The ω -3 poly-unsaturated fatty acids and the function of the brain and retina in infants

Lotte Lauritzen, Camilla T. Damsgaard, Anders D. Andersen & Kim. F. Michaelsen

Abstract

The central nervous system of human infants has a uniquely high content of docosahexaenoic acid (DHA, 22:6ω-3), which is accreted during the brain growth spurt that occurs during the first year of life. Based on results from randomized controlled trials on visual acuity it is presently agreed that preterm infants have a conditional need for preformed DHA, but the data for term infants are inconclusive. The term infant studies are in general performed more recently and with higher levels of α-linolenic acid (LNA, 18:3ω-3) in the control formulas. A meta-regression analysis of the data has shown that differences in the dose of ω-3 poly-unsaturated fatty acid (PUFA) are an important factor in explaining the inconsistencies in the functional outcomes. Thus, the data on both term and preterm infants are in agreement with a classical dose-response relationship, but it is unknown at this stage whether dietary LNA could meet the ω-3 PUFA requirements. Moreover, the potential long-term implications of the early improvements in visual function are not known. ω-3 PUFA intake in the first year of life is also believed to affect infant cognitive development, although this question remains unresolved. Breast-feeding has been shown to confer a long-term advantage in cognitive performance of approximately 3 IQ-points relative to that in formula-fed subjects, but this difference could be due to confounding as well as specific components of human milk. The DHA-content of human milk depends on the maternal fish intake, which in many countries does not support optimal levels of DHA in the milk. Most maternal fish oil supplementation trials report no advantage to infant mental development during the first year of life, but many of these studies find positive associations between breast-milk DHA and neuro-developmental outcomes, mirroring the results of observational studies. Some of these studies indicate possible negative effects of ω-3 LCPUFA, e.g. on language development, but the interpretation is complicated by lack of knowledge of the long-term predictive role of the employed early tests on cognitive development. Apart from direct mental effects, changes in the fatty acid composition of the central nervous system may also influence other types of behavior and body functions, such as the regulation of blood pressure. Early intake of ω-3 PUFA has been shown to affect blood pressure later in life in both LCPUFA-supplemented formula-fed infants and ω-3 PUFA deficient rats. This line of research is up-coming and we expect the next decade will provide us with new aspects of the effect of ω-3 PUFA that need to be taken into consideration when making dietary recommendations for PUFA-intake during growth.

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Department of Human Nutrition,University of Copenhagen, Rolighedsvej 30, DK-1958 Frederiksberg C, Denmark • ll@life.ku.dk

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Introduction: ω-3 PUFA and the developing brain

Humans have a very high growth rate during the first year of life, particularly in the central nervous system (CNS). The CNS has a uniquely high content of docosahexaenoic acid (DHA, 22:6ω-3) that accretes during its growth spurt. Infancy is characterized by a high growth velocity of the head and brain, which increases in weight from 200 g at birth to 1 kg at 1 year of age, when the growth spurt starts to fade out (Martinez, 1992), and DHA is accreted in this period. This accretion is not only due to the increase in brain size but also to an increase in the relative content of DHA (Martinez, 1992). It has been estimated that approximately half of the DHA accumulation within the body of a breast-fed infant appears in the brain (Cunnane *et al.,* 2000), which has been calculated to accumulate as much as 4 g DHA during the first year of life (Cockburn, 1997). In order to judge the magnitude of this accumulation one has to consider both the intake and the efficiency of incorporation.

The efficiency of DHA-incorporation from dietary alfa-linolenic acid (LNA, 18:3ω-3) appears to be extremely low in rats, partly due to recycling of carbon into non-essential components in the brain (Cunnane *et al.,* 1999). A number of primate studies (Sheaff Greiner *et al.,* 1996; Sheaff Greiner *et al.,* 1997; Su *et al.,* 1999) have shown that dietary DHA is incorporated in the CNS about 10 times more efficiently than LNA, but that only a small fraction of the supplied ω-3 fatty acids are incorporated, regardless of the source. A number of studies investigating the brain fatty acid composition of infants that died from sudden infant death syndrome (SIDS) show that brain DHA-accretion confers a dietary ω-3 PUFA demand (Farquharson *et al.,* 1992; Farquharson *et al.,* 1995; Jamieson *et al.,* 1999; Makrides *et al.,* 1994). Also, an increase in the relative DHA-content in the brain was observed in breast-fed infants, whereas no increase was seen in formula-fed infants (Makrides *et al.,* 1994). This indicates that the infant formulas did not sustain brain DHA-accretion, at least not to the same extent as breast-milk. The important question is whether the differences in DHA-accretion in the CNS affect brain function.

ω-3 PUFA in breast-milk and infant formula

Infant formula is designed to match breast-milk in its overall fatty-acid composition, i.e. its content of saturated, mono-unsaturated and poly-unsaturated fats (PUFA). However, as the composition of breast-milk is variable, no defined standard exists for the composition of formula, especially not with respect to the content of specific PUFAs. The PUFA-content of formulas has changed over the years as evidence of the importance of ω-3 PUFA has increased. A decade ago most infant formulas contained linoleic acid (18:2ω-6) and LNA as the only sources of PUFA. In the early studies from the 1980-1990'ies, which examined the functional effects of infant formula, the LNA-content was as low as 0.2 % of the energy or less (Faldella *et*

al., 1996; Uauy *et al.,* 1990). Today many formulas contain some long-chain (LC)PUFA, most often both DHA and arachidonic acid (20:4ω-6). In 2003, all term infant formulas on the Danish market contained linoleic acid and LNA in a ratio of approximately 8:1 (Straarup *et al.,* 2006). Human milk always contains some DHA and other LCPUFAs, but the amount of ω-3 LCPUFA varies greatly with diet (Brenna *et al.,* 2007; Lauritzen *et al.,* 2001). Breast-milk from women with special diets, such as vegans who ingest no LCPUFA and Eskimos who consume large amounts of ω-3 LCPUFA, differs only 3-fold in their content of linoleic acid, arachidonic acid and LNA, but differs more than 15-fold in their content of DHA. Reports on the DHA-content of breast-milk from individual women range from 0.1 to 3.5 % of the fatty acids (FA%) (Brenna *et al.*, 2007; Koletzko and Rodriguez-Palmero, 1999).

ω-3 PUFA and visual acuity

Research on functional CNS-effects of ω-3 PUFA-intake in infancy has mainly focused on visual acuity. This is due to: 1) The exceptional high levels of DHA in the retina, 2) Visual acuity being a well-described symptom of ω-3 PUFA-deficiency (Neuringer *et al.,* 1984), and 3) Visual acuity develops rapidly during early infancy and is relatively easy to measure (Lauritzen *et al.,* 2004a). Visual acuity is normally assessed by the general physician as the ability to read letters or symbols from a certain distance. This method is however not applicable to infants, who instead are presented with stripes in order to judge their tendency to gaze at these (the Teller card method). Alternatively infant visual acuity can be determined as the visually evoked electric potentials in their visual cortex by the use of electrophysiological methods (VEP or SWEEP-VEP) (Neuringer *et al.,* 1994).

As reviewed in (Lauritzen *et al.,* 2001), several randomized controlled trials have shown an improvement in visual acuity in formula-fed preterm infants after addition of DHA to formula. A meta-analysis showed a slower visual acuity maturation in preterm infants (born <37th week of gestation) given formulas with a LNA-content of less than 2 FA% compared to those given human milk or formulas with DHA, alone or in combination with ω-6 LCPUFA (SanGiovanni *et al.,* 2000b). The difference in visual acuity between infants who were fed formulas with or without DHA was observed at 2 and 4 months of age. Only a few studies compared visual acuity between breast-fed and formula-fed preterm infants (Birch *et al.,* 1992; Birch *et al.,* 1993), but these studies and a number of similar studies in term infants have shown better visual acuity at 4 months with breast-feeding (SanGiovanni *et al.,* 2000a). Since many randomized controlled trials in preterm infants show positive effects on infant visual acuity when DHA is added to infant formula, it is generally agreed that preterm infants have a conditional need for preformed DHA (e.g. (Fleith and Clandinin, 2005)). In contrast, the data from term infants is not believed to be conclusive.

