9]. By comparison, mean daily intakes of EPA and n-3 DPA were 56 and 25 mg, respectively [9]. However, the median intake of DHA was only 15 mg/day [9], but differences between mean and median intakes reflecting the non-normal distribution of the intake data. Median daily intakes of EPA and n-3 DPA were 8 and 6 mg, respectively [9]. In a more recent study using an updated nutrient composition database, the mean daily intake for DHA was 100 mg, which was 40% of n-3 LCPUFA intake [10]. Again, median intakes were lower, being about 50% of the mean. An interesting observation from these studies is that meat (including poultry) provides 40–45% of EPA + n-3 DPA + DHA consumed by typical Australian adults, with fish and seafood products providing 45–50% [10].

### Table 1. Typical DHA content of selected fish, other seafood and meats

<table>
<thead>
<tr>
<th>Food</th>
<th>DHA, g/100 g food</th>
<th>Typical adult portion size, g</th>
<th>DHA, g/portion</th>
<th>Contribution of DHA to EPA + n-3 DPA + DHA, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mackerel</td>
<td>1.10</td>
<td>160</td>
<td>1.75</td>
<td>55</td>
</tr>
<tr>
<td>Canned pilchards</td>
<td>1.20</td>
<td>110</td>
<td>1.32</td>
<td>45</td>
</tr>
<tr>
<td>Canned sardines</td>
<td>0.68</td>
<td>100</td>
<td>0.68</td>
<td>40</td>
</tr>
<tr>
<td>Salmon</td>
<td>1.3</td>
<td>100</td>
<td>1.3</td>
<td>60</td>
</tr>
<tr>
<td>Trout</td>
<td>0.83</td>
<td>120</td>
<td>1.0</td>
<td>55</td>
</tr>
<tr>
<td>Herring</td>
<td>0.69</td>
<td>120</td>
<td>0.82</td>
<td>50</td>
</tr>
<tr>
<td>Cod</td>
<td>0.16</td>
<td>120</td>
<td>0.19</td>
<td>65</td>
</tr>
<tr>
<td>Haddock</td>
<td>0.10</td>
<td>120</td>
<td>0.12</td>
<td>65</td>
</tr>
<tr>
<td>Plaice</td>
<td>0.10</td>
<td>130</td>
<td>0.13</td>
<td>35</td>
</tr>
<tr>
<td>Canned tuna</td>
<td>0.14</td>
<td>45</td>
<td>0.06</td>
<td>75</td>
</tr>
<tr>
<td>Crab</td>
<td>0.45</td>
<td>85</td>
<td>0.38</td>
<td>45</td>
</tr>
<tr>
<td>Prawns</td>
<td>0.04</td>
<td>60</td>
<td>0.02</td>
<td>35</td>
</tr>
<tr>
<td>Mussels</td>
<td>0.16</td>
<td>40</td>
<td>0.06</td>
<td>25</td>
</tr>
<tr>
<td>Beef</td>
<td>&lt;0.01</td>
<td>90</td>
<td>&lt;0.01</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Lamb</td>
<td>0.02</td>
<td>90</td>
<td>0.02</td>
<td>25</td>
</tr>
<tr>
<td>Pork</td>
<td>0.01</td>
<td>90</td>
<td>&lt;0.01</td>
<td>&lt;25</td>
</tr>
<tr>
<td>Chicken</td>
<td>0.03</td>
<td>100</td>
<td>0.03</td>
<td>50</td>
</tr>
</tbody>
</table>

Data are taken from the British Nutrition Foundation [7]. Note that both DHA content and portion size may vary.
DHA is also present in fish body (aka fish), fish liver, algal and krill oils, in concentrated supplements and in pharmaceutical grade preparations designed for controlling blood triglyceride levels. Table 2 summarises the DHA content of typical preparations of these sources. It is clear that the use of supplements, whether these are over-the-counter or prescribed, can substantially increase the intake of DHA. DHA-rich oils from algae or tuna are used in infant formulas.

### DHA Status

#### DHA Concentration in Different Metabolic and Anatomical Compartments

DHA is transported in the bloodstream as a component of lipoproteins (within triglycerides, phospholipids and cholesteryl esters) or as a nonesterified ('free') fatty acid (largely due to release from adipose tissue stores or from spill-over of lipase-mediated hydrolysis of circulating lipoproteins). DHA can be stored in adipose tissue esterified into triglycerides. DHA is found in all cell membranes esterified into phospholipids and other complex lipids. Table 3 lists some reported concentrations of EPA and DHA in different metabolic or anatomical compartments in humans [11–23]. It is evident that the absolute and proportional contribution of EPA or DHA to the total fatty acids present within any of the compartments listed in table 3 differs both between the two fatty acids and between compartments. Most often, DHA is present at a higher concentration than EPA. This is especially true in specific regions of the brain and eye, where DHA makes a significant contribution to the fatty acid complement and EPA is virtually absent.

#### DHA is Highly Concentrated in the Human Brain and Eyes

More than 50% of the dry weight of the human brain is lipid, particularly structural lipid (i.e. phospholipids). The most abundant fatty acids in the brain are DHA, arachidonic acid and adrenic acid [24, 25]. The human brain and retina contain an especially high proportion of DHA relative to other tissues and little EPA [24–26] (table 3). For example, DHA was reported to contribute an average of 18% of fatty acids to adult human brain grey matter [14], while Makrides et al. [15] 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 [15]. 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 grey matter phosphatidylserine [25] and an average of 22% of fatty acids in retina phosphatidylcholine [26]. DHA contributes 50–70% of the fatty acids present in the rod outer segments of the retina [26]. These rod outer segments contain the eyes' photoreceptors.

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 1,100 g at 18 months of age [27]. Over this period, the DHA content of the brain increases from 900 μg/g (90 mg in total) to 3,000 μg/g (3,300 mg total) [28, 29]. This represents a 35-fold increase in total brain DHA. The estimated rate of accretion of DHA into the human brain in the last trimester of pregnancy is 15–22 mg/week [30]. This is also the most active period of brain cell division. Thus, it is believed that an adequate supply of DHA during this period is essential for normal growth, neurological and visual development and function, and learning behaviour [29].

#### DHA Concentration during Pregnancy

There is a significant linear relationship between the DHA contents of maternal and umbilical cord plasma phospholipids. This suggests that maternal plasma phos-
Table 3. Typical EPA and DHA concentrations reported in different compartments in humans

<table>
<thead>
<tr>
<th>Population</th>
<th>Compartment</th>
<th>Units</th>
<th>EPA</th>
<th>DHA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generally healthy men and women aged 18–45 years; UK</td>
<td>Plasma triglycerides</td>
<td>μmol/lb</td>
<td>4</td>
<td>10</td>
<td>West and Calder, unpublished data</td>
</tr>
<tr>
<td>Generally healthy men and women aged 20–80 years; UK</td>
<td>Plasma triglycerides</td>
<td>% of fatty acids</td>
<td>0.3</td>
<td>0.8</td>
<td>[11]</td>
</tr>
<tr>
<td>Generally healthy men and women aged 18–45 years; UK</td>
<td>Plasma phospholipids</td>
<td>μmol/lb</td>
<td>40</td>
<td>120</td>
<td>West and Calder, unpublished data</td>
</tr>
<tr>
<td>Generally healthy men and women aged 20–80 years; UK</td>
<td>Plasma phospholipids</td>
<td>% of fatty acids</td>
<td>1.2</td>
<td>3.6</td>
<td>[11]</td>
</tr>
<tr>
<td>Healthy pregnant women aged 18–40 years; week 38 of pregnancy; UK</td>
<td>Plasma phospholipids</td>
<td>% of fatty acids</td>
<td>0.4</td>
<td>3.8</td>
<td>[12]</td>
</tr>
<tr>
<td>Newborn infants (umbilical cord); healthy pregnancies; UK</td>
<td>Plasma phospholipids</td>
<td>% of fatty acids</td>
<td>0.3</td>
<td>6.4</td>
<td>[12]</td>
</tr>
<tr>
<td>Generally healthy men and women aged 18–45 years; UK</td>
<td>Plasma cholesteryl esters</td>
<td>μmol/lb</td>
<td>55</td>
<td>40</td>
<td>West and Calder, unpublished data</td>
</tr>
<tr>
<td>Generally healthy men and women aged 20–80 years; UK</td>
<td>Plasma cholesteryl esters</td>
<td>% of fatty acids</td>
<td>1.0</td>
<td>0.6</td>
<td>[12]</td>
</tr>
<tr>
<td>Generally healthy men and women aged 18–45 years; UK</td>
<td>Plasma nonesterified fatty acids</td>
<td>μmol/lb</td>
<td>0.9</td>
<td>3.4</td>
<td>West and Calder, unpublished data</td>
</tr>
<tr>
<td>Generally healthy men and women aged 20–80 years; UK</td>
<td>Plasma nonesterified fatty acids</td>
<td>% of fatty acids</td>
<td>0.4</td>
<td>1.6</td>
<td>[12]</td>
</tr>
<tr>
<td>Generally healthy men and women aged 20–80 years; UK</td>
<td>Red blood cells</td>
<td>% of fatty acids</td>
<td>2.3</td>
<td>5.2</td>
<td>[12]</td>
</tr>
<tr>
<td>Generally healthy men and women aged 20–80 years; UK</td>
<td>Platelets</td>
<td>% of fatty acids</td>
<td>1.1</td>
<td>2.0</td>
<td>[12]</td>
</tr>
<tr>
<td>Generally healthy men aged 18–40 years; UK</td>
<td>Blood neutrophils</td>
<td>% of fatty acids</td>
<td>0.6</td>
<td>1.6</td>
<td>[13]</td>
</tr>
<tr>
<td>Generally healthy men and women aged 20–80 years</td>
<td>Blood mononuclear cells (mainly lymphocytes)</td>
<td>% of fatty acids</td>
<td>0.8</td>
<td>1.9</td>
<td>[11]</td>
</tr>
<tr>
<td>Men (mean age 68 years) with no evidence of dementia</td>
<td>Brain grey matter</td>
<td>% of fatty acids</td>
<td>NR</td>
<td>18</td>
<td>[14]</td>
</tr>
<tr>
<td>Men (mean age 68 years) with no evidence of dementia</td>
<td>Brain white matter</td>
<td>% of fatty acids</td>
<td>NR</td>
<td>4</td>
<td>[12]</td>
</tr>
<tr>
<td>Breast-fed term infants who had died at a mean age of 4 months; Australia</td>
<td>Cerebral cortex</td>
<td>% of fatty acids</td>
<td>&lt;0.1</td>
<td>8</td>
<td>[15]</td>
</tr>
<tr>
<td>Breast-fed term infants who had died at a mean age of 4 months; Australia</td>
<td>Retina</td>
<td>% of fatty acids</td>
<td>0.1</td>
<td>12</td>
<td>[15]</td>
</tr>
<tr>
<td>Men (mean age 55 years) who had received a heart transplant; USA</td>
<td>Cardiac muscle</td>
<td>% of fatty acids</td>
<td>0.2</td>
<td>1.5</td>
<td>[16]</td>
</tr>
<tr>
<td>Mainly men (mean age 60 years) undergoing cardiac surgery; Australia</td>
<td>Cardiac muscle      phospholipids</td>
<td>% of fatty acids</td>
<td>0.5</td>
<td>4.8</td>
<td>[17]</td>
</tr>
<tr>
<td>Healthy men (mean age 21 years); UK</td>
<td>Skeletal muscle</td>
<td>% of fatty acids</td>
<td>0.6</td>
<td>1.5</td>
<td>[18]</td>
</tr>
<tr>
<td>Healthy men and women aged 25–45 years; USA</td>
<td>Skeletal muscle      phospholipids</td>
<td>% of fatty acids</td>
<td>0.7</td>
<td>1.9</td>
<td>[19]</td>
</tr>
<tr>
<td>Men and women aged 38–41 years undergoing surgery; Chile</td>
<td>Liver</td>
<td>% of fatty acids</td>
<td>0.4</td>
<td>6.8</td>
<td>[2]</td>
</tr>
</tbody>
</table>
Phospholipids are an important source of DHA for the fetus and that maternal plasma phospholipid DHA concentration determines DHA supply to the fetus. An increase in maternal plasma DHA concentration occurs during pregnancy [31] (fig. 3), and this increase precedes the increase in DHA accretion by the brain. The extent of the increase differs among pregnant women in different countries [32], perhaps indicating dietary differences during the pre-pregnancy period. Al et al. [33] reported that maternal plasma phospholipid DHA content was lower in women who had had multiple pregnancies than in those in their first pregnancy. This may indicate that maternal body stores are important in maintaining plasma DHA status but that these may be eroded by multiple pregnancies.

