# Omega 3 fatty acids on child growth, visual acuity and neurodevelopment

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### Abstract

The aim of this review is to evaluate the effects of omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA) supplementation in pregnant and lactating women and infants during postnatal life, on the visual acuity, psychomotor development, mental performance and growth of infants and children. Eighteen publications (11 sets of randomized control clinical trial [RCTs]) assessed the effects of the n-3 LCPUFA supplementation during *pregnancy* on neurodevelopment and growth, in the same subjects at different time points; 4 publications (2 data sets from RCTs) addressed physiological responses to n-3 LCPUFA supplementation during *pregnancy & lactation* and 5 publications (3 data sets from RCTs) exclusively during *lactation*. Some of these studies showed beneficial effects of docosahexaenoic acid (DHA) supplementation during pregnancy and/or lactation especially on visual acuity outcomes and some on long-term neurodevelopment; a few, showed positive effects on growth. There were also 15 RCTs involving term infants who received infant formula supplemented with DHA, which met our selection criteria. Many of these studies claimed a beneficial effect of such supplementation on visual, neural, or developmental outcomes and no effects on growth. Although new well designed and conducted studies are being published, evidence from RCTs does not demonstrate still a clear and consistent benefit of n-3 LCPUFA supplementation during pregnancy and/or lactation on term infants growth, neurodevelopment and visual acuity. These results should be interpreted with caution due to methodological limitations of the included studies.

**Key words: omega-3 fatty acids: pregnancy: lactation: infant formula: term infants: neurodevelopment: visual acuity: growth**

## Introduction

Docosahexaenoic acid (DHA) and arachidonic acid (AA) are important to foetal and infant growth and development. Observational and intervention studies provide consistent evidence that maternal dietary and circulating DHA is an important determinant of foetal blood concentrations of DHA[1–6]. Although linoleic acid (LA) and α-linolenic acid (ALA), and preformed long chain polyunsaturated fatty acids (LCPUFAs), can be transported through the placenta, there is a preferential transfer of the latter forms[6,7]. Several studies have assessed the effect of prenatal LCPUFA dietary supplementation on pregnancy outcome, aiming to improve it—i.e., preventing preeclampsia, prolonging gestation, preventing preterm birth, and improving foetal growth[3,8–9].

On the other hand, the importance of DHA in central nervous system (CNS) development is one of the most intensely studied areas [10–14]. DHA functions in neurogenesis, neurotransmission and protection against oxidative stress[15]. AA is also important for infant growth and development. n-3 and n-6 LCPUFA are critical for infant and child brain development; they are involved in numerous neuronal processes, ranging from effects on membrane fluidity to gene expression regulation[16]. Brain accumulation of DHA starts *in utero*, with quantitatively marked deposition in the second half of gestation[17–19], coinciding with the growth spurt in the grey matter[19]. Deficiencies and imbalances of LCPUFAs are associated with impairments in cognitive and behavioural performance[19–20].

Fish intake during pregnancy and a higher n-3 LCPUFA status at birth were associated with a better visual development in infants born at term[20–22]. However, levels of DHA and eicosapentaenoic acid (EPA) are often low in the Western diet[23–24]. It remains controversial whether LCPUFA supplementation to pregnant and breastfeeding mothers is beneficial for the development of their infants[25–26], optimal doses for efficacy and long-term effects at different developmental ages remain to be determined. Today, there are in excess of 40 perinatal randomized controlled trials involving LCPUFA interventions assessing different aspects of early childhood development and growth[29].

The earliest publications in human infants from the early 1990s showed that preterm infants fed a formula supplemented with n-3 LCPUFA, mainly as DHA, had improved retinal sensitivity and visual acuity compared with preterm

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infants fed the standard un-supplemented formulas of the day, which were low in n-3 PUFA (most were lacking alpha-linolenic acid) and were rich in n-6 PUFA[28−30]. Other intervention studies has also provide evidence that dietary DHA improves visual, mental, and motor skill development in some preterm and term infants fed supplemented formula[31−34]. In a non-randomised observational study, term infants fed breast milk have been found to have more mature visual acuities and correlated to higher erythrocyte DHA levels than those receiving formula[31]. Evidence to suggest that breast-fed infants have a long term IQ advantage over those who have been fed formula has been evident in the literature for many years[35−37]. Moreover, we realize that the majority of comparisons between breast fed and formula-fed infants are confounded by genetic polymorphisms that affect LCP metabolism and socioeconomic factors which affect the outcomes of most studies[38−40].

The present review was undertaken to systematically assess the evidence of short and long-term effects of n-3 LCPUFA supplementation during pregnancy and/or postnatal life on the visual acuity, psychomotor development, mental performance and growth of children.

## Methods

### Criteria for considering studies for this review (Table 1)

*Types of interventions: inclusion criteria.* All relevant RCTs with LCPUFA intervention in healthy pregnant women,

lactating mothers and healthy full-term infants ($\geq 37$ weeks gestation at birth) were elegible for inclusion.From the randomised control trials RCTs or quasi-RCTs found in literature, only the ones designed to study the effects of n-3 LCPUFA supplementation on any standardized measures of growth, psychomotor development, mental performance and visual acuity in the offspring were selected. A trial was defined as quasi-random if the method used to allocate study pregnant women or infants to the study group was either not statistically random or was not clearly stated.

Interventions during pregnancy, lactation and with infant formula for full-term infants with DHA plus AA or DHA alone were included to be compared to those unsupplemented or receiving placebo during pregnancy or postnatally.

LCPUFA supplements could be from any source including fish oil, egg triglycerides or fungal oils. Trials in which precursor essential FAs (α-linolenic and linoleic acids) were used in the intervention group were not included, because intake of the precursors is far less effective with respect to LCPUFA deposition in fetal brain.

Studies involving prenatal and lactating mothers supplementation were assessed independently from studies with postnatal supplementation of infant formula.

The selection of interventions with infant formula in full-term infants had the following criteria: 1) Study formula was commenced within two weeks after birth; 2) Study formula was the only source of milk from the time of randomisation until at least 8 weeks of age; 3) A minimum of three months follow-up data on clinical outcomes of interest was available.

*Types of interventions: exclusion criteria.* Trials in women with high-risk pregnancies were not included, with high-risk pregnancy being defined as one in which a condition places the mother, the developing foetus, or both at higher-than-normal risk for complications during or after pregnancy and birth (e.g., a preterm delivery during an earlier pregnancy, intrauterine growth retardation, pregnancy-induced hypertension, or multiparity).

We excluded studies if the title and abstract were not relevant; however, we obtained articles for all potentially relevant studies if the abstract contained insufficient information to warrant exclusion. All areas of disagreement were discussed by the reviewers to achieve a consensus, and taken also into account the quality assessment.

Trials which used breast milk in addition to study formula during the first 8 weeks of life were not included to address the effect of early intervention with LCPUFA in formula-fed term infants. Trials reporting on only biochemical outcomes were also excluded.

*Types of outcome measures.* Visual acuity: measured either using Teller cards, visual evoked potentials (VEP), electroretinography (ERG), electroencephalography (EEG) and stereoacuity. Neurodevelopmental outcomes: General Quotient, Intelligent Quotient, Psychomotor Scores, Behavior and other measures of Cognitive Functions. Growth: weight, length, head circumference and/or BMI.

**Table 1.** Inclusion and exclusion criteria established for the systematic review

| Inclusion criteria | – RCTs designed to study the effects of n-3 LCPUFA supplementation on growth, visual acuity and neurodevelopment |
| --- | --- |
| | – Studies involving supplementation with DHA plus AA or DHA alone in healthy pregnant women |
| | – Studies involving supplementation with DHA plus AA or DHA alone in healthy lactating mothers |
| | – Studies involving supplementation with DHA plus AA or DHA alone of infant formula in healthy full-term infants ($\geq 37$ weeks gestation at birth) |
| | – Study formula commenced within two weeks after birth |
| | – Study formula as only source of milk from the time of randomisation until at least 8 weeks of age |
| | – A minimum of three months follow-up data available on clinical outcomes of interest. |
| Exclusion criteria | – Trials in women with high-risk pregnancies |
| | – Trials in which precursor essential FAs (α-linolenic and linoleic acids) were used in the intervention group. |
| | – Title or abstract not relevant |
| | – Trials which used breast milk in addition to study formula during the first 8 weeks of life |
| | – Trials reporting on only biochemical outcomes |
| | – Certain publication types (Letters to the editor, abstracts and proceedings from scientific meetings) |

RCTs: Randomized clinical trials; DHA: Docosahexaenoic acid; AA: Arachidonic Acid; FAs: Fatty acids.

