Review:
DHA supplementation in infants and children
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Dr. Mallikarjuna H.B.
MBBS, MD (Paediatrics), DCH, IYCF,
Professor Of Paediatrics, M. S. Ramaiah Medical College, Bangalore.
Executive Board Member Of CIAP 2018,
International Course Director & National Trainer For IYCF Courses

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Exclusive human milk feeding for the first 6 months of life, with continued breastfeeding for up to 2 years of life or longer, is recognized as the normative standard for infant feeding. Human milk is uniquely suited to the human infant, both in its nutritional composition and in the non-nutritive growth factors, immune factors, hormones, and other bio-active components that can act as biological signals and confer protection against illness in infancy and later in life. After 6 months, complimentary foods are needed to furnish the nutrients that are likely to become limiting.

From a nutritional perspective, infancy is a critical and vulnerable period. At no other stage of life is a single food adequate as the sole source of nutrition. This phenomenon occurs when immaturity in tissues and organs involved in nutrient metabolism (i.e., the gastrointestinal tract, liver, and kidneys) limits the ability of an infant to respond to excesses or deficiencies in nutrient intakes. Human milk is species specific, and many of the nutrients it contains are secreted as bound components that can offer protection from digestion and facilitate absorption and utilisation.

Human milk provides variability in sensory experience to the infant, based on the diet of the month. It comes with a changing mix of more than 40 different aromas. New-borns recognise these complex aromas and respond. The aromas in the human milk also influence the child’s preferences for certain foods based on the dietary habits of the mother. Therefore, the diet of the mother and breastfeeding plays an important role in developing healthy eating habits.

The composition of human milk is highly variable and tailored to the nutritional needs of the baby. It varies with mothers diet and lifestyle, baby’s health and development status and feeding stages.

During the last few decades, there has been considerable interest in the roles of long chain polyunsaturated fatty acids (LCPUFAs) such as docosahexaenoic acid (DHA) and arachidonic acid (ARA) in infant growth and development.(Forsyth, Gautier et al. 2017) They are indispensable for normal growth, neurodevelopment, vision and overall health.(Harris and Baack 2015) In utero LCPUFA accretion occurs mainly during the last trimester of pregnancy, when maternal levels are high with rapid growth and development of brain of fetus. Premature infants born before this process is complete, are relatively deficient in DHA. (Agostoni, Marangoni et al. 2008) Moreover, DHA levels in very low birth weight infants (VLBWs) remains low due to inadequate fat stores, ineffective conversion from precursor fatty acids and a limited nutritional supply.(Lapillonne, Groh-Wargo et al. 2013)Research data suggest that LCPUFA supplementation improves neurodevelopmental and visual outcomes in this high risk population.

Worldwide, 24% of all preterm birth (PTB) (<37 weeks) occur in India, where vegetable-based diets low in DHA are common.(Carlson, Gajewski et al. 2017)The rapid accretion of LCPUFA by the infant brain during the first 1,000 days of life underlined the potential importance of these fatty acids during this critical period of growth and development. The evidence that infants who received breast milk, which contains LCPUFA, had higher concentrations of DHA in their red blood cell membrane and in the cerebral cortex compared to infants on infant formula devoid of these fatty acids, was the main driver for clinical intervention studies.(Makrides, Neumann et al. 1994), (Farquharson, Jamieson et al. 1995. New evidence demonstrates that benefits of DHA supplementation extend beyond the brain. Experimental studies and a several clinical trials validate the role of improved LCPUFA provision in prevention of diseases specific to premature infants, including retinopathy of prematurity (ROP), necrotizing enterocolitis (NEC) and bronchopulmonary dysplasia (BPD). In developing countries, intakes of DHA and ARA from complementary foods are low. Therefore the government health
agencies need to adopt pragmatic strategies that will ensure that there is adequate provision of LCPUFA especially to the most vulnerable infants. (Forsyth, Gautier et al. 2017)

**Composition of Human Milk**

Human milk is a complex biological fluid composed of thousands of constituents in several compartments: an aqueous phase with true solutions (87%), colloidal dispersions of fat globules (4%), fat-globule membranes, and live cells. Human-milk constituents can be broadly categorized according to their physical or physiologic properties. These categories of constituents include:

**Table 1: Composition of human milk**

<table>
<thead>
<tr>
<th>Proteins</th>
<th>Non Proteins nitrogen</th>
<th>Carbohydrates</th>
<th>Lipids</th>
<th>Others</th>
<th>Vitamins</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-Lactalbumin</td>
<td>a-amino nitrogen</td>
<td>Lactose</td>
<td>Fat-soluble vitamins (A, D, E, and K</td>
<td>Cells</td>
<td>Water Soluble</td>
</tr>
<tr>
<td>P-Lactoglobulin</td>
<td>Creatine</td>
<td>Oligosaccharides</td>
<td>Carotenoids</td>
<td>Epithelial Leukocytes</td>
<td>Biotin Choline Folate</td>
</tr>
<tr>
<td>Caseins</td>
<td>Creatinine</td>
<td>Glycopeptides</td>
<td>Fatty acids</td>
<td>Lymphocytes</td>
<td>Inositol Niacin Pantothenic acid</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Glucosamine</td>
<td>Bifictus factors</td>
<td>Phospholipids</td>
<td>Macrophages</td>
<td>Riboflavin Thiamin</td>
</tr>
<tr>
<td>Growth factors</td>
<td>Nucleic acids</td>
<td>Parenteral fats</td>
<td>Sterols and hydrocarbons</td>
<td>Neutrophils</td>
<td>Vitamin B1, Vitamin B2 Vitamin</td>
</tr>
<tr>
<td>Hormones</td>
<td>Nucleotides</td>
<td></td>
<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>Polyamines</td>
<td></td>
<td></td>
<td></td>
<td>Minerals and ionic constituents</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>Urea</td>
<td></td>
<td></td>
<td></td>
<td>Bicarbonate Calcium</td>
</tr>
<tr>
<td>Secretory IgA and other immunoglobulins</td>
<td>Uric acid</td>
<td></td>
<td></td>
<td></td>
<td>Chloride Citrate Magnesium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Phosphate Potassium</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Sodium Sulfate</td>
</tr>
</tbody>
</table>

The composition and volume of human milk secreted are influenced by factors such as genetic individuality; maternal nutrition; stage of gestation and lactation; and techniques of sampling, storage and measurement.

**The nutritional components of human milk are derived from three sources:**

i. The nutrients of milk originate by the synthesis in the lactocyte

ii. Some are dietary in origin

iii. Some originate in the maternal stores

Overall, the nutritional quality of human milk is highly conserved, but attention to maternal diet is important for some vitamins and the fatty acid composition of human milk.

Individual donor milk samples from term mothers range at least from 0.6 to 1.4 g/dL for total protein, 1.8 to 8.9 g/dL for fat, 6.4 to 7.6 g/dL for lactose, and 50 to 115 kcal/dL for energy. Furthermore, the typical composition of preterm milk differs from that of term milk.

**Lipids**

Lipids comprise the major energy-yielding fraction of human milk, 97% to 98% of which are triglycerides. The constituent Fatty Acids (FAs) represent approximately 88% of milk fat. They are by far the most variable constituents in milk. The total fat content of human milk varies from 30 to 50g/L, and the corresponding energy contribution is approximately 45% to 55% of total kilojoules. The assimilation of fatty acids by young infants is crucial not only for energy to support growth but also for
the synthesis and development of retinal and neural tissues.

Human milk is a rich source of the essential FAs, linoleic acid (C18:20-6, 8-17%) and a-linolenic acid (C18:20-3, 0.5-0.8%), and their long-chain derivatives, arachidonic acid (C20:406, 0.5-0.7%) and docosahexaenoic acid (C22:60-3, 0.245%).

**Lipid classes in mature milk**
- Triacylglycerols 97–98%
- Diacylglycerols
- Monoacylglycerols
- Nonesterified fatty acids
- Phospholipids 1.1%
- Phosphatidylinositol
- Phosphatidylserine
- Phosphatidylethanolamine
- Phosphatidylcholine
- Sphingomyelin
- Cholesterol 0.7 – 1.3%

**Important fatty acids**
- Arachadonic acid – immunomodulator function
- Linoleic acid – Is used for deriving other essential fatty acids when they are not supplemented in diet
- Linolenic acid
- Palmitic acid – better absorption of fats and calcium and for protein acylation
- Stearic acid
- Myristic acid – protein acylation
- Capric acid – bactericidal
- Oleic acid

**Fatty acids biochemistry**
Naturally occurring fatty acids contain 4 to 24 carbon atoms in a molecule. According to the number of carbon atoms present, fatty acids are divided into:
- Long (18 or more)
- Medium (8 to 12)
- Short (4 to 6) chain fatty acids.