Even though maternal and fetal blood DHA concentrations are highly correlated, DHA is concentrated in the fetal circulation and in fetal tissues [34], a process sometimes referred to as biomagnification (fig. 4). Placental adaptation to ensure efficient DHA transfer from the maternal to the fetal circulation is an important part of the biomagnification process; this is discussed in detail elsewhere [34]. Furthermore, there are observations that the placenta can synthesise DHA from ALA [34], which would allow for in situ provision of DHA to help meet the demands for DHA imposed by pregnancy.

Table 3 (continued)

<table>
<thead>
<tr>
<th>Population</th>
<th>Compartment</th>
<th>Units</th>
<th>EPA</th>
<th>DHA</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men and women aged 23–63 years undergoing surgery; Chile</td>
<td>Liver phospholipids</td>
<td>% of fatty acids</td>
<td>4.8</td>
<td>15.1</td>
<td>[21]</td>
</tr>
<tr>
<td>Patients with inflammatory bowel disease; UK</td>
<td>Colonic mucosa</td>
<td>% of fatty acids</td>
<td>0.3</td>
<td>1.7</td>
<td>[22]</td>
</tr>
<tr>
<td>Generally healthy men and women aged 20–80 years; UK</td>
<td>Subcutaneous adipose tissue</td>
<td>% of fatty acids</td>
<td>0.2</td>
<td>0.2</td>
<td>[11]</td>
</tr>
<tr>
<td>Generally healthy men (mean age 34 years); Iran</td>
<td>Spermatozoa</td>
<td>% of fatty acids</td>
<td>0.6</td>
<td>9.6</td>
<td>[23]</td>
</tr>
</tbody>
</table>

Data are taken from the selected references and are not exhaustive. NR = Not reported.

- Blood collected after an overnight fast. Absolute n-3 PUFA concentrations will be affected by the concentrations of blood lipids and lipoproteins; these data are for healthy non-obese adults.
The Effect of Increased DHA Intake on DHA Status

Increasing the Intake of DHA Increases Its Status in Many Body Compartments

Increased intakes of DHA (and EPA) from fish or from n-3 supplements are reflected in increased proportions of both fatty acids in blood lipid, blood cell and many tissue compartments. This has been reported many times for total plasma or serum lipids or for the complex lipid components of plasma or serum (i.e. triglycerides, phospholipids and cholesteryl esters) and is also well described for erythrocytes, platelets and leukocytes (see [35] for references). There are also descriptions of increased proportions of DHA (and EPA) in human tissues, including skeletal muscle [18, 19], heart [16], gut mucosa [22] and adipose tissue [11, 36] when their intake is increased. These compartments all show a dose- and time-dependent incorporation of both EPA and DHA [11, 13, 35, 36], but the precise pattern depends upon the specific location [37, 38] (fig. 5). Pools that are turning over rapidly show faster incorporation of both EPA and DHA than slower turning-over pools. Thus, plasma lipids 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. However, Makrides et al. [15] demonstrated that the DHA concentration of the cerebral cortex of breast-fed infants who would have received DHA via breast milk is higher than that of infants who had not been breast fed.

The higher status of EPA and DHA achieved through increased intake 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, the intake returns to the earlier lower levels, then EPA and DHA statuses decline, eventually returning to earlier levels (fig. 5). This is well described for blood lipids, platelets, leukocytes and erythrocytes [35]. However, just as the incorporation of EPA into different pools is faster than the incorporation of DHA, the loss of EPA is faster than the loss of DHA [35]. 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.

Increasing Maternal and Fetal DHA Status during Pregnancy

With the recognition that maternal supply of DHA to the fetus is important and that fetal and infant DHA status is a major contributor to optimal visual and brain development and function, investigations of ways to increase maternal DHA status have been made. Clearly the simplest way to do this would be to increase maternal DHA intake from fish or from supplements. Regular consumption of fatty fish resulted in higher maternal erythrocyte DHA content [39, 40]. Connor et al. [41] reported that consumption of sardines and fish oil by women from week 30 of pregnancy resulted in higher DHA contents of maternal plasma and erythrocytes 4 weeks later than observed in women taking placebo. In that study, a total of 3 g n-3 LCPUFAs including 1.1 g DHA were supplied per day. A similar intake of n-3 LCPUFAs (2.7 g/day) and of DHA (1.1 g/day) provided as fish oil from week 30 of pregnancy resulted in higher DHA contents of maternal plasma phospholipids and of umbilical cord vein and artery at birth [42]. Fish oil providing 3.3 g/day n-3 LCPUFAs (including 2.2 g/day as DHA) from week 20 of pregnancy resulted in higher DHA contents of maternal erythrocytes at weeks 30 and 37 of pregnancy and at 6 weeks after delivery [43]. Furthermore, the DHA content of cord blood erythrocytes was 40% higher than in the placebo group [43]. A much lower intake of DHA (200...
mg/day) from week 15 of pregnancy resulted in higher DHA content of maternal plasma erythrocytes at week 28 of pregnancy and at birth, although there was no effect on DHA content of cord blood plasma or erythrocytes [44].

Increasing the Status of DHA in Human Breast Milk

After a baby is born it is important that a supply of preformed DHA is continued. Breast milk DHA content can be increased by maternal supplementation with fish oil [45], DHA-rich oil [46, 47] or n-3 LCPUFA-rich eggs [47] or by increased maternal fatty fish consumption [48]. Harris et al. [45] gave lactating women 5 g/day fish oil for 28 days, 10 g/day fish oil for 14 days or 47 g/day fish oil for 8 days. Levels of DHA in breast milk were 0.1% of total fatty acids at baseline and 0.5, 0.8 and 4.8% of fatty acids after 5, 10 and 47 g/day fish oil, respectively. Hawkes et al. [46] gave lactating women placebo, ‘low’-dose DHA (300 mg/day) or ‘high’-dose DHA (600 mg/day) from day 3 after delivery for 4 weeks. They found that the DHA content of maternal plasma, maternal mononuclear cells, breast milk and breast milk cells increased in relation to DHA intake. In another low-dose approach, Jensen et al. [47] compared fish oil, algal oil and eggs as a source of DHA for incorporation into breast milk: lactating women (2 weeks after giving birth) received about 200 mg/day DHA from the oils or eggs for 4 weeks. This resulted in an increase in DHA in maternal plasma phospholipids (from 2.5% to about 4% of total fatty acids) and in breast milk (from about 0.2% to about 0.4% of total fatty acids). Furthermore, infant plasma phospholipid DHA increased from about 3.6% to about 5% of total fatty acids. This demonstrates that increased consumption of DHA by lactating women results in increased DHA in breast milk, subsequently elevating infant DHA status. The Salmon in Pregnancy Study investigated the effect of consumption of two portions of salmon per week from week 20 of pregnancy until delivery [12]. This intervention resulted in higher breast milk DHA than seen in women in the control group who did not eat fatty fish [48] (fig. 6).

