Introduction:

Through its website, www.dhaomega3.org, The DHA/EPA Omega-3 Institute located within the University of Guelph Research Park, (Guelph, ON, Canada) has focused its efforts for many years on the provision of evidence-based information on the relationship between the dietary consumption of long-chain omega-3 fatty acids throughout the human lifecycle and health outcomes. The present report (key Q and A's) is provided so as to briefly summarize current knowledge on LCPUFA (long-chain polyunsaturated fatty acids) with an emphasis on DHA omega-3 fatty acid (docosahexaenoic acid) as an important nutrient for enhancing the health and well-being of infants in both the short-term and well into childhood. Additional commentary is provided on the important role of ARA omega-6 fatty acid (arachidonic acid). Human milk is the preferred basis of nutrition for infants and infant formula feeding is employed when the former is not possible. Evidence-based research from epidemiological and interventional/clinical trials as published in peer-reviewed journals which evaluated the health benefits of dietary LCPUFA as ingested by infants from breast milk or infant formula will be addressed.

Qu: What is DHA?

Ans: ‘DHA’ is the acronym for ‘docosahexaenoic acid’ – the unique long-chain polyunsaturated fatty acid within the omega-3 family having 22 carbons in chain length and 6 unsaturation sites (6 double bonds between adjacent carbon atoms) within the molecular structure (also designated as 22:6 n-3 in scientific papers). The ‘omega-3’ or ‘n-3’ designation indicates that the first of the 6 double bonds begins at the 3rd carbon down when counting from the methyl end of the fatty acid.

Qu: Where is DHA found in the human body?

Ans: DHA is found in all tissues and cells throughout the human body and is by far the most abundant omega-3 fatty acid in cellular membranes. It is exceptionally abundant in nerve tissue – notably the brain and retina of the eye which relates to its important physiological roles in supporting optimal cognitive and visual functioning, respectively, amongst other functions. It is interesting to note that only very minute amounts of the short-chain omega-3 fatty acid known as LNA (alpha-linolenic acid, 18:3 n-3) are found in these neural tissues. Based on determinations of fetal fatty acid contents of various body organs during gestational stages, the
brain alone represented 23 % of the infant's DHA at 35-40 weeks.


**Qu: Does DHA have any special physico-chemical properties which underlie its important functioning in cell membranes?**

**Ans:** Yes! DHA omega-3 fatty acid has numerous unique properties which other unsaturated fatty acids cannot duplicate. The multiple unsaturation sites within the molecule provide flexibility via the resultant folding over and curved conformation. The six double bonds in the DHA structure results in a dramatic lowering of its melting point (to minus 44 degrees C or minus 47 degrees F). Thus, at human body temperature of 37.0 degrees C (98.6 degrees F), DHA is in a fluid form within cell membranes (bound to phospholipid) and in constant and extremely rapid motion which also mediates its unique functionality. DHA is necessary for various functions in the central nervous system, including neuroprotection, nerve regeneration, and neuronal membrane bio- and synaptogenesis in memory and vision.


**Qu: Are there important metabolites derived from DHA that can play important functions related to health in the body?**

**Ans:** Yes! DHA has a myriad of biological functions which involve its metabolic conversion to key bioactive products including resolvins and neuroprotectins. For example, The DHA-derived mediator (neuroprotectin D1) protects the brain and retina against cell injury-induced oxidative stress and dampens inflammation while promoting its resolution, and restoring homeostasis.

Qu: What are the dietary fatty acid types that can contribute to the availability of DHA in the body for physiological and cellular functioning to support human health?

Ans: There are two main dietary determinants of DHA status within tissues and cells of the human body. One of these is the dietary intake of the short-chain fatty acid as LNA (a precursor of DHA) and DHA itself. In order for LNA (as found in plant/seed oil sources such as canola oil and soybean oil) to generate DHA in the human body, it must be metabolically converted by a series of enzymatic reactions (so-called desaturation and elongation reactions) located mainly within the liver to form DHA which can then be released into the circulation for subsequent uptake by other tissues.

Qu: How limited is the efficiency of conversion in adults and infants of the short – chain omega-3 fatty acid (as alpha-LNA) as present in plant-based sources to the long-chain DHA omega-3 fatty acid?

Ans: It is well established that the human body has a very limited ability to convert LNA via the various desaturation/elongation processes all the way to DHA. As example, reviews of the topic have concluded the overall conversion efficiencies to be less than 5 % in adults (mixed genders) and well below 1 % in males.

Evidence using isotopic tracer studies on premature infants aged 3 or 7 months consuming short-chain omega-3 fatty acid as LNA plus long-chain omega-3 fatty acid (as DHA) found that the amount of synthesized DHA (from the precursor LNA) amounted to only 8 % and 5 % of the DHA derived from direct dietary DHA intakes at 3 and 7 months, respectively. Corresponding studies on term infants indicate that the overall conversion of LNA to DHA within the body is in the order of only approximately 1 %. Furthermore, the conversion efficiency % (from LNA to DHA) is highly variable between individual infants with some approaching near zero (non-detectable limits). Thus, the most direct means by far for providing DHA to the body during infancy and throughout the human life cycle is to provide preformed dietary DHA directly.