## Search methods for identification of studies

The search strategy included the use of a validated filter for identifying RCTs[41] which was combined with a topic specific strategy using the following PubMed's MeSH terms: (FA *OR* omega-6 *OR* omega-3 *OR* n-6 *OR* n-3 *OR* eicosapentaenoic acid *OR* EPA *or* docosahexaenoic acid *OR* DHA *OR* arachidonic acid *OR* AA *OR* LCPUFA *OR* long-chain FA *OR* essential FA *OR* fish oil) *AND* (mother supplementation *OR* pregnancy *OR* lactation *OR* breastfeeding *OR* infant formula) AND (Infant *OR* Newborn *OR* Neonate) *AND* (growth *OR* weight *OR* length *OR* height *OR* head circumference) AND (cognitive function OR development *OR* neurodevelopment *OR* psychomotor development *OR* memory *OR* attention *OR* language *OR* intelligence OR cognition OR visual function *OR* visual acuity *OR* electroretinogram *OR* visual evoked potentials *OR* VEP *OR* behaviour *OR* neurobehaviour) AND (Clinical Trial *OR* Randomised Controlled Trial *OR* Review). The reference lists of identified studies and key review articles, including previously published reviews, as well as, major epidemiological studies and position papers, were also searched for all studies that assessed the effects of n-3 LCPUFA supplementation on child growth, neurodevelopment and/or visual function. We performed a computerized literature search of MEDLINE (from 1966 to April 2011), EMBASE (from 1980 to April 2011), LILACS (from 1980 to April 2011) and the Cochrane Central Register of Controlled Trials (CENTRAL, the Cochrane Library (issue 4, 2011). We imposed no limit with respect to the language of publication, but certain publication types (i.e., letters to the editor, abstracts, and proceedings from scientific meetings) were excluded.

## Quality assessment of trials

The assessment of the quality of the studies that met the inclusion criteria was made separately by CC and MVE, with the knowledge of the authors or journals being assessed. The subsequent strategies associated with good-quality studies were evaluated: generation of allocation sequences and allocation concealment; blinding of investigators, participants, outcome assessors, and data analysts (yes, no, or not reported); intention-to-treat analysis (yes or no); and comprehensive follow-up. The generation of allocation sequences was considered adequate if the resulting sequences were unpredictable, such as computer-generated random numbers, and inadequate if the resulting sequences were predictable. The quality of allocation concealment was considered appropriate only if the randomization method applied unable the investigator or the participant to recognize or influence the intervention group before enrollment in the study. Allocation concealment was considered unclear when researchers claimed to have applied randomization methods but gave no description about it; it was considered inadequate when the randomization methods were unsuitable.

In relation to blinding methods, we have analyzed: double-blind (neither patients, care providers or assessors knew which treatment was being performed), single-blind (either patients or care providers or assessors were aware of treatment), and open (all parties were aware of treatment). For studies with an intention-to-treat analysis, a positive finding on the reviewers' part meant that the authors had specifically reported undertaking this type of analysis or that our own study confirmed this finding or both. On the contrary, a negative finding either meant that authors did not report the use of intention-to-treat analysis, that we could not confirm its use on study assessment, or both. We have established the percentage of participants excluded or lost to follow-up in order to evaluate how complete was patient follow-up. Such was considered adequate if ≥80% of participants were included in the final analysis; if no losses to follow-up were reported we assumed that the study completeness was adequate. Furthermore, we categorized the risk of bias by the number of criteria judged inadequate in each study: low risk of bias (≤1 inadequate criterion), medium risk of bias (≤3 inadequate criteria), and high risk of bias (>3 inadequate criteria).

## Data collection

Two review authors (CC and MVE) assessed eligibility of studies for inclusion independently. CC and MVE initially screened the title, abstract, and key words of every report identified by the search strategy; the reviewers then retrieved the full text for potentially relevant trials and for reports whose relevance was not clear. Three of us (CC, MVE and TA) independently applied the inclusion criteria to each potentially relevant trial to ascertain its eligibility. If differences in opinion existed, they were resolved by discussion. Differences in the inclusion of trials according to their quality were resolved after consultation with the other review authors (HS and RU). Data from each study were extracted by all of the reviewers using standardized data extraction forms prepared by CC and MVE. After extraction, all data were compared to minimize the possibility of errors.

## Data synthesis (Statistical methods)

The mean difference (MD) between the treatment and control groups was selected to represent the difference in continuous outcomes with a 95% confidence interval (CI). This was feasible when the mean values of the outcomes, the standard deviations of the outcomes, and the number of participants in whom the outcome was assessed in each of the 2 groups were available. If not, the analyses reported by the authors of the original articles are presented.

## Results

Recently published systematic reviews and meta-analyses on this topic were identified[25-26,42-47] and subsequently relevant references of published RCTs were found.

## Included studies

All RCTs included in this systematic review were fully peer-reviewed publications.

### Included RCTs analyzing the effects of LCPUFA intervention on mothers during pregnancy and/or lactation (Figure 1)

From a total of 105 existing RCTs designed to analyze the effect of LCPUFA intervention on mothers during pregnancy and/or lactation, 16 studies met the selection criteria. The included trials described 11 study populations for neurodevelopment outcome and 13 study populations assessed the growth outcome (*including a total of 4524 participants*), in infants and children born from supplemented pregnant and/or fed from their supplemented mothers during lactation. The general characteristics of these RCTs Table 2.

The time of neurodevelopment and physical growth assessment varied from birth to 7 years. The visual evaluations were performed during the age range of 0 to 5 years, and they differed from one another with regard to the vision-testing protocols and the number of vision testers.

Eighteen publications (including 11 sets of RCTs) assessed the effects of the n-3 LCPUFA supplementation during *pregnancy* on neurodevelopment and physical growth, on the same groups of patients and at different time points[48–65], 4 publications (2 sets of RCTs) explored the effect of n-3 LCPUFA supplementation during *pregnancy & lactation*[66–69] and 5 publications (3 sets of RCTs) exclusively during *lactation*[70–74].

Six publications were focused on analyzing the supplementation to mothers during *pregnancy* with n-3 LCPUFA on *child neurodevelopment*[48,53–56,64], 5 papers reported results on *visual acuity*[48–51,64], and 14 articles examined the effect on *growth* as an outcome[48–52,54,57–64].

3 articles, from the same RCT study population, explored the effect of n-3 LC-PUFA supplementation during *pregnancy & lactation* on the offspring neurodevelopment[66–68] and 4 published RCTs explored the effect on infant and children growth[66–69].

5 publications (3 sets of RCTs) analyzed the effect of supplementing *lactating mothers* on their offspring neurodevelopment[70–74], which was assessed using different tests and neurophysiologic procedures, as summarized in Table 2.

### Included RCTs analyzing the effects of LCPUFA supplemented infant formulas (Figure 2)

From 21 identified RCTs to analyze the effects of LCPUFA supplemented *infant formulas* on neurodevelopment and physical growth, 15 met criteria and thus were selected for inclusion in this review. The included RCTs described 15 study populations for neurodevelopment outcome and 13 study populations assessed the physical growth outcome (*including a total of 2061 infants and children*), from birth to 2 years of age. The general characteristics of these RCTs are shown in Table 3.

Seventeen publications (7 sets of RCTs) assessed the effects of the n-3 LCPUFA supplementation of infant formula on neurodevelopment and physical growth, in the same groups of patients and at different time points[32–34,75–89]. Another 9 RCTs which reported the complete study in a single publication were included in this review[90–97].

Thirteen papers reported the effect of n-3 LCPUFA supplementation of infant formula on visual acuity[34,75,78,80,83–86,88,90,92,96–97], 17 publications analyzed the effect on neurodevelopment[32–33,75,77–89,91–92,95,97] and 13 articles reported the effect on physical growth from different RCTs[33,76,80–84,86,88,90–94].

### LCPUFA supplementation during pregnancy and lactation

*Visual acuity (results are summarized in Table 4)*. Malcolm *et al.* found no significant differences in implicit times, amplitudes or parameters of the stimulus-response function in the electroretinogram in the first week of postnatal life comparing infants born to mothers receiving, from 15 weeks of
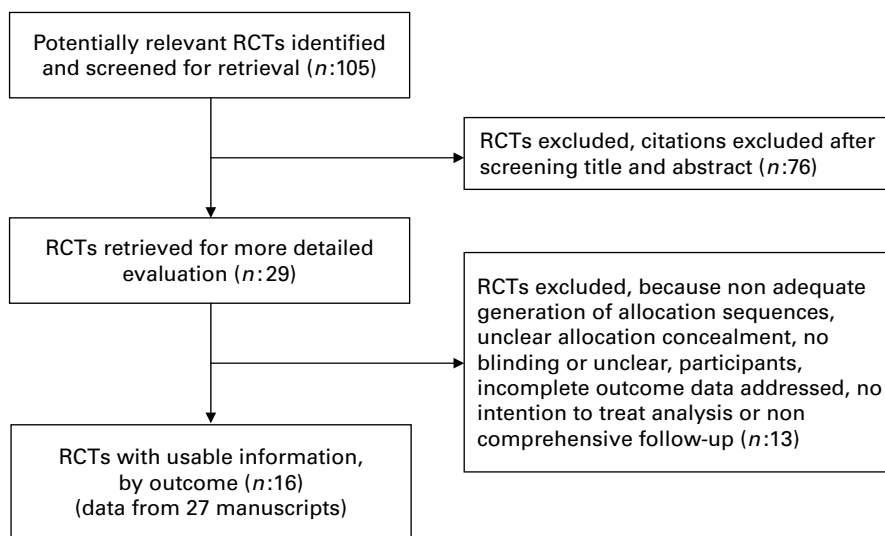
**Fig. 1.** Flow chart of the included RCTs analyzing the effects of LCPUFA intervention on mothers during pregnancy and/or lactation.