Mechanisms of Action of DHA

Cell Membranes Containing DHA within Phospholipids Have Unique Functional Properties

Phospholipids are quantitatively the major lipid component of cell membranes. Fatty acids in the phospholipids play important roles assuring the correct environment for membrane protein function, maintaining membrane order (‘fluidity’), and influencing the formation of signaling platforms termed lipid rafts. Membrane phospholipids are substrates for the generation of second messengers like diacylglycerols, lysophospholipids and PUFAs themselves [49]. DHA is a constituent of cell membrane phospholipids and imparts unique physical and chemical properties on the phospholipid and any signalling molecules that are produced from it [49]. Because of its highly unsaturated nature, DHA adopts a three-dimensional shape that is different from that of other common membrane fatty acids (fig. 1). This shape strongly influences membrane order and has an impact on membrane protein function and on the assembly of lipid rafts (fig. 7). Hence, the DHA content of a cell membrane can have a significant influence on cellular behaviour and responsiveness to signals, which may be electrical, chemical, hormonal or antigenic in nature. Within the brain, DHA has important actions in regulating intracellular signaling [50]. Perhaps the single best example of the unique role that cell membrane DHA has in a physiological function relates to the role of the rod in the retinal photoreceptor [51]. The cells of the rod outer segment have an exceptionally high content of DHA in their membranes (50–70% of fatty acids) [26]. The DHA is a component of phospholipids that cluster around the protein rhodopsin which receives the light signal. When the signal is received, rhodopsin undergoes a conformational change that initiates a signal transduction cascade. The physical nature of DHA within the membrane facilitates the conformational change [51]. Studies with rhodopsin imbed-
DHA Can Act via Cell Surface and Intracellular Receptors

Peroxisome Proliferator-Activated Receptors

Peroxisome proliferator-activated receptors (PPARs) are transcription factors; they regulate gene expression and so play a role in cell and tissue responses to the environment. There are several isoforms of PPARs; PPAR-α and PPAR-γ are the most well understood. PPARs act by forming a heterodimer with the retinoic X receptor, the ligand for which is cis-9-retinoic acid. PPAR-α is expressed mainly in the liver and is involved in regulating hepatic metabolic responses. The transcription of genes encoding several key enzymes of β-oxidation and of lipid-protein metabolism has been shown to be regulated by PPAR-α. Thus, activation of PPAR-α results in partitioning of fatty acids towards hepatic oxidation and away from triglyceride synthesis. PPAR-γ is expressed in adipose tissue, where it is involved in regulating adipocyte differentiation and metabolic responses of adipocytes including promoting insulin sensitivity, and in inflammatory cells, where it is involved in regulating the production of inflammatory mediators, having an anti-inflammatory action. PPARs are activated by noncovalent binding of ligands which include n-3 LCPUFAs and various lipid mediators. DHA can induce and activate PPARs [53] and upregulate a number of PPAR target genes [54], which may be at least partly responsible for the ability of DHA to lower fasting plasma triglyceride concentrations [55], to increase insulin sensitivity [56] and to reduce inflammation [57].

G-Protein-Coupled Receptors

Several membrane-associated G-protein-coupled receptors (GPRs) are able to bind fatty acids differentially according to structural features of those fatty acids. GPR40 and GPR120 can both bind long-chain fatty acids and are active in signal transduction. Oh et al. [58] reported that GPR120 is highly expressed on adipocytes and on inflammatory macrophages. Macrophage GPR120 was shown to be involved in anti-inflammatory signalling, inhibiting activation of the prototypical pro-inflammatory transcription factor nuclear factor kappa B [58]. DHA (and EPA) promoted GPR120-mediated gene activation, and several anti-inflammatory actions of DHA did not
occur in GPR120 knockdown cells. Oh et al. [58] also demonstrated that DHA promoted the translocation of the glucose transporter GLUT4 to the surface of cultured adipocytes and that this was associated with enhanced glucose uptake. These effects were abolished by GPR120 knockout, suggesting that GPR120 mediates at least some of the metabolic actions of DHA.

**DHA is a Substrate for Biosynthesis of Bioactive Mediators**

PUFAs including linoleic acid, dihomo-γ-linolenic acid (20:3n-6), arachidonic acid, EPA, n-3 DPA, n-6 DPA (22:5n-6) and DHA all give rise to bioactive lipid mediators formed through the cyclooxygenase and lipoxygenase pathways [57]. These mediators have many actions, but are most well recognised for their roles in inflammation, immunity, platelet reactivity and smooth muscle contraction. Historically, most attention has been given to the prostaglandins, thromboxanes and leukotrienes, produced from arachidonic acid. Like EPA, DHA has some actions to reduce the production of these mediators, which may be an important mechanism by which DHA can affect inflammation, immunity, blood clotting and so on. More importantly, however, new families of bioactive lipid mediators produced from both EPA and DHA have been discovered. These mediators are able to induce resolution of inflammation (i.e. *turn inflammation off*) and to promote immune function, thereby assisting with host defence and diminishing the pathological effect of inflammation [59, 60]. They include the resolvins produced from EPA (E-series) and DHA (D-series) and protectins and maresins produced from DHA. Protectins are also referred to as neuroprotectins when generated within neural tissue, where they appear to have an important role [59, 60]. The synthesis of resolvins, protectins and maresins involves the cyclooxygenase and lipoxygenase pathways, with different epimers being produced in the presence and absence of aspirin [59, 60]. Figure 7 shows the outline of the pathway of DHA conversion to these mediators. D-series resolvins and protectins have been described in human plasma [61], adipose tissue [62] and breast milk [63]. The biological effects of resolvins, protectins and maresins have been examined extensively in cell culture and in animal models [59, 60]. Resolvins D1 and D2 and protectin D1 have each been demonstrated to have potent anti-inflammatory and inflammation-resolving actions in these model systems [59, 60], acting through specific GPRs.

**Conclusions**

DHA is a long-chain, highly unsaturated n-3 fatty acid. It has a structure that gives it unique physical and functional properties. DHA is metabolically related to other n-3 fatty acids: it can be synthesised via EPA from the plant essential fatty acid ALA. However, this pathway does not appear to be very efficient in many individuals, although the conversion of ALA to DHA is much better in young women than in young men. It also appears that young infants may be more efficient converters of ALA to DHA than many adults, although the conversion rate is variable among infants. Many factors have been identified that affect the rate of conversion of ALA to DHA. These factors include n-6 fatty acid levels, availability of several trace elements, sensitivity to insulin, female sex hormone status and polymorphisms in, and epigenetic modification of, the genes encoding the various enzymes involved in the pathway. The implication of poor conversion is that DHA needs to be consumed preformed. Along with EPA and n-3 DPA, DHA is found in fairly high amounts in seafood, especially fatty fish, and in various forms of n-3 supplements. The amount of DHA in seafood and in supplements varies. Breast milk contains DHA. DHA is found esterified into complex lipids within the bloodstream, in adipose stores and in cell membranes. Its concentration in different compartments varies greatly. The brain and eye have high DHA contents compared to other organs. DHA is especially concentrated in the grey matter of the brain and in the rod outer segments of the retina. In the brain DHA is involved in neuronal signalling, while in the eye it is involved in visual quality. DHA is accumulated in the brain and eye late in pregnancy and in early infancy. A lower DHA content is linked to poorer cognitive development and visual function. Thus, it is vital that pregnant and lactating women as well as infants consume sufficient preformed DHA to support brain and eye development and function.

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It is vital that pregnant and lactating women as well as infants consume sufficient preformed DHA to support brain and eye development and function.
DHA intake. DHA affects cell and tissue physiology and function through numerous mechanisms, which are summarised in figure 7. These include alterations in membrane structure and function, in membrane protein function, in cellular signalling and in lipid mediator production. In addition to the effects on neuronal signalling and vision, DHA reduces inflammation, improves immune function and optimises cellular metabolism. Through these effects DHA acts to lower the risk of insulin resistance, metabolic syndrome, hyperlipidemia and cardiovascular disease.

Appendix

Table: Glossary of terms

- Saturated fatty acid: A fatty acid with no double bonds in its hydrocarbon (acyl) chain
- Unsaturated fatty acid: A fatty acid with at least 1 double bond in its hydrocarbon (acyl) chain
- Polyunsaturated fatty acid (PUFA): A fatty acid with at least 2 double bonds in its hydrocarbon (acyl) chain
- Long-chain PUFA (LCPUFA): A fatty acid with 20 or more carbons in its hydrocarbon (acyl) chain
- Omega-3 (also ω-3 or n-3) PUFAs: A family of PUFAs with the terminal double bond on carbon number 3 counting the methyl carbon as carbon number 1
- Omega-6 (also ω-6 or n-6) PUFAs: A family of PUFAs with the terminal double bond on carbon number 6 counting the methyl carbon as carbon number 1
- Docosahexaenoic acid (DHA): An n-3 LCPUFA; has 22 carbons and 6 double bonds in its hydrocarbon (acyl) chain

References


Disclosure Statement

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Docosahexaenoic Acid

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The importance of DHA during CNS development has led to the hypothesis that early diet modulation of DHA may alter the normal trajectory of brain development.

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Docosahexaenoic Acid and Neurodevelopmental Outcomes of Term Infants

by Suzanne Meldrum and Karen Simmer

Key insights
Although there is ample evidence highlighting the importance of docosahexaenoic acid (DHA) in the development and function of the central nervous system (CNS), clinical data on the effects of dietary DHA on neurocognitive outcomes remains inconclusive. Despite the existence of clear dietary intake recommendations for pregnant and lactating women, DHA levels are insufficient even across the populations of many developed countries.

Current knowledge
DHA and arachidonic acid are long-chain polyunsaturated fatty acids (LCPUFA) essential for development and overall health. High concentrations of DHA are found within the lipid bilayer of neurons, affecting membrane fluidity and neuronal transmission. DHA also modulates the function of other membrane-bound proteins, such as enzymes, ion channels and receptors. Deposition of DHA in the brain peaks during the third trimester of pregnancy and during the first year of life, coinciding with the period of rapid brain growth. During fetal development, circulating maternal DHA is sequestered by the growing fetus. The DHA requirements of infants are met through the diet, either from breast milk or formula.

Practical implications
Despite the positive findings from some observational studies, many randomized controlled intervention trials have failed to demonstrate a conclusive benefit of maternal DHA supplementation on infant neurodevelopment. Few trials have evaluated supplementation during the lactation period. In contrast, many trials have been conducted on LCPUFA supplementation of infant formula. Regardless of the time period of the intervention, there is a large degree of heterogeneity between the studies with respect to the DHA dose, the intervention period and outcomes assessed. These trials do not demonstrate a benefit of DHA supplementation of healthy infants on child development. The individual response to prenatal DHA levels may be influenced by other factors, such as genetic background, smoking, maternal education and birth weight. This suggests that future efforts should focus on characterizing the responders to DHA to clarify the role of optimal dose and timing of potential interventions.

Recommended reading
Docosahexaenoic Acid and Neurodevelopmental Outcomes of Term Infants

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Key Messages
- Supplementation with docosahexaenoic acid (DHA) or fish oil during pregnancy and/or lactation does not improve child development.
- Supplementation of healthy infants with DHA or fish oil does not improve child development.

Key Words
Docosahexaenoic acid · Long-chain polyunsaturated fatty acid · Fish oil · Child development

Abstract
Docosahexaenoic acid (DHA), a long-chain polyunsaturated fatty acid, is essential for normal brain development. DHA is found predominantly in seafood, fish oil, breastmilk and supplemented formula. DHA intake in Western countries is often below recommendations. Observational studies have demonstrated an association between DHA intake in pregnancy and neurodevelopment of offspring but cannot fully adjust for confounding factors that influence child development. Randomised clinical trials of DHA supplementation during pregnancy and/or lactation, and of term infants, have not shown a consistent benefit nor harm on neurodevelopment of healthy children born at term. The evidence does not support DHA supplementation of healthy pregnant and lactating women, nor healthy infants.