Qu: What are the natural dietary sources of pre-formed DHA omega-3 fatty for infants?

Ans: Breastfeeding has long been recognized as the preferred feeding mode in providing optimal nutrition for healthy term infants. DHA is found in all breast milk in the world and at widely-varying levels depending to a considerable extent on the DHA intakes (from fish/seafood, other foods, supplements) of the lactating mothers. Algal oil containing natural DHA (non-synthetic) has long been approved and often used as a source of DHA in infant formula when breastfeeding is not possible for whatever reason(s). Algal oil containing DHA represents a ‘veggie’ (non-mammalian) source of DHA.


Qu: What is the origin of DHA as found in maternal breast milk?

Ans: The DHA in breast milk is derived in part from the dietary intake of LNA and its conversion (albeit very limited) to DHA plus the dietary intake of pre-formed DHA from fish/seafood, DHA-enriched foods, and supplemental DHA (present in certain algal oils and in fish oils). Consequently, the levels of DHA in breast milk (total fat) from vegan women lacking pre-formed dietary DHA have been found to be approximately one-third the levels found in the breast milk from omnivorous women (consuming some DHA in addition to LNA in their regular diets). Clinical trials with lactating mothers employing marked increases in daily LNA intakes (eg., from LNA-rich plant seed oil sources) over several weeks have failed to significantly elevate DHA levels in either blood or breast milk. However, increasing intakes of DHA (from fish/fish oil or algal oil sources) for the lactating mother have consistently resulted in both early and correspondingly much higher levels of DHA available for the breast-fed infant.


Qu: Is there a relationship between the intake of DHA omega-3 fatty acid by lactating
mothers and the DHA status in their infant child?

**Ans:** Yes! The amounts of DHA consumed on a regular basis by lactating mothers (as fish/seafood, DHA-enriched foods, DHA supplements) have shown a close positive relationship to not only the DHA status in their breast milk but also to the corresponding levels of DHA in the circulating blood of their breast-fed infants including key tissues (eg., brain). Studies on infants having died of ‘cot death’ showed much higher levels of DHA in the membrane phospholipid components of their brain tissue (cerebral cortex) if they had been consuming breast milk with 0.4 % of milk fat as DHA as compared to those consuming infant formula devoid of DHA. These marked differences in brain DHA levels were found even when comparing breast milk with 0.4 % of the fat as DHA to DHA-deficient formula with much higher levels of alpha-linolenic acid (LNA) than the former source. These compositional and other studies (see below) have supported the importance of dietary long-chain polyunsaturated fatty acids, particularly DHA, for normal development of the infant cerebellum.


**Qu:** Is there a similar relationship between DHA levels as present in infant formula and the levels of DHA circulating in the body of infants?

**Ans:** Yes! Numerous clinical trials have shown such a close and positive relationship. For example, the so-called DIAMOND Study fed four groups of term infants an identical infant formulae (including 1.61-1.68 % of fatty acids as LNA omega-3 plus 0.64 % as AA omega-6) differing only in the levels of algal DHA therein - 0.00, 0.32, 0.64, and 0.96 % of milk fat as DHA, respectively. Over the following 12 months, there were corresponding higher levels of DHA in the bloodstream of the infants with increased DHA intakes (as compared to the non-supplemented group) by 149, 204, and 256 %, respectively. When the formula for preterm infants was supplemented with long-chain polyunsaturated fatty acids (LCPUFA) including DHA in amounts typical for human milk fat, the corresponding blood levels of these fatty acids were found to be similar to that found during breast milk feeding.

**Qu:** What is the form and bioavailability (absorbability) of DHA omega-3 fatty acid from breast milk or infant formula?

**Ans:** Almost all the fat (fatty acids including DHA omega-3) in breast milk and in infant formula is in the triglyceride form. The absorption coefficients for DHA based on various clinical trials with preterm infants have ranged from 69 - 98 % and 74 - 84 %, respectively. As reviewed, the addition of LCPUFA (incl. DHA) in infant formulas for term infants, with appropriate regard for quantitative and qualitative qualities, has been concluded to be safe and will enable the formula-fed infant to achieve the same blood LCPUFA status as that of the breast-fed infant.


**Qu:** Are the levels of DHA omega-3 fatty acid in breast milk from different parts of the world similar?

**Ans:** No! Major surveys of the DHA status in human breast milk collected worldwide have shown dramatic differences. These DHA levels have ranged from 0.06 % to 1.40 % of total fatty acids in milk fat. As expected, regions with higher intakes of fish/seafood tended to exhibit considerably higher levels of DHA available for the breastfed infant. These worldwide surveys have found the average (mean) level of DHA to be 0.32-0.37 % of total fatty acids. Based on energy intakes of 100 kcal/kg body weight and WHO target body weights (avg. for both genders), an assumed milk fat level of 3.6 % fat would provide average (worldwide) DHA intakes per term infant (ages 3 - 6 months) of 101-146 mg/day with the global ranges in dietary intakes as low as only 19-24 mg DHA /day or as high as 442-551 mg DHA/day in some regions. It is also noted that wide differences in DHA levels within a country or region also exist due to maternal dietary patterns, ethic differences, other factors (eg., use of DHA supplements).