**Table 2.** Characteristics of the included Randomized Clinical Trials (RCTs) related to analyze the effect of supplementation during pregnancy and lactation on growth and neurodevelopment

| N° | Author/publication year | Intervention (n-3 LC-PUFA dose/day) | Control | Period | Growth parameters & Neurological examination | | Growth & Neurodevelopment outcomes |
|---|---|---|---|---|---|---|---|
| | | | | | Anthropometry/Test | Age | |
| **Supplementation during pregnancy** | | | | | | | |
| 1 | Smithers et al. 2011[65]/Australia | Three 500-mg/d capsules of DHA-rich FO concentrate, providing: DHA: 800 mg/d EPA: 100 mg/d (n = 89) | Three 500-mg/d vegetable oil capsules without DHA (n = 93) | From <21 weeks of pregnancy until delivery | Sweep VEP | 4 mo | DHA supplementation in women with singleton pregnancies does not enhance infant visual acuity in infants at 4 mo of age. |
| 2 | *Makrides et al. 2010[64]/Australia | As above (n = 351) | As above (n = 375) | As above | BSID-III | 18 mo | No differences between groups |
| 3 | Campoy et al. 2011[56]/Spain, Germany, Hungary | FO (500 mg/d DHA +150 mg/d EPA) (n = 37), or FO + 400 μg 5-MTHF (n = 37) | Placebo (n = 45) or 400 μg/d 5-MTHF (n = 35) | From 20 weeks of gestation until delivery | K-ABC | 6 ½ y | No differences between groups in K-ABC Children whose mothers had higher DHA percentages in erythrocyte PE at delivery were more likely to have a MPC score of the Kaufman ABC over the 50th percentile at 6·5 y. |
| 4 | Escolano-Margarit et al. 2011[55]/Spain, Germany, Hungary | FO (500 mg/d DHA +150 mg/d EPA) (n = 43), or FO + 400 μg 5-MTHF (n = 37) | Placebo (n = 47) or 400 μg/d 5-MTHF (n = 40) | From 20 weeks of gestation until delivery | Hempel test Touwen test | 4 y 5 ½ y | No differences between groups No differences between groups Better neurological optimality scores in children at the age of 5·5 y with increasing DHA levels in cord blood at delivery |
| 5 | Courville et al. 2011[59]/USA | DHA cereal-based 5 bars/ week DHA: 300 mg (EPA:DHA = 1:8) Mean intake: 214 mg DHA/d (n:22) | Placebo (n:25) Maize oil (n:22) | From 20–24 weeks until delivery | Weight, Length, HC | At birth | No statistical differences between supplemented groups |
| 6 | Stein et al. 2011[62]/Mexico | Algal oil capsules DHA: 400 mg/day (Median DHA intake: 55 mg/d) (n = 370) | Placebo (n = 369) | From 18–22 weeks until delivery | Weight, Length, HC | 18 mo | Heterogeneity of associations by maternal gravidity for weight, length and head circumference. (n:739; 76 % of the birth cohort) Offspring of supplemented primigravide mothers had higher length than controls. |
| 7 | Ramakrishnan et al. 2010[58]/Mexico | Algal oil capsules DHA: 400 mg/day (Median DHA intake: 55 mg/d) (n: 540) | Placebo (n:554) | From 18–22 weeks until delivery | Weight, Length, HC | At birth | Offspring of supplemented primigravide mothers were heavier (n:370) and had larger head circumferences than controls (n:603). |

**Table 2.** *Continued*

| N° | Author/publication year | Intervention (n-3 LC-PUFA dose/day) | Control | Period | Anthropometry/Test | Age | Growth & Neurodevelopment outcomes |
|---|---|---|---|---|---|---|---|
| | | | | | Growth parameters & Neurological examination | | |
| 8 | Dunstan *et al.* 2008[48]/Australia | Fish oil capsules: DHA: 2·2 g/d + EPA: 1·1 g/d (n = 52) | Olive oil capsules: n-9 oleic acid 2·7 g/d (n = 46) | From 20 weeks gestation until delivery | Weight and Length GMDS PPVT CBCL | At birth 2½ y | No differences in growth between groups. GMDS: supplemented group (S): higher eye hand coordination. No differences for mean general quotient and other subscales. S: Significant positive correlation between eye and hand coordination and n-3 LC-PUFA in cord blood. S: Inverse correlation between eye and hand coordination and performance score with AA in cord blood. PPVT: No differences; CBCL: No differences. |
| 9 | Innis & Friesen 2008[51]/Canada | Capsules with DHA from the *Crythecodinium cohnii* (DHA ≈ 0·4 g/d; n = 67) | Soybean and corn oil capsules: LA 0·53 g, ALA 0·08 g/d (n = 68) | From 14–16 weeks gestation until delivery | Weight and Length VEPc Teller acuity Card | At birth 60 ± 3 days | No differences in growth between groups. No differences between groups; Differences between boys and girls; Increased risk of low visual acuity among 60-d-old infants of mothers following their usual diet compared with infants of DHA supplemented women. |
| 10 | Judge *et al.* 2007b[53]/USA | DHA-containing: Cereal-based bars (300 mg as low EPA fish oil: EPA:DHA 1:8); (≈DHA 214 mg/wk) (n = 16) | Cereal-based Placebo bars with Corn oil (n = 14) | From 24 weeks gestation until delivery | 2 step problem solving test from Willatts Fagan Test of Infant Intelligence | 9 m | Problem solving: S Group better performance. Fagan Test of Infant Intelligence: No differences (recognition memory abilities). |
| 11 | Judge *et al.* 2007a[52]/USA | As above (n = 14) | As above (n = 15) | As above | Growth Teller acuity cards | At birth 4 m & 6 m | No differences in growth between groups. Better Visual Acuity at 4 m, no differences at 6 mo |
| 12 | Tofail *et al.* 2006[54]/Bangladesh | Fish oil capsules containing DHA 1·2 g/d + EPA 1·8 g/d (n = 200) | Soy oil capsules: LA: 2·25 g/d LNA: 0·27 g/d (n = 200) | From 25 weeks gestation until delivery | Weight & Length BSID II Psychosocial stimulation at home (HOME) | At birth 10 m | No differences between groups |
| 13 | Sanjurjo *et al.* 2004[63]/Spain | Fat (2 g/d); 200 mg DHA/d + 40 mg EPA/d | Placebo | From weeks 27–28 of gestation to delivery | Weight | At birth | No differences between groups |