The Role of Docosahexaenoic Acid in the Neurodevelopment of the Fetus and Infant

Long-chain polyunsaturated fatty acids (LCPUFA), including docosahexaenoic acid (DHA) and arachidonic acid are essential for overall health including normal growth, vision and, specific to this review, neurodevelopment. Peak brain deposition of DHA occurs during the third trimester of gestation and during the first year, coinciding with rapid brain growth [1]. Infants are provided with DHA during early development of the central nervous system (CNS) predominantly through placental transfer during gestation and through dietary intake postnatally [2]. Despite intake recommendations for pregnant and lactating women existing between 200–300 mg per day [3, 4], intake in Western nations may be insufficient due to the high demands of the fetus and inadequate maternal intake [5]. Postnatally, while breast milk
and/or infant formula may contain DHA, the amount provided will vary based on maternal dietary intake and brand of formula, respectively. Countries that typically experience low levels of DHA within breast milk are Western nations such as the USA (0.2%), Canada (0.14%) and Australia (0.25%) [6].

There have been a number of expert reviews detailing the evidence for the roles of DHA during early CNS development [7–11], and each highlights the importance of DHA for optimal function. DHA is found at high concentrations within the lipid bilayer of neurons, playing a functional role in fluidity/flexibility of the membrane affecting neuronal transmission [12]. Furthermore, evidence suggests that DHA also has demonstrable effects upon the activities of membrane-bound enzymes, ion channels and receptors, gene expression and neuronal inflammation via eicosanoid precursors [13]. Specific to neurodevelopment, the high proportion of DHA in neural membranes suggests that DHA is important for membrane biogenesis and, consequently, such events as neurogenesis, neuronal migration and outgrowth [7, 8].

The importance of DHA during CNS development has led to the hypothesis that early diet modulation of DHA may alter the normal trajectory of brain development, and subsequently affect the functional skills derived from CNS structures. Research has been undertaken investigating how and in what quantities DHA is supplied to the infant CNS, and whether such quantities are sufficient to support optimal brain function.

**Associations between DHA Intake (Maternal and Neonatal/Infant) and Neurodevelopment of the Fetus and Infant**

Observational trials provide data showing that maternal and infant omega-3 (n-3) LCPUFA consumption and/or status may positively influence infant neurodevelopment. Significant positive associations have been reported for specific outcomes in a number of maternal studies [14–22], with a smaller number observing negative associations [18, 20, 22, 23], or no associations [21–25]. Benefits for a wide range of outcomes have been found such as verbal intelligence quotient, language, motor and neurological development and social behaviour. Despite some consistency of results, study methodology is variable, particularly for the sample size, the measurement of DHA via food frequency questionnaire or specific cell fractions and the impact of confounding factors including socioeconomic status.

The largest study to date was conducted by Hibbeln et al. [17] in 2007, whereby seafood consumption <340 g/week at 32 weeks’ gestation was associated with an increased risk of lowest-quartile verbal intelligence in the offspring at 8 years. The strength of this study lies in the large sample size of 11,875 participants, far greater than the majority of other trials. The study concluded that pregnant women should not reduce seafood consumption during pregnancy, and by doing so may lose benefits to offspring neurodevelopment.

A recent study of this design was conducted by Julvez et al. [26]. In a population characterised by high seafood consumption (Spain), moderate positive associations were found between seafood consumption during the first and third trimesters of pregnancy and child neuropsychological development at 5 years. Yet, following adjustment for cord-blood LCPUFA levels, only part of the significance remained, suggesting that the beneficial effect was not only derived from DHA consumption. Herein lies the difficulty in the interpretation of such trials. Such observational studies are unable to establish causality because of the difficulty in adjusting for the complex confounding factors that also influence early child development.

**Observational studies are unable to establish causality because of the difficulty in adjusting for the complex confounding factors that also influence early child development**

To explore this, Gould et al. [27] recently conducted an association study to explore what maternal factors influenced DHA status during pregnancy. Their results suggested that responsiveness to prenatal DHA was related to the characteristics of the specific population groups studied. In the Australian cohort examined by the authors, birth weight, maternal education and smoking were seen to interact with DHA status during pregnancy and neurodevelopment. Such findings suggest that either future trials power their studies sufficiently to be able to evaluate subgroup analyses post hoc, or that responders to DHA supplementation are identified prior to supplementation. Either way will require significant changes to the design or sample sizes of future observational trials.

For infant and childhood association studies, results are more mixed, with studies showing a combination of
negative or no association [18, 28, 29], compared to positive [16, 30, 31]. This may highlight the importance of the maternal period for DHA accretion.

**Randomised Controlled Trials of Maternal LCPUFA Supplementation and Infant Neurodevelopment**

A number of randomised controlled trials (RCTs) have been undertaken to ascertain if maternal LCPUFA supplementation is an effective strategy for improving offspring neurodevelopment. A recent meta-analysis conducted by Gould et al. [32] included 11 RCTs totalling 5,272 participants and observed no significant differences between the supplemented groups for cognitive, language or motor development. A single finding for enhanced cognitive scores for children aged 2.5 years was detected; yet, this effect was ascertained from 2 trials with a high risk of bias and the authors advised caution in interpretation. The methodological quality of the trial was generally observed as poor, particularly the sample sizes, the high attrition rates and statistical design.

The largest maternal supplementation study to date was conducted by Makrides et al. [33] in 2010: 726 pregnant women were supplemented with 800 mg/day of either fish oil or placebo during the second half of pregnancy. The study had a high level of methodological rigour with low attrition. No effects of DHA treatment during pregnancy on neurodevelopment were identified, either at 18 months or in a more recent follow-up at 4 years. Further, in a subset of this cohort, 158 children were assessed at 27 months for attention, working memory and inhibitory control. No significant differences were observed between the groups, except with a small number of inconsistent effects which were attributed to chance.

Since 2013, two new RCTs have been published, both of which found no statistically significant effects of maternal DHA supplementation. Ramakrishnan et al. [34] recruited 730 women in Mexico supplemented daily with either 400 mg DHA or placebo capsules from 18–22 weeks’ gestation until delivery and found no effects on neurodevelopment at 18 months of age using the Bayley Scales of Infant Development (BSID) (Spanish version). Hurtado et al. [35] recruited 110 pregnant women to an RCT of 400 mg DHA per day from 28 weeks’ gestation until the end of lactation, assessing neurodevelopment at 12 months of age. No differences were observed between groups, also according to the BSID. However, attrition in the study was high and only ~40% of the study participants were assessed for neurodevelopment.

Overall, results from maternal supplementation RCTs still appear inconclusive. Yet, it can be noted that controlled studies have not shown the same consistent positive findings that observational trials have yielded. While this may be a result of heterogeneity in study design, and in many cases insufficient methodological quality, it remains possible that supplementation with DHA during pregnancy may not confer any neurological benefit to the offspring. Particular difficulties in the design of maternal RCTs are identifying what dose should be administered, when neurodevelopmental testing should take place and what specific neurodevelopmental tests should be administered.

**Particular difficulties in the design of maternal RCTs are identifying what dose should be administered, when neurodevelopmental testing should take place and what specific neurodevelopmental tests should be administered**

**RCTs of Supplementation during Lactation and Infant Neurodevelopment**

The investigation of DHA supplementation during lactation for improved infant and child neurodevelopment has been less specifically examined in the recent literature. A number of maternal supplementation trials have continued to supplement mothers during lactation [36], but such a design makes it difficult to identify during which time period supplementation was beneficial. Overall, studies including both maternal and lactation supplementation with DHA have not observed any significant effects on neurodevelopment.

A Cochrane analysis conducted by Delgado-Noguera et al. [37] in 2010 reviewed RCTs of LCPUFA supplementation during lactation, and included 6 trials totalling 1,280 women. Supplementation trials of both pregnancy and lactation, along with lactation only, were included. No significant differences were observed as a result of supplementation for children’s neurodevelopment. Yet, following a sensitivity analysis, a significant benefit was observed for psychomotor development at 5 years based on only 1 trial [38]. The trials were ascribed as having a low risk of bias and an overall rigor-
ous methodology, and the authors suggested that more trials were required for more conclusive findings to be reached.

Only three trials have investigated lactation specifically [38–40]. Similar to the trials of maternal supplementation, the optimal concentration of DHA within breast milk to confer any neurological benefit is as yet unknown. Breast milk DHA levels vary worldwide, with concentrations of as high as 1% noted in countries with high fish intake such as Japan [6]. Using five different doses of DHA supplementation for 12 weeks after birth, Gibson et al. [39], in 1997, observed little further increase in infant plasma or erythrocyte DHA levels when breast milk was above 0.8% of total fatty acids. In this study, higher mental development index scores using the Bayley scales were associated with higher breast milk DHA status at 12 months; yet, this effect was not observed at 2 years.

In a high-dose trial, Lauritzen et al. [40] in Denmark supplemented 122 mothers with low fish intake to fish oil or placebo supplementation for the first 4 months of lactation (800 mg DHA and 600 mg eicosapentaenoic acid). A third group of breastfeeding women with high fish intake were included as a reference. It was observed that vocabulary at 1 year was lower in the children of the fish oil-supplemented group compared to children in the control group, but this finding was not observed at 2 years. In contrast, in 2005, Jensen et al. [38] conducted a study supplementing breastfeeding mothers using DHA algal oil (approx. 200 mg DHA per day) for 4 months after delivery. Despite maternal DHA phospholipid contents increasing to 75% higher than baseline, neurodevelopment and vision were unaffected during the first 12 months. However, at 30 months of age, the supplemented group had a higher Bayley psychomotor development index (but not a higher mental development index). Again at 5 years of age, children whose mothers received DHA versus placebo performed significantly better on the sustained attention subscale of the Leiter International Performance Scale.

In summary, for trial of DHA supplementation during lactation, further trials are necessary in order to definitively conclude if supplementation can improve infant and child neurodevelopment. Sufficient statistical power, long-term follow-up and an investigation of optimal dosage are required.