Various reviews of the trials in term infants report that only half of the studies show a faster visual acuity maturation after DHA-addition, while the other half show no effects (Eilander *et al.,* 2007; Lauritzen *et al.,* 2001; SanGiovanni *et al.,* 2000a). However, it is important to keep in mind that as evidence has accumulated, the ω-3 PUFA-content of formulas has increased (Lauritzen *et al.* 2001). The first three preterm infant trials used formulas (with 0.25-0.5 FA% LNA) that would now be considered to be ω-3 PUFA-deficient. The studies in term infant are in general performed more recently and with considerably higher levels of LNA in the control formulas (around 2-3 FA%). The DHA-content that was used in the experimental formulas has also varied. One of the early trials with preterm infant used large amounts of fish oil (as much as 0.5-1 FA% ω-3 LCPUFA) (Carlson *et al.,* 1991), whereas lower amounts (down to 0.1-

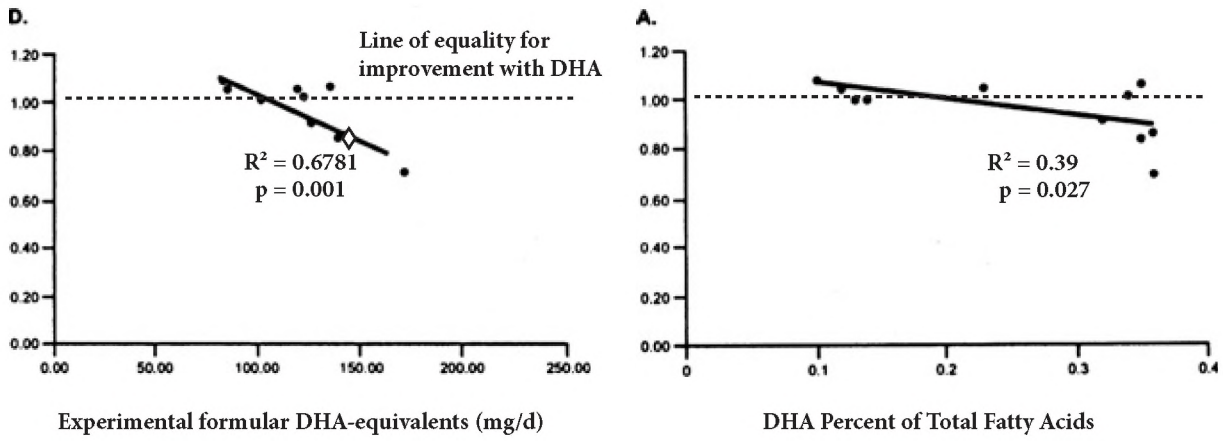

Fig.1. *Left:* Dose-response: term infant DHA intake *vs.* visual acuity improvement. The plot shows the results from the meta-regression analysis of Uayu *et al.* (2003). The experimental formula content is given in DHA-equivalents (mg/d) assuming a 10% conversion of LNA to DHA. The result from the study of Birch *et al.* (2005) (◊) has been superimposed on the fit from the meta-regression analysis. *Right:* the results of a crude analysis that do not consider formula content of LNA.

0.2 FA% DHA) were used in the trials with term infants (Auestad *et al.*, 2001; Carlson *et al.*, 1996). The more recent studies seem to have realized the need for larger DHA-doses and provided 0.3-0.4 FA% DHA in their experimental formulas. The difference between the results of the term and preterm infant trials are much less obvious when we look at only the most recent trials, that have used similar standard formulas and similar doses of DHA (Auestad *et al.*, 2001; Birch *et al.*, 2005; Innis *et al.*, 2002; O'Connor *et al.*, 2001; Wezel-Meijler *et al.*, 2002).

In a review of the effect of ω-3 PUFA in the function of the brain and retina in infants, we hypothesized that the differences in the dose of ω-3 PUFA (both LNA and DHA) could be an important factor in explaining the inconsistencies in the functional outcomes in the term infant trials (Lauritzen *et al.*, 2001). This has later been confirmed in a meta-regression analysis (Uauy *et al.*, 2003). Uauy and his coworkers found a highly significant association between the observed effects on visual acuity and the combined intake of LNA and DHA in the term infant trials. The group tested different theoretical proportions (0-10 %) of LNA-conversion to DHA and the best fit was achieved with a DHA-equivalence factor of 10% for LNA. This is in agreement with data on DHA-incorporation in the CNS of primates. The meta-regression analysis included seven trials that assessed visual acuity at 4 months of age (Fig. 1) and explained as much as 68 % of the variation between the results of the randomized controlled trials that tested formulas with and without DHA in term infants (p=0.001) (Uauy *et al.*, 2003). Also, the results of a more recent trial (Birch *et al.*, 2005) fits nicely with the regression (Fig. 1). Thus, in our opinion the present data from both term and preterm infants are in agreement with a classical dose-response relationship, suggesting that the dietary need may be around 100 mg/d or 0.4 FA% DHA in the milk, regardless of gestational age at birth. At this stage it is unknown whether LNA-intake alone could meet the ω-3 PUFA-requirements of infants. To our knowledge only one study has investigated the effects of a formula with a LNA-content above 4 FA% (Innis *et al.*, 1997).

Since breast-milk DHA varies with maternal

diet, a way to look at the developmental effects of infant DHA-intake is to perform randomized trials with lactating mothers. We and others have conducted such trials in which the DHA-content of breast milk was manipulated by supplementing the lactating mothers with fish oil in order to look for functional effects on visual acuity and other outcomes in the infants (Table 1). The breast-milk DHA content in our trial correlated significantly with the maternal intake of ω-3 LCPUFA and was raised from a mean of 0.4 FA% in the olive oil-supplemented (control) group to around 1 FA% in the fish-oil supplemented group (Lauritzen *et al.*, 2004b). In our study, the fish-oil supplement did not have any immediate effect on the SWEEP-VEP visual acuity of the infants at 2, 4 or 9 months of age, i.e. no significant differences were observed between the two randomized groups. However, we did see a significant association between the DHA content of infant erythrocytes (a well-accepted biomarker of DHA intake) and their visual acuity at 4 months. The erythrocyte DHA-content together with the degree of breast-feeding, gestational age and number of siblings were found to account for 24 % of the variance in infant visual acuity (around 4 % by DHA alone). The low degree of explanation is in part due to noise in the SWEEP-VEP testing of visual acuity in the infants. When the random variation of the SWEEP-VEP method is removed, the effect of DHA is estimated to increase to a much larger fraction of the variance (Lauritzen *et al.*, 2004a).

The two other randomized controlled trials looking at the effects of ω-3 LCPUFA-supplementation during lactation did not find any group-differences in infant visual function (Gibson *et al.*, 1997; Jensen *et al.*, 2005) and neither did a maternal supplementation trial in which ω-3 LCPUFA-supplements were given during pregnancy or during both pregnancy and lactation (Malcolm *et al.*, 2003a). Accordingly, the most obvious conclusion would be that maternal DHA-supplementation does not markedly affect infant visual development (Eilander *et al.*, 2007). However, the pregnancy trial is questionable as no biochemical effect of the intervention was observed in breast milk or infant blood at delivery (Malcolm *et al.*, 2003a). In contrast, a more recent trial in which mothers were supplemented with DHA during pregnancy did find an effect on infant visual acuity at 4 months, although not at 6 months of age (Judge *et al.*, 2007a). This study was performed in the US and used a very low DHA-dose. Since both intake of DHA and thus the breast-milk content of DHA are lower in the US than in Scandinavia, this study may indicate that the trials should be re-evaluated in order to look at dose-response-relations, like the formula trials. However, the new pregnancy DHA-supplementation trial was only a small study and the result could be a chance-finding. Data from one of the published trials with maternal DHA supplementation in pregnancy (Malcolm *et al.*, 2003a; Malcolm *et al.*, 2003b) and a new Canadian trial (Innis, 2007a) show associations between visual functions (ERG, VEP-latency and visual acuity) in infants and biochemical biomarkers of DHA intake and a few observational studies in breast-fed infants have also observed that DHA in breast-milk or infant erythrocytes was positively associated with visual acuity (Innis *et al.*, 2001; Jørgensen *et al.*, 2001; Makrides *et al.*, 1993). This supports the association found in our maternal supplementation trial and underlines the need for a dose-response approach when evaluating results. Furthermore, the evidence indicates that the optimal milk DHA level is higher than that found in breast-milk from women with a low intake of ω-3 LCPUFA.