<table>
<thead>
<tr>
<th></th>
<th>Human milk</th>
<th>Cow's milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long - chain</td>
<td>95%</td>
<td>83%</td>
</tr>
<tr>
<td>Medium - chain</td>
<td>5%</td>
<td>5%</td>
</tr>
<tr>
<td>Short - chain</td>
<td>0%</td>
<td>12%</td>
</tr>
</tbody>
</table>

**Importance of Essential LCPUFAs**

Essential LCPUFAs are vital constituents of the phospholipid bi-layer of cell membranes. They contribute to structural integrity and function throughout the body. They have highly specialized
functional roles in the brain and retina making them critical for normal signal transduction, neurotransmission and neurogenesis. In tissues throughout the body, they are released from membranes by phospholipases for transformation to important hormones, eicosanoids, lipoxins and resolvins that mediate inflammation, immune function, platelet aggregation and lipid homeostasis. They also play role as local signaling molecules and transcription regulators of genes involved in inflammation, development and metabolism. Their abundant presence, association and multidimensional functionality demonstrates extremely important role of LCPUFAs for normal growth, development, and overall health.

Essential LCPUFAs cannot be synthesized in the body due to lack required enzymes and must be obtained from dietary food. There are 2 types of LCPUFAs

1. Omega 3:
   a. Docosahexaenoic acid (DHA) 22-carbon LCPUFAs obtained directly from oily fish.
   b. Its precursor is alpha – linolenic acid (ALA). ALA is found in flaxseed, canola, walnuts and soy.

2. Omega 6:
   a. Arachidonic acid (ARA) 20-carbon LCPUFAs obtained directly from meat and eggs
   b. Its precursor is linoleic acid (LA). LA is found in vegetable oils, nuts and seeds.

ARA is found throughout the body in phospholipid membranes and upon activation serves as a precursor to prostaglandins, thromboxane and leukotrienes. (Harris and Baack 2015)

ALA can be converted to eicosapentaenoic acid (EPA) and DHA to very small extent. These are nutritionally less abundant. It is these omega-3 LCPUFAs that are rapidly and preferentially incorporated into cell membranes. They serve important functional and structural roles in the brain and retina. They also have anti-inflammatory and metabolic signalling functions in other tissues. A balanced condition of these pathways is crucial for normal immune function and clotting. Any excess can lead to inflammatory response. (Harris and Baack 2015).

The parent FAs of the n-3 and n-6 FA series, the EFAs, ALA and LA, respectively, are converted to longer-chain, more unsaturated FAs by the same series of desaturases and elongases. Also, although the desaturases and elongases have a definite preference for the 0-3 FAs, competition exists between the two series for these enzymes. Thus, a high intake of ALA relative to LA results in higher levels of 0-3 LC-PUFAs, including DHA, and lower levels of 0-6 LC-PUFAs, including AA; however, although the hepatic activity of A-6 desaturase, which is thought to be the rate-limiting enzyme for synthesis of LC-PUFAs, is equal to or more than adult levels early in gestation, and term and preterm infants can convert ALA to DHA and LA to AA.

The conversion levels vary in accordance to the levels of other fatty acids and the ratio of n-6:n-3 PUFAs consumed in the diet. n0-6 PUFA intake in western diets are typically high, supplying approximately 16 times more n-6 PUFA than n-3 typically due to high intake of beef, pork, poultry, wheat germ and various cooking oils. In contrast, the diets of our paleolithic ancestors are thought to have contained roughly equal ratios of n-3:n-6. Subsequently there has been sufficient debate as to whether modern contain sufficient diets of n-3 FA to support optimal growth across the life span of the individual especially through pregnancy and lactation.

<table>
<thead>
<tr>
<th>Table 3: Comparison of human and cows milk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ABSORPTION OF FATS</strong></td>
</tr>
<tr>
<td>Infant</td>
</tr>
<tr>
<td>Breast-fed</td>
</tr>
<tr>
<td>Cow’s milk/proprietary formulae containing a mixture of animal and vegetable fats</td>
</tr>
</tbody>
</table>

(Harris and Baack 2015)
**Table 4. Fat content of human and cow’s milk per 100 ml milk**

<table>
<thead>
<tr>
<th></th>
<th>Fat (g)</th>
<th>Cholesterol (mg)</th>
<th>Energy (kcal)</th>
<th>Total saturated fatty acids (mg)</th>
<th>Total mono-unsaturated fatty acids (mg)</th>
<th>Total poly-unsaturated fatty acids (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human milk Mean (Range)</td>
<td>4.2 (3.7-4 g)</td>
<td>16 (12-23)</td>
<td>70 (65-75)</td>
<td>2001</td>
<td>1612</td>
<td>317</td>
</tr>
<tr>
<td>Cow’s milk</td>
<td>3.9</td>
<td>14</td>
<td>67</td>
<td>2330</td>
<td>1244</td>
<td>107</td>
</tr>
</tbody>
</table>

**Table 5: Composition of lipids in prepartum and postpartum milk**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Prepartum</th>
<th>Postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Early</td>
<td>Late</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>--</td>
<td>2</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Lipid (g/dL)</td>
<td>1.15</td>
<td>1.28</td>
</tr>
<tr>
<td>Phospholipid (mg/dL)</td>
<td>37</td>
<td>40</td>
</tr>
<tr>
<td>% of total lipid</td>
<td>3.2</td>
<td>3.1</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>--</td>
<td>--</td>
</tr>
</tbody>
</table>

**Table 6: Percentage of total lipids at lactation Day**

<table>
<thead>
<tr>
<th>Lipid Class</th>
<th>Percentage of Total Lipids at Lactation Day</th>
<th>Immediate Extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Total lipid, % in milk</td>
<td>2.04 ± 1.32</td>
<td>2.89 ± 0.31</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Monoacylglycerol</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Free fatty acids</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>1.3 (34.5)</td>
<td>0.7 (20.2)</td>
</tr>
<tr>
<td>1,2-Diacylglycerol</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>1,3-Diacylglycerol</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>97.6</td>
<td>98.5</td>
</tr>
<tr>
<td>Cholesterol esters (mg/L)</td>
<td>39</td>
<td>41</td>
</tr>
<tr>
<td>Number of women</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 7. Factors influencing human milk fat content and composition**

<table>
<thead>
<tr>
<th>Factors</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of gestation</td>
<td>Shortened gestation increases the long-chain polyunsaturated fatty acids secreted.</td>
</tr>
<tr>
<td>Stage of lactation</td>
<td>Phospholipid and cholesterol contents are highest in early lactation.</td>
</tr>
<tr>
<td>Parity</td>
<td>High parity is associated with reduced endogenous fatty acid synthesis.</td>
</tr>
<tr>
<td>Volume</td>
<td>High volume is associated with low milk fat content.</td>
</tr>
<tr>
<td>Feeding</td>
<td>Human milk fat content progressively increases during a single nursing.</td>
</tr>
<tr>
<td>Maternal diet</td>
<td>A diet low in fat increases endogenous synthesis of medium chain fatty acids (C6 to C10).</td>
</tr>
<tr>
<td>Maternal energy status</td>
<td>A high weight gain in pregnancy is associated with increased milk fat.</td>
</tr>
</tbody>
</table>
DHA is predominant in fish and seafood and consumption of these foods in Indian children is virtually lacking. In vegetarians the predominant polyunsaturated fatty acid is linolenic acid (LA) – an omega 6 fatty acid. Alpha linolenic acid (ALA) which is an omega 3 fatty acid is not so abundant in vegetarian diets. ALA is a precursor of EPA and DHA. The ideal ratio of LA to ALA should be 3:1 in the diet, however most diets have a ratio of 10:1 or 15:1. Under these circumstances, omega 6 fatty acids inhibit the conversion pathway of omega 3 fatty acids. Thus decreasing LA content has been found to increase the ALA conversion to EPA and DHA.