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**The optimal concentration of DHA within breast milk to confer any neurological benefit is as yet unknown**

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**DHA Supplementation of Term Infants (Formula and Direct Supplementation) and Neurodevelopment**

RCTs of LCPUFA supplementation of infant formula have been conducted for the past 30 years and came about due to the lack of LCPUFA in commercial infant formulas compared to breast milk. While a relatively large number of trials have been conducted, there is a large degree of heterogeneity between studies, in particular regarding the dose of DHA, the length of supplementation and the method of assessment [41, 42]. Furthermore, the more recent identification of the genetic control of PUFA metabolism has raised the question of individual variability for DHA requirements. Two recent meta-analyses have been completed, one including 15 trials [43] and another evaluating 12 [44]. Both found no beneficial effects of supplementation on either mental or psychomotor development. Yet, Koletzko et al. [45], in a systematic review including 5 trials not evaluated by the previous meta-analyses, commented on the lack of consistency of RCTs, but noted a trend towards a greater likelihood of benefit with higher dosages (≥0.32% DHA and ≥0.66% arachidonic acid) and a longer duration of higher postnatal LCPUFA supplementation (up to 1 year of age).

Regarding the method of neurodevelopmental assessment, Sun et al. [42] lately assessed the validity and reliability of such measures used in LCPUFA supplementation trials. Using 29 articles, they noted that the methods of assessment all lacked predictive validity for future neurocognitive performance, and that measures of intellectual ability are usually not subject to change in the short term. They identified that a ‘well-designed, valid and clinical outcome assessment that measures neurocognitive function in neonates and infants is essential to provide the scientific evidence required for future clinical trials’. Yet, it was noted by the authors that no such measures exist, and therefore future collaboration and research towards such goals should be completed. Until this has taken place, determining the impact of DHA supplementation for infants may remain elusive.

**Conclusion**

There is no definite evidence that DHA supplementation in pregnancy, lactation or infancy improves the neurodevelopment of healthy term infants. If future trials are...
to provide certainty, they will need to recruit large sample sizes to identify potential subgroup analyses, such as maternal factors, infant gender and genetics, identify the optimal dose for supplementation and assess using well-designed and valid measures.

References


Disclosure Statement
The authors have no conflicts of interest to disclose.
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The balance between the metabolites of n-3 and n-6 fatty acids plays an important role in the maintenance of normal gestation length and is a critical element in cervical ripening and the initiation of labor.

Key insights
Globally, early preterm birth is a leading cause of death in children under 5 years of age. Initial observations on the longer duration of pregnancies and reduced incidence of preterm birth in fish-eating communities prompted further research into the role of omega-3 (n-3) long-chain polyunsaturated fatty acids (LCPUFA) in improving gestation length. The prenatal period is a vulnerable window that is highly sensitive to n-3 LCPUFA deficiency.

Current knowledge
The majority of preterm births occur spontaneously and are due to multiple factors that trigger the normally quiescent uterus to undergoing contractions and labor. Currently, there is no strategy that can be used as a primary prevention for widespread clinical use. The n-3 LCPUFA such as docosahexaenoic acid (DHA) and eicosapentaenoic acid are dietary agents that can modulate several clinical conditions via their anti-inflammatory actions. These may be relevant for modulating the inflammatory cascades that underpin the maternal response to the fetus during the birth process.

Practical implications
The most compelling evidence to support the efficacy of DHA supplementation in reducing preterm birth came from the DOMInO trial. Supplementation of n-3 LCPUFA during the last half of pregnancy in 2,399 women resulted in a 50% reduction in the incidence of early preterm birth. These findings have been supported by those from several other trials and meta-analyses. In general, supplementation with marine oil or n-3 LCPUFA in pregnancy is safe and well tolerated, but further work needs to be done to clarify the optimal dosage and timing of n-3 supplementation during pregnancy.

Recommended reading
Docosahexaenoic Acid and Preterm Birth

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Key Messages
• Preterm birth is one of the leading causes of infant deaths worldwide. There is a growing need to identify safe, effective, easily available primary prevention strategies to prevent preterm delivery, especially ‘early preterm birth’ before 34 weeks of gestation.
• Epidemiological and randomised controlled trial evidence exists to support an effect of increased omega-3 long-chain polyunsaturated fatty acid intake during pregnancy on length of gestation.

Key Words
Preterm birth · Omega-3 · Pregnancy · Nutritional supplements · Fatty acids · Primary prevention

Abstract
Preterm birth accounts for more than 85% of all perinatal complications and deaths. There are many short- and long-term consequences of being born too soon. These infants often require intensive care and are at increased risk of early morbidities often with life-long sequelae. Approximately 50% of all preterm births have unknown or unclear causes, and there are no effective primary prevention strategies in widespread clinical use. Epidemiological studies have observed an increased length of gestation in populations with high fish consumption. These findings have led to randomised controlled trials of omega-3 (n-3) long-chain polyunsaturated fatty acid (LCPUFA) supplementation which show that these dietary agents may delay the timing of birth and may have value as a prophylactic intervention in some women. This review presents the available evidence and discusses the relationship between prenatal n-3 LCPUFA supplementation during pregnancy and the incidence of preterm birth.

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Introduction
Preterm birth (before 37 completed weeks of gestation) complications are now the leading cause of under-five deaths globally, accounting for 17% of all such deaths [1] and more than 85% of all perinatal complications [2]. It is estimated that 11% of births (15 million babies each year worldwide) are premature (table 1), with half of these due to unknown or unclear causes [3–5]. Advances in perinatal and neonatal care have resulted in greatly improved survival rates for preterm babies; however, there is an increasing awareness that, even in high-income countries, many of these children suffer short- and long-term consequences of being born too soon [6]. These infants often require intensive care and are at increased risk of early morbidities such as respiratory distress syndrome, intraventricular haemorrhage, necrotising enterocolitis, blindness and cerebral palsy. In early childhood, developmental difficulties may emerge in preterm children and later manifest as low educational achievement, high unemployment and other deficits in social
and emotional wellbeing, as well as having wider societal and economic impacts [7, 8].

One quarter of preterm births are due to medical intervention following pregnancy-related complications including pre-eclampsia, intra-uterine growth restriction or maternal disease [9]. The remainder of preterm births occur spontaneously and are due to multifactorial processes causing the uterus to change from quiescence to active contractions and birth. The precise cause of spontaneous preterm labour is of unknown aetiology in up to half of all cases [10]. Whilst tocolytic drugs to inhibit uterine contractions are available in an emergency situation to stop or delay premature labour, there are currently no effective primary prevention strategies for preterm birth in widespread clinical use. More than 10 Cochrane systematic reviews have investigated treatments ranging from dietary supplements to therapeutic drugs such as betamimetics, magnesium sulphate and calcium channel blockers. Despite the wide range of treatments investigated in the reviews, none have been shown to be effective in reducing the risk of spontaneous preterm birth.

### n-3 LCPUFA and Preterm Birth

In recent decades, omega-3 (n-3) long-chain polyunsaturated fatty acids (LCPUFA), specifically docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), have emerged as efficacious dietary agents that moderate a number of clinical conditions through their known anti-inflammatory activity. This action may also apply to pregnancy and the inflammatory cascade of labour where the maternal immune response to the fetus is crucial. The feto-placental unit is supplied with n-3 and omega-6 (n-6) LCPUFA from the maternal circulation, which is influenced by maternal dietary intake and endogenous synthesis. The prostaglandins derived from n-6 arachidonic acid (AA, 20:4n-6) within the utero-placental unit in normal pregnancy are countered by local production of prostaglandins derived from n-3 LCPUFA within the same tissues. The balance between the metabolites of n-3 and n-6 fatty acids plays an important role in the maintenance of normal gestation length and is a critical element in cervical ripening and the initiation of labour [11]. If local production of n-6-derived prostaglandins within the feto-placental unit is too high, or local accumulation of n-3 LCPUFA is too low, the cervix may prematurely ripen and uterine contractions increase, which may in turn lead to preterm birth, particularly in susceptible women [12].

### Maternal DHA Intake

Modern Western diets are low in n-3 LCPUFA and high in n-6 fatty acids, leading to a predominance of AA in tissues. The prenatal period is a time of increased risk for n-3 LCPUFA deficiency as DHA is preferentially transferred from maternal tissue stores to the developing fetus [13]. The World Health Organization recommends an intake of 300 mg/day of n-3 LCPUFA for pregnant women [14]; however, women in many low-, middle- and high-income countries do not achieve this amount, with the exception of coastal countries where fish and other

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**Table 1. Estimated preterm birth rates and total number of preterm births for 2010**

<table>
<thead>
<tr>
<th>Region</th>
<th>Livebirths, n</th>
<th>Estimated mean preterm birth rate, %</th>
<th>Preterm births, n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Developed regions</td>
<td>14,300,000</td>
<td>8.6</td>
<td>1,233,200</td>
</tr>
<tr>
<td>Eastern Asia</td>
<td>17,400,000</td>
<td>7.2</td>
<td>1,262,200</td>
</tr>
<tr>
<td>Latin America</td>
<td>10,200,000</td>
<td>8.4</td>
<td>852,800</td>
</tr>
<tr>
<td>Northern Africa</td>
<td>3,542,100</td>
<td>7.3</td>
<td>259,200</td>
</tr>
<tr>
<td>Oceania</td>
<td>263,200</td>
<td>7.4</td>
<td>19,500</td>
</tr>
<tr>
<td>South-Eastern Asia</td>
<td>38,700,000</td>
<td>13.3</td>
<td>5,159,300</td>
</tr>
<tr>
<td>Sub-Saharan Africa</td>
<td>32,100,000</td>
<td>12.3</td>
<td>3,936,800</td>
</tr>
<tr>
<td>Western Asia</td>
<td>4,855,300</td>
<td>10.1</td>
<td>488,200</td>
</tr>
<tr>
<td>Caribbean</td>
<td>682,800</td>
<td>11.2</td>
<td>76,500</td>
</tr>
<tr>
<td>Caucasus and Central Asia</td>
<td>1,643,000</td>
<td>9.2</td>
<td>151,300</td>
</tr>
<tr>
<td>Total worldwide</td>
<td>135,000,000</td>
<td>11.1</td>
<td>14,936,700</td>
</tr>
</tbody>
</table>

Source: Blencowe et al. [32].
marine foods are easily accessible, affordable and commonly consumed [15]. Whilst marine foods are rich sources of DHA and EPA, nutritional advice for pregnant women regarding marine oil consumption is complicated with warnings that suggest limiting overall fish consumption to avoid potential methyl-mercury or polychlorinated biphenyls exposure. As a result, the intake of these long-chain n-3 fats is well below the required intake to achieve normal rises in the feto-placental unit for the maintenance of a full-term pregnancy, highlighting a potential insufficiency for the feto-placental unit. This has been suggested to contribute to the increasing rate of preterm birth in developed countries, as well as in the developing countries where diets have become more Westernised with lower intakes of fish and higher intake of n-6-rich vegetable oils [16].