Apart from the optimal dose, another unresolved matter is whether or not the effect of ω-3 LCPUFA on visual acuity is long-lasting. Only a few randomized trial have looked at the effects on visual acuity beyond the first year of life (Auestad *et al.*, 2003; Birch *et al.*, 2007; Singhal *et al.*, 2007).

Study	a) Experimental supplement b) Control oil c) Period of supplementation	DHA in breast-milk (FA%)	Performed assessments (n in all random groups)	Observed group-differences (relative to control)	Description of observed associations with DHA in breast-milk or RBC
Supplementation in pregnancy					
Malcolm *et al.*, 2003a; Malcolm *et al.*, 2003b	a) 250 mg/d ω-3 LCPUFA/d (fish oil) b) High oleic acid sunflower oil c) Gestation wk 15 to delivery	a) 0.2 nmol/l b) 0.3 nmol/l at 0 wks	1) Infant plasma & RBC at 0 wks (53) 2) ERG within 1 wk (41 or 44) 3) Flash VEP at 0, 2.5 & 6 mo (55, 52, 51) 4) t-VEP 2.5 & 6 mo (?)	1) NS: RBC-DHA increased by 5% 2) No differences 3) No differences 4) No differences in acuity or parameters	2) Significant associated with RBC ω-3 LCPUFA status at birth 3) No associations 4) Association between peak latency and RBC-DHA at birth
Tofail *et al.*, 2006	a) 3 g/d ω-3 LCPUFA (fish oil) b) Soy oil c) Gestation wk 25 to delivery	Not assessed	1) BSID at 10 mo (249) 2) Infant behavior at 10 mo (249)	1) No differences 2) No differences	No biochemical measures of the intervention included
Judge *et al.*, 2007a Judge *et al.*, 2007b	a) 340 mg/d ω-3 LCPUFA (functional foods) b) Corn oil c) Gestation wk 24 to delivery	Not assessed	1) Teller at 4 & 6 mo (30, 26) 2) Fagan at 9 mo 3) PS at 9 mo	1) Better acuity at 4, but not at 6 mo. 2) No differences 3) Higher score	No biochemical measures of the intervention included
Supplementation in pregnancy and lactation					
Helland *et al.*, 2001 Helland *et al.*, 2003	a) 2.2 g/d (cod liver oil) b) Corn oil c) Gestation wk 18 to 12 wks post partum	a) 1.2 b) 0.47 at 12 wks	1) Infant plasma PL at 0, 4 & 12 wks (74, 82, 75) 2) EEG at 2 days & 12 wks (148, 122) 3) Fagan at 6 & 9 mo (262, 245) 4) K-ABC at 4 y (84)	1) ω-3 PUFA increased by 60% at 4 wks 2) No differences 3) No differences 4) Higher score	2) Mature and immature 2d-EEG was associated with differences in ω-3 LCPUFA status at birth 3) No associations 4) Association with ω-3 LCPUFA status at 0 & 4 wks and maternal DHA-intake.

Table 1. Overview of randomized trials on the visual and cognitive effects in children after maternal fish oil-supplementation during pregnancy and/or lactation.

Study	a) Experimental supplement b) Control oil c) Period of supplementation	DHA in breast-milk (FA%)	Performed assessments (n in all random groups)	Observed group-differences (relative to control)	Description of observed associations with DHA in breast-milk or RBC
Supplementation in lactation					
Makrides *et al.*, 1996 Gibson *et al.*, 1997	a) 0-1.3 g/d DHA (algal oil) b) None used c) Day 5 to 12 wks post partum	a) 0.21-1.13 at 12 wks	1) Infant plasma & RBC at 12 wks (52) 2) VEP at 12 & 16 wks (26, 36) 3) BSID at 1 & 2 y (51,49)	1) RBC-DHA increased by >70% 2) No differences	1) Asymptotic dose-response with milk-DHA 2) No association with milk or RBC-DHA 3) MDI positively associated with milk & RBC-DHA at 1y, but not at 2 y.
Jensen *et al.*, 2005	a) 200 mg/d DHA (algal oil) b) Soy/Corn oil (50:50) c) Day 5 to 4 mo post-partum	a) 0.35 b) 0.20 at 4 mo	1) Infant plasma PL at 4 mo (159) 2) Teller at 4 & 8 mo & SWEEP-VEP at 4 mo (147,147, 160) 3) t-VEP at 4 & 8 mo (168, 153) 4) CAT, CLAMS, DQ at 12 & 30 mo (165, 147) 5) BSID at 30 mo (133)	1) PL-DHA increased by 35% 2) No differences 3) Lower amplitude 4) No differences 5) No difference in MDI, but higher PDI	2-5) No significant correlation with any visual or neuro-developmental outcome and infant PL-DHA at 4 mo
Lauritzen *et al* 2004 & 2005	a) 1.3 g/d ω-3 LCP-UFA (functional foods) b) Olive oil c) 1 wk to 4 mo post-partum	a) 1.34 b) 0.41 at 4 mo	1) Infant RBC at 4 mo (78) 2) SWEEP-VEP at 2 & 4 mo (88, 97) 3) PS at 9 mo (86) 4) CDI at 1 and 2 y (89, 71) 5) Follow-up with blood pressure, immune function and body composition at 2.5 y	1) RBC-DHA increased by 40% 2) No differences 3) Higher score in girls 4) Lower 1y-comprehension, mostly in boys and less sentence complexity in boys at 2y	1) Asymptotic dose-response with milk-DHA 2) Association between visual acuity and RBC-DHA at 4 mo 3) No association with RBC-DHA at 4 mo 4) Active vocabulary at 1 y adversely associated with RBC-DHA at 4 mo, but no asscociattions at 2y

Table 1. (continued)

BSID: Bayley Scales of Infant Development, **MDI**: Mental Developmental Index, **PDI**: Psychomotor Development Index, **CAT**: Clinical Adaptive Test, **CLAMS**: Clinical Linguistic and Auditory Milestone Scale, **DQ**: Developmental Quotient on Gesell Scale Gross Motor scale, **ERG**: Electroretinogram, **K-ABC**: Kaufman Assessment Battery for Children, **NS**: Not significant, **PL**: Phospholipids, **PS**: Problem solving assessed by the Infant Planning Test, **RBC**: Erythrocyte, **SWEEP-VEP**: Swept visual evoked potential assessment of visual acuity, **t-VEP**: Transient visual evoked potential assessment of visual acuity, latency or amplitude.

In one of their trials Birch and her co-workers found that children who during the intervention from 0-4 months of life had received standard unsupplemented formula still had poorer visual acuity at 12 month (Hoffman *et al.*, 2000) and 4 years of age (Birch *et al.*, 2007), whereas Auestad *et al.* and her co-workers found no effect on visual acuity neither in infancy nor at 3½ years of age (Auestad *et al.*, 2003). In the largest long-term randomized trial so far, no effect of formula LCPUFA-supplementation was observed on the proportion of children with low visual acuity at 4-6 years of age (Singhal *et al.*, 2007). Many of the trials have measured visual acuity at different ages during the intervention period, typically at 2, 4, 6, 9 and 12 months. The results from these studies appear to separate into two types. Some studies (e.g. (Carlson *et al.*, 1993)) and two meta-analyses (SanGiovanni *et al.*, 2000a; SanGiovanni *et al.*, 2000b) indicate that the unsupplemented children catch up, since significant differences are generally observed at 2 and 4 months, but not at older ages. Other studies show a more permanent effect, with persistent differences in visual development between the supplemented and unsupplemented groups, or even increased differences over time (e.g. (Birch *et al.*, 2007)). The meta-analysis of term infant trials indicate that the ability to detect differences in visual acuity may depend on the method of visual acuity assessment (SanGiovanni *et al.*, 2000a). It has been suggested that the differences are explained by differences in sensitivity of the visual acuity test or by differences in the amount of DHA included in the experimental formula (Cheatham *et al.*, 2006). However, in our opinion the observed inconsistencies in the persistency of the effect are not explained by gestational age at birth, DHA-dose or visual acuity test-type, as there is no systematic difference in these variables between the studies that found an effect of DHA and those that did not. However, the inconsistent results could be due to other aspects of testing. In our experience, the children are harder to test with the SWEEP-VEP method when they are older than 8 months of age, resulting in noise and improper measurements, probably because the children are impatient and easier distracted during the test at this age. Therefore, we propose that the lack of a persistent effects of DHA despite continued DHA supplementation could be due to testing-noise. Even if the effect of DHA on visual acuity should prove to be truly transient, two studies report that other aspects of visual function later in childhood may depend on infant nutrition (Singhal *et al.*, 2007; Williams *et al.*, 2001). Both of these studies found that breast-feeding (Singhal *et al.*, 2007) was associated with a greater likelihood of achieving a mature foveal stereoacuity at 3-6 years of age (Singhal *et al.*, 2007; Williams *et al.*, 2001). Furthermore, one of these studies found that children whose mothers ate oily fish during pregnancy were also more likely to have a high degree of stereoacuity at 3½ years of age (Williams *et al.*, 2001), whereas the other study observed no effect of formula LCPUFA-supplementation on foveal stereoacuity (Singhal *et al.*, 2007). Even if the visual effect turns out not to be persistent, an accelerated maturation of visual acuity could maybe affect maturation of higher cognitive functions or it could reflect general differences in the information processing that may also affect other CNS functions.