**Qu:** What is the relationship between DHA levels in breast milk and health outcomes in the infant?
**Ans**: Published research has revealed a significant positive association between breast milk DHA content and visual acuity thereby supporting a cause-and-effect relationship. Maternal intakes of 80 mg DHA/day (commonplace in North America and other regions) and breast milk levels below 0.2 % of total fatty acids have been associated with the risk of sub-optimal infant development. A positive association has been reported between the DHA status in breast milk and infants’ scores on the NBAS (Neonatal Behavioral Assessment Scale). Recent analyses of breast milk from 28 countries indicated that, greater than socio-economic factors, the DHA omega-3 levels were concluded to make a highly significant contribution to childhood math scores and a predictor of cognitive performance. It is also noted that, after adjusting the average level of breast milk DHA to 0.35 % of milk fat (via DHA supplementation of the lactating mothers), and comparing such to breast milk having DHA at 0.20 %, infants (at 30 months of age) consuming the higher DHA level for 4 months postpartum exhibited a significantly better performance on the Bayley PDI (Psychomotor Development Index). The Bayley PDI reflects motor skills/hand-eye coordination. It is noted that breast milk levels of DHA at approx. 0.20 % of milk fat (and somewhat lower) are commonplace in North America and other global regions. At five years of age, those children who had earlier received breast milk with 0.35 % of milk fat as DHA (rather than 0.20 %) performed better on a test for sustained attention. It is noted that term infants (at 3 months age) receiving milk fat with 0.35 % DHA would ingest approx. 102-136 mg DHA/day on average (with energy intakes ranging from 90-120 kcal/kg/day) in contrast to only 58-78 mg DHA/day with 0.20 % DHA. It is also noteworthy that epidemiological studies as reviewed recently supported an inverse relationship between DHA levels in breast milk and the development of atopic disease in children with a family history of atopic disease.


**Qu**: What have recent review articles indicated about the presence of LCPUFA (incl. DHA) in infant formula and health outcomes which focused on cognition and visual performance?

**Ans**: Firstly, it needs to be emphasized that breast milk is recommended for infants whenever possible. Several years ago, the addition of LCPUFA (incl. non-synthetic/natural DHA omega-3) to infant formula was employed and approved based on its safety, clinical trials showing infant health benefits (when compared to formula containing only short-chain omega-3 as LNA), and the presence of DHA in all breast milk in the world. While different levels of DHA have been
added to infant formula in the published clinical trials, a substantial proportion of such have employed levels of DHA ranging from 0.32 – 0.36 % of milk fat. The latter range is in keeping with the reported average worldwide levels of 0.32 – 0.37 %.

Since the highest levels of DHA (as % of total tissue/cellular fatty acids) in the body are found in the cerebral cortex and retina, wherein the mechanisms of action of DHA have been studied extensively in supporting optimal mental and visual acuity, respectively, numerous clinical trials have evaluated the effect of formula supplemented with LCPUFA (incl. DHA) on infant cognition and visual performance. Early well-controlled trials reported a significant benefit of an early dietary supply of LCPUFA (incl. DHA at 0.35 % of milk fat via infant formula) on mental development in term infants at certain stages (as measured by the Mental Development Index). Other trials, with varying research protocols, LCPUFA levels, etc., have found a benefit in some and no significant effect in others which has led to questioning if certain measures of neurodevelopment may be benefitted but not others. An earlier Cochrane review (in 2011) of various trials on this topic in preterm infants indicated that 3 of 7 studies reported benefit of LCPUFA supplementation on neurodevelopment in different infant populations at different postnatal ages. A more recent 2014 review (of trials wherein the average level of DHA in the supplemented formula was 0.30 % of milk fat and average treatment duration was 8 months) concluded overall that, compared to the placebo (control) groups, the presence of DHA significantly improved cognitive development in infants, including the Mental Development Index, the Psychomotor Development Index, and language, motor, and cognitive abilities. Comparative studies on term infant formulae differing only in the DHA contents (0.00-control group, 0.32, 0.64, or 0.96 % of milk fat as DHA) fed over a 12-month duration to term infants indicated a significantly enhanced cognitive development in the children at 18 months of age following ingestion of DHA as compared to the controls. Levels above 0.32 % DHA did not provide any further benefit. While very few long-term follow-up trials (over several years) have been conducted, children aged 6 years were found to exhibit significantly faster abilities for processing information if fed formula containing LCPUFA (versus lacking LCPUFA) early in their infancy. While not the focus of the present article, there is a large body of literature in support of the importance of optimal DHA provision for cognition throughout the human lifespan.