Epidemiological Evidence

The first observations linking n-3 LCPUFA intake and pregnancy duration were made following population comparisons in the early 1980s. The longer duration of pregnancies observed in the genetically similar fish-eating community of the Faroe Islands compared to Danish women led to the suggestion that fatty acids from marine food, a rich source of EPA and DHA, could delay spontaneous delivery [17]. Subsequent cohort studies have also demonstrated a positive association between fish or n-3 LCPUFA intake and the duration of gestation [18–20]. A systematic review of 151,880 mother-child pairs from 19 population-based European birth cohort studies showed that women who ate fish >1 time/week during pregnancy had a lower risk of preterm birth than women who rarely ate fish [21]. The apparent consistency of these associations appears promising; however, it is not possible to infer a causal link between increased n-3 LCPUFA exposure (via fish) in pregnancy and increased gestational length because of the difficulty in excluding the possibility of residual confounding environmental factors, or that there may be constituents of fish other than n-3 LCPUFA driving these associations.

Evidence from Randomised Controlled Trials

Initial randomised controlled trials (RCTs) investigating the effect of n-3 LCPUFA supplementation for the prevention of preterm birth used high-dose EPA-rich fish oil and largely targeted women with higher-risk pregnancies. A 2006 Cochrane review of marine oil supplementation during pregnancy included data from 6 trials and measured a variety of maternal and neonatal health outcomes including preterm birth [22]. Two of these trials (n = 860 women in total) measured preterm birth outcomes of women with high-risk pregnancies. A meta-analysis showed that the fish oil intervention resulted in an approximate 2-day increase in the mean duration of gestation, a nonsignificant 8% reduction in preterm birth <37 weeks’ gestation and a significant 31% reduction in early preterm birth (<34 weeks’ gestation). More recent trials of prenatal n-3 supplementation have included women with normal-risk pregnancy and have intervened with DHA because of the association with early childhood developmental outcomes [23–25]. The strongest evidence to support the efficacy of DHA supplementation to reduce preterm birth comes from the DOMInO trial, the largest trial to date of n-3 LCPUFA supplementation in pregnancy (n = 2,399) [23]. This trial was designed to assess the effect of n-3 LCPUFA supplementation (predominantly as DHA) during the last half of pregnancy on the prevalence of postnatal depression in women and on early childhood neuro-developmental outcomes. A preplanned secondary analysis showed a reduction in early preterm birth (<34 weeks) in the DHA-supplemented group compared with the control group, corresponding to a 50% reduction in the incidence of early preterm birth (1.09 vs. 2.25%, adjusted RR 0.49, 95% CI 0.25–0.94, p = 0.03). These findings concur with results of the 2006 Cochrane review, and when combined in an updated meta-analysis including two other trial recent trials (n = 3670) [24, 26], the numbers of included women increase 2.5- to 3-fold and the effect sizes remain remarkably similar and are increasingly compelling (table 2).

Collectively, data from the Cochrane systematic review [22] combined with more recent reviews [27–29] imply that the effects of n-3 LCPUFA are not attributable only to prostaglandins arising from EPA, as newer trials have used DHA-based supplementation. Clearly the mechanisms underlying the effects of n-3 LCPUFA on the duration of gestation are complex, and this is perhaps not surprising when one considers that multiple mechanisms often underpin important biological pro-
cesses, and it is likely that birth is no exception. Doses of n-3 LCPUFA supplementation used in RCTs during pregnancy range from 133 to 3,000 mg/day, and the intervention period has commenced between 16 and 30 weeks’ gestation until delivery or beyond. Overall, systematic reviews show that supplementation with marine oil or n-3 LCPUFA is safe in pregnancy and is generally well tolerated. Rates of serious adverse events were similar between treatment groups and occurrence of side effects (e.g. vomiting, nausea and diarrhoea) was generally similar except for belching and bad taste, which occurred more frequently in the marine oil-supplemented groups. No differences have been shown in the incidence of antepartum hospitalisation, caesarean section, eclampsia or other serious maternal morbidity between treatment and control groups [22, 27, 28]. However, a number of trials did report a significant increase in the incidence of post-term inductions or post-term pre-labour caesarean section in women supplemented with n-3 LCPUFA until delivery compared with control. This is a concern because post-term birth is also associated with increased perinatal mortality and morbidity in both infants and mothers [30]. Animal studies demonstrate that as the duration, dose and timing of n-3 LCPUFA supplement administration are altered, the levels accumulated within the feto-placental unit change in a timed dose-response relationship [11]. It has been shown that within 6 weeks, people who stop taking fish oil supplements have their plasma and red cell n-3 LCPUFA return to levels which are similar to those of the general population of low fish consumers [31]. Current trials are investigating the optimal timing of n-3 LCPUFA supplementation targeting the critical period before 34 weeks when preterm delivery has the poorest outcomes and ceasing treatment to reduce the risk of post-term intervention (Australian New Zealand Clinical Trials Registry number 12613001142729).

### Conclusion

Preventing preterm birth remains one of the most challenging issues in obstetric and neonatal care. Whilst a level of concordance is evident between epidemiological studies and RCTs, inconsistencies remain. This is perhaps not surprising when one considers that maternal n-3 LCPUFA exposure in observational studies is most likely based on a lifetime of exposure compared to a defined period of n-3 LCPUFA supplementation in RCTs. The evidence to date consistently demonstrates that n-3 LCPUFA supplementation during pregnancy increases the mean duration of gestation by 2 days and produces a 40–50% reduction in early preterm birth (<34 weeks’ gestation) [22, 23]. Future directions for research in this area include refinement of dosing and timing of n-3 LCPUFA supplementation. It is important to identify and evaluate (by RCT) other agents and sustainable food-based dietary interventions that are thought to influence similar pathways. In addition, further studies to understand the mechanisms surrounding individual response to n-3 LCPUFA supplementation and the relationship to immune profile/inflammatory indicators, as well as social and clinical characteristics will be vital in optimising future treatment recommendations.

### Disclosure Statement

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**Table 2. Cochrane review of n-3 LCPUFA for the prevention of preterm birth**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Effect of n-3 LCPUFA treatment relative to control</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>2006 Cochrane review [22]</td>
</tr>
<tr>
<td></td>
<td>Combined studies [22, 23, 27, 28]</td>
</tr>
<tr>
<td>Mean difference in gestation</td>
<td>2.5 (95% CI 1.0 – 4.1)</td>
</tr>
<tr>
<td>length, days</td>
<td>1,621 women from 3 trials</td>
</tr>
<tr>
<td></td>
<td>2.0 (95% CI 1.1 – 3.0)</td>
</tr>
<tr>
<td></td>
<td>4,289 women from 5 trials</td>
</tr>
<tr>
<td>Relative risk of preterm birth</td>
<td>0.92 (95% CI 0.79 – 1.07)</td>
</tr>
<tr>
<td>&lt;37 weeks’ gestation</td>
<td>1,916 women from 5 trials</td>
</tr>
<tr>
<td></td>
<td>0.92 (95% CI 0.80 – 1.04)</td>
</tr>
<tr>
<td></td>
<td>5,586 women from 8 trials</td>
</tr>
<tr>
<td>Relative risk of early preterm</td>
<td>0.69 (95% CI 0.49 – 0.99)</td>
</tr>
<tr>
<td>birth &lt;34 weeks’ gestation</td>
<td>860 women from 2 trials</td>
</tr>
<tr>
<td></td>
<td>0.60 (95% CI 0.44 – 0.81)</td>
</tr>
<tr>
<td></td>
<td>3,560 women from 4 trials</td>
</tr>
</tbody>
</table>

CI = Confidence interval.
References


DOI: 10.1159/000448263
Experimental studies as well as recent clinical trials show that providing larger amounts of DHA than currently and routinely provided is associated with better neurological outcomes at 18 months to 2 years

Long-Chain Polyunsaturated Fatty Acids and Clinical Outcomes of Preterm Infants

by Alexandre Lapillonne and Sissel J. Moltu

**Key insights**

Infants who are born prematurely have unique nutritional requirements due to their immaturity. The standard nutritional management of premature infants results in deficiencies in long-chain polyunsaturated fatty acids (LCPUFAs), particularly docosahexaenoic acid (DHA). Providing higher levels of DHA than routinely given is associated with better neurological and clinical outcomes.

**Current knowledge**

Infants who are born premature or extremely premature have a high risk of morbidities and mortality. During their initial hospitalization, parenteral nutrition is given to meet their nutritional needs. LCPUFAs play important roles in perinatal growth and development and are therefore an important component of nutrition for these infants. In premature infants, the endogenous capacity for synthesizing DHA and eicosapentaenoic acid is not sufficient to meet their requirements for these critical LCPUFAs. Limited supply of LCPUFAs through external nutritional sources further exacerbates the problem.

**Practical implications**

Currently, the nutritional management of preterm infants results in an early and severe deficit in DHA. The smallest and most premature infants are especially vulnerable; these infants are the most likely to benefit from high-dose DHA supplementation. Due to their immaturity, premature infants are at risk of concomitant diseases such as bronchopulmonary dysplasia, retinopathy of prematurity, necrotizing enterocolitis, and white matter injury of the brain. Supplementation with LCPUFAs including DHA has been used to alleviate these risks, possibly as a consequence of their anti-inflammatory effects. Despite some conflicting findings, the main message from clinical studies indicates that supplementing with adequate levels of LCPUFAs is an important step towards optimizing the clinical outcomes of premature infants.

**Recommended reading**

Long-Chain Polyunsaturated Fatty Acids and Clinical Outcomes of Preterm Infants

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Key Messages

- Recent clinical trials show that providing larger amounts of docosahexaenoic acid (DHA) than currently and routinely provided is associated with better neurological outcomes at 18 months to 2 years.
- There is growing evidence that omega-3 long-chain polyunsaturated fatty acids, particularly DHA, may reduce the incidence or severity of neonatal morbidities by affecting different steps of the immune and anti-inflammatory response.