ω-3 PUFA and cognitive function

Apart from visual function, the intake of ω-3 LCPUFA in the first year of life is also believed to affect the cognitive development of the infants. In a meta-analysis, breast-feeding was shown to confer a long-term advantage in cognitive performance of approximately 3 IQ-points relative to formula-feeding (Anderson *et al.*, 1999). The effect in preterm infants was much stronger than in term infants, supporting that the effect is caused

by ω-3 LCPUFA. In a recent WHO meta-analysis the effect was 4.9 IQ points (Horta *et al.*, 2007). One study showed that breast-feeding also had an effect of the same magnitude in young adults (Mortensen *et al.*, 2002). An effect of breast-feeding of 3-5 IQ-points is not large compared to the effect of genetic and social conditions, but a shift in IQ in of this magnitude in a population may have a large impact on the number of disadvantaged children. Breast-feeding is a choice of the mother and is also determined by socioeconomic factors. These must be controlled for before we can conclude if the milk itself has causal effects on cognitive function. Proper adjustment for confounding requires collection of information on many potential confounding factors, including parental IQ and measures of parenting skills and the home environment. In a recent analysis of a large cohort from the US, which included sibling pair analysis and control for maternal IQ, the conclusion was that there was no effect of breast-feeding on IQ (Der *et al.*, 2006). However, mean duration of breast-feeding was only 3 months and more than half of the children were not breast-fed. Furthermore, it was based on a US population, where breast-milk DHA levels are likely to be sub-optimal (Brenna *et al.*, 2007). Thus, although there is a plausible mechanism for an effect of breast-feeding on cognitive development through breast-milk DHA content, there is still a discussion about the potential influence of residual confounding by factors such as the quality of parental care, which goes along with a healthy diet.

Epidemiological studies have described positive associations between maternal intake of marine foods and verbal IQ measured at 8 years of age (Hibbeln *et al.*, 2007). However, permanent adverse associations with brain function have also been described, especially in countries with a high intake of marine mammals with high levels of methyl-mercury (Debes *et al.*, 2006; Gochfeld and Burger, 2005). Interestingly, in populations exposed only through fish intake, similar levels of maternal methyl-mercury exposure are not associated with adverse outcomes (Davidson *et al.*, 2006). Benefits from fish consumption are confounded by socioeconomic variables and by the replacement of other foods that could potentially influence the outcome. Thus, additional studies with better dose-construction and preferably bio-monitoring of both ω-3 LCPUFA-status and neurotoxin exposure are needed in order to evaluate the composite benefit-risk dose-curve of fish on infant CNS development (Gochfeld and Burger, 2005). Furthermore, differences caused by breast-feeding *versus* formula-feeding could be confounded by socio-economic factors or other specific components of human milk, rather than being a true effect of ω-3 LCPUFA (McCann and Ames, 2005). Therefore, randomized controlled intervention trials are needed to prove any causal effects of ω-3 LCPUFA.

Some randomized trials support an association between IQ and ω-3 PUFA intake, but the results are diverse. Rather than making definite conclusions, we will therefore exemplify and discuss some of the relevant issues in the field. The two most recent reviews conclude that 1) evidence from several types of studies, particularly animal studies, suggest that changes in brain DHA-concentrations are positively associated with changes in cognitive or behavioral performance (McCann and Ames, 2005) and 2) that randomized trials in preterm formula-fed infants indicate a beneficial effect of LCPUFA on cognitive development, but that further studies are needed to assess the effect in term formula-fed infants (Eilander *et al.*, 2007). Many of the studies investigating the effects of DHA on cognitive function have used tests such as the Griffiths Scales or the Bayley Scales of Infant Development (BSID), which were originally developed to identify children with serious developmental problems (Cheatham *et al.*, 2006). These well-established but very general tests may not be

well-suited for detecting small differences within the normal range of behavior, the development of which in early infancy is characterized by memory and speed of information processing (Cheatham *et al.*, 2006). A couple of studies have investigated the effects of infant DHA-supplementation on specific developmental measures of information processing and detection of novelty (Auestad *et al.*, 2001; O'Connor *et al.*, 2001; Willatts *et al.*, 1998b). By use of a specific problem solving test, the Infant Planning Test, designed for children at exactly the age at which they were tested (Willatts, 1999), Willatts and his coworkers found that infants, who were fed infant formula supplemented with LCPUFA during the first 4 months of life had better overall test-scores at 10 months than those who were given standard formula (Willatts *et al.*, 1998a). In this test the child had to overcome some physical barriers (e.g. remove a cloth) in order to find a toy and the effect was mainly seen in the final step of the test (Willatts *et al.*, 1998a) as the less mature children never made it to this step. It has been suggested that the cognitive effect of dietary DHA may be easier detectable at older ages, when cognitive tests are more sensitive and reliable (e.g. by (Eilander *et al.* 2007)). One of the term trials on LCPUFA-supplemented infant formulas found increased performance in the Wechsler Preschool and Primary Scale of Intelligence-test at a long-term follow-up at 4 years of age (Birch *et al.*, 2007).

Six of the seven randomized trials with ω-3 LCPUFA during pregnancy and/or lactation tested the cognitive effects in the children (Table 1). Most of the maternal fish oil-supplementation trials report no clear advantage to infant mental development during the first two years of life, but some of the studies found positive associations between DHA in breast-milk and neuro-developmental outcomes, mirroring the results of observational studies (Innis, 2007b). Our maternal fish oil-supplementation trial showed better problem solving at 9 months in the intervention group, but only in girls (Lauritzen *et al.*, 2005). In the lactation trial by Gibson's group, they found an associations between RBC and milk DHA-levels and mental function, measured by global tests of infant development, although this was only observed at 1 year and not at 2 years of age (Gibson *et al.*, 1996). Jensen *et al.* found a positive effect of a low dose of DHA during lactation on the psychomotorical score on the Bayley scale at 1½ years of age (Jensen *et al.*, 2005) and a recent maternal supplementation-trial found an improvement of Infant Planning Test scores in infants 9 months after the pregnant mothers had ingested DHA-enriched functional foods (Judge *et al.*, 2007b). Data from a Norwegian trial with cod liver oil-supplementation during pregnancy and lactation showed that the children from the cod liver oil-group did significantly better in the over-all score when they examined their problem solving abilities at 4 years of age (Helland *et al.*, 2003). They used the K-ABC test, which consists of a number of tasks on sequential problem solving, simultaneous problem solving and non-verbal abilities. In contrast, results from a Dutch observational study do not provide evidence for a positive association between LCPUFA-status at birth or at 7 years (Bakker *et al.*, 2003) and cognitive performance at 7 years of age, but showed a significant negative correlation between perinatal DHA status and internalizing problem behavior (Krabbendam *et al.*, 2007).