Mammalian cell lactocytes are not capable of LCPUFA de novo and hence, it needs to be derived from maternal circulation – either from diet or from the body stores. This is evidenced by the low circulating levels of LCPUFA in pregnant and lactating women. Although newborns are able to convert PUFAs to LCPUFAs, the amount is not sufficient to meet their requirement. To ensure sufficient supply of LCPUFAs to infants, breast feeding women are advised to consume higher preformed DHA and AA.

**Daily requirement of DHA**

While exact dose of DHA to be consumed is not known, the following are recommended:

Pregnant and Nursing women: A workshop sponsored by the National Institutes of Health and International Society for the study of fatty acids and lipids (NIH/ISSFAL) recommended an intake of 300mg/day of DHA.

Children and Healthy Adults” NIH/ISSFAL have recommended an intake of 220 mg/day of DHA.

**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 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 and this increase precedes the increase in DHA accretion by the brain.

The maternal plasma phospholipid DHA content is lower in women who 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, a process sometimes referred to as biomagnification. Placental adaptation to ensure efficient DHA transfer from the maternal to the fetal circulation is an important part of the biomagnification process. Furthermore, there are observations that the placenta can synthesise DHA from ALA, which would allow for in situ provision of DHA to help meet the demands for DHA imposed by pregnancy.

Large epidemiological observational studies have found a significant association between low maternal sea food consumption (<340gm/week) and sub optimal neuro-cognitive outcomes in childhood. Children aged 6 months to 8 years, whose mothers consumed low sea foods diets during pregnancy had lower verbal IQ, displayed less pro-social behaviour and had poorer social and communication skills compared to those whose mothers consumed high sea food diets. So, it is well established that maternal fish oil supplementation during pregnancy substantially increases fetal DHA concentration at the time of birth.

The phenomenon of increasing LC-PUFA in fetal and infant blood and tissues relative to that of their mother has been described as “bio-magnification”, but could also be interpreted as a natural consequence of a dual liver system i.e., the combined PUFA metabolism and conversion of LA and ALA to AA and DHA in both the mother and the fetus/infant. Both term and preterm infants have been shown to convert stable isotope labelled LA and ALA to AA and DHA, respectively, and the synthesis has been shown to decrease with post-conceptional age. The desaturase capacity has been estimated to be in the order of 40 mg/ (kg x day) of AA and 13 mg/ (kg x day) of DHA in neonates born in the 32nd week of gestation, but to decrease to around 14 and 3 mg/ (kg x day) at 1 month past expected term. This synthetic rate may still provide a substantial contribution to fulfill infant needs, which, based on maintenance of plasma DHA homeostasis, have been estimated to be around 5 mg/ (kg x day) of DHA. However, this does not exclude that exogenous sources of DHA are needed in the diet to fulfill the requirements of the growing infant.

Fat digestion in the early neonatal period is not fully developed, primarily as a result of pancreatic insufficiency. Digestion of milk lipids in nursing neonates is achieved by a concert of enzymes. The first is lingual lipase, which initiates hydrolysis in the stomach; the second is gastric lipase; the third is bile salt-stimulated lipase, which is indigenous to human milk; and the fourth is pancreatic lipase. Compared with adults, fat digestion is markedly aided by gastric lipase in infants and, in breastfed
infants, by the bile salt-dependent lipase of human milk. The stereometric structure of triglycerides can influence the hydrolysis rate and thus absorption. The predominant FA in human milk, palmitic acid (C16:0), is preferentially found in the stereospecific number 2 position of the triglyceride, so it is primarily absorbed as the 2-monoglycerides.

The DHA Gap of Prematurity

The high long-chain polyunsaturated fatty acids (FAs) secreted in the milk of women who deliver prematurely may reflect the enhanced need for these essential FAs by premature infants. These FAs that are normally stored by the fetus in late gestation are required to function in growth and brain development.

During early life, there is limited metabolic capacity to convert ALA to DHA. As DHA cannot be synthesized de novo by the developing fetus, it is largely dependent on a maternal source. Most DHA accumulation occurs during the third trimester of pregnancy when the growth and brain development are rapid. (Kuipers, Luxwolda et al. 2012) There is surge in circulating lipids under the influence of hormonal changes during pregnancy producing a hyperlipidemic state. Estrogen further enhance conversion of precursor ALA to DHA, sustaining preferential uptake. Fatty acid (FA) transport across the placenta is both passive and active. Passive transport is directly dependent on maternal blood levels of FA. The active transport occurs via FA transport proteins which are up-regulated during pregnancy which preferentially transport LCPUFAs to the fetal blood stream. Thus in fetal life, infancy and early childhood, DHA should be acquired from dietary sources to maintain optimal health.

In preterm infants DHA accumualation remains incomplete leading to disturbance in normal LCPUFA accretion. Thus, resulting in lower DHA levels in preterm infants than their term counterparts. (Agostoni, Marangoni et al. 2008) Likewise, in very preterm infants (<28 weeks gestation) this deficit persists or worsens due to decreased adipose stores and inability to convert precursor ALA to DHA and poor nutritional provision of preformed LCPUFA. (Martin, Dasilva et al. 2011) The preterm infants often does not reach full enteral feedings until after several weeks of age. Thus makes them depend heavily on parenteral nutrition early in life.

The intravenous lipid emulsions which are commercially available provide essential precursor FAs only, rather than preformed DHA. These formulation are inadequate to maintain DHA levels in very low birth weight (VLBW) infants due to decreased desaturase conversion and increased demands during rapid growth and neuro-development. These reasons are specific to premature infants resulting in persistently low DHA levels. Moreover the complications of prematurity or illness further delay the advancement of feedings further aggravating the DHA deficit. (Harris and Baack 2015)

Nutritional options available during full enteral feedings in the neonatal intensive care unit (NICU) provide extremely variable daily allowances of DHA. These do not account for the relative deficits of premature infants. Mother’s own milk is the recommended diet for all infants. It supplies both ARA and DHA. However, analysis of breast milk indicates there is a wide variation in DHA content (from

![Figure 3: DHA concentration in maternal blood plasma lipids in late pregnancy and in umbilical cord blood plasma at birth](image-url)
0.06–1.4%) based on regional, individual dietary and lactation differences. (Lauritzen, Jorgensen et al. 2002, Brenna, Varamini et al. 2007)

Lactating mothers who deliver prematurely have milk with higher DHA content than those who deliver at term. (Berenhauser, Pinheiro do Prado et al. 2012) Alternatives to mother’s own milk include donor human milk and commercially available infant formula. Donor human milk is a good source of LCPUFA and its pasteurization does not alter DHA concentrations. However, the overall fat content is generally lower. There is variable DHA provision between banks. (Berseth, Harris et al. 2014)

Body stores of DHA:

Over the first 6 months of life, DHA accumulates at about 10 mg/d in the whole body of breast–fed infants with 48% of that amount appearing in the brain. To achieve that rate of accumulation, breast–fed infants need to consume a minimum of 20 mg DHA/d. Virtually all breast milk provides a DHA intake of at least 60 mg/d though it may be variable. Thus a store of about 1050 mg of DHA in body fat at term birth is present.

Role of DHA in Vision and Neuro-development

The importance of nutrition for visual and cognitive development was recognised, based on the findings that breastfed infants had a higher IQ compared to the formula fed infants. (Lucas, Morley et al. 1992) This was also supported by a correlation between the fat content in breast milk and improved neurodevelopment at 12 months of age. (Agostoni, Marangoni et al. 2008)

Several formula supplementation studies followed for term and then preterm babies. Although findings from these studies support, but do not demonstrate a conclusive benefit from LCPUFA supplementation for term infants. (Drover, Hoffman et al. 2011) The current findings about the “DHA gap of prematurity”, subsets of premature infants demonstrate improvements in visual and neurodevelopmental outcomes. (Harris and Baack 2015) The finding of clinical studies are are varied and dependent upon diet variability, regional differences, dose, timing, and sensitivity of outcome measurements for each particular study.

Nonetheless there is increasing support for the need to adequately remedy the DHA deficit in premature infants, term infants and children.