Key Words
Docosahexaenoic acid · Enteral nutrition · Parenteral nutrition · Polyunsaturated fatty acids · Premature infant

Abstract

Long-chain polyunsaturated fatty acids (LCPUFAs) play specific roles during the perinatal period and are very important nutrients to consider. The possible effects of LCPUFAs, particularly docosahexaenoic acid (DHA), on various clinical outcomes of preterm infants are discussed in this paper. Since DHA accumulates in the central nervous system during development, a lot of attention has focused on the effects of DHA on neurodevelopment. Experimental studies as well as recent clinical trials show that providing larger amounts of DHA than currently and routinely provided is associated with better neurological outcomes at 18 months to 2 years. This early advantage, however, does not seem to translate into detectable change in visual and neurodevelopmental outcomes or behavior when assessed in childhood. There is growing evidence that, in addition to effects on development, omega-3 LCPUFAs may reduce the incidence or severity of neonatal morbidities by affecting different steps of the immune and anti-inflammatory response. Studies in preterm infants suggest that the omega-3 LCPUFAs may play a significant role by reducing the risk of bronchopulmonary dysplasia, necrotizing enterocolitis and possibly retinopathy of prematurity and sepsis. Overall, evidence is increasing to support the benefits of high-dose DHA for various health outcomes of preterm infants. These findings are of major clinical relevance mainly because infants born preterm are at particularly high risk for a nutritional deficit in omega-3 fatty acids, predisposing to adverse neonatal outcomes. Further studies are warranted to address these issues as well as to more precisely determine the LCPUFA requirement in order to favor the best possible outcomes of preterm infants.

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**Introduction**

Preterm birth is the leading cause of child mortality in high- and middle-income countries. The risk of morbidity and mortality in infants born very and extremely premature has been well documented [1]. Fortunately, improved pre- and postnatal care has led to reduced mortality and morbidity. However, the risk of severe medical disabilities increases sharply with decreasing gestational age (GA) at birth [1].

During the initial part of hospitalization, immature infants need parenteral nutrition to meet their nutritional requirements. Afterwards, nutrition is supplied via the enteral route using either enriched breast milk or preterm formula. However, replacing the nutrition provided by the placenta is difficult, and postnatal malnutrition and growth failure are commonly seen in preterm infants.

The brain is particularly vulnerable to the influences of nutrition between 24 and 42 weeks of gestation [2]. Thus, fetal and neonatal malnutrition may have global or isolated effects on the developing brain, depending on the requirements of the particular nutrients at the time of the deficit [2]. Both early and enhanced supply of energy, protein and lipids have shown to be beneficial for growth and neurodevelopment [3, 4]. Moreover, optimized nutrition has been shown to mediate disease severity [5].

The main target for feeding preterm infants is to achieve growth that resembles normal fetal growth rates [6]. In recent recommendations, this goal has been extended to achieving satisfactory functional development. The estimated amounts of nutrients necessary for growth similar to that of the fetal model are based on estimates obtained by factorial as well as empirical methods [6].

It is important to consider long-chain polyunsaturated fatty acids (LCPUFAs) due to their specific roles during the perinatal period. Therefore, the aim of this paper is to review the possible effects of LCPUFAs, particularly docosahexaenoic acid (DHA), on various clinical outcomes of preterm infants.

**LCPUFA Metabolism during the Perinatal Period**

The PUFAs linoleic acid and α-linolenic acid are essential fatty acids that must be provided exogenously [6, 7]. Linoleic acid is converted to arachidonic acid (AA) and α-linolenic acid to docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Particularly AA and DHA accumulate rapidly during the last trimester and the first postnatal months, i.e. the period of rapid growth and brain development [6, 7]. DHA is the main lipid of the central nervous system and comprises as much as 30–50% of neuronal plasma membranes by weight [7, 8]. However, fetal brain accretion amounted to only 1.1 and 4.65% of the total body accretion of the omega-6 (n-6) and omega-3 (n-3) fatty acids, respectively [9]. Since most LCPUFAs accumulate in the white adipose tissue and to a lesser extent in the lean mass and in the liver, the DHA accumulation in other organs as well as the accumulation of other LCPUFAs is important to take into account when determining requirements.

AA and DHA are transferred by special transport molecules across the placenta to the fetus. The placenta provides the fetus with a selectivity which favors the transfer of DHA over all other fatty acids including AA [10]. There is evidence from stable isotope studies that AA and DHA synthesis can occur in premature infants. It has also been deduced from these tracer studies that the rate of AA synthesis is significantly greater than that of DHA, suggesting that the fetus has a greater ability to regulate its own levels of AA, not those of DHA, by de novo synthesis or reuptake by the placenta [10]. Overall, these data suggest that DHA supply may be more critical than that of AA during the perinatal period.

Studies conducted by using the LCPUFA precursors labeled with stable isotopes indicate that LCPUFA synthesis occurs even in small preterm infants and appears to be higher than in term infants. However, based on estimates of LCPUFA endogenous synthesis rates, premature infants appear to have an endogenous capacity for conversion of α-linolenic acid to DHA and EPA that cannot fulfill the requirement based on the fetal accretion rate [8, 11]. Furthermore, extremely premature infants are at increased risk of developing deficits in n-3 LCPUFAs due to the lack of adipose stores and limited provision of preformed LCPUFAs through nutritional sources.

The in utero accretion rate of DHA is estimated to be 43 and 212 mg/kg/day for DHA and AA, respectively [12]. It has been shown that current nutritional management of preterm infants leads to an early and severe DHA deficit, increasing with decreasing GA [13]. Depletion of DHA may lead to reduced visual function and alterations in behavior or cognitive performance [14], whereas DHA and AA supplementation has shown positive effects on growth, visual function and mental development in randomized controlled trials (RCT) [15–18].

**Vision and Brain Development**

In experimental studies, poor accumulation of retinal and brain DHA leads to abnormal retinal physiology, poor visual acuity, increased duration of visual fixation,
and increased stereotyped behaviors and locomotor activity [14]. The evidence most relevant to the issue of causality showed that control performance levels can be restored when DHA is added to the diets of animals in which brain DHA concentration had been severely reduced. Nevertheless, the magnitude of these effects is not large, despite the fact that the studies were conducted under profound dietary restriction.

Studies in preterm infants indicate possible benefits for retinal and cognitive development, as suggested by greater retinal sensitivity to photic stimulation assessed by electroretinography, more mature visual acuity, and short-term effects on global developmental outcomes at 6–18 months after DHA supplementation of preterm infant formula in controlled clinical studies [19, 20].

With regard to neurodevelopment in preterm infants, recent meta-analyses suggest that benefits of formula supplementation with LCPUFA are less clear [21]. Among many possible explanations for the difficulty in demonstrating clinical benefits of LCPUFA supplementation in preterm formulas by meta-analysis are the extreme variability in study designs and the selection of relatively mature and healthy preterm infants which are likely less DHA deficient than very preterm infants. Furthermore, the meta-analyses include studies comparing some LCPUFA supplementation versus no supplementation and do not include studies comparing two doses of LCPUFAs. Interestingly, the amount of LCPUFAs used in early studies was chosen to produce the same concentration of AA and DHA in formula as in term breast milk (i.e. 0.2–0.4% fatty acids). This may not be a wise approach for preterm infants and, particularly, for very and extremely preterm infants because the amount of DHA provided by ingesting breast milk is below the in utero accretion rate [13].

Three studies report outcome data in preterm infants fed milk with a higher DHA content of 0.5–1.7% of total fatty acids. The first study, which examined the effect of providing DHA supplementation (0.50% of total fatty acids) for up to 9 months after term, showed that DHA improved growth in the whole cohort of preterm infants and improved mental development in boys [22]. In the second study, the effect of the supplementation during hospitalization of human milk with oils that provided an extra 32 mg of DHA and AA per 100 ml was assessed [15]. At the 6-month follow-up evaluation, the intervention group performed better than the control group in the problem-solving subscore of the Ages and Stages Questionnaire, and in the electrophysiologic assessment of event-related potentials, suggesting better recognition memory. At 20 months’ postnatal age, no differences in the mental and motor development scores of the Ages and Stages Questionnaire or in the Mental Developmental Index (MDI) score of the Bayley Scales of Infant Development were observed, but the intervention group had better results at 20 months at the free-play sessions, suggesting positive effects from supplementation on functions related to attention. Finally, plasma DHA concentration at discharge was positively correlated with the Bayley MDI and with ‘sustained attention’ [18]. Long-term follow-up, at 8 years of age, showed no effects on white matter microstructure, behavioral outcome, and cognitive functions [23, 24]. The third study was designed to compare the effects of a high versus standard DHA intake during hospitalization (i.e. 1 vs. 0.35% total fatty acids as DHA) while AA intake was kept constant (0.5% total fatty acids) [17, 25]. Visual acuity was improved significantly at 4 months corrected age. At 18 months, there were no overall differences in MDI or in the Psychomotor Developmental Index of the Bayley Scales, but fewer infants were classified as having an MDI score of less than 70. Infants who weighed <1,250 g and were fed the high-DHA diet had a higher MDI score than controls (mean difference = 4.6, 95% CI 0.1–9.0, p < 0.05), but the difference was not significant when GA at delivery, sex, maternal education, and birth order were taken into account. Girls, but not boys, fed a high-DHA diet had higher MDI scores and were less likely to have mild or significant developmental delay than control girls. Finally, the early advantage seen on visual and cognitive functions did not translate into any clinically meaningful change in visual and neurodevelopmental outcomes or behavior when assessed in childhood [26–28].

Overall, these studies show that providing larger amounts of DHA supplements is associated with better neurological outcomes at 18 months to 2 years (fig. 1). One study suggested that the smallest babies are the most vulnerable to DHA deficiency and likely to reap the greatest benefit from high-dose DHA supplementation. The observation that a nonsignificant difference in mean MDI translated to fewer infants with a low MDI score suggests
that a high dose of DHA is more efficient, or is only efficient, in certain subgroups of infants, probably those at high risk of DHA deficiency.

**LCPUFAs as Immunonutrients**

Immature infants are at risk of experiencing concomitant diseases due to immaturity, among these: bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC), white matter injury of the brain (WMI), persistent ductus arteriosus, and sepsis. Perinatal infections or inflammation processes seem important in the pathogenesis of several of these comorbidities [29, 30]. Recent studies have demonstrated that immature infants have elevated levels of inflammatory cytokines during the neonatal period, and that up-regulated cytokine expression is positively associated with BPD, ROP, WMI, and impaired neurodevelopmental outcomes [30–32]. A proposed mechanism behind the upregulated immune response is sustained activation (ongoing endogenous exposures) and/or impaired resolution of inflammation [31].

There is growing evidence that in addition to structural effects on growth and organ development, n-3 LCPUFAs, particularly DHA and EPA, may reduce the incidence or severity of BPD, ROP, NEC, and WMI by affecting different steps of the immune response [33, 34]. LCPUFAs have the capacity to influence the immune response by several means.