One of the highly discussed issues is the timing of the "open-window"-period, *i.e.* what stage in infant or fetal life is most vulnerable to effects of deficiencies in the DHA-supply (Eilander *et al.*, 2007). However, most of the positive results on cognitive development so far are observed in post-partum maternal supplementation trials (Eilander *et al.*, 2007). This is in contrast to the general belief that the earlier during development, the more sensitive is the brain. This belief is based on the assump-

tion that DHA is affecting the formation of nerves and synapses early in fetal life, and that once the CNS is established, the effect is limited. It could, however, be hypothesized that early post-natal life is a more vulnerable period, because although the relative growth of the fetus is large in pregnancy, the absolute amount of DHA that is accreted in the brain is much larger during the first months after birth. Randomized trials with follow-on formulas or DHA-enriched baby foods in term breast-fed infants after weaning have found positive effects on visual acuity (Birch *et al.*, 2002; Hoffman *et al.*, 2004). This suggests that the critical period, in which the dietary DHA-supply can affect the maturation of cortical function, extends beyond fetal life and the early lactation period. Furthermore, a combined analysis of some of the LCPUFA-trials has demonstrated that the duration of the LCPUFA-supplementation period was positively associated with improvements in visual acuity (Morale *et al.*, 2005). Thus, it is likely that CNS function is affected by the LCPUFA-intake throughout the first year of life (Morale *et al.*, 2005).

Some of the studies on the effect of early LCPUFA-intake and infant cognitive function indicate possible negative effects of ω-3 LCPUFA, e.g. on language development. However, the interpretation of these results is complicated by the lack of detailed knowledge on infant cognitive development and the long-term predictive role of the employed early tests on later cognitive development (Cheatham *et al.*, 2006). Our maternal supplementation trial showed a negative effect of fish oil on language development at the follow-up examinations at 1 and 2 years of age (Lauritzen *et al.*, 2005). Language development was examined with MacArthurs parent-assessed Communicatory Development Inventory (CDI)-questionnaire, in which the parents report the number of words that the child is able to understand and say. The active and passive vocabulary at 1 year was found to be lower in the children from the fish oil-supplemented group and at 2 years of age there was still a negative effect in boys, who are known to mature at a slower rate than girls. A similar inverse relationship between ω-3 LCPUFA and language acquisition assessed by the CDI-questionnaire was shown in an observational study and in a randomized trial in preterm formula-fed infants (Auestad *et al.*, 2003; O'Connor *et al.*, 2001; Scott *et al.*, 1998). This has been taken as adverse effects, but we and others have found slower language development in children of parents with higher education (Lauritzen *et al.*, 2005), who are likely to have better verbal and educational skills later in life. Fast language development may intuitively be interpreted as a sign of high cognitive function, but it is also possible that a slower language development reflects a different state of mind – e.g. that the children so to speak "think before they speak".

The point is that there are many things we do not know about infant cognitive and verbal development. John Colombo's group found that the attention-score was differently related to the DHA-content in umbilical cord blood (a measure of the DHA-status at birth) at two different ages (Colombo *et al.*, 2004). In the their test the child was given a complicated electronic toy with wheels, buttons, sounds etc. and the investigator assesses the time the child spends looking at the toy. Those with high DHA-status paid less attention to the toy than those with low DHA at 12 months, whereas it was opposite at 18 months, resulting in a significant age×DHA-effect. From other tests the group could show that long duration of attention at 12 months was positively associated with less mature behavior and reflects low processing efficiency, whereas it at 18 months was associated with more mature behavior and reflected the ability to stay focused. These results indicate that effects on cognitive function manifest differently at different ages. Colombo speculated that the effect of e.g. DHA could be evident only on the function that is most demanding to the infant at that spe-

cific age– i.e. staying awake, holding attention and IQ later in childhood (Wainwright and Colombo, 2006).

ω-3 PUFA and other central nervous system effects

The CNS regulates many functions in the body *via* the autonomic nervous system. Thus, apart from direct mental effects, changes in the DHA-content in the brain could also influence other types of behaviors and body functions, such as the development of blood pressure control. It has been speculated that perinatal ω-3 PUFA intake may have a programming effect, reducing the risk of cardiovascular diseases in later life (Das, 2003). Compared with formula-feeding breast-feeding has been shown in two large systematic reviews and meta-analyses to be associated with a 1.2-1.4 mm Hg lower systolic blood pressure later in life (Horta *et al.*, 2007; Martin *et al.*, 2005). Moreover, in a unique study, Singhal *et al.* randomized preterm infants to either banked breast-milk or preterm infant formula and found that blood pressure at 13-16 years of age was negatively associated with the intake of breast-milk (Singhal *et al.*, 2001). This latter study indicates an effect of specific components in the milk rather than confounding factors often related to voluntary breast-feeding. Not surprisingly only a few randomized trials have examined long-term programming effects of such specific breast-milk components in humans. However, an early intake of ω-3 PUFA has been shown to affect blood pressure later in life in both LCPUFA-supplemented formula-fed infants (Forsyth *et al.*, 2003) and ω-3 PUFA-deficient rats (Armitage *et al.*, 2003). Forsyth *et al.* found that infants receiving dietary supplementation with LCPUFA added to infant formula had lower blood pressure at 6 years of age than those who had been given the standard control formula (Forsyth *et al.*, 2003). This is in accordance with data from the rat study, showing permanently increased blood pressure in offspring that had been exposed to ω-3 PUFA-deficiency during early life (Armitage *et al.*, 2003; Weisinger *et al.*, 2001). The control-formula group in Forsyth's study may also have been ω-3 PUFA-deficient, as the formula did not contain the currently recommended 2 FA% LNA and had a very high ω-6/ω-3 PUFA-ratio (>16:1) (The Commission of the European Communities, 2006). We do not know whether the blood-pressure lowering effect in that study was caused by ω-3 LCPUFA, since the formula in Forsyth's study contained both DHA and arachidonic acid (20:4ω-6). Our maternal fish oil-supplementation trial did not show any convincing effects on blood pressure at 2½ years (Larnkjær *et al.*, 2006) but we did find a tendency towards an effect on heart rate (Larnkjær *et al.*, 2006). Furthermore, we recently found an acute blood-pressure lowering effect of 3 months fish-oil supplementation in healthy infants at 12 months of age (Damsgaard *et al.*, 2006). As with the effects of ω-3 LCPUFA on cognitive functions, the evidence on blood pressure shows many interesting indications, but we need more randomized controlled trials in order to make firm conclusions about the long-term effects of ω-3 LCPUFA on blood pressure regulation.

Despite the lack of firm evidence, plausible mechanisms exist. Programming depends on some sort of imprinting or memory mechanism and it is most likely that long-term vascular effects would be mediated via the development and regulation of the autonomous nervous system. Changes in the hypothalamic-pituitary-adrenal axis have been suggested as a possible mechanism of early programming (Wintour *et al.*, 2003). Pharmacological studies in preterm infants have shown that antenatal treatment with glucocorticoids such as β-methasone may persistently influence the hypothalamic-pituitary-adrenal regulation (Davis *et al.*, 2006). An effect of ω-3 PUFA on the au-

tonomic control of heart rhythm, blood pressure and other physiological functions may therefore provide a common link between possible effects on later health and on the CNS development. The observed long-term effect of ω-3 LCPUFA-supplementation on blood pressure (Forsyth *et al.*, 2003) and heart rate (Larnkjær *et al.*, 2006) could be interpreted as an indicator of accelerated CNS maturation. Exactly how DHA affects CNS functions remains to be elucidated, but a number of potential mechanisms are supported by data from *in vitro* studies and animal experiments (Innis, 2007b).

Conclusions

In summary, early intake of ω-3 LCPUFA from human milk and infant formula has been shown to affect DHA accretion in the tissues, but it is presently unclear to what extent neural function is affected by tissue composition and what the optimal levels of DHA in various tissues are. Human milk DHA-content depends on maternal diet, but often provides a better supply of ω-3 PUFA than formulas. Formula-fed infants have been shown to have poorer visual acuity than breast-fed infants and the visual acuity in formula-fed infants has been shown to be associated with the overall ω-3 PUFA intake. Dietary LNA appears to correspond to 1/10 of preformed DHA and the optimal intake in preterm and term infants may be around 100 mg/d DHA. The potential long-term implications for visual function are unknown, but other CNS functions may be affected. Breast-feeding is associated with a cognitive advantage relative to formula. However, both observational studies relating maternal fish intake with child IQ, randomized studies investigating cognitive effects of DHA-enriched formula, and studies with ω-3 LCPUFA in pregnancy/lactation are inconclusive with regard to the mental benefits for the infants. Changes in CNS ω-3 LCPUFA may also influence behavior and physiological function, such as blood pressure. Some studies indicate possible negative effects, e.g. on language, but we lack randomized trials and knowledge about cognitive development and the predictive value of early tests of cognitive and verbal function and markers of health. Hopefully, the next decade will provide new evidence that will contribute to elucidate the dietary needs for PUFA during optimal growth and development.