Regarding impacts on visual acuity, results of clinical intervention trials on term infants receiving LCPUFA - enriched formula (containing DHA) versus deprived formula (controls) have given mixed results. The earlier 2011 Cochrane review reported beneficial effects on visual acuity in 4 of 9 studies. It has been pointed out that formulae with levels of LCPUFA close to mean (average) worldwide levels may well be more likely to exhibit benefit. A more recent meta – analyses (in 2013) on 19 studies (randomized controlled trials) including preterm and term infants concluded evidence for a significant benefit of LCPUFA supplementation on infants’ visual acuity at 2, 4, and 12 months of age when visual acuity was assessed by using visual evoked potential and at 2 months of age by using behavioral methods. The reduced power of the earlier Cochrane review(s) to assess differences because of the approaches taken were indicated. Different levels of LCPUFA for assessing any benefits on visual acuity have rarely
been employed in single clinical trials. However, the DIAMOND Study in term infants using formulae with 0.00, 0.32, 0.64, or 0.96 % of milk fat as DHA found that, while 0.32 % DHA improved visual acuity relative to the control group (0.00 % DHA), higher amounts of DHA did not further improve visual performance.


**Qu:** In addition to the aforementioned benefits of dietary LCPUFA (incl. DHA) in support of better cognitive functioning and visual performance during infancy, have controlled clinical trials revealed other health benefits of LCPUFA for infants ?

**Ans:** Yes ! A recent (2016) review of published trials supported the essentiality of DHA in the postnatal maternal and infant diet for the development of the infants' immune system early in life. Relative to formula not supplemented with LCPUFA, intervention studies have demonstrated an improvement in many markers of immune function which appear to result in beneficial health outcomes including a lowering of the risk of developing allergic and atopic disease early in life. A recently-published clinical trial (2016) found that LCPUFA supplementation during infancy significantly reduced the risk of skin and respiratory allergic diseases in childhood.

An earlier and large clinical trial found that infants receiving formula supplemented with LCPUFA exhibited a much lower incidence of bronchiolitis/bronchitis at 5, 7, and 9 months when compared to those receiving formula devoid of LCPUFA. A more recent clinical trial found a significantly lower incidence of common respiratory symptoms as well as diarrhea in healthy infants receiving the LCPUFA-supplemented formula.

Qu: In the clinical/interventional studies wherein LCPUFA inclusion in infant formula (containing the short-chain PUFA as linoleic acid omega-6 plus alpha-linolenic acid omega-3) resulted in enhanced health outcomes in term infants, what dietary levels/intakes of DHA were commonly present.

Ans: In the majority of these trials wherein the LCPUFA-containing formula was compared to the corresponding control formula (lacking LCPUFA), the DHA was often present at levels ranging from 0.32-0.36 % of the total fatty acids. Formula milk fat levels of 3.6 % with 0.32 % of total fatty acids as DHA would (on average) provide at least 100 mg DHA (or more) per term infant daily based on WHO target body weights at 3 to 12 months of age and energy intakes of at least 100 kcal/kg body wt./day. For infants meeting WHO body weights when approaching 24 months of age and ingesting 100 kcal/day, average DHA intakes from such formula would approach 200 mg/infant/day.

Qu: What are the target/recommended daily intakes of DHA omega-3 fatty acid for term infants from various health agencies and international organizations?

Ans: In 2010, EFSA (the European Food Safety Authority) recommended intakes of DHA omega-3 fatty acid for term infants of approx. 20-50 mg /day for young infants up to 6 months of age and 100 mg DHA/day from 6-24 months. Subsequently in 2013, EFSA considered 100 mg DHA/day to be adequate for the majority of infants from birth and up to 24 months. In a recent review, the Early Nutrition Academy Workshop (2014) targeted a supply of at least 100 mg DHA/day in infant formula for term infants. Based on target body weights according to infant age (including both genders) set by the WHO (World Health Organization) and energy needs of 100-120 kcal/kg body weight/day, and average milk fat levels of 3.6 %, the required weight % of milk fat as DHA which would be needed to reach a DHA intake of 100 mg/day can be calculated. With energy needs of 100-120 kcal/kg body weight/day, DHA would need to be at least 0.29 - 0.34 % of milk fat for infants aged 3 months. While breast milk is the normal and unequalled food for infants, it is noteworthy that breast milk levels of DHA in numerous regions/countries globally fall well short of providing target dietary intakes. Interestingly, a major recently-published study (2016) from Europe found that term infants (ages 3 months) who were ingesting 77 kcal/kg/day on average with breast milk fat levels averaging only 2.9 % resulted in average intakes of DHA of only 50 mg/day. These low DHA intakes were found with the average breast milk fat levels of DHA being 0.26 wt. % of the milk fat. The authors stated that the DHA intakes of these breast-fed infants, and those as determined for the infants aged 6 months, were markedly lower than the advisable intakes of 100 mg DHA/day due in part to the lower milk fat content and insufficient DHA intakes by the breastfeeding European women. It is noted that if the DHA levels had been 0.32 wt. % of breast milk fat, the average DHA intake of
the 3-month old infants would still have been only 62 mg/day.