Certain LCPUFAs serve as precursors for the synthesis of eicosanoids (e.g. dihomo-γ-linolenic acid (20:3n-6), AA, and EPA) and docosanoids (e.g. DHA). AA is a precursor of pro-inflammatory mediators (such as leukotrienes of the n-4 series), and of prostaglandins and thromboxanes of the n-2 series, which increase the vascular tone and promote platelet aggregation. AA is also a precursor of lipoxins which are inflammation-resolving mediators. In contrast, prostaglandins and thromboxanes of the n-3 series and leukotrienes of the n-5 series, formed from EPA, have many antagonistic effects such as a reduction in platelet aggregation and vascular tone as well as less inflammatory effects. Metabolites from EPA can modulate inflammation by decreasing the production of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) through the peroxisome proliferator-activated receptor pathways, which in turn inhibits the nuclear transcription factor κB (NF-κB), and increasing the production and secretion of anti-inflammatory eicosanoids as interleukin-10 [33]. Resolvins, protectins, and maresins formed from n-3 LCPUFAs evoke anti-inflammatory and pro-resolving mechanisms, and enhance microbial clearance [34].

**Bronchopulmonary Dysplasia**

BPD is defined as persistent oxygen dependency at 36 weeks’ postmenstrual age and is, along with postnatal growth restriction, the most common morbidity of prematurity. BPD occurs mostly in infants born before 28 weeks' gestation.
weeks of gestation [1]. BPD is characterized by diffuse fibrosis of the lung and impaired alveolar development [35]. Although the pathogenesis is multifactorial, intrauterine and postnatal growth restriction is an independent risk factor, possibly by affecting pulmonary alveolar and vessel growth [35].

Lipids have been considered toxic in acute respiratory failure since they may induce or intensify gas exchange abnormalities. The historical pure soybean lipid emulsion induces an increase in intrapulmonary shunt with reduction of the PaO$_2$/FiO$_2$ ratio concordant with an increase in pulmonary blood pressure and vascular resistances [36]. In contrast there is some evidence from experimental studies that n-3 LCPUFAs may be beneficial in conditions associated with pulmonary hypertension through production of epoxides [37]. This, in turn, may reduce the need for mechanical ventilation and the risk of BPD.

The beneficial effect of DHA on lung function is supported by animal studies. For example, in a rat model of hyperoxia-induced lung injury, DHA supplementation was shown to decrease leukocyte infiltration in the pups of DHA supplemented nursing dams [38]. Separate studies have also demonstrated that exposure to high DHA increases the production of dipalmitoylphosphatidylcholine, the major surfactant lipid in the fetal and neonatal lung [39]. Some studies suggest that the use of a fish oil lipid emulsion or of a fish oil mixed lipid emulsion may reduce the risk of BPD and that adequate n-3 LCPUFA status may protect newborns from lung injuries induced by hyperoxia [40]. Along with sufficient early supply of protein and energy to promote growth, n-3 PUFAs seem to protect against lung injury or reduce BPD severity by a DHA-dependent activation of the peroxisome proliferator-activated receptor pathways, thereby accelerating lung maturation, pneumocyte growth and vasoproliferation [41].

In severe sepsis and in patients with acute lung injury, fatty acids from fish oil were found to attenuate the initial injurious hyperinflammatory state [42]. The bronchoalveolar lavages of adult patients with acute respiratory distress syndrome receiving n-3 fatty acids and γ-linoleic acid show an important decrease in global cell count, in polymorphonuclear cell percentage, IL-8 and leukotriene B$_4$ concentrations. The specific change in immune response was associated with an improvement of the PaO$_2$/FiO$_2$ ratio, a reduction in mechanical ventilation need and duration, a decrease risk of complications, and a decreased length of stay in the intensive care unit [43].

Studies in preterm infants suggest improved lung development and a reduced incidence of BPD with fish oil supplementation [35]. The best evidence of the effects of n-3 fatty acids on the prevention of BPD comes from a large randomized trial assessing the effects of 60 mg/kg/day DHA compared with a standard dose of 20 mg/kg/day DHA on neurodevelopmental outcome of 657 preterm infants [17]. The study was not designed to test BPD; however, in exploratory analyses in the subgroup of infants less than 29 weeks’ GA, there was a significant reduction in infants requiring supplemental oxygen at 36 weeks’ postmenstrual age (RR 0.76, 95% CI 0.58–1.00, p = 0.05).

A recent meta-analysis targeting the role of LCPUFA supplementation in preterm infants born before 33 weeks of gestation found potential protective effects of n-3 fatty acids on BPD [44]. The effects were found for all interventions (RR 0.88, 95% CI 0.74–1.05) and in the subgroup of RCT that exclusively supplemented with n-3 LCPUFA (RR 0.84, 95% CI 0.66–1.13).

**Retinopathy of Prematurity**

In the retina, DHA is especially enriched in rod photoreceptor outer segments and essential for their differentiation, survival, and signal transduction [45]. ROP is a disorder of vascular development of the retina and it is the main reason for visual impairment in extreme premature infants. As for the lung, both nutritional and inflammatory factors seem to be important mediators in disease progression. Dietary n-3 LCPUFA reduces pathologic retinal neovascularization in oxygen-induced retinopathy in mice [46]. Two studies, one observational and one not blinded, reported a reduction in the need for laser therapy for ROP in very premature infants using fish oil supplementation in the lipid emulsion [47, 48]. Another study of infants with a GA of 28–31 weeks reported less ROP in fish oil-supplemented infants but no difference in need for treatment of proliferative disease [49]. Two other randomized controlled studies did not show any beneficial of fish oil lipid emulsion on ROP [50, 51].

While many studies have focused on DHA and its importance for vision and cognitive development, few studies have addressed the role of AA during fetal and neonatal life and after preterm birth. Just like DHA, AA is an important component of cell membranes where a change in composition results in changed function [52]. AA is an important precursor of factors, which appear essential for angiogenesis and thereby may play a significant role in the pathogenicity of ROP [52].
**White Matter Injury**

The most common brain injuries in premature infants are intraventricular hemorrhage and periventricular leukomalacia; with severe brain injury being defined as the presence of either intraventricular hemorrhage grade ≥3 or cystic periventricular leukomalacia. Indeed, WMI is associated with poor nutritional status as well as inflammation [29]. n-3 LCPUFAs, particularly DHA, are essential nutrients in brain development. In addition to being an important building block, DHA is also a substrate for neuroprotectin D1, which inhibits the pro-inflammatory cytokine production in human glial cells. In the same line of evidence, some experimental studies have shown that providing DHA may prevent neonatal brain injury by inhibiting oxidative stress and apoptosis of neuron cells [53]. Since most extremely premature infants receive insufficient amounts of DHA during neonatal hospitalization, providing adequate amounts of essential fatty acids to extremely premature infants, including preformed DHA and EPA, from birth onward, may improve neurodevelopmental outcome by mediating brain inflammation.

**Sepsis and Noninfectious Inflammation**

Very preterm infants are susceptible to sepsis, possibly as a result of attenuated innate immune responses [31]. Interestingly, these infants also show signs of sustained systemic inflammation with elevated pro-inflammatory cytokines. Sepsis may be defined as ‘the host’s deleterious and nonresolving systemic inflammatory response to microbial infection’ [54]. The host response is similar to the activation triggered by noninfectious tissue injuries like trauma, burns and ischemic reperfusion events [30], making it difficult to distinguish them from another. The newly identified alarmin molecule High Mobility Group Box 1 (HMGB1), which has been recognized as an important mediator of sepsis [30], is also thought to play an important role in lung injury and the pathogenesis of BPD [55]. HMGB1, an activator of NF-κB, is released by necrotic, but not apoptotic cells, and sustains the inflammatory process after the resolution of the early stage of inflammation [55]. As mentioned, one of the anti-inflammatory potentials of n-3 LCPUFAs is the ability to inhibit the activation of NF-κB [33], and thereby possibly modulate an inappropriate inflammatory response. These pathophysiological observations are in accordance with the observed association between low DHA and AA concentrations and the increased incidence of sepsis in preterm infants [58].

**Necrotizing Enterocolitis**

NEC is a serious disease of the gastrointestinal tract in very preterm infants and may lead to intestinal failure or death. The pathogenesis is multifactorial, but as with the other above-mentioned neonatal comorbidities, numerous inflammatory mediators seem to play a role in disease progression, among them HMGB1 [56, 57]. Several experimental models of NEC have demonstrated LCPUFA modulated reduction in both incidence and severity of bowel disease through multiple pathways associated with intestinal inflammation and necrosis [58–61]. The protective effects of DHA are multifactorial. Local cell membrane phospholipids play a structural role in protecting the integrity of intestinal cells and alterations in LCPUFA content is important in bacterial translocation and intracellular fluid shifts associated with cell stress signaling that initiates NEC [58].

Despite promising animal studies, results in preterm infants are mixed. In part, this may also be due to limited sample sizes, variable timing and dosing of DHA supplementation and similar confounding as described above. In an RCT, premature infants who were fed a DHA-supplemented formula had a decreased incidence of NEC compared to those who were fed a formula devoid of LCPUFAs [62]. Recently, a systematic review of n-3 LCPUFAs for extremely preterm infants found a trend toward a reduction in the risk of NEC (RR 0.50, 95% CI 0.23–1.10) [44].

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**Conclusion**

Since DHA accumulates in the central nervous system during development, a lot of attention has initially focused on the effects of DHA on neurodevelopment. Experimental studies as well as recent clinical trials show that providing larger amounts of DHA than currently and routinely provided is associated with better neurological outcomes at 18 months to 2 years. This early advantage, however, does not seem to translate into detectable change in visual and neurodevelopmental outcomes or behavior when assessed in childhood. However, this does...
not mean that supplementing with adequate amounts of LCPUFA during the perinatal period is not necessary.

Beside the effects on somatic development, there is growing evidence that n-3 LCPUFAs, particularly DHA and EPA, may reduce the incidence or severity of the most common comorbidities of prematurity by affecting different steps of the immune and anti-inflammatory response. These findings are of major clinical relevance mainly because infants born preterm, especially the smallest ones, are at particularly high risk for a nutritional deficit in n-3 fatty acids, predisposing to adverse neonatal outcomes. Further studies are warranted to address these issues as well as to more precisely determine the LCPUFA requirement in order to favor the best possible outcomes of preterm infants.

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