**Table 2.** *Continued*

| N° | Author/publication year | Intervention (n-3 LC-PUFA dose/day) | Control | Period | Growth parameters & Neurological examination Anthropometry/Test | Age | Growth & Neurodevelopment outcomes |
|---|---|---|---|---|---|---|---|
| 14 | Smuts et al. 2003a[60]/USA | High-DHA eggs: DHA: 135 mg/egg; mean intake = 206 ± 112 mg/DHA/d (n = 27) | Ordinary eggs (n = 25): DHA: 18 mg/egg; mean intake = 23 ± 9 mg/d; Low-egg intake group (n = 26) mean DHA intake = 7 ± 9 mg/d | Possibly 3rd trimester (no information provided); mean: ≈13 wk | Weight, Length, HC | At birth | No differences between groups |
| 15 | Smuts et al. 2003b[61]/USA | High-DHA eggs (n = 176): DHA: 133 mg/egg; mean intake 7·2 ± 3·4 eggs/wk = 137 ± 65 mg/DHA/d | Ordinary eggs (n = 174): DHA: 33 mg DHA/egg; intake = 7·3 ± 3·4 eggs/wk = 34 ± 16 mg DHA/d | From weeks 24–28 of gestation to delivery | Weight, Length, HC | At birth | DHA intake correlated with birth weight |
| 16 | Malcolm et al. 2003b[49]/UK | Fish oil capsules: 0·1 g of DHA in 0·323 g oil per capsule DHA 0·2 g/d (EPA:DHA 1:6) (n = 50) | Sunflower oil capsules 0·323 g/d (81 % oleic acid) (n = 50) | From 15 weeks of gestation until delivery | Visual assessment Transient flash VEP Pattern-reversal stimuli VEP | 1, 50 & 66 weeks post-conceptional age | No differences between groups Peak latency of the P100 was 4·6 ms shorter for infants with 1 % higher DHA status in umbilical cord. Association between DHA status of infants at term and early postnatal pattern-reversal VEP |
| 17 | Malcolm et al. 2003a[50]/UK | As above | As above | As above | Weight, Length & HC VEP Scotopic Electroretinography | At birth 1 week of life | No differences in growth between groups No differences between groups. Infants in the highest quartile for cord blood DHA had higher retinal sensitivity compared with infants in the lowest quartile. |
| 18 | Olsen et al. 1992[57]/Denmark | Fish oil (4 g/d): DHA: 920 mg/d + EPA: 1280 mg/d Vit E: 2 mg/d ≈2·7 g n-3 PUFA/d | Olive oil (4 g/d) or no oil supplement | From week 30 of gestation until delivery | Weight Length | at birth | No statistical differences between groups |
| **Supplementation during pregnancy and lactation** | | | | | | | |
| 1 | Helland et al. 2008[68]/Norway | Cod liver oil Containing DHA: 1·183 g/d + EPA: 0,803 g/d + vitamins A, D and E (n = 82) | Corn oil containing LA: 4·747 g/d + ALA: 0,092 g/d + vitamins A, D and E (n = 61) | From 17–19 weeks of gestation until 3 m after delivery | Weight, Height, BMI K-ABC | 7 y | No significant differences in growth No significant differences in scores on the K-ABC between groups. |
| 2 | Lucia et al. 2007[69]/Germany | Supplement consisting Fish oil: DHA 200 mg + Vitamins and minerals + 4·5 g FOS (n = 43) | (n = 74) Basic supplement containing: Vitamins & minerals or Vitamins & minerals & 4·5 g FOS | From 21st week of gestation until 3 mo after delivery | Weight, Length, HC | 1, 3, 21 mo | Significant lower BMI and birth weight in babies from DHA supplemented mothers, at 1, 3 and 21 mo |

**Table 2.** *Continued*

| N° | Author/publication year | Intervention (n-3 LC-PUFA dose/day) | Control | Period | Anthropometry/Test | Age | Growth & Neurodevelopment outcomes |
|---|---|---|---|---|---|---|---|
| | | | | | **Growth parameters & Neurological examination** | | |
| 3 | Helland *et al.* 2003[67]/Norway | As above (n = 48) | As above (n = 52) | As above | K-ABC | 4 y | Higher Mental Processing Composite scores of the K-ABC at 4 y in children from DHA and EPA supplemented mothers. MPC 4 years correlated with maternal intake of DHA and EPA. |
| 4 | Helland *et al.* 2001[66]/Norway | As above (n = 175) | As above (n = 166) | As above | Weight, Length, HC EEG Fagan Test of Infant Intelligence | At birth 2 d, 3 mo 6 mo, 9 mo | No differences in growth between groups No differences in EEG scores or Fagan scores. Neonates with mature EEG (N = 70) had a higher concentration of DHA in umbilical plasma phospholipids than neonates with immature EEG (N = 51) on the second day of life. |
| **Supplementation during lactation** | | | | | | | |
| 1 | Cheatham, *et al.* 2011[73]/Denmark | Microencapsulated Fish oil added to müsli bars or to homemade cookies or as capsules containing n-3 LC-PUFA 1·5 g/d (DHA ≈ 0·9 g/d); low content of EPA (n = 36) | Microencapsulated Olive oil added to müsli bars or to homemade cookies or as capsules (n = 28) | For 4 months after delivery | Stroop task Strength and Difficulties Questionnaire | 7 y | Negative effect on pro-social behavior score in boys Speed of processing scores were predicted by maternal n-3 LCPUFA scores (negative relation) and maternal education (positive relation) Stroop scores: working memory and inhibitory control were predicted by infant erythrocyte DHA status at 4 months (negative relation). |
| 2 | Jensen *et al.* 2010[74]/USA | Algal oil capsules (≈ 200 mg/d DHA) (n = 114) | Vegetable oil capsules: LA: 56·3 %, ALA: 3·9 % (n = 113) | For 4 months after delivery | Gross and fine motor function; Perceptual/visual-motor function; Attention, Executive Function; Verbal skills; Sweep VEP, transient VEP and Bayley-Lovie cards | 5 y | No differences in visual function between groups Children whose mothers received DHA versus placebo performed significantly better on the Sustained Attention Subscale of the Leiter International Performance Scale. |
| 3 | Jensen *et al.* 2005[70]/USA | As above | As above | | Teller Acuity Card Sweep VEP BSID | 4 and 8 m 12 and 30 m | Lower amplitude in babies from DHA supplemented mothers Higher PDI at 30 mo of age in children from DHA supplemented mothers. |

**Table 2.** *Continued*

| N° | Author/publication year | Intervention (n-3 LC-PUFA dose/day) | Control | Period | Growth parameters & Neurological examination | | Growth & Neurodevelopment outcomes |
| --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | | Anthropometry/Test | Age | |
| 4 | Lauritzen *et al.* 2005[72]/Denmark | Microencapsulated Fish oil (müsli bars or homemade cookies or as capsules) containing n-3 LC-PUFA 1·5 g/d (DHA ≈ 0·9 g/d); low content of EPA (*n* = 48) | Microencapsulated Olive oil added to müsli bars or to homemade cookies or as capsules (*n* = 38) | For 4 months after delivery | Problem solving ability Infant linguistic development {MacArthur CDI forms for infant (8−16 mo) & toddler (16−30 mo)} | 9 m 1 and 2 y | No significant differences on problem solving between groups. Passive vocabulary was lower in children aged 1 of the FO compared to OO group. Word comprehension at 1 y was inversely associated with erythrocyte-DHA at 4 mo. Small effect of DHA levels in breast-milk on early language development. |
| 5 | Lauritzen *et al.* 2004[71]/Denmark | As above (*n* = 53) | As above (*n* = 44) | As above | Infant visual acuity (measured by Sweep VEP) | 2 and 4 mo | No differences in the Sweep VEP patterns between groups. Higher red blood cell levels of n-3 LCPUFA was associated to better visual acuity at 4 mo of age |

FO: Fish Oil; OO:Olive oil; HC: Head circumference; LCPUFAs: Long chain polyunsaturated fatty acids; LA: Linoleic acid; ALA: α-linolenic acid; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; GMDS: Griffiths Mental Development Scales; PPVT: Peabody Picture Vocabulary Test; K-ABC: Kaufman ABC; MPC: Mental Processing Composite; CBCL: Child Behavior Checklist; BSID: Bayley Scales of Infant Development; PDI: Psychomotor Development Index; MDI: Mental Development Index; MacArthur: CDI: Communicative Development Inventory; VEP: Visual evoked potentials; EEG: Electroencephalography. *Included term and preterm infants.
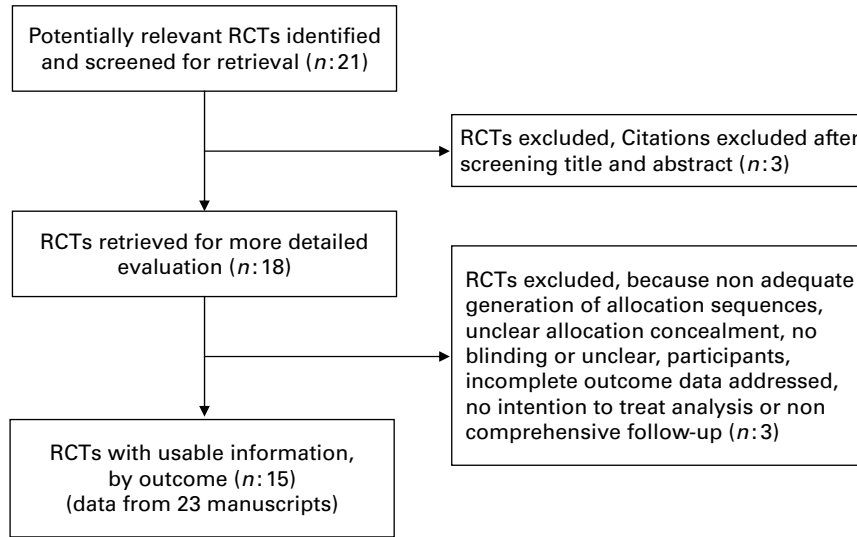
**Fig. 2.** Flow chart of the included RCTs analyzing the effects of LCPUFA supplemented infant formulas on visual acuity, neurodevelopment and growth.

pregnancy til delivery, 200 mg of DHA/day supplements during pregnancy and those receiving placebo[49–50]. There were also no differences in the measures of transient flash VEP waveform at birth and at the ages of 50 and 66 weeks postconceptional age (PCA); However, positive associations were found between infants' DHA status at birth and the sensitivity and maturity of the rod photoreceptors responses at birth[49], as well as, with the maturity of the pattern-reversal VEP at the ages of 50 and 66 weeks[50].