Qu: What maternal dietary intakes of DHA and would be needed to ensure that the levels of DHA in breast milk reach targets that would provide at least 100 mg DHA/term infant/day?

Ans: Increasing short-chain omega-3 fatty acid intakes (as LNA) by lactating mothers has been proven to be highly ineffective for improving DHA levels in breast milk whereas the direct consumption of pre-formed DHA from food/supplemental sources is highly efficient. For example, dramatic increases of daily LNA intakes to well above current recommended targets over a multi-week period failed to increase DHA levels in breast milk. In contrast, ingesting DHA results in early and pronounced dose-response improvements in DHA levels in breast milk. Various human trials in lactating mothers have indicated that ongoing average intakes of DHA (including diet providing approx. 80 mg/day plus 200 mg via supplement) totalling close to 300 mg/day can, within two weeks, ensure that breast milk DHA levels can reach at least 0.32 % of total fatty acids for the large majority. Based on target body weights (WHO), energy intakes of at least 100 kcal/kg body weight, and milk fat levels of 3.6 % yielding average fat intakes of 33 – 51 gms/day would provide DHA intakes of 106-163 mg/day for term infants aged 3-12 months (with DHA at 0.32 % of milk fat). Higher total daily DHA intakes during lactation (approaching 500 mg/day) can be expected to increase breast milk DHA levels to be approx. 0.50 % of total fatty acids). With energy intakes and/or milk fat levels lower than the aforementioned in some sectors, higher dietary intakes of maternal DHA and correspondingly higher DHA levels in breast milk would ensure the provision of target DHA intakes of at least 100 mg/day for term infants. For example, human milk fat levels of 2.7 % versus 3.6 % would require DHA to be 0.43 % of total fatty acids in the former in order to provide equivalent amounts of DHA for term infants. DHA intakes during lactation of at least 400 mg/day have been found to yield DHA levels in breast milk in the range of 0.45-0.50 % of total fatty acids. These higher levels are found in several countries globally.

Qu: Based on clinical research/evidence, is it likely that the DHA requirements for the premature infant are even greater than such for the term infant?

Ans: Preterm infants are recognized as being at particularly high risk for an insufficient supply of DHA to support normal growth, neurodevelopment, and health because of the interruption in the normal process for the accretion of DHA in the body. Daily enteral DHA supplementation can alleviate deficiency in premature infants. Clinical trials/reviews in preterm infants have indicated that deficient intakes of DHA are associated with delayed cognitive development and visual impairment and other health concerns. A higher DHA status in the first few weeks of life has been associated with improved microstructural brain development and improved outcomes in preterm born children.

Based on current knowledge, adequate intakes of DHA from the time of preterm birth in terms of absolute amounts of 55-60 mg/kg body weight/day to expected term have been suggested so as to support normal DHA status and to provide better neurocognitive and visual functions. Such targets would generally require higher levels of DHA than that for term infants – approaching 1.0 to as high as 1.5 % of milk fat for the ELBW (extremely low birth weight infants). It has been reported that providing enteral feeds containing DHA at approx. 1 % total fatty acids relative to DHA at 0.30 % to premature infants improved the MDI (Mental Development Index) scores of girls. Some inconsistency in the results from various randomized clinical trials in showing a benefit of LCPUFA (incl. DHA) supplementation of preterm infant formula on visual acuity have been attributed to the use of relatively low doses of DHA (0.2 to 0.4 % of total fatty acids). A higher amount of DHA (1.0 % of fatty acids) versus a lower level (0.3 % DHA) provided for preterm infants (mixed genders) via human milk or formula feeding has shown a significantly better performance in visual acuity with the higher level at four months of CA (corrected age). The high-dose DHA supplementation of preterm infants (approx. 1 % of milk fat as DHA ) relative to a lower dose was also found to reduce the incidence of BPD (bronchopulmonary dysplasia) in boys and in all infants with birth weights below 1250 grams and to reduce the incidence of reported hay fever in boys at either 12 or 18 months.


**Qu: Does the higher DHA requirement for the premature infant have implications for advice to mothers who are providing breast milk via donor milk banks?**

**Ans:** Yes! The generally lower than desirable levels of DHA in donor breast milk for premature infants reflects the low dietary intakes of the women. Donor milk banks and donors should be encouraged to improve regular and specific DHA intakes to ensure that milk fat DHA levels fully support optimal intrauterine accretion targets for the infant. For example, maternal supplementation with 1000 mg algal DHA/day over 14 days was found to elevate overall levels of DHA in donor milk to 0.65 % of total fatty acids (range from 0.4 to 1.1 %). Higher levels of maternal DHA supplementation can bring DHA levels in breast milk to 1 % of milk fat. Worldwide levels of DHA at 1.0 – 1.4 % of milk fat have been found in areas with relatively high intakes of DHA from fish/seafood.