DHA, Docosahexaenoic acid; ARA, Arachidonic acid; GA, Gestational age; BW, Birth weight; IQ, Intelligence quotient; PDI, Bayley’s Scale of Infant Development – Psychomotor Developmental Index; MDI, Bayley Scale of Infant Development – Mental Developmental Index; MCDI, MacArthur Communicative Development Inventory; SDQ, Strengths and Difficulties Questionnaire; STSC, Short Temperament Scale for Children.

*Wechsler Abbreviated Scale of Intelligence.
‡Children’s Memory Scale (CMS) word paired scores, a test of hippocampal function.

It is challenging to study DHA related neurodevelopmental outcomes in infants. Generally, Bayley’s
Table 1. DHA intervention studies and neurodevelopmental outcomes in premature infants

<table>
<thead>
<tr>
<th>Reference</th>
<th>Population</th>
<th>Intervention</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Belfort, Rifas-Shiman et al. 2011)</td>
<td>N=614</td>
<td>DHA</td>
<td>Greater weight and BMI gain to term were associated with better MDI and PDI</td>
</tr>
<tr>
<td>(Westerberg, Schei et al. 2011)</td>
<td>n=92</td>
<td>Ongoing follow up at 20 months</td>
<td>Improved free-play Duration of Focused Attention &amp; Summary Attention Rating Score over 20 months</td>
</tr>
<tr>
<td>(Isaacs, Ross et al. 2011)</td>
<td>n=107</td>
<td>Ongoing follow up at 10 years</td>
<td>Improved verbal IQ - full scale IQ, vocabulary, similarity and word-pair learning scores in formula fed only infants.</td>
</tr>
<tr>
<td>(Smithers, Collins et al. 2010)</td>
<td>n=128/125</td>
<td>Ongoing follow up at 26mo/3-5 yr.</td>
<td>No difference in comunication (MCDI) at 26 mo. or behaviour (SDQ and STSC) by 3-5 years.</td>
</tr>
<tr>
<td>(Makrides, Gibson et al. 2009)</td>
<td>n=657</td>
<td>1% to term vs. 0.3%</td>
<td>4-5 points higher MDI in &lt;1250 g and all girls</td>
</tr>
<tr>
<td>(Henriksen, Haugholt et al. 2008)</td>
<td>n=141, BW&lt;1500 g receiving breast milk (mother’s or donor)</td>
<td>32 mg DHA and 31 mg ARA/100 ml breast milk per day for average 63 days</td>
<td>Improved Ages and Stages problem solving scores at 6 months</td>
</tr>
<tr>
<td>(Fang, Kuo et al. 2005)</td>
<td>n=28, GA:30-37 weeks</td>
<td>0.05% DHA for 25 weeks</td>
<td>7 point higher MDI ans 4 point higher PDI at 6 months 11 point higher MDI and 8 point higher PDI at 12 months</td>
</tr>
<tr>
<td>(Clandinin Van Aerde et al. 2005)</td>
<td>n=361</td>
<td>0.3% to 12 months</td>
<td>7 point higher MDI in boys at 18 months</td>
</tr>
<tr>
<td>(Fewtrell, Abott et al. 2004)</td>
<td>n=238</td>
<td>0.34% DHA / 0.68% ARA</td>
<td>No difference in MRI myelination scores at 3 and 6 mo. or MDI/PDI at 3, 6, 12 and 24 months</td>
</tr>
<tr>
<td>(van Wexel-Meiller, van der Knaap et al. 2002)</td>
<td>n=42</td>
<td>0.34% DHA / 0.68% ARA</td>
<td>No difference in MRI myelination scores at 3 and 6 mo. or MDI/PDI at 3, 6, 12 and 24 months</td>
</tr>
<tr>
<td>(O Connor, Hall et al. 2001)</td>
<td>n=470</td>
<td>0.3% DHA to term 0.2% to 1 year</td>
<td>9 points higher on PDI in &lt;1250 g at 12 months</td>
</tr>
<tr>
<td>(Carlson and Werkman 1996)</td>
<td>n=459</td>
<td>0.1% DHA / 0.6% ARA</td>
<td>Improved visual attention at 12 months by the Fagan Test of Infant Intelligence</td>
</tr>
<tr>
<td></td>
<td>BW: 747 g-1245 g</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Scales of Infant Development (BSID) including the Mental Developmental Index (MDI) and Psychomotor Developmental Index (PDI) which are well standardized and validated test of overall infant development, is used to evaluate the short term outcomes. This scale is also normally used in neonatal follow up clinics. Some researchers are of the opinion that this test may not be a sensitive indicator of hippocampal or specific learning dysfunction including difficulty with perceptual organization, distractibility, processing speed and inattention at school age, which are common in very premature infants and are improved with increased DHA status. (Anderson and Doyle 2003), (Colombo, Carlson et al. 2013)

Similarly, there are speculations regarding sensitivity and specificity of visual acuity tests that are administered at a very young age ranging from Teller cards to Visual Evoked Potentials (VEP). Although meta-analysis does not conclusively demonstrate a significant effect of LCPUFA formula supplementation on infant cognition, this conclusion should be cautiously considered, especially for premature infants. (Qawasmi, Landeros-Weisenberger et al. 2012) Meta-analysis which combines the data from formula supplementation studies has limitations because of dose-related variability that was inadvertently introduced with timing and administration methods.

In most randomized controlled trials, LCPUFA provision in intervention formulas was inconsistent (DHA 0.2–1.0% of total FAs). Many trials were designed to provide the amount of DHA found in world-wide term human milk (0.32%) which may address the needs of healthy, full-term babies but cannot begin to meet the deficit found in very premature infants. (O'Connor, Hall et al. 2001)

Table 2. DHA intervention studies and viral outcomes in premature infants

<table>
<thead>
<tr>
<th>Reference</th>
<th>Population</th>
<th>Intervention</th>
<th>Visual Test</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Molly, Stokes et al. 2016)</td>
<td>N=104</td>
<td>1% high DHA group or a DHA (0.2–0.3% control group)</td>
<td>Visual acuity, contrast sensitivity, vernier acuity, binocular stereopsis and visual perception</td>
<td>No evidence of differences between the high-DHA groups in any of the visual-processing measures</td>
</tr>
<tr>
<td>(O'Connor, Hall et al. 2001)</td>
<td>N=470 GA: &lt;33 weeks BW: 750-1800 g</td>
<td>0.3% DHA to term 0.2% to 1 year</td>
<td>VEP Teller cards</td>
<td>VEP better at 6 months</td>
</tr>
<tr>
<td>(Carlson, Werkman wt al. 1993)</td>
<td>N=67 BW: 725-1400 g</td>
<td>0.5% DHA</td>
<td>Teller cards</td>
<td>Better at 2 and 4, but not different at 6, 9, 12 months</td>
</tr>
<tr>
<td>(Birch, Birch et al. 1992)</td>
<td>N=73 BW: 1000-1500 g</td>
<td>1% DHA for 4 months</td>
<td>ERG/VEP Teller cards</td>
<td>ERG and VEP better at 3 weeks, VEP improved at 4 months</td>
</tr>
</tbody>
</table>

DHA - Docosahexaenoic acid; EPA - Eicosapentaenoic acid; GA - Gestational age; BW - Birth Weight; ERG - Electroretinography; VEP - Visual Evoked Potential
premature infants. Preterm human milk typically has higher DHA content than term human milk to support the infant’s requirement. Moreover, DHA supplementation through formula is reliant on enteral intake which is widely variable in very premature infants. Thus, the length of time that VLBWs receive little to no enteral, preformed DHA, may introduce significant bias.

Breastfeeding is another confounding factor in clinical trials because extremely variable levels of DHA are present in human milk dependent on regional, dietary, lactation and storage factors. (Berseth, Harris et al. 2014), (Brenna, Varamini et al. 2007) Several of the formula supplementation clinical trials permitted breastfeeding even in the “control group” that was getting non-supplemented formula, and breast milk FA levels are rarely reported in such trials. In fact, the most convincing study demonstrating DHA related improvements in both the MDI and PDI required that 80% or more of the infant’s diet be either supplemented or non-supplemented formula rather than breast milk. (Clandinin, Van Aerde et al. 2005) Hence, in formula supplementation clinical trials, “dosing” is difficult to precisely define, and the provision may have been too low and started too late for the preterm population.