Innis *et al.* found no significant differences in the Teller acuity cards at the age of 60 days PCA between infants born from mothers receiving DHA supplements during pregnancy and those who didn't. However, infants in the placebo group were more likely to have a lower visual acuity than those born to DHA-supplemented mothers[51]. Judge *et al.*[52] showed significant main effects for visual acuity at 4 months of age in those infants whose mothers were supplemented during pregnancy (DHA group), and no differences at 6 months of age; however, in the regression analysis the authors show better visual acuity in the offspring of the DHA supplemented group mothers at 4 months. Smithers *et al.*[65] reported results from the DOMInO trial showing that in those infants whose mothers were supplemented during pregnancy with 800 mg/d of DHA +100 mg of EPA, and were fed only breast milk, the VEP acuity at 4 mo of age was not different compared to the control group. Lauritzen *et al.* reported no significant differences in Sweep VEP at 2 and 4 mo in babies whose mothers received fish oil supplementation during lactation; however, higher red blood cell n-3 LCPUFA content were associated with better visual acuity at 4 mo of age[71].

Studies based on lactating mothers' supplementation with n-3 LCPUFA have shown also some controversial results. Jensen *et al.* demonstrated that supplementation to mothers during lactation with 200 mg/d of DHA determines lower amplitude in the Sweep VEP in their infants at 4 & 8 months[70], but these differences in visual function (*Sweep*

*VEP, transient VEP and Bayley-Lovie cards*) were not confirmed at 5 years[74].

*Neurodevelopment.* The main results are shown in Table 5.

Four studies assessed neurological outcome in the first 2 years of life after n-3 LCPUFA supplementation in pregnancy, none reported significant effects on performance in neurologic tests in the supplemented group; two reported no differences between the supplemented and control group performance in the Fagan Test of Infant Intelligence (FTII) at 6 and 9 months of age[53,66] and three reported no differences in the BSID at 10th and 18th months[54,70,64]. Helland *et al.*[66] reported no differences in electroencephalogram (EEG) maturity at 2 days and 3 months after birth between groups, but observed that higher EPA and DHA levels were associated with more mature EEG patterns. Judge *et al.*[53] reported a positive effect of supplementation on the 2 step problem solving test at 9th months post birth. Dunstan *et al.* reported better eye and hand coordination in the Griffiths Mental Development Scales (GMDS) in the supplemented group at 2.5 years, a significant positive association with n-3 LCPUFA and an inverse correlation with AA in cord blood[48]. Based on our evaluation of the data to date, 3 trials have reported long term effects of supplementation to date. Helland *et al.* reported better performance in the K-ABC mental development test in the supplemented group compared to control at 4 years of age, but this effect was not observed when IQ was measured at 7 years. The authors also report a significant positive correlation between IQ at 4 years and DHA levels in infant plasma at 4th weeks of life as well as an association between maternal DHA levels at 35 week gestation and IQ in the children at 7 years[67–68]. The NUHEAL trial reported no differences in neurological outcome of children assessed with Hempel (4 years) and Touwen examinations (5.5 years) between children born to mothers receiving fish oil supplements and those who didn't; but, the authors also demonstrated better neurological scores in children at 5.5 yrs with increasing higher DHA levels in cord

**Table 3.** Characteristics of the included Randomized Clinical Trials (RCTs) related to analyze the effect of infant formula supplementation on growth and neurodevelopment in term infants

| N° | Author/publication year/country | Intervention (n-3 LC-PUFA dose/day) | Control | Period | Growth & Neurological examination Anthropometry and Neurological Test | Age | Growth & Neurodevelopment outcomes |
|---|---|---|---|---|---|---|---|
| 1 | Drover et al. 2011[75]/USA | Infant formula supplemented with DHA + AA from single-cell algal (Crypthecodinium cohnii) & fungal (Mortierella alpina) oils, at different dosis: DHA: 0·32 % (Enfamil LIPIL®) (n = 29), 0·64 % (n = 32) or 0·96 % (n = 28) + AA: 0·64 % LA: 16·9 %-17·5 % ALA: 1·61 %-1·68 % | Infant formula with DHA: 0 %® AA: 0 % (Enfamil with Iron) (n = 28) LA: 16·9 %-17·5 % ALA: 1·61 %-1·68 % | First 12 mo of life, sole source of nutrition until ≈ 4 mo of age | BSID II | 18 mo | No significant differences in MDI, PDI or BRS between the supplemented groups. MDI scores of DHA-supplemented children were higher than those who did not received DHA supplementation (pooling those supplemented with DHA). |
| 2 | Birch EE et al. 2010[76]/USA | Infant formula supplemented with DHA + AA from single-cell algal (Crypthecodinium cohnii) & fungal (Mortierella alpina) oils, at different dosis: DHA: 0·32 % (Enfamil LIPIL®) (n = 83), 0·64 % (n = 85) or 0·96 % (n = 88) + AA: 0·64 % LA: 16·9 %-17·5 % ALA: 1·61 %-1·68 % | Infant formula with DHA: 0 %® AA: 0 % (Enfamil with Iron) (n = 86) LA: 16·9 %-17·5 % ALA: 1·61 % − 1·68 % | First 12 mo of life, sole source of nutrition until ≈ 4 mo of age | Weight, Length, weight/length and HC Sweep VEP Acuity | 1·5, 4, 6, 9, 12 mo | No differences in physical growth between groups Infants fed control formula had significantly poorer visual evoked potential visual acuity at 12 mo of age than did infants who received any of the DHA-supplemented formulas (0·32 % to 0·64 %) (P< 0·001). |
| 3 | De Jong C, et al. 2010[77]/The Netherlands | Infant formula supplemented with: DHA: 0·30 % + AA: 0·45 % (n = 146) | Standard Infant formula (n = 169) Breastfed reference group (n = 160) | From birth to 2 mo of life | Touwen | 9 y | No significant differences in neurological function between groups. Breast-fed children have a slightly better neurodevelopmental outcome than formula fed. |
| 4 | Birch EE, et al. 2007[78]/USA | Infant formula supplemented with(microalgal/microfungal LCP): DHA: 0·36 % + AA: 0·72 % (n = 17) Or only with: DHA: 0·36 % (n = 16) | Standard Infant formula (n = 19) Breastfed reference group (n = 32) | From birth to 2 mo of life | HOTV visual acuity Amblyopia Treatment Study (ATS); Electronic Visual Acuity (EVA) Wechsler preschool & primary scale of Intelligence; and WPPSI-R | 4 y | No differences in visual acuity between DHA and DHA + AA-supplemented groups compared to breast fed infants. Control group showed poorer visual acuity than breast-fed one. IQ maturation is similar in the supplemented and control groups to that of breast-fed infants. |
| 5 | Bouwstra H et al. 2005[79]/Netherlands | Infant formula supplemented with: DHA: 0·30 % + AA: 0·45 % (n = 146) | Standard Infant formula (n = 169) Breastfed reference group (n = 160) | From birth to 2 mo of life | Hempel BSID | 6,12,18 mo 18 mo | No significant differences in MDI nor in PDI between groups |
| 6 | Birch EE et al. 2005[90]/USA | Infant formula supplemented with: DHA: 0·36 % + AA: 0·72 % (n = 51) | Standard Infant formula (n = 52) No breastfed reference group | From birth to 52 weeks of age | Weight, length Sweep VEP Acuity Random dot stereoacuity | 6,17,26, 39,52 w | No differences in growth parameters between groups Infants in the LCPUFA group had a better visual acuity than control group at 6,17,26 and 52 w Steroacuity was significantly better in the LCP-supplemented group at 17wk, but not at 39 wk. |