**Qu: Have there been any lengthy follow-up trials looking at the potential benefit of early infant intakes of LCPUFA (incl. DHA) several years later in childhood?**

**Ans:** Yes – a few! However, there is a need for more long-term (several year) follow-up studies to monitor health outcomes of both term and preterm infants in relation to their early intake/status with respect to LCPUFA. Such prolonged benefits several years later have been indicated in trials with both term and preterm infants. A long-term follow-up study found that children at age 6 years were faster at processing information if they received LCPUFA as term infants relative to controls lacking LCPUFA. While early benefits of a higher DHA level (1.0 % of fatty acids) in preterm formula versus lower (0.2 to 0.3 %) on mental development have been reported, no clinically meaningful differences in language development or behaviour were apparent between 3- and 5-year corrected age. A very long-term follow-up study (9 years) found significant benefits for children aged 10 years who were receiving preterm formula containing LCPUFA (incl. DHA at 0.5 % of fat) versus control (no DHA) on several cognitive measures and benefits for girls in literary measures.
Qu: What about the accumulation and abundance of the LCPUFA as ARA omega-6 (arachidonic acid, 20:4 n-6) in the developing nervous tissue of infants?

Ans: Arachidonic acid (ARA) and docosahexaenoic acid (DHA) acids are the most abundant polyunsaturated fatty acids in the brain, where they both provide numerous biological functions and effects. ARA represents approximately 10-11% of the total fatty acids in the central nervous system (cerebral cortex and retina) of the human infant (breast-fed or formula-fed). The ARA levels are moderately higher and slightly lower overall than corresponding DHA levels in the brain and retina, respectively. The total fetal body amounts and accretion rates for ARA at 25-40 weeks gestational age are at least twice that for DHA. While DHA levels ranged from 7.0 - 7.8% of total fatty acids, ARA ranged from 9.2 - 10.9%.

Qu: What are the major sources of ARA in breast milk and in infants?

Ans: Maternal body stores of ARA are considered to be the major source of milk ARA with little contribution from the conversion of linoleic acid omega-6 (LA) to ARA. The direct intake of preformed ARA is a very important source of ARA for the infant with some ARA derived via endogenous synthesis. Various studies in infants have shown very limited conversion rates of LA (linoleic acid) to ARA (fractional rates of conversion ranging from only 0.4 to 2.7%). Such low rates of endogenous synthesis of ARA have therefore been deemed inadequate to sustain blood levels of ARA. Breast milk-fed infants consuming ARA exhibited higher levels of circulating ARA than those fed formula lacking ARA. Feeding formula with preformed ARA led to significantly higher levels of circulating ARA which were similar to those in the breast-fed infants.


Qu: What are some of the unique properties and key functions of ARA in the body?

In addition to playing a key structural role in maintaining membrane integrity and function via its unique physico-chemical properties in nervous tissue and elsewhere, ARA mediates synaptic transmission in the brain, as well as regulating inflammation, cell-signaling, blood flow, and other cellular processes via its conversion to bioactive metabolites (eicosanoids, others). While the focus of ARA in the body is often on its functions in the brain and retina, it should be pointed out that metabolites of ARA play key roles in other tissues and cells. For example, the generation of prostanoids in the kidney from ARA (via the action of cyclooxygenase enzyme activity) plays an important role in maintaining renal function, body fluid homeostasis, and blood pressure. ARA and its various metabolites support important vascular and immune functions. Recent evidence from animal models indicates that dietary arachidonic acid supplementation during the prenatal and postnatal periods prevents retinal degeneration. It is noteworthy that ARA (20:4 n-6) is the immediate precursor of the longer-chain omega-6 fatty acid (formed via elongation of ARA) known as ADA, adrenic acid (22:4 n-6). High ADA levels (23.5 % of the total fatty acids) similar to ARA (18.4 %) and DHA (19.8 %) are present in the infant brain phospholipid (alkenyacyl PE) at 0-6 months. ADA is suggested to play an important role in myelination in neural tissues.


Qu: What are the levels of ARA in the LCPUFA found in global breast-milk and in many intervention trials with commercial infant formula?

Ans: Two major studies on the worldwide levels of ARA in human breast milk have reported the average levels to be 0.47 % and 0.55 % of the total fatty acids. Interestingly, the variability in ARA levels was considerably less than such for DHA. This somewhat stable level of ARA in human milk provides support for the structural and functional needs of nervous tissue during a period of active growth and development. For a term infant consuming 840 ml/day, the estimated daily ARA intake would be approx. 162-190 mg. Various international organizations have recommended that, if breastfeeding is not feasible for whatever reason, ARA should be added to infant formula at a level which is at least equal to that of added DHA. Some have advised levels of ARA at 0.4 to 0.6 % of total fatty acids which would encompass levels which
are close to the aforementioned worldwide levels. A considerable portion of the intervention trials with formulae in term infants have employed levels of DHA at 0.32 - 0.36 % of total fatty acids plus ARA levels at 0.34 – 0.72 % of total fatty acids.


Qu: Have there been many clinical trials in infants wherein infant formula with DHA (without and with ARA) have been directly compared in relation to various health outcomes ?