Significant visual and neuro-developmental benefits have been found from improved DHA provision in preterm infants especially with the utility of more specific learning assessment tools or higher and more reliable supplementation methods. It was found that breastfed premature infants had significantly higher DHA blood levels. Correlation was established between improved attention, impulsivity and processing speed at 5 years of age and higher DHA blood levels. (Tanaka, Kon et al. 2009). It was found that VLBW infants supplemented with 32 mg DHA and 31 mg ARA in addition to DHA containing breast milk (0.7%) had a better problem solving and recognition memory by event-related potentials at 6 months of age. (Henriksen, Haugholt et al. 2008) On follow up of this cohort showed improved attention at 20 months of age. (Westerberg, Schei et al. 2011) It can be inferred that subsets of premature infants fed formula with added DHA and ARA had better neuro-cognitive function, visual attention, visual evoked response time and visual acuity than premature infants fed non-supplemented formula and effects persisted later in life.

**The DHA Deficiency and Additional Health Risks of Prematurity**

It is now recognised that the positive effects of DHA may go well beyond brain development and function. VLBW infants are more vulnerable to risk of inflammatory mediated diseases that dramatically increase the morbidity and mortality of prematurity. The evidence suggest that DHA supplementation decreases the incidence and severity of several health risks including NEC, BPD and ROP. The adequate balance of LCPUFAs may also reduce the risk of late onset sepsis. (Martin, Dasilva et al. 2011). Experimental animal study on the structural and mechanical bone parameters, showed that high omega-3 levels contribute to superior trabecular and cortical structure, as well as to stiffer bones and improved bone quality. (Koren, Simsa-Maziel et al. 2014)

**Retinopathy of Prematurity (ROP)**

Retinopathy of prematurity (ROP) also known as retrolental fibroplasia (RLF), is a disease of the eye affecting prematurely born infants. It is produced by abnormal vascular development of the retina, is the leading cause of visual impairment and blindness. Several factors can determine whether the disease progresses, including overall health, birth weight, and the stage of ROP at initial diagnosis. Supplemental oxygen exposure, while a risk factor, is not the main risk factor for development of this disease. Restricting supplemental oxygen use reduces the rate of ROP, but may raise the risk of other hypoxia-related systemic complications, including death.
In premature infants there is a state of relative hyperoxia as compared to fetuses still developing in utero, which down regulates vascular endothelial growth factor. This can lead to obliteration of the developing microvasculature in the retina resulting in down regulation of vessels. On the other side there is increasing metabolic demand which leads a relative hypoxic state with overcompensation of angiogenichormones resulting in a second phase of rapid neovascularization. The abnormal vascular growth in this second stage may invade the vitreous placing traction on the retina.

The retina contains rods and cones that have membranes highly enriched with DHA. Infants born prematurely are at risk of DHA insufficiency, because they may not have benefited from a full third trimester of the mother’s lipid stores. (SanGiovanni and Chew 2005) DHA supplementation decreases the severity of ROP in VLBW infants. (Pawlik, Lauterbach et al. 2011) DHA incites cytoprotective, angiogenic regulation and neuroprotective mechanisms. Maternal supplementation with omega-3 LCPUFA demonstrates a decrease in both the primary oxygen-induced vaso-obliteration and the secondary neovascularization abnormalities associated with oxygen induced retinopathy in nursing mouse pups. An experimental study showed that increasing omega-3-PUFA tissue levels decreased the avascular area of the retina by increasing vessel regrowth after injury, thereby reducing the hypoxic stimulus for neovascularization. (Connor, SanGiovanni et al. 2007) The bioactive ω-3-PUFA-derived mediators’ neuroprotectinD1, resolvind1 and resolvinE1 protected against neovascularization. The protective effect of omega-3-PUFAs and their mediators was mediated in part, through suppression of tumor necrosis factor-α.

Supplementing ω-3-PUFA may be of benefit in preventing retinopathy. Clinical trials to evaluate the effect of supplementing neonatal diet with omega-3-PUFA are ongoing. The inflammatory cytokine was found in a subset of microglia that was closely associated with retinal vessels. These findings indicate that increasing the sources of omega-3-PUFA or their bioactive products reduces pathological angiogenesis. (Connor, SanGiovanni et al. 2007) Recent studies demonstrated the protective effects of omega-3 LCPUFAs against ROP in VLBW infants. (Pawlik, Lauterbach et al. 2011)

In a clinical study infants weighing <1250 g at birth were randomly allocated to 2 groups: an
A randomized controlled study was conducted in 80VLBW infants receiving parenteral nutrition from the first day of life were evaluated. One of the two lipid emulsions were used in the study infants: Group 1 (n=40) received fish-oil based lipid emulsion and Group 2 (n=40) soybean oil based lipid emulsion (Intralipid®). It was found that the maternal and perinatal characteristics were similar in both groups. The median (range) duration of parenteral nutrition [14days (10-28) vs 14 (10-21)] and hospitalization [34days (20-64) vs 34 (21-53)] did not differ between the groups. Laboratory data including complete blood count, triglyceride level, liver and kidney function tests recorded before and after parenteral nutrition also did not differ between the two groups. In Group 1, two patients (5.0%) and in Group 2, 13 patients (32.5%) were diagnosed with retinopathy of prematurity (OR: 9.1, 95% CI 1.9-43.8, p=0.004). One patient in each group needed laser photocoagulation, without significant difference. Multivariate analysis showed that only receiving fish-oil emulsion in parenteral nutrition decreased the risk of development of retinopathy of prematurity [OR: 0.76, 95% CI (0.06-0.911), p=0.04]. (Beken, Dilli et al. 2014)

With the current knowledge it can be concluded that there is no significant difference of development of ROP with breast milk and formula feed with DHA.

### b. Necrotizing Enterocolitis (NEC)

Necrotizing enterocolitis is a medical condition primarily seen in premature infants, where portions of the bowel undergo necrosis. It threatens the life and long-term health of 5–10% of VLBW infants. NEC carries a 15–30% mortality for premature infants and those who survive are at risk for recurrent strictures and bowel obstruction, mal absorption, failure to thrive from short bowel syndrome, parenteral nutrition associated liver disease and central line infections. (Harris and Baack 2015)
DHA provides protective effects medicated by several factors. Local cell membrane phospholipids help in protecting the integrity of intestinal cells. Alterations in LCPUFA content is essential in bacterial translocation and intracellular fluid shifts associated with cell stress signaling that initiates NEC. LCPUFAs in phospholipid membranes also serve as precursor molecules for eicosanoid production; they are integral in modulating inflammatory cell signaling, gene expression and transcription of key regulators in inflammatory and endotoxin translocation. The evidence is building up to support the role of DHA in NEC protection. (Caplan, Russel et al. 2001)

In a randomized, double-masked, clinical study, hospitalized preterm infants fed a commercial (control) preterm formula or an experimental formula with egg phospholipids were compared for neuro-development. Infants fed with experimental formula developed significantly less stage II and III NEC compared with infants fed the control formula (2.9 versus 17.6%, p < 0.05). Similar rates of bronchopulmonary dysplasia compared with the control formula, the experimental formula provided 7-fold more esterified choline, arachidonic acid (AA, 0.4% of total fatty acids), and docosahexaenoic acid (0.13%). Phospholipids are constituents of mucosal membranes and intestinal surfactant, and their components, AA and choline, are substrates for intestinal vasodilatory and cytoprotective eicosanoids (AA) and the vasodilatory neurotransmitter, acetylcholine (choline), respectively. One or more of these components of egg phospholipids may have enhanced one or more immature intestinal functions to lower the incidence of NEC in this study. (Carlson, Montalto et al. 1998). Several animal 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. (Caplan, Russel et al. 2001)

c. Bronchopulmonary dysplasia (BPD)

Bronchopulmonary dysplasia (formerly chronic lung disease of infancy) is a serious lung disease of premature infants caused due to arrested alveolarization in developing lungs exposed to mechanical ventilation, oxygen, and other inflammatory mediators before normal development is complete.

It is more common in infants with low birth weight and those who receive prolonged mechanical ventilation. It affects about 32% of premature babies and 50% of VLBW infants. (Harris and Baack 2015)

It results in significant morbidity and mortality. The definition of BPD has continued to evolve since then primarily due to changes in the population, such as more survivors at earlier gestational ages, and improved neonatal management including surfactant, antenatal glucocorticoid therapy, and less aggressive mechanical ventilation.