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References

Anderson, J. W., B. M. Johnstone, and D. T. Remley. Breast-feeding and cognitive development: a meta-analysis, *Am. J. Clin. Nutr.* **70**, 525-535 (1999).

Armitage, J. A., A. D. Pearce, A. J. Sinclair, A. J. Vingrys, R. S. Weisinger and H. S. Weisinger. Increased blood pressure later in life may be associated with perinatal n-3 fatty acid deficiency, *Lipids* **38,** 459-464 (2003).

Auestad, N., R. Halter, R. T. Hall, M. Blatter, M. L. Bogle, W. Burks, J. R. Erickson, K. M. Fitzgerald, V. Dobson, S. M. Innis, L. T. Singer, M. B. Montalto, J. R. Jacobs, W. Z. Qiu, and M. H. Bornstein. Growth and development in term infants fed long-chain poly-unsaturated fatty acids: A double-masked, randomized, parallel, prospective, multivariate study, *Pediatrics* **108**, 372-381 (2001).

Auestad, N., D. T. Scott, J. S. Janowsky, C. Jacobsen, R. E. Carroll, M. B. Montalto, R. Halter, W. Z. Qiu, J. R. Jacobs, W. E. Connor, S. L. Connor, J. A. Taylor, M. Neuringer, K. M. Fitzgerald, and R. T. Hall. Visual, cognitive, and language assessments at 39 months: A follow-up study of children fed formulas containing long-chain poly-unsaturated fatty acids to 1 year of age, *Pediatrics* **112**, E177-E183 (2003).

Bakker, E. C., A. J. A. Ghys, A. D. M. Kester, J. S. H. Vles, J. .

Dubas, C. E. Blanco, and G. Hornstra. Long-chain polyunsaturated fatty acids at birth and cognitive function at 7y of age, *Eur. J. Clin. Nutr.* **57**, 89-95 (2003).

Birch, E. E., D. G. Birch, D. Hoffman, L. Hale, M. Everett, and R. D. Uauy. Breast-feeding and optimal visual development, *J. Ophthalmol. Strabismus* **30**, 33-38 (1993).

Birch, E. E., D. G. Birch, D. R. Hoffman and R. Uauy. Dietary essential fatty acid supply and visual development, *Invest. Ophthalmol. Visual Sci.* **33**, 3242-3253 (1992).

Birch, E. E., S. Garfield, Y. Castaneda, D. Hughbanks-Wheaton, R. Uauy, and D. Hoffman. Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain poly-unsaturated fatty acid-supplemented infant formula, *Early Hum. Dev.* **83**, 279-284 (2007).

Birch, E. E., S. Garfield, D. R. Hoffman, R. Uauy, and D. G. Birch. A randomized controlled trial of early dietary supply of long- chain poly-unsaturated fatty acids and mental development in term infants, *Dev. Med. Child Neurol.* **42**, 174-181. (2000).

Birch, E. E., D. R. Hoffman, Y. S. Castaneda, S. L. Fawcett, D. G. Birch, and R. D. Uauy. A randomized controlled trial of long-chain poly-unsaturated fatty acid supplementation of formula in term infants after weaning at 6 wk of age, *Am. J. Clin. Nutr.* **75**, 570-580 (2002).

Birch, E. E., Y. S. Castaneda, D. H. Wheaton, D. G. Birch, R. D. Uauy, and D. R. Hoffman. Visual maturation of term infants fed long-chain poly-unsaturated fatty acid-supplemented or control formula for 12 mo, *Am. J. Clin. Nutr.* **81**, 871-879 (2005).

Brenna, J. T., B. Varamini, R. G. Jensen, D. A. Diersen-Schade, J. A. Boettcher, and L. M. Arterburn. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide, *Am. J. Clin. Nutr.* **85**, 1457-1464 (2007).

Carlson, S. E., R. J. Cooke, P. G. Rhodes, J. M. Peeples, S. H. Werkman, and E. A. Tolley. Long-term feeding of formulas high in linolenic acid and marine oil to very low birth weight infants: phospholipid fatty acids, *Pediatr. Res.* **30**, 404-412 (1991).

Carlson, S. E., A. J. Ford, S. H. Werkman, J. M. Peeples, and W. W. Koo. Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin, *Pediatr. Res.* **39**, 882-888 (1996).

Carlson, S. E., S. H. Werkman, P. G. Rhodes, and E. A.Tolley. Visual acuity development in healthy preterm infants: effect of marine-oil supplementation, *Am. J. Clin. Nutr.* **58**, 35-42 (1993).

Cheatham, C. L., J. Colombo, and S. E. Carlson. n-3 fatty acids and cognitive and visual acuity development: methodologic and conceptual considerations, *Am. J. Clin. Nutr.* **83**, 1458S-1466S (2006).

Cockburn, F. Fatty acids in early development, *Prenat. Neonat. Med.* **2**, 108-115 (1997).

Colombo, J., K. N. Kannass, D. J. Shaddy, S. Kundurthi, J. M. Maikranz, C. J. Anderson, O. M. Blaga, and S. E. Carlson. Maternal DHA and the development of attention in infancy and toddlerhood, *Child Develop.* **75**, 1254-1267 (2004).

Cunnane, S. C., V. Francescutti, J. T. Brenna, and M. A. Crawford. Breasts-fed infants achieve a higher rate of brain and whole body docosahexaenoate accumulation than formula-fed infants not consuming dietary docosahexaenoate, *Lipids* **35**, 105-111 (2000).

Cunnane, S. C., C. R. Menard, S. S. Likhodii, J. T. Brenna, and M. A. Crawford. Carbon recycling into de novo lipogenesis is a major pathway in neonatal metabolism of linoleate and alpha-linolenate, *Prostagland. Leuk. Essent. Fatty Acids* **60**, 387-392 (1999).

Damsgaard, C. T., L. Schack-Nielsen, K. F. Michaelsen, M. B. Fruekilde, O. Hels, and L. Lauritzen. Fish Oil Affects Blood Pressure and the Plasma Lipid Profile in Healthy Danish Infants, *J. Nutr.* **136**, 94-99 (2006).

Das, U. N. A perinatal strategy to prevent coronary heart disease, *Nutrition* **19**, 1022-1027 (2003).

Davidson, P. W., G. J. Myers, B. Weiss, C. F. Shamlaye, and C. Cox. Prenatal methyl mercury exposure from fish consumption and child development: A review of evidence and perspectives from the Seychelles Child Development Study, *Neurotoxicology* **27**, 1106-1109 (2006).

Davis, E. P., E. L. Townsend, M. R. Gunnar, S. F. Guiang, R. C. Lussky, R. F. Cifuentes, and M.K. Georgieff. Antenatal betamethasone treatment has a persisting influence on infant HPA axis regulation, *J. Perinatol.* **26**, 147-153 (2006).

Debes, F., E. Budtz-Jorgensen, P. Weihe, R. F. White, and P. Grandjean. Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years, *Neurotoxicol. Teratol.* **28,** 536-547 (2006).

Der, G., G. D. Batty, and I. J. Deary. Effect of breast feeding on. intelligence in children: prospective study, sibling pairs analysis, and meta-analysis, *Br. Med. J.* **333,** 945-948A (2006).

Eilander, A., D. C. Hundscheid, S. J. Osendarp, C. Transler, and P. L. Zock. Effects of n-3 long chain poly-unsaturated fatty acid supplementation on visual and cognitive development throughout childhood: A review of human studies, *Prostagland. Leuk. Essent. Fatty Acids* **76**, 189-203 (2007).

Faldella, G., M. Govoni, R. Alessandroni, E. Marchiani, G. P. Salvioli, P. L. Biagi, and C. Spano. Visual evoked potentials and dietary long chain poly-unsaturated fatty acids in preterm infants, *Arch. Dis. Child. Fetal Neonatal. Ed.* **75,** F108-F112 (1996).