**Table 3.** *Continued*

| N° | Author/publication year/country | Intervention (n-3 LC-PUFA dose/day) | Control | Period | Growth & Neurological examination — Anthropometry and Neurological Test | Growth & Neurological examination — Age | Growth & Neurodevelopment outcomes |
|---|---|---|---|---|---|---|---|
| 7 | Ben XM et al. 2004[91]/China | Infant formula supplemented with: DHA + AA. The LCPUFA content of the formula was not clear. ($n = 69$) | Standard Infant formula ($n = 52$) Breastfed reference group ($n = 26$) | From birth to 6 mo of life | Growth BSID | 6 mo 3,6 mo | No significant differences in weight, length or head circumference between groups at 6 mo No significant differences in MDI nor in PDI between groups |
| 8 | Auestad N et al. 2003[80]/USA | Infant formula supplemented with: DHA: 0·13 % + AA: 0·45 % ($n = 46$) Or only with: DHA: 0·20 % ($n = 43$) | Standard Infant formula ($n = 45$) Breastfed reference group ($n = 63$) | First 12 mo of life, sole source of nutrition until ≈ 4 mo of age | Weight, length and HC Sweep VEP Acuity Teller cards Stanford Binet IQ McArthur CDI Peabody Picture Vocabulary Test | 39 mo | No differences in growth between groups No significant differences in Sweep VEP between groups at 12 mo No statistical differences in visual acuity using Teller cards at 3y No differences between groups in the Stanford Binet IQ at 39 mo No differences in cognitive and language assessment at 39 mo |
| 9 | Lucas A et al. 2002[81]/England | Infant formula supplemented with: DHA: 0·32 % + AA: 0·30 % ($n = 154$) | Standard Infant formula ($n = 155$) Breastfed reference group ($n = 138$) | From birth to 6 mo of life | Weight, length and HC BSID Knobloch, Passama-nick and Sherrads' tests | 6, 9, 18 mo 18 mo 9 mo | No significant differences in weight, length or head circumference between groups at 4,6,9,18 mo No significant differences in MDI nor in PDI between groups |
| 10 | Auestad N et al. 2001[92]/USA | Infant formula supplemented (egg/TG): DHA: 0·13 % + AA: 0·45 % ($n = 80$) or (fish/fungus oil: DHA: 0·13 % + AA: 0·45 % ($n = 82$) | Standard Infant formula ($n = 77$) Breastfed reference group ($n = 82$) | First 12 mo of life, sole source of nutrition until ≈ 4 mo of age | Growth Teller acuity cards Fagan test of infant intelligence BSID McArthur's CDI Parental reporting of infant temperament | 1, 2, 4, 6, 9, 12 mo 2, 4, 6, 12, 14 mo 6,9 mo 6, 12 mo 6,12 mo 9, 14 mo 6, 12 mo | No significant differences in weight, length or head circumference between groups at 4, 6 mo No significant differences in visual acuity between groups using Teller cards No statistical differences between groups in the Fagan Test No significant differences in MDI nor in PDI between groups at 6 mo or 1 y. |
| 11 | Hoffman DR et al. 2000[34]/USA | Infant formula supplemented with(microalgal/microfungal LCP): DHA: 0·36 % + AA: 0·72 % ($n = 27$) Or only with: DHA: 0·36 % ($n = 26$) | Standard Infant formula ($n = 26$) Breastfed reference group ($n = 29$) | From birth to 17 weeks of life | Sweep VEP Acuity ERG | 6,17,26,52 weeks 6w, 17w, 1y | Better visual acuity at both 17 and 52 weeks of age. More mature electroretinographic responses at 6 weeks of age. Supplementation leads to better visual function later in life (52w). |
| 12 | Lapillonne A et al. 2000[93]/France | Infant formula supplemented with: DHA: 0·31 % ($n = 12$) | Standard Infant formula ($n = 12$) No breastfed reference group | From birth to 4 mo of life | Weight, Length and HC | 2,4 mo | No differences in weight, length or head circumference between groups |
| 13 | Agostoni C et al. 2000[82]/Italy | Infant formula supplemented with: DHA: 0·30 % + AA: 0·44 % ($n = 29$) | Standard Infant formula ($n = 31$) Breastfed reference group ($n = 30$) | From birth to 4 mo of life | Bruner-Lezine test | 24 mo | No differences on Developmental Quotient (DQ) at 24 mo |

**Table 3.** *Continued*

| N° | Author/publication year/country | Intervention (n-3 LC-PUFA dose/day) | Control | Period | Anthropometry and Neurological Test | Age | Growth & Neurodevelopment outcomes |
|---|---|---|---|---|---|---|---|
| | | | | | **Growth & Neurological examination** | | |
| 14 | Birch EE et al. 2000[83]/USA | Infant formula supplemented with (microalgal/microfungal LCP): DHA: 0·36 % + AA: 0·72 % (n = 19) Or only with: DHA: 0·36 % (n = 17) | Standard Infant formula (n = 20) Breastfed reference group (n = 20) | From within five days of birth till 17 weeks of life | Weight Sweep VEP Acuity BSID II | 4, 12 mo 18 mo | No differences in growth between groups Better VEP acuity at 4 mo was associated with a better MDI and PDI score at 18 mo. 7 points increase of MDI in the DHA supplemented groups. |
| 15 | Makrides M et al. 2000[84]/Australia | Infant formula supplemented with: DHA: 0·34 % + AA: 0·34 % (n = 28) Or only with: DHA (0·34 %) (n = 27) | Standard Infant formula (n = 28) Breastfed reference group (n = 63) | From the 5 days to 4 mo of life | Weight, length, head circumference Steady state VEP BSID | 6,16,34 weeks 1,2 y 1y & 2y | No differences in growth parameters between groups at 4 mo Better VEP visual acuity in the supplemented group at 4 mo, but no differences at 7–8 mo No significant differences in MDI nor in PDI between groups |
| 16 | Morris G et al. 2000[94]/Wales | Infant formula supplemented with: DHA: 0·20 % + AA: 0·40 % (n = 54) | Standard Infant formula (n = 55) No breastfed reference group | From birth to 4 mo of life | Growth | 6 weeks; 3,6,12 mo | No differences in weight between groups at 6 mo |
| 17 | Lucas A et al. 1999[33]/England | Infant formula supplemented with: DHA: 0·32 % + AA: 0·30 % (n = 154) | Standard Infant formula (n = 155) Breastfed reference group (n = 138) | From birth to 6 mo of life | Weight, Length, Head circumference, mid-upper arm circumference, sum skinfolds BSID Knobloch, Passamanick and Sherrads' tests | 6,9,18 mo 18 mo 9 mo | No differences in weight, length or head circumference between groups at 6 mo No significant differences in MDI nor in PDI between groups No significant differences between groups in Knobloch, Passamanik and Sherrards Development Screening Inventory |
| 18 | Makrides M et al.1999[85]/Australia | Infant formula supplemented with: DHA: 0·34 % + AA: 0·34 % (n = 28) Or only with: DHA (0·34 %) (n = 27) | Standard Infant formula (n = 28) Breastfed reference group (n = 63) | From birth to 4 mo of life | Weight, length, head circumference | 6,16,34 weeks and 1 y & 2 y | No differences in growth parameters |
| 19 | Birch EE et al. 1998[86]/USA | Infant formula supplemented with: DHA: 0·36 % + AA: 0·72 % (n = 27) Or only with: DHA: 0·36 % (n = 26) | Standard Infant formula (n = 26) Breastfed reference group (n = 29) | From within five days of birth till 17 weeks of life | Growth Sweep VEP Acuity Forced-choice preferential looking acuity (FPL) | 6,17,26,52 wk 17,52 wk | No differences in weight, length or head circumference between groups at 6 mo Infants in the LCPUFA group had a better visual acuity than control group |
| 20 | Scott DT et al. 1998[87]/USA | Infant formula supplemented with: DHA: 0·13 % + AA: 0·45 % (n = 46) Or only with: DHA: 0·20 % (n = 43) | Standard Infant formula (n = 45) Breastfed reference group (n = 63) | First 12 mo of life, sole source of nutrition until ≈ 4 mo of age | BSID McArthur CDI Peabody Picture Vocabulary Test | 1y | No significant differences in MDI nor in PDI between groups DHA group scored lower on the Vocabulary Comprehension and Production Scales Significant negative correlations between DHA levels and vocabulary outcomes. |