Omega-3 LCPUFA may protect against chronic inflammatory lung disease in premature infants. (Tiesset, Pierre et al. 2009) Along with earlier stated anti-inflammatory mechanisms, other protective properties in the lung are mediated through the PPAR pathways, of which DHA is a known ligand. PPAR agonists accelerate lung maturation and prevent hyperoxia induced lung injury by stimulating development of Type II, surfactant producing pneumocytes and vasoproliferation in the lung. (Rehan, Sakurai et al. 2010)

Omega-3 LCPUFA supplementation decreases endotoxin- and Pseudomonas-induced lung injury by
alteration of both pro- and anti-inflammatory molecules associated with BPD. These anti-inflammatory actions lead to improved bacterial clearance, reduced lung injury, and increased survival after infection. (Tissot, Pierre et al. 2009)

In a multicenter, randomized controlled trial comparing the outcomes for preterm infants <33 weeks’ gestation who consumed expressed breast milk from mothers taking either tuna oil (high-DHA diet) or soy oil (standard-DHA) capsules it was found there was a reduction in BPD in boys (relative risk [RR]: 0.67 [95% confidence interval (CI): 0.47-0.96]; P=.03) and in all infants with a birth weight of <1250 g (RR: 0.75 [95% CI: 0.57-0.98]; P=.04). There was no effect on duration of respiratory support, admission length, or home oxygen requirement. There was a reduction in reported hay fever in all infants in the high-DHA group at either 12 or 18 months (RR: 0.41 [95% CI: 0.18-0.91]; P=.03) and at either 12 or 18 months in boys (RR: 0.15 [0.03-0.64]; P=.01). There was no effect on asthma, eczema, or food allergy. (Manley, Makrides et al. 2011)

In a retrospective cohort study of 88 infants born at <30 weeks’ gestation. It was found that DHA and ARA levels declined rapidly in the first postnatal week, with a concomitant increase in linoleic acid levels. Decreased DHA level was associated with an increased risk of Chronic Lung Disease (CLD) (OR, 2.5; 95% CI, 1.3-5.0). Decreased arachidonic acid level was associated with an increased risk of late-onset sepsis (hazard ratio, 1.4; 95% CI, 1.1-1.7). The balance of fatty acids was also a predictor of CLD and late-onset sepsis. An increased linoleic acid:DHA ratio was associated with an increased risk of CLD (OR, 8.6; 95% CI, 1.4-53.1) and late-onset sepsis (hazard ratio, 4.6; 95% CI, 1.5-14.1). (Martin, Dasilva et al. 2011)

Finding additional innovative ways to administer DHA to infants on early mechanical ventilation or oxygen exposure during critical periods of pulmonary development may further reduce lung disease in this at risk population. However recent negative trial also lead to speculation of role of DHA in BPD. Enteral DHA supplementation at a dose of 60 mg per kilogram per day did not result in a lower risk of physiological bronchopulmonary dysplasia than a control emulsion among preterm infants born before 29 weeks of gestation and may have resulted in a greater risk. (Collins, Makrides et al. 2017)

**DHA supplementation in pregnancy and it’s impact**

Pregnancy is a period of additional demands of Omega-3 fatty acids due to high maternal transference of EPA (20:5 n-3) and DHA (22:6 n-3) to the fetus for brain growth and subsequent cognitive development. (Vaz, Farias et al. 2017)
At present there are only two studies which have evaluated the effects of increased prenatal DHA exposure on DNA methylation in the neonate. One study demonstrated that maternal DHA supplementation induced small changes in global DNA methylation and methylation at IGF2/H19 imprinted genes in cord blood (Lee, Barraza-Villarreal et al. 2013) while another did not find any substantial effect of prenatal DHA supplementation. (Amarasekara, Noakes et al. 2014) Hence more genome-wide methylation studies are required to determine the impact of prenatal DHA supplementation on the epigenome of the infant, and whether this could underlie the effects of maternal DHA supplementation on infant and child outcomes.

Pregnant women consuming more DHA also provide more DHA to their fetus and after delivery, have higher milk DHA during lactation. It is well established that the biosynthesis of DHA from α-linolenic acid is very limited, especially under several conditions including caloric deprivation, protein inadequacy, and corticosteroids, which inhibit the δ6-desaturase and, therefore, DHA synthesis. In fact, there have been many attempts to increase DHA status and milk DHA by feeding α–linolenic acid without success. (Carlson, Gajewski et al. 2017) A study demonstrated that extremely low milk DHA in women consuming a placebo, but milk DHA increased to 0.5–0.7% in the group assigned to receive a dietary supplement of 1000 mg of DHA/day. (Valenne, Morrow et al. 2013)

Results of randomized trials on the effects of prenatal DHA on infant cognition are mixed, but most trials have used global standardized outcomes, which may not be sensitive to effects of DHA on specific cognitive domains. Several randomized trials of maternal DHA supplementation during pregnancy and/or lactation have been conducted with mixed results; some did not report advantages while some showed positive effects on problem solving. (Judge, Harel et al. 2007) Of studies that followed infants into the preschool period, two reported significant benefits on IQ and neuro-developmental measures while another did not. (Helland, Smith et al. 2003) Positive effects of prenatal or postnatal DHA supplementation or status on attention in infancy and early childhood has been documented in several but not all studies. In clinical study women were randomized to 600 mg/d DHA or a placebo for the last two trimesters of pregnancy it was found that infants of supplemented mothers maintained high levels of sustained attention (SA) across the first year; SA declined for the placebo group. The supplemented group also showed significantly reduced attrition on habituation tasks, especially at 6 and 9 months. (Colombo, Gustafson et al. 2016)

DHA supplementation during pregnancy in obese mothers may have long-lasting effects on offspring measures of adiposity. (Foster, Escaname et al. 2017)

The Institute of Medicine does not set a Dietary Reference Intake (DRI) for DHA in pregnancy; however, the FAO/WHO and expert groups suggest an intake of 200 mg of DHA per day for pregnancy and lactation. (Simopoulos, Leaf et al. 2000) Many US prenatal vitamins now contain 200 mg of DHA. Intake less than 600 mg DHA have not found a reduction in pre-term birth. (Smuts, Borod et al. 2003) DHA is a nutrient and intake and status are inherently variable. Women with low DHA status may require more DHA to reach an intake to reduce preterm birth.
Current Dietary recommendation of DHA for Infants

Breast milk is the optimal nutrition for all infants and contains all essential FAs, including preformed DHA. Because of the rapid growth and development that occurs during the first year of life, the dietary provision of lipids for infants is extremely important. The DHA levels found in human milk are dependent upon maternal intake and blood levels, the current consensus by experts in the field is that pregnant and lactating women should receive at least 200 mg/day of DHA through diet or a safe alternative supplement. (Koletzko, Lein et al. 2008) This recommendation is supported by the World Association of Perinatal Medicine, the Early Nutrition Academy, and the Child Health Foundation. The consensus group also recommends that if formula is substituted, it should contain between 0.2–0.5% of total FAs as DHA and an equal or greater amount of ARA to support infant growth and development. (Koletzko, Lein et al. 2008) Many formula supplementation studies show that LCPUFA blood levels do not reach those of breastfed infants with the addition of precursor FAs alone (i.e., ALA and LA), and the addition of only DHA and not ARA to formulas may be associated with a decreased growth rate. (Clandinin, Van Aerde et al. 2005)

Recommendations by the Child Health Foundation task force established the importance of dietary LCPUFA provision for infants and prompted the addition of DHA and ARA to commercialized infant formulas. However, the optimal daily DHA intake is yet unknown. Using the average breast milk content as the standard dietary provision of LCPUFA for all infants is problematic because DHA content in human milk varies a great deal. A meta-analysis including 65 studies and 2474 women around the world found the mean level of DHA to be 0.32 ± 0.22% (range: 0.06–1.4%, median 0.26%). (Brenna, Varamini et al. 2007) DHA concentration varies with maternal dietary fish intake, socioeconomic status and dietary supplementation. LCPUFA levels also vary with duration of breastfeeding, freezing/storage and between preterm and term milk. Overall, Indian women tend to have DHA levels below the world wide mean, presumably due to their typically low fish intake. Additionally, breast milk is designed to meet the needs of a normal term infant, but may not account for needs at earlier developmental time points or to make up for relative deficiencies noted in newborns at-risk for deficiency (premature, small for gestational age, infants born to diabetic mothers).