Farquharson, J., F. Cockburn, W. A. Patrick, E. C. Jamieson, and R. W. Logan. Infant cerebral cortex phospholipid fatty-acid composition and diet, *Lancet* **340**, 810-813 (1992).

Farquharson, J., E. C. Jamieson, K. A. Abbasi, W. J. A. Patrick, R. W. Logan, and F. Cockburn. Effect of diet on the fatty acid composition of the major phospholipids of infant cerebral cortex, *Arch. Dis. Child.* **72**, 198-203 (1995).

Fleith, M. and M. T. Clandinin. Dietary PUFA for preterm and term infants: Review of clinical studies, *Crit. Rev. Food Sci. Nutr.* **45,** 205-229 (2005).

Forsyth, J. S., P. Willatts, C. Agostoni, J. Bissenden, P. Casaer, and G. Boehm. Long chain poly-unsaturated fatty acid supplementation in infant formula and blood pressure in later childhood: follow up of a randomised controlled trial, *Br. Med. J.* **326**, 953-936 (2003).

Gibson, R. A., M. A. Neumann, and M. Makrides. Effect of dietary docosahexaenoic acid on brain composition and neural function in term infants, *Lipids* **31** Suppl, S177-S181 (1996).

Gibson, R. A., M. A. Neumann, and M. Makrides. Effect of increasing breast milk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants, *Eur. J. Clin. Nutr.* **51,** 578-584 (1997).

Gochfeld, M. and J. Burger. Good fish/bad fish: A composite benefit-risk by dose curve, *Neurotoxicology* **26**, 511-520 (2005).

Helland, I. B., O. D. Saugstad, L. Smith, K. Saarem, K. Solvoll, T. Ganes, and C. A. Drevon. Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women, *Pediatrics* **108**, U23-U32 (2001).

Helland, I. B., L. Smith, K. Saarem, O. D. Saugstad, and C. A. Drevon. Maternal Supplementation With Very-Long-Chain n-3 Fatty Acids During Pregnancy and Lactation Augments Children's IQ at 4 Years of Age, *Pediatrics* **111**, e39-e44 (2003).

Hibbeln, J. R., J. M. Davis, C. Steer, P. Emmett, I. Rogers, C. Williams, and J. Golding. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study, *Lancet* **369,** 578-585 (2007).

Hoffman, D. R., E. E. Birch, D. G. Birch, R. Uauy, Y. S. Castaneda, M. G. Lapus, and D. H. Wheaton. Impact of early dietary intake and blood lipid composition of long-chain poly-unsaturated fatty acids on later visual development, *J. Pediat. Gastroenterol. Nutr.* **31,** 540-553 (2000).

Hoffman, D. R., R. C. Theuer, Y. S. Castaneda, D. H. Wheaton, R. G. Bosworth, A. R. O'Connor, S. E. Morale, L. E. Wiedemann, and E. E. Birch. Maturation of Visual Acuity Is Accelerated in Breast-Fed Term Infants Fed Baby Food Containing DHA-Enriched Egg Yolk, *J. Nutr.* **134**, 2307-2313 (2004).

Horta, B. L., R. Bahl, J. C. Martines, and C. G. Victora. *Evidence on the long-term effect of breastfeeding. Systematic reviews and meta-analyses.* World Health Organization Report, Geneva, Switzerland (2007).

Innis, S. M. Human milk: maternal dietary lipids and infant development, *Proc. Nutr. Soc.* **66**, 397-404 (2007a).

Innis, S. M. Dietary (n-3) Fatty Acids and Brain Development, *J. Nutr.* **137**, 855-859 (2007b).

Innis, S. M., D. H. Adamkin, R. T. Hall, S. C. Kalhan, C. Lair, M. Lim, D. C. Stevens, P. F. Twist, D. A. Dieresen-Schade, C. L. Harris, K. L. Merkel, and J. W. Hansen. Docosahexaenoic acid and arachidonic acid enhance growth with no adverse effects in preterm infants fed formula, *J. Pediatr.*

140, 547-554 (2002).

Innis, S. M., S. S. Akrabawi, D. A. DiersenSchade, M. V. Dobson, and D. G. Guy. Visual acuity and blood lipids in term infants fed human milk or formulae, *Lipids* **32,** 63-72 (1997).

Innis, S. M., J. Gilley, and J. Werker. Are human milk long-chain poly-unsaturated fatty acids related to visual and neural development in breast-fed term infants? *J. Pediatr.* **139**, 532-538 (2001).

Jamieson, E. C., J. Farquharson, R. W. Logan, A. G. Howatson, W. J. A. Patrick, L. T. Weaver, and F. Cockburn. Infant cerebellar gray and white matter fatty acids in relation to age and diet, *Lipids* **34**, 1065-1071 (1999).

Jensen, C. L., R. G. Voigt, T. C. Prager, Y. L. Zou, J. K. Fraley, J. C. Rozelle, M. R. Turcich, A. M. Llorente, R. E. Anderson, and W. C. Heird. Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants, *Am. J. Clin. Nutr.* **82**, 125-132 (2005).

Jørgensen, M. H., O. Hernell, E. Hughes, and K. F. Michaelsen. Is there a relation between docosahexaenoic acid concentration in mother's milk and visual development in term infants? *J. Pediat. Gastroenterol. Nutr.* **32**, 293-296 (2001).

Judge, M. P., O. Harel, and C. J. Lammi-Keefe. A docosahexaenoic acid-functional food during pregnancy benefits infant visual acuity at four but not six months of age, *Lipids* **42**, 117-122 (2007a).

Judge, M. P., O. Harel, and C. J. Lammi-Keefe. Maternal consumption of a docosahexaenoic acid-containing functional food during pregnancy: benefit for infant performance on problem-solving but not on recognition memory tasks at age 9 mo, *Am. J. Clin. Nutr.* **85**, 1572-1577 (2007b).

Koletzko, B. and M. Rodriguez-Palmero. Poly-unsaturated fatty acids in human milk and their role in early infant development, *J. Mammary Gland Biol. Neoplasia* **4,** 269-284 (1999).

Krabbendam, L., E. Bakker, G. Hornstra, and J. van Os. Relationship between DHA status at birth and child problem behaviour at 7 years of age, *Prostagland. Leuk. Essent. Fatty Acids* **76,** 29-34 (2007).

Larnkjær, A., J. H. Christensen, K. F. Michaelsen, and L. Lauritzen. Blood pressure and heart rate variability in children 2 years after fish oil supplementation of their lactating mothers, *J. Nutr.* **136**, 1539-1544 (2006).

Lauritzen, L., H. S. Hansen, M. H. Jørgensen, and K. F. Michaelsen. The essentiality of long-chain *n*-3 fatty acids in relation to development and function of the brain and retina, *Prog. Lipid Res.* **40**, 1-94 (2001).

Lauritzen, L., M. H. Jørgensen, and K. F. Michaelsen. Test-retest reliability of swept visual evoked potential measurements of infant visual acuity and constrast sensitivity, *Pediat. Res.* **55**, 701-708 (2004a).

Lauritzen, L., M. H. Jørgensen, T. B. Mikkelsen, I. M. Skovgaard, E. M. Straarup, S. F. Olsen, C. E. Høy, and K. F. Michaelsen. Maternal fish oil supplementation in lactation: Effect on visual acuity and *n*-3 fatty acid content of infant erythrocytes, *Lipids* **39**, 195-206 (2004b).

Lauritzen, L., M. H. Jørgensen S. F. Olsen, E. M. Straarup, and K. F. Michaelsen. Maternal fish oil supplementation in lactation: effect on developmental outcome in breast-fed infants, *Reprod. Nutr. Dev.* **45**, 535-547 (2005).

Makrides, M., M. A. Neumann, R. W. Byard, K. Simmer, and R. A. Gibson. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants, *Am. J. Clin. Nutr.* **60,** 189-194 (1994).

Makrides, M., M. A. Neumann, and R. A. Gibson. Effect of maternal docosahexaenoic acid (DHA) supplementation on breast-milk composition, *Eur. J. Clin. Nutr.* **50,** 352-357 (1996).

Makrides, M., K. Simmer, M. Goggin, and R. A. Gibson. Erythrocyte docosahexaenoic acid correlates with the visual response of healthy, term infants, *Pediat. Res.* **33**, 425-427 (1993).