Ans: No ! Almost all intervention trials in infants have employed a comparison of infant formula lacking LCPUFA with a comparative formula containing both LCPUFA (containing DHA plus ARA). Early studies in preterm infants reported that formula with ARA plus DHA provided higher levels of ARA in the red blood cell phospholipids at the end of preterm formula feeding as compared to formula containing DHA alone. Further, the former formula (ARA plus DHA) resulted in a higher body weight at certain stages (as compared to DHA alone) with ARA levels in the red blood cell phospholipid being positively correlated to body weight and length at some stages of infant development. Feeding formula to term infants which contained DHA (0.35 % of fatty acids but no ARA) resulted in much lower levels of ARA in blood plasma phospholipid as compared to breastfed infants. The addition of ARA (0.34 % of fatty acids) resulted in a marked improvement in circulating ARA levels which approached levels found in the breastfed infants. There is a research need going forward for clinical trials which better clarify the health benefits to infants (short- and long-term into later childhood) of ARA in addition to DHA. However, because of its abundance in all body tissues/cells, in all breast milk, and known functions in the body, intentionally excluding ARA from intervention trials using infant formulae poses a most serious ethical dilemma.


Qu: What is the bioavailability to infants of ARA from breast milk or from infant formula ?

Ans: A very recent study in preterm infants (aged 2 and 6 weeks) reported average fatty acid
absorption coefficients ranging between 86-96 % overall for ARA.


**Qu:** What is the recommendation of Health Canada for infant nutrition ?

**Ans:** Breastfeeding – exclusively for the first six months, and sustained for up to two years or longer with appropriate complementary feeding is important for the nutrition, immunologic protection, growth, and development of infants and toddlers. The addition of the fatty acids, docosahexaenoic acid (DHA) and arachidonic acid (ARA), to infant formula has long been permitted. Health Canada has indicated that clinical studies show that infant formulas with DHA and ARA support normal growth and development in healthy term and pre-term infants. Health Canada approved a DHA-containing oil from a unicellular algal source and an ARA-containing oil from a unicellular fungus as acceptable sources of DHA and ARA for infant formulas.


**Overall Summary:**

1) Based on average global estimates of DHA omega-3 fatty acid (docosahexaenoic acid, 22:6 n-3) levels in breast milk and population studies plus controlled intervention trials (in both breast-fed and formula-fed term infants) supporting various health benefits to term infants (incl. cognitive, visual, immune/allergic, respiratory/bronchitis, diarrhea, other) fed milk containing 0.32-0.36 % of total fatty acids as DHA (along with ARA), DHA levels in breast milk or formula of at least 0.32 % total fatty acids is advised. Recommended infant intakes for DHA should consider any and all potential health benefits without rendering judgements based on any single health outcome.

2) Some reviews and meta-analyses have determined the majority of published studies to support an overall benefit of DHA presence in breast milk and infant formula for diverse infant health outcomes while others have determined such to have been established in a minority of studies with many showing ‘no effect’. Some ‘no effect’ studies have been accounted in the
aforementioned reviews by the use of lower supplemental levels of DHA and differences in the design/criteria/parameters employed in the trials. Other uncontrolled factors include the marked individual differences in the initial DHA status of the infants enrolled in such studies (ie, wide discrepancies in DHA delivery to the infant from the mother during gestation and DHA status of the infant).

3) It should be recognized that the traditional/historical approach to setting nutrient requirements has been based on ensuring that, even if a minority of the population may be expected to receive a health benefit from setting a nutrient requirement, such should be established. This rationale can be applied to infant nutrition studies (on LCPUFA) where factors such as large inter-individual differences in DHA status at birth, very low and wide differences in short-chain omega-3 as LNA conversion to DHA, and other confounding variables exist. It is noted that the Recommended Dietary Allowance (RDA) is defined as: the average daily dietary intake level that is sufficient to meet the nutrient requirement of nearly all (97 to 98 percent) healthy individuals in a group.

4) Various organizations/sources have recommended that term infants should be targeted to receive at least 100 mg DHA per day. Based on expected energy intakes and WHO body weights (both genders combined), most infants (ages 3 months and older) ingesting milk (breast milk or formula) with a fat content of at least 3.6 % and 0.32 % of total fatty acids as DHA would consume 100 mg of DHA or more daily.

5) Breast milk is the preferred source of infant nutrition. Higher levels of DHA in the breast milk fat for term infants have been positively related to various health outcomes. In order to bring breast milk levels of DHA to at least 0.32 % of milk fat, various trials have indicated that regular supplementation with 200 mg DHA/day over and above DHA ingestion from background dietary sources (often in the range of 80 mg DHA/day) can be expected to bring milk fat DHA levels to at least 0.32 % of milk fat. Thus, a recommended maternal intake totalling 300 mg DHA/day during lactation from dietary sources and via supplementation if needed is advisable. It is noted that, because of the slower turnover of DHA in the body as compared to many other nutrients, consideration to a ‘weekly DHA requirement’ such as 2100 mg/week could be given provided that DHA sources are consumed no more than 3-4 days apart if such a strategy would help compliance in meeting targeted DHA intakes (especially considering the reluctance of many to consume fish on a daily basis).