Managing the DHA deficit of Prematurity

At present, LCPUFAs are provided to premature infants through infant formula or human milk fortifier supplements. This content appears to meet the routine needs of term infants, but is not adequate to remedy the DHA deficit found in premature infants. Recommendations on dietary intakes of ARA and DHA in early life are few and predominantly relate to findings obtained in developed countries. For this reason outcome studies show more conclusive benefit with DHA provision at a higher range or supplementation in addition to dietary sources. (Henriksen, Haughlot et al. 2008) (Makrides, Gibson et al. 2009) Formulas supplemented with an algal DHA source have been shown to be safe and well-tolerated, but a higher DHA dose (>0.32%) may be necessary to correct the relative deficiency and to optimize the benefits for VLBW infants.

The European Food Standard Authority (EFSA) has recommended a DHA level of 0.3% total fatty acid in infant formula for infants aged 0-12 months, (Westerberg, Schei et al. 2011) and an intake of a minimum of 100 mg DHA/day for older infants and young children. (van Wezel-Meijler, van der Knaap et al. 2002) The Food and Agricultural Organisation of the United Nations (FAO) recommended that during the period of 0-6 months a daily requirement of 0.1-0.18% energy for DHA (equivalent to a mean intake of 102 mg/day); for 6-24 months, a DHA intake of 10-12 mg/kg body weight; 2-4 years, DHA and EPA intake of 100-150 mg/day; increasing to DHA and EPA of 200-250 mg at age 6-10 years. (Martin, Dasilva et al. 2011)
Regarding ARA there are few explicit dietary recommendations; an expert group recommended 140 mg ARA/day during the first months of life. (Tiehet, Pierre et al. 2009) Belgian Health Council dietary recommendation for young children of ARA is 0.10-0.25% energy (approximately 102-258 mg/day) and DHA is 0.10-0.40% energy (approximately 102-413 mg/day). (Freedman, Weinstein et al. 2002)

Addition of DHA to commercialized human milk fortifiers is used to increase calories, protein and mineral content of human milk and meet the needs of VLBW infants. By 34 to 35 weeks of gestation, when babies develop a coordinated suck and swallow, they are typically given enteral feedings through a feeding tube. Until that time, mothers pump and freeze their milk which is then thawed and fortified with human milk fortifier for feedings. This dosing method supplya higher daily DHA dose.

So far no DHA supplementation study has attempted to “normalize” the DHA status of preterm infants throughout the critical first weeks of postnatal development (i.e., achieve levels found in term babies). The route and dose provided through either infant formula or breast milk with fortifier relies on the variable ability of the infant’s gastrointestinal system to handle full enteral feedings. Thus, this approach is unreliable for providing sufficient DHA for catch up. Many VLBWs are not fed completely by enteral route for several weeks or longer, and routinely available intravenous lipids do not contain preformed omega-3 FAs. Due to these factors, the average accumulation of DHA during the first month of life in a very preterm infant is roughly 50% of the expected in utero accretion. (Lapillonne, Groh-Wargo et al. 2013)

New parenteral products are being developed to provide improved LCPUFA balance by IV route until full enteral provision can be accomplished. Investigated results of parenteral interventions are highly anticipated. An alternative approach could be the direct enteral provision of DHA, independent of diet. This potentially cost-effective method would allow early intervention even before the infant reaches full feedings or fortification. Daily enteral dosing can be easily adjusted, is independent of the need for invasive intravenous access and may be continued beyond parenteral nutrition needs. Although there may be benefits to either parenteral or enteral supplementation, careful evaluation of potential adverse consequences or unintended alterations to the balance of the omega-6: omega-3 ratio will be required.

Now the necessity for supplemental DHA in the premature infant is established. VLBW infants rapidly become and remain DHA deficient for an extended period of time due to ineffective conversion from precursor fatty acids, lower fat stores, and a limited nutritional provision of DHA after birth. Optimizing LCPUFA provision postnatally may not only improve vision and neurodevelopment in VLBW infants, but may also reduce the morbidity and mortality from BPD, NEC, and ROP.

### B. DHA supplementation in children

Despite known health benefits of omega 3 LCPUFAs and fish intake, children and adolescents have low intake. (Gopinath, Moshtaghian et al. 2017) The brain and prefrontal cortex continue to develop until the late 20s. (van der Wurff, von Schacky et al. 2016) It is thus crucial to study the role of Omega-3 LCPUFA in children and adolescence as they play an important role in brain development and functioning. Moreover, higher DHA intake has been associated with changes in the functional activity of the prefrontal cortex in boys aged 8–10 years. (McNamara, Able et al. 2010)

Omega 3 LCPUFAs supplementation at 2 g/day increases blood levels substantially, more so in smaller children. A possible U-shaped response curve should be explored. (Arnold, Young et al. 2017) Supplementation effects: Compared to placebo, 2 g Ω3 per day increased EPA blood levels sevenfold and DHA levels by half (both p < 0.001). Body weight correlated inversely with increased EPA (r = -
0.52, p = 0.004) and DHA (r = -0.54, p = 0.003) and positively with clinical mood response. Mediation: EPA increase baseline-to-endpoint mediated placebo-controlled global function and depression improvement: the greater the EPA increase, the less the placebo-controlled omega 3 improvement. (Arnold, Young et al. 2017)

Recently, a study of children and adolescents with type 1 diabetes, showed that a dietary pattern characterized by lower intake of vegetables and fish was associated with wider retinal venular calibre in children and adolescents with type 1 diabetes. (Keel, Itsiopoulose et al. 2016) There is a debate whether long-chain polyunsaturated fatty acids (LCPUFA) improve cognitive performance. LCPUFAs are involved in many aspects of brain functioning, for example, neuronal membrane fluidity and neurotransmission. (van der Wurff, von Schacky et al. 2016)

**Intelligence, Attention Span and Behaviour:**

Studies have shown that pre-term infants, born without the benefit of the maternal delivery of DHA during the period of most rapid brain growth, the last trimester of pregnancy, did not perform as well on cognitive mental tests later in life. Specific behavioural and learning problems have also been shown to correlate significantly with low DHA levels. Duration of breastfeeding and colostrum PUFA levels were associated with children’s IQs in the EDEN cohort study. (Bernard, Armand et al. 2017)

**Attention-deficit hyperactivity disorder (ADHD):**

This disorder is associated with deficits in erythrocyte and plasma DHA and reductions in prefrontal cortex (PFC) blood flow and gray matter volume. (McNamara, Able et al. 2010) There is evidence that n-3 PUFA supplementation monotherapy improves clinical symptoms and cognitive performances in children and adolescents with ADHD, and that these youth have a deficiency in n-3 PUFA levels. However, the effect is not clinically significant if supplementation is not given for prolonged duration and in adequate doses. (Anand and Sachdeva 2016) A meta-analysis provide further support to the rationale for using n-3 PUFA as a treatment option for ADHD. (Chang, Su et al. 2017)

**Dyslexia:**

A learning disorder marked by impairment of the ability to recognize and comprehend written words, has been correlated with suboptimal DHA levels. (Montgomery, Burton et al. 2013)

**Autism spectrum disorder (ASD):**

Autism is a neurodevelopmental disability with an increasing prevalence. Traditional medicine does not offer any cures for autism. Patients with autism spectrum disorders tend to have greater prevalence of gastrointestinal disorders. Many autism patients have food aversion and are picky eaters. Many parents have undertaken complementary and alternative treatments especially nutritional interventions. Omega-3 fatty acid (n-fatty acids) supplementation has been advocated by many experts. Omega-3 fatty acid supplements are part of 12-step autism treatment program. Because of the limited number of included studies and small sample sizes, no firm conclusions can be drawn. However, the limited data currently available suggest that ω-3 FA supplementation does not enhance the performance of children with ASD.