**Table 3.** *Continued*

| N° | Author/publication year/country | Intervention (n-3 LC-PUFA dose/day) | Control | Period | Anthropometry and Neurological Test | Age | Growth & Neurodevelopment outcomes |
|---|---|---|---|---|---|---|---|
| | | | | | **Growth & Neurological examination** | | |
| 21 | Willatts P *et al.* 1998[95]/Scotland | Infant formula supplemented with: DHA: 0·15−0·25 % + AA: 0·30−0·40 % (*n* = 34) | Standard Infant formula (*n* = 38) No breastfed reference group | From birth to 4 mo of life | Problem solving tests | 10 mo | LCPUFA-supplemented infants had significantly more intentional solutions than infants who received control formula |
| 22 | Auestad N *et al.* 1997[88]/USA | Infant formula supplemented with: DHA: 0·13 % + AA: 0·45 % (*n* = 46) Or only with: DHA: 0·20 % (*n* = 43) | Standard Infant formula (*n* = 45) Breastfed reference group (*n* = 63) | First 12 mo of life, sole source of nutrition until ≈ 4 mo of age | Weight, Length, HC Sweep VEP Acuity Teller cards | 1,2,4,6,9,12 mo | No differences in growth between the two groups. No significant differences in visual acuity between groups using Sweep VEP acuity or Teller cards in all ages |
| 23 | Agostoni C *et al.*1997[32]/Italy | Infant formula supplemented with: DHA: 0·30 % + AA: 0·44 % (*n* = 29) | Standard Infant formula (*n* = 31) Breastfed reference group (*n* = 30) | From birth to 4 mo of life | Bruner-Lezine test | 4,12,24 mo | No differences on Developmental Quotient (DQ) at 12 mo |
| 24 | Carlson SE *et al.*1996[96]/USA | Infant formula supplemented with: DHA: 0·10 % + AA: 0·43 % (*n* = 19) | Standard Infant formula (*n* = 20) Breastfed reference group (*n* = 19) | From birth to 1 y of life | Visual Acuity Teller acuity cards | 2,4,6,9,12 mo | No significant differences in visual acuity between groups using Teller cards |
| 25 | Agostoni C *et al.*1995[89]/Italy | Infant formula supplemented with: DHA: 0·30 % + AA: 0·44 % (*n* = 29) | Standard Infant formula (*n* = 31) Breastfed reference group (*n* = 30) | From birth to 4 mo of life | Bruner-Lezine test | 4 mo | Higher Developmental Quotient (DQ) at 4 mo |
| 26 | Makrides M *et al.* 1995[97]/Australia | Infant formula supplemented with: DHA: 0·35 % + EPA and GLA (*n* = 13) | Standard Infant formula (*n* = 19) Breastfed reference group (*n* = 28) | From birth until 30 weeks of life | VEP Acuity BSID | 16, 30 weeks 1 y | Infants in the LC-PUFA group had better visual acuity at 4 mo No significant differences in MDI nor in PDI between groups |

HC: Head Circumference; LCPUFAs: Long chain polyunsaturated fatty acids; LA: Linoleic acid; GLA: γ-Linoleic acid; ALA: α-linolenic acid; DHA: Docosahexaenoic acid; EPA: Eicosapentaenoic acid; IQ: Intelligence Coefficient; GMDS: Griffiths Mental Development Scales; PPVT: Peabody Picture Vocabulary Test; K-ABC: Kaufman ABC; CBCL: Child Behaviour Checklist; BSID: Bayley Scales of Infant Development; PDI: Psychomotor Development Index; MDI: Mental Development Index: BRS: Behavior rating scales; CDI: Communicative Development Inventory; FO: Fish Oil; OO:Olive oil; TG: triglycerides; VEP: Visual evoked potentials; EEG: Electroencephalography.

**Table 4.** Significant effects found of n-3 LCPUFA supplementation during Pregnancy or Lactation Child Visual Development

| Outcome | N | Mean difference [95 % CI] |
|---|---|---|
| *Supplementation during pregnancy* | | |
| *Judge et al. 2007*[52] | | |
| Visual acuity scores at 4 months | 30 | 0·5 [−0·4, 1·4]* |
| *Malcolm et al. 2003*[50] | | |
| *Mean peak latency of transient flash VEP* | | |
| N3 at 50 weeks PCA | 34 | −35·5 [−69·4, −1·6]* |
| *Mothers' supplementation during lactation* | | |
| *Jensen et al. 2005*[70] | | |
| Teller acuity card at 4 months | 147 | 0·30 (0·09, 0·51)* |
| Teller acuity card at 8 months | 147 | −1·2 (−1·38, −1·02)* |

CI = confidence interval, PCA = post-conceptional age. VEP = visual evoked potential, cpd = cycles per degree, ms: miliseconds, μV = microvoltium, * = significant difference (P< 0·05).

blood[55]. Moreover, children whose mothers had higher DHA content in erythrocyte phosphatidylethanolamine (PE) at delivery were more likely to have a Mental Processing Composite (MPC) score of the Kaufman ABC over the median at 6·5 yrs. Likewise higher AA/DHA ratio in maternal erythrocyte PE at delivery was associated with greater proportion of low MPC scores (below the median)[56].

Lauritzen *et al.* demonstrated that passive vocabulary at 1 yr of age was lower in children whose mothers were supplemented with fish oil during lactation, compared to those babies whose mothers received olive oil; in the same study the authors reported that word comprehension at 1 yr of age was inversely associated with erythrocyte-DHA content at 4 mo additionally a small effect of breast-milk DHA was noted on early language development[71]. Jensen *et al.* in 2005 report a benefit of DHA supplementation during lactation at 2·5 years of age, despite no differences in infancy[70]. The supplemented group from this cohort of children was reported to perform better on a test of sustained attention at the age of 5 years[74]. Recently, Makrides *et al.*[64] have shown that mean cognitive composite scores and mean language composite scores of children in the DHA group of mothers supplemented during pregnancy did not differ from children in the control group.

Cheatham *et al.*[74] reported that early fish oil supplementation during lactation could have a negative effect on later cognitive abilities, suggesting a need to target an optimun DHA level, below and above which, there could be detrimental consequences to brain development.

*Growth*. Most studies included in this review showed no statistical differences after supplementation with DHA during pregnancy and/or during lactation in birth weight, length, weight for length or ponderal index at different ages[54,58–59,61–62,64].

Smuts *et al.*[61] showed no significant differences between groups, but demonstrated that DHA intake correlates with birth weight. Tofail *et al.*[54] investigated the effect of fish oil (1·2 g DHA and 1·8 g eicosapentaenoic acid, EPA 20:5 n-3, per day) from 25 weeks gestation until birth on growth and development in 249 mother-infant pairs from a very poor area of Bangladesh. Birth weight, length, head circumference and ponderal index did not differ between groups at birth or at 10 months. Ramakrishnan *et al.*[58] and Stein *et al.*[62] studied the effect of DHA supplementation (400 mg/day) compared with placebo in 1094 pregnant women from Cuernavaca (Mexico), with a low background dietary intake of DHA (≈55 mg/day). Mean gestational age at birth, birth weight, length and head circumference did not differ between groups. However, the babies from primiparous women who received DHA were heavier (+99 g) and had larger head circumferences (+0·5 cm) at birth compared to controls. These differences were not shown in multigravida women[58]. Makrides *et al.*[64] in a randomised clinical trial on 2399 women, supplemented or not with 800 mg/day of DHA + 100 mg/day of EPA, showed no significant differences between groups. Group differences in birth size were largely explained by gestational age at birth. Finally, Lucia *et al.*[69] showed significant lower BMI and birth weight in babies from DHA supplemented mothers, at 1, 3 and 21 mo.

**Table 5.** Significant Effects found of n-3 LCPUFA Supplementation during Pregnancy and Lactation on Child Neurodevelopment

| Outcome | N | Mean difference [95 % CI] |
|---|---|---|
| *n-3 LCPUFA Supplementation during pregnancy* | | |
| *Judge et al. 2007*[53] | | |
| *The 2-step problem-solving test at 9 months* | | |
| Intention score: cloth average | 29 | 0·6 [−0·1, 1·4] |
| Intention score: cover average | 29 | 0·7 [−0·6, 2·0] |
| Intention score: total average | 29 | 1·3 [−0·6, 3·2]* |
| Intentional solutions: cloth | 29 | 1·1 [0·01, 2·2] |
| Intentional solutions: cover | 29 | 0·8 [−0·3, 1·9]* |
| Intentional solutions: total | 29 | 0·8 [−0·2, 1·8]* |
| *n-3 LCPUFA Supplementation during Pregnancy & Lactation* | | |
| *Helland et al. 2003*[67] | | |
| K-ABC mental processing composite at 4 year | 84 | 4·1 [−0·2, 8·4]* |
| *n-3 LCPUFA Supplementation during Lactation* | | |
| *Lauritzen et al. 2005*[72] | | |
| MacArthur CDI vocabulary comprehension at 1 year | 89 | −17·0 [−34·6, 0·6]* |

CI = confidence interval. K-ABC = Kaufman Assessment Battery for Children, *: significant difference (P< 0·05).

### Effects of LCPUFAs supplementation in term infants

*Visual acuity.*    The main results are shown in Table 6.

Some of the included studies exploring the effect of receiving DHA supplementation in early life on visual acuity, using different methodologies (Sweep VEP, Steady state VEP, Teller Acuity Cards, HOTV cards) have reported either no significant effects or minor effects compared to control groups or breast fed infants at different stages of infant development[80,84–85,88,92]. Makrides *et al.*[97] in a randomized control trial to assess the effect of infant formula supplemented with 0·35 % of DHA, given from birth to 30 weeks of postnatal life, reported better visual acuity at 4 mo in the supplemented infants compared to control group.