Malcolm, C. A., D. L. McCulloch, C. Montgomery, A. Shepherd, and L. T. Weaver. Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: a double blind, prospective, randomised trial, *Arch. Dis. Child. Fetal Neonat. Ed.* **88**, F383-F390 (2003a).

Malcolm, C.A., R. Hamilton, D. L. McCulloch, C. Montgomery, and L. T. Weaver. Scotopic Electroretinogram in Term Infants Born of Mothers Supplemented with Docosahexaenoic Acid during Pregnancy, *Invest. Ophthalmol. Visual Sci.* **44**, 3685-3691 (2003b).

Martin, R. M., D. Gunnell, and G. D. Smith. Breastfeeding in infancy and blood pressure in later life: Systematic review and meta-analysis, *Am. J. Epidemiol.* **161**, 15-26 (2005).

Martinez, M. Tissue levels of poly-unsaturated fatty acids during early human development, *J. Pediatr.* **120**, S129-S138 (1992).

McCann, J. C. and B. N. Ames. Is docosahexaenoic acid, an n-3 long-chain poly-unsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals, *Am. J. Clin. Nutr.* **82**, 281-295 (2005).

Morale, S. E., D.R. Hoffman, Y. S. Castaneda, D. H. Wheaton, R. A. Burns, and E. E. Birch. Duration of long-chain poly-unsaturated fatty acids availability in the diet and visual acuity, *Early Hum. Dev.* **81**, 197-203 (2005).

Mortensen, E. L., K. F. Michaelsen, S. A. Sanders, and J. M. Reinisch. The association between duration of breastfeeding and adult intelligence, *J. Am. Med. Assn.* **287**, 2365-2371 (2002).

Neuringer, M., W. E. Connor,C. van Petten, and L. Barstad. Dietary Omega-3 Fatty Acid Deficiency and Visual Loss in Infant Rhesus Monkeys, *J. Clin. Invest.* **73**, 272-276 (1984).

Neuringer, M., S. Reisbick, and J. Janowsky. The role of n-3 fatty acids in visual and cognitive development: current evidence and methods of assessment, *J. Pediatr.* **125**, S39-S47 (1994).

O'Connor, D. L., R. Hall, D. Adamkin, N. Auestad, M. Castillo, W. E. Connor, S. L. Connor, K. Fitzgerald, S. Groh-Wargo, E. E. Hartmann, J. Jacobs, J. Janowsky, A. Lucas, D. Margeson, P. Mena, M. Neuringer, M. Nesin, L. Singer, T. Stephenson, J. Szabo, and V. Zemon. Growth and development in preterm infants fed long-chain poly-unsaturated fatty acids: A prospective, randomized controlled trial, *Pediatrics* **108**, 359-371 (2001).

SanGiovanni, J. P., C. S. Berkey, J. T. Dwyer, and G. A. Colditz. Dietary essential fatty acids, long-chain poly-unsaturated fatty acids, and visual resolution acuity in healthy fullterm infants: a systematic review, *Early Hum. Dev.* **57**, 165-188 (2000a).

SanGiovanni, J. P., S. Parra-Cabrera, G. A. Colditz, C. S. Berkey, and J. T. Dwyer. Meta-analysis of dietary essential fatty acids and long-chain poly-unsaturated fatty acids as they relate to visual resolution acuity in healthy preterm infants, *Pediatrics* **105**, 1292-1298 (2000b).

Scott, D. T., J. S. Janowsky, R. E. Carroll, J. A. Taylor, N. Auestad, and M. B. Montalto. Formula supplementation with long-chain poly-unsaturated fatty acids: Are there developmental benefits? *Pediatrics* **102**, E591-E593 (1998).

Sheaff Greiner, R. C., J. Winter, P. W. Nathanielsz, and J. T. Brenna. Brain docosahexaenoate accretion in fetal baboons: Bioequivalence of dietary alpha-linolenic and docosahexaenoic acids, *Pediatr. Res.* **42**, 826-834 (1997).

Sheaff Greiner, R. C., Q. Zhang, K. J. Goodman, D. A. Giussani, P. W. Nathanielsz, and J.T. Brenna. Linoleate, alpha-linolenate, and docosahexaenoate recycling into saturated and mono-unsaturated fatty acids is a major pathway in pregnant or lactating adults and fetal or infant rhesus monkeys, *J. Lipid Res.* **37**, 2675-2686 (1996).

Singhal, A., T. J. Cole, and A. Lucas. Early nutrition in preterm infants and later blood pressure: two cohorts after randomised trials, *Lancet* **357**, 413-419 (2001).

Singhal, A., R. Morley, T. J. Cole, K. Kennedy, P. Sonksen, E. Isaacs, M. Fewtrell, A. Elias-Jones, T. Stephenson, and A. Lucas. Infant nutrition and stereoacuity at age 4-6 y, *Am. J. Clin. Nutr.* **85**, 152-159 (2007).

Straarup, E. M., L. Lauritzen, J. Faerk, C. E. Høy, and K. F. Michaelsen. The stereospecific triacylglycerol structures and fatty acid profiles of human milk and infant formulas, *J. Pediat. Gastroenterol. Nutr.* **42**, 293-299 (2006).

Su, H. M., L. Bernardo, M. Mirmiran, X. H. Ma, P. W. Nathanielsz, and J. T. Brenna. Dietary 18:3n-3 and 22:6n-3 as sources of 22:6n-3 accretion in neonatal baboon brain and associated organs, *Lipids* **34**, S347-S350 (1999).

The Commission of the European Communities. Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC, http://eur-lex.europa.eu/JOHtml.do?uri=OJ:L:2006:401:SOM:EN:HTML *Official J. Eur. Union*, **L401 49**, 1-33 (2006).

Tofail, F., Q. Kabir, J. D. Hamadani, F. Chowdhury, S. Yesmin, F. Mehreen, and S. N. Huda. Supplementation of fish-oil and soy-oil during pregnancy and psychomotor development of infants, *J. Health Populat. Nutr.* **24**, 48-56 (2006).

Uauy, R., D. R. Hoffman, P. Mena, A. Llanos, and E. E. Birch. Term infant studies of DHA and ARA supplementation on neurodevelopment: results of randomized controlled trials, *J. Pediatr.* **143**, S17-S25 (2003).

Uauy, R.D., D.G. Birch, E.E. Birch, J. E. Tyson, and D. R. Hoffman. Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-weight neonates, *Pediatr. Res.* **28**, 485-492 (1990).

Wainwright, P. E. and J. Colombo. Nutrition and the development of cognitive functions: interpretation of behavioral studies in animals and human infants, *Am. J. Clin. Nutr.* **84**, 961-970 (2006).

Weisinger, H.S., J.A. Armitage, A.J. Sinclair, A.J. Vingrys, P.L. Burns, and R.S. Weisinger. Perinatal omega-3 fatty acid deficiency affects blood pressure later in life, *Nat. Med.* **7**, 258-259 (2001).

Wezel-Meijler, G., M. S. van der Knaap, J. Huisman, E. J. Jonkman, J. Valk, and H.N. Lafeber. Dietary supplementation of long-chain poly-unsaturated fatty acids in preterm infants: effects on cerebral maturation, *Acta Paediat.* **91,** 942-950 (2002).

Willatts, P. Development of means-end behavior in young infants: Pulling a support to retrieve a distant object, *Develop. Psychol.* **35**, 651-667 (1999).

Willatts, P., J. S. Forsyth, M. K. DiModugno, S. Varma, and M. Colvin. Effect of long-chain poly-unsaturated fatty acids in infant formula on problem solving at 10 months of age, *Lancet* **352**, 688-691 (1998a).

Willatts, P., J. S. Forsyth, M. K. DiModugno, S. Varma, and M. Colvin. Influence of long-chain poly-unsaturated fatty acids on infant cognitive function, *Lipids* **33**, 973-980 (1998b).

Williams, C., E. E. Birch, P. M. Emmett, and K. Northstone. Stereoacuity at age 3.5 y in children born full-term is associated with prenatal and postnatal dietary factors: a report from a population-based cohort study, *Am. J. Clin. Nutr.* **73**, 316-322 (2001).

Wintour, E. M., K. Johnson, I. Koukoulas, K. Moritz, M. Tersteeg, and M. Dodic. Programming the cardiovascular system, kidney and the brain - A review, *Placenta* **24,** S65-S71 (2003).