6) Attention should be given to the fact that the fat content of breast milk shows considerable variability (by geographical locale, maternal diet and age, stage of lactation) with levels often well below 3.6-4.4 % of milk fat and approaching 2.5 % in various global regions and under
certain conditions. Thus, a nursing infant from the latter region consuming 750-900 milk/day would fall well short of a target of 100 mg DHA per day despite the breast milk fat level being 0.32 % of milk fat. Such a shortfall could be readily overcome by increasing maternal DHA intakes so to correspondingly increase DHA levels in breast milk to be well above 0.32 % of milk fat. In view of the considerable prevalence of breast milk fat levels globally being well below 3.6 %, it is reasonable to suggest that a target average total daily intake of 500 mg DHA/day during lactation as a ‘DHA insurance strategy for infants’ be considered to yield an expected DHA level in milk fat of approx. 0.50 % of total fatty acids with the large majority of term infants thereby ingesting at least 100 mg DHA/day regardless of unknown and lower milk fat levels. Many countries worldwide have current breast milk levels of DHA in the range of 0.50 % or higher.

7) Premature infants are at particularly high risk of DHA deficiency such that significantly higher levels of DHA in milk fat (provided via donor milk if possible or formula) than normally considered optimal for term infants are advised. In the case of donor breast milk provided through milk banks, educational information needs to be provided on the much higher intakes of DHA (via diet and/or supplementation) that are required to provide the higher target DHA levels in milk fat for the premature infant.

8) ARA (arachidonic acid omega-6) and DHA (docosahexaenoic acid omega-3) both provide distinct structure-functional relationships, cellular functions, and are immediate precursors for the generation of key bioactive/metabolic products which underlie their key physiological roles in nervous tissue and other cells/organs. As found for DHA, ARA is present at substantial levels in worldwide breast milk and its addition at such levels to infant formula is known to maintain circulating ARA levels to be similar to such as found in breast-fed infants.

9) Worldwide breast milk levels of DHA have been found to average 0.32-0.37 % of milk fat and 0.47-0.55 % for ARA. The vast majority of clinical trials with infant formula have compared formula (control) with short-chain omega-3 (as LNA) plus omega-6 (as LA) with the identical formula enriched with LCPUFA containing DHA plus ARA. A considerable portion of controlled clinical trials in infants which have shown various health benefits with the presence of LCPUFA have employed enriched formula with DHA and ARA levels similar to the aforementioned mean levels in global breast milk. Until such time that future research can better delineate and differentiate the contribution of ARA to the observed health benefits of LCPUFA inclusion, it would be highly impetuous to suddenly remove ARA from infant formula and face the risk of compromising the health outcomes of infants worldwide who cannot be breast-fed for various reasons.

10) It is advised that, based on current knowledge as elaborated upon herein, both DHA and
ARA levels in infant formulae for term infants should be based on such levels as found in average global breast milk compositions which have also been employed in a significant number of clinical trials wherein health benefits have been reported. For the premature infant, considerably higher levels of DHA in both donor breast milk and formula are highly recommended.

**Qu: Who is the author of the above material?**

**Ans:** The sole author of this material is Dr. Bruce Holub (University Professor Emeritus, Dept. of Human Health and Nutritional Sciences, University of Guelph, Guelph, ON). Following his undergraduate degree at the University of Guelph (Chemistry and Biology), he completed his Ph. D. from the Dept. of Biochemistry, Faculty of Medicine, University of Toronto (major-Biochemistry, minor – Nutrition) followed by post-doctoral work (MRC Fellow) in the Department of Biological Chemistry, University of Michigan Medical School. He then joined the faculty of the Univ. of Guelph. Prof. Holub has served as President of the Nutrition Society of Canada. His research, via epidemiological and intervention/clinical trials, is focused on dietary omega-3 fatty acids as DHA plus EPA from fish/fish oils or as DHA from algal oil for mothers, infants (via breast milk or infant formula), and toddlers as well as adults in support of optimal health outcomes throughout the life cycle including the prevention/management of chronic disorders. He has authored over 200 papers in scientific journals and has given over 400 invited lectures in 20 countries. Prof. Holub also serves as Scientific Director for the DHA/EPA Omega-3 Institute and its analytical laboratory which performs omega-3 fatty acid analyses within the Univ. of Guelph Research Park including a freely-accessible website that provides current evidence-based health and research information on DHA/EPA from fish/seafood, fish oils, algal oils, enriched supplements and functional foods containing DHA/EPA omega-3 fatty acids at www.dhaomega3.org.

Dr. Holub declares that, as the sole independent author of the above text, he has not subjected this material to any scrutiny or recommendations for any alterations. Further, he has not personally received any financial remuneration for his writing, any sharing of such, and posting for educational purposes at www.dhaomega3.org. Dr. Holub can be reached via email at bholub@uoguelph.ca or via phone at (519)-824-4120, ex. 53721.