**Asthma:**

A cross-sectional study showed that oral administration of natural anti-inflammatory products such as ω-3 is a promising complementary approach to managing asthma. A total of 39 patients among 50 volunteers completed this 3-month study. They took a soft gel capsule containing 180mg EPA and 120mg DHA daily. After treatment with ω-3, symptom score improved in 28 (72%) patients. The results of spirometry showed remarkable improvement in FEV1/FVC (P=0.044) and PEF (P<0.0001)
after treatment, but considering a cut-off of 80%, real improvement was observed in 32% of patients with PEF<80% which raised above the cut-off after ω-3 treatment (P=0.004) whereas, FEV1/FVC changes were above the cut-off value in 89% of the patients. After treatment, IL-17A and TNF-α levels decreased significantly (both P=0.049). (Farjadian. Moghtaderi et al. 2016)

**Nonalcoholic fatty liver disease (NAFLD):**

It is one of the most important causes of chronic liver disease in children and adults. Recently, therapeutic supplementation with DHA demonstrated an anti-inflammatory and insulin-sensitizing effect in children with NAFLD. The anti-inflammatory effects of DHA depend on its ability to alter phospholipid composition of the cell membrane, to disrupt lipid rafts and to hamper the transcriptional activity of nuclear factor-x03BA;B that controls the expression of proinflammatory cytokines. These effects of DHA are due to the interaction with the G-protein-coupled receptor 120 (GRP120), a lipid-sensing receptor highly expressed in activated macrophages. In fact, DHA may activate GPR120 expression in macrophages causing anti-inflammatory effects, and insulin-sensitizing and antidiabetic effects in vivo. A diet low in n-3 polyunsaturated fatty acids, as well as the presence of genetic factors, may induce a reduction in the GRP120 signal and the activation of Kupffer cells and inflammation during NAFLD. Therefore, it is conceivable that DHA/GRP120 may play a key role in slowing the progression of liver damage in patients with NAFLD. (Delia Corte, Mosca et al. 2016)

**Formula supplementation vs maternal supplementation**

Clinical studies demonstrated that formula-fed infants had lower plasma and tissue levels of DHA and other LC-PUFAs than did breastfed infants. Because formulas contain only the essential FAs (EFAs), LA and ALA, whereas human milk contains these EFAs and DHA, AA, and other LC-PUFAs, investigators assumed that the ability of infants to desaturate and elongate the two EFAs was limited. Investigators have suggested that the better scores of breastfed versus formula-fed infants on standardized tests of cognitive or behavioral development at school age or later are caused by the presence of these FAs in human milk.99,51

When infants were offered feeds in which 60 per cent of the fatty acids were in the form of linoleic (C18:2) acid, a rapid increase in the amount of linoleic acid in the adipose tissue was noted. Such a change is accompanied by alteration in the phospholipid composition of cell membranes, especially of the erythrocytes. It was found that in pre-term babies fed infant formulae rich in polyunsaturated fatty acids, the erythrocyte cell membrane becomes at risk of peroxidation. Iron added to the formulae generates free radicals which initiate the process of peroxide hemolysis of the erythrocyte. To avoid this, large amounts of

| Table 11: Fatty acid composition of human and cow’s milk, several proprietary infant feeding formulae and commonly uses vegetable fats in their manufacture |
|---------------------------------|--|--|--|--|--|--|--|--|--|
|                                | Saturated |                  |                  |                  |                  |                  |                  |                  |                  |
|                                |           |                  |                  |                  |                  |                  |                  |                  |                  |
| Fatty acids                    | c10:0     | c12:0            | c14:0            | c16:0            | c18:0            | c16:1            | c18:1            | c18:2            | c18:3            |
| Human milk                     | 1.3       | 5                | 7                | 25               | 9.3              | 3.8              | 33               | 6.7              | 1.4              |
| Cow’s milk                     | 2.7       | 3.3              | 10.8             | 25               | 10.8             | 2.6              | 27               | 1.3              | 1.4              |
| SMA                            | 1         | 10               | 6                | 16               | 11               | 1                | 29               | 24               | 2                |
| Nativa                         | 2.9       | 9                | 9                | 22               | 7                | 1                | 35               | 13               | 1                |
| Almiron B                      | 0         | 0                | <1               | 11               | 2                | <1               | 27               | 58               | 2                |
| Farilacid                      | 2         | 2                | 9                | 25               | 14               | 2                | 35               | 7                | 1                |
| Frisolac                       | <1        | 6                | 3                | 32               | 4                | 0                | 38               | 16               | 0                |
| Similac                        | 2         | 19               | 7                | 9                | 3                | 0                | 19               | 40               | <1               |
| Milumil                        | 1         | 4                | 7                | 35               | 8                | 1                | 32               | 10               | 0                |
| Nan                            | 2         | 4                | 11               | 31               | 9                | 2                | 24               | 16               | 1                |
| Pelargon                       | 2         | 2                | 8                | 24               | 11               | 1                | 30               | 16               | 1                |
| Humana 1 and 2                 | 1         | 1                | 3                | 23               | 8                | <1               | 44               | 13               | <1               |
| Oleo oils                      | –         | 0.2              | 3                | 3               | 20               | –                | 45.5             | 3.0              | 0.5              |
| Corn oil                       | –         | –                | 13               | 4                | –                | 29.0             | 54.0             | –                | –                |
| Coconut oil                    | 6.9       | 19.5             | 8.5              | 2                | –                | 6.0              | 1.5              | –                | –                |
| Soya oil                       | –         | –                | Trace            | 11.0             | 4                | –                | 25.0             | 51.0             | 9.0              |
| Cottonseed oil                 | –         | –                | 1.0              | 29.0             | 4                | 2                | 24.0             | 40.0             | –                |

a. Expressed as g/100 g total fat.
b. Expressed as g/100 g of the oil.
vitamin E (an antioxidant) are needed.

**Differences between formula supplemented with long-chain polyunsaturated fatty acids and human milk**

Important differences exist between LC-PUFA-supplemented formulas and human milk. First, the LC-PUFAs in human milk are components of the milk triglycerides and are present primarily in the sn-1 and sn-2 positions of the triglycerides rather than in all three positions as has been reported for single-cell triglycerides. Also, human-milk triglycerides rarely contain more than one molecule of AA or DHA, whereas the single-cell oils may contain as many as three. These differences may not be problematic with respect to overall bioavailability of LC-PUFAs, but they raise questions as to whether the importance of LCPUFAs in human milk can be determined from studies of LC-PUFA supplemented versus unsupplemented formulas. Similar questions are raised by the phospholipid supplements. Although LC-PUFAs from phospholipids seem to be at least as well, or better, absorbed as LCPUFAs from triglycerides, human milk contains minimal amounts of phospholipid.

Another important difference may be the presence in human milk but not supplemented formulas of LC-PuFAs other than DHA and AA. Because these FAs can be converted endogenously to DHA and AA and affect endogenous conversion of ALA and LA to DHA and AA, respectively, their presence may be at least partially responsible for the apparent need for greater amounts of DHA and AA in formula to result in the same plasma lipid content of these FAs observed in infants fed human milk.

**Conclusion**

DHA is an omega 3 essential fatty acid indispensable for growth, development and functions of brain and retina. In infants and children, better mental processing scores, psychomotor development and stereo acuity are associated with DHA intake. DHA fortified food helps to maintain plasma phospholipids DHA content in children. Diet being poor in DHA in pre-schoolers and non-breast fed infants, it is evident that maintaining a continuum of DHA during the early life period in developing countries is challenging. But by enabling an optimum supply from both breast milk (or supplemented infant food) and complementary foods during this period, an adequate DHA intake should be achievable. Nevertheless, further investigations are warranted to assess the long-term effects of omega-3 PUFAs on the later immune-defense and health status during early growth and development. Future studies are required to support the recommendation of DHA supplementation in healthy children.

**References**


Dear Doctor,
Worldwide, 24% of all preterm birth occur in India, where vegetable-based diets low in DHA are common. The rapid accretion of long chain polyunsaturated fatty acids (LCPUFA) by the infant brain during the first 1,000 days of life underlined the potential importance of these fatty acids during this critical period of growth and development. Research data reveal that LCPUFA supplementation improves neurodevelopmental and visual outcomes in this high risk population.

It is indeed a pleasure to present to you this QMR issue by Dr. Mallikarjuna H.B., renowned pediatrician. In this issue, he is enlightening us on ‘DHA supplementation in infants and children’.

I sign off by once again reminding you to continue sending in your comments and suggestion regarding the QMR. Do write to me at balaji.more@raptakos.com with your write ups, notes or tidbits on various topics of interest that can make for informative and interesting reading.

With best regards,

Dr. Balaji More
Vice President - Medical