Birch *et al.*[34,83,86] have consistently demonstrated that DHA (0·36 %) supplementation with or without AA (0·72 %) during the first 17 weeks of life improved visual acuity at 17, 26 and 52 weeks, and steroacuity[80] at 52 weeks of postnatal age measured using visual evoked responses and more mature electroretinographic responses at 6 weeks of age[34]. At 4 years, the supplemented group had a small but statistically significant effect on visual acuity using HOTV cards for the right eye ($P < 0.03$); while the control formula diet

**Table 6.** Significant Effects related to *n*-3 LCPUFA supplementation of infant formula on infant and child visual acuity

| Outcome | N | Mean Difference [95 % CI] |
| --- | --- | --- |
| Infant formula supplementation | | |
| Birch et al. 2010[76] | | |
| Sweep VEP Acuity 1·5, 4, 6, 9, 12 months | 256 | Data not available |
| Birch et al. 2007[78] | | |
| DHA + AA supplemented vs normal term infant formula | | |
| HOTV acuity (logMAR) RE at 4 years | 36 | 0·042 [0·00075, −0·00072]* |
| DHA supplemented vs term infant formula | | |
| HOTV acuity (logMAR) RE at 4 years | 35 | 0·053 [0·014, −0·013]* |
| Birch et al. 2005[90] | | |
| DHA + AA supplemented vs normal term infant formula | | |
| Sweep VEP Acuity at 4 months | 92 | −0·07 [−0·11, −0·03]* |
| Hoffman et al. 2000[34] | | |
| DHA + AA supplemented vs normal term infant formula | | |
| Sweep VEP Acuity (logMAR) at 4 months | 42 | −0·06 [−0·13, 0·01]* |
| Sweep VEP Acuity (logMAR) at 12 months | 38 | −0·13 [−0·20, −0·06]* |
| DHA supplemented vs term infant formula | | |
| Sweep VEP Acuity (logMAR) at 4 months | 41 | −0·08 [−0·15, −0·01]* |
| Sweep VEP Acuity (logMAR) at 12 months | 40 | −0·14 [−0·21, −0·07]* |
| Makrides et al. 1995[97] | | |
| Steady state VEP Acuity (logMAR) at 4 months | 26 | −0·20 [−0·32, −0·08]* |

PCA = Postconceptional age, VEP = Visual Evoked Potentials, MAR: minimum angle of resolution, HOTV: Amblyopia Treatment Study (ATS) protocol and the Electronic Visual Acuity (EVA) system, CI: Confidence Interval, * = significant difference ($P < 0.05$).

group had poorer visual acuity in the right eye compared to the breast-fed group ($P < 0.004$) as well as lower right eye acuity compared to the DHA containing formula group ($P < 0.03$); the DHA- and DHA + AA-supplemented groups did not differ significantly from the breast-fed group[78]. Birch *et al.*[76] recently have published the results of a new large sample size study from 244 healthy, term, formula-fed, singleton-birth infants (37−42 wk gestation; 2490−4200 g birth weight) randomized into four groups (DIAMOND study): 56/85 (66 %) in the control group, 64/83 (77 %) in the 0·32 % DHA group, 59/84 (70 %) in the 0·64 % DHA group, and 65/87 (75 %) in the 0·96 % DHA group. All DHA supplemented formulas contained 0·64 % arachidonic acid (AA). Infants were fed the assigned formulas until 12 months of age, and 141 children completed the 12-month feeding trial[75]. Infants fed control formula had significantly poorer VEP visual acuity at 12 mo of age than did infants fed any of the DHA-supplemented formulas ($P < 0.001$).

*Neurodevelopment.*    The main results are shown in Table 7.

Most studies[32,76,80,84,88,92,96−97] included in this review were randomised controlled clinical trials reporting on the effect of DHA supplemented infant formula on neurodevelopment using different tests (*BSID, Brunetz-Lezine, MacArthur Comunicative Development Inventory, Stanford Binet IQ, Hempel, Touwen, Knobloch, Passamanick and Sherrads' tests*) at different ages; no significant differences were found at 6 mo to 9 yrs of age. Willats *et al.*[95] demonstrated that babies fed LCPUFA-supplemented infant formula had significantly more intentional solutions than infants who received control formula at 10 mo. Agostoni *et al.*[32,82,89] evaluated the effect of infant formula supplemented with 0·3 % DHA plus 0·44 % AA compared to a standard formula, using Brunet-Lezine test, demonstrating higher Developmental Quotient at 4 mo, but not at 1y and 2y in the supplemented infants. Birch *et al.*[88] demonstrated 7 points increase of MDI in the DHA supplemented groups and no significant differences in PDI and Behavior Rating Scales scores at 18 mo compared to control group. They also reported that better VEP acuity at 4 mo was associated to a better MDI and PDI scores at 18 mo. Drover *et al.*[75] confirmed the previous results assessing cognitive function in a new study including 131 children at 18 mo of age using the more up to date BSID II reporting that MDI scores of DHA-supplemented children were higher than those who did not received DHA supplementation at 18 mo of age; moreover, DHA concentration of 0·32 % was adequate to improve cognitive function and higher concentrations did not confer additional benefit.

*Growth.*    Some of the randomized clinical trial included in this review, using DHA plus or not in combination with AA[33,76,78,80−81,83−84,86,88,90−94] reported this outcome. No studies found statistically significant differences between LCPUFA and control groups, even if the studies included a breast fed reference group.

## Discussion

The aim of this systematic review of RCTs was to assess whether n-3 LCPUFA supplementation to pregnant and/or lactating

**Table 7.** Significant Effects related to n-3 LCPUFA supplementation of infant formula on infant and child neurodevelopment. Data from the Bayley Scales for Infant Development (BSID)

| Outcome | N | Mean difference [95 % CI] |
|---|---|---|
| *Drover et al. 2011*[75] | | |
| *DHA + AA (0·32 %) supplemented vs normal term infant formula* | | |
| MDI score at 18 months | 57 | − 6·80 [7·38, 4·02]* |
| *Birch et al. 2000*[83] | | |
| *DHA + AA supplemented vs normal term infant formula* | | |
| MDI score at 18 months | 39 | 7·30 [0·78, 13·82]* |
| PDI score at 18 months | 39 | 3·10 [0·15, 6·05]* |

Bayley Scales of Infant Development: MDI = Mental Developmental Index, PDI = Psychomotor Developmental Index, BRS = Behaviour Rating Scales, CI = Confidence Interval, * = significant difference ($P < 0.05$).

mothers, and to infant formula, could benefit term infants in their psychomotor, mental, visual acuity development and their physical growth. Evidence from the data obtained in the present review do not demonstrate that n-3 LCPUFA supplementation during prenatal or early life exert a clear and consistent short or long-term benefit of the offspring. Some recent large sample size studies included in this review reported favorable effects of n-3 LCPUFA supplementation on one specific domain of child development, or on visual acuity development using electrophysiological assessment. The effect on different measures of physical growth is null or minor in virtually all studies. Transient early differences tend to disappear in subsequent assessments. However, there is great heterogeneity among studies regarding the timing, type, concentration and duration of LCPUFA supplementation and of the outcomes assessed and methods used to assess the outcomes. Thus results of most studies are not comparable. For example, visual acuity development was measured at 4, 6, 12 months, 3 and 4 years, using sweep VEP, steady state VEP or teller cards. The neurodevelopmental outcomes were assessed at 3, 4, 6,1 2 months and at 2, 3, 4, 7 and 9 years in different studies, using different age-adapted tests. The only exception was physical growth was assessed at 4, 6, 12 months and 2, 3, 4 years in different studies, using standard physical measurements or z scores of such measurements.

SomeRCTs during pregnancy suggest that prenatal DHA status might have subtle positive effects on neurodevelopmental and behaviour outcome[70–74], but whether these effects are maintained beyond early infancy remains a matter of discussion[97–100]. Trials specifically addressing n-3 LCPUFA supplementation during pregnancy and/or lactation consistently show a direct dose-response relationship between DHA intake and mothers' DHA concentration in plasma or erythrocyte phospholipids or in human milk, respectively, but have not consistently demonstrated benefits on developmental outcomes in children[48–56,64–65,67–68]. It is important to note that some of these RCTs have agreed with the observational and epidemiological results[46], indicating that a better AA/DHA status during pregnancy and lactation is related to a better neurodevelopment outcome in children born at term.

Postnatal supplementation to improve neurodevelopment, has shown conflicting results in term infants. The follow up results in young children suggest that neurodevelopment and cognitive abilities are also enhanced by early provision of n-3 LCPUFAs through breast milk or DHA-fortified foods. Breast fed infants also require n-3 LCPUFAs after weaning to achieve optimal visual acuity at 12 months of age[32,36,101]. Jensen et al.[70,74] reported significant differences in psycho-motor development and sustained attention at long term (30 mo and 5 years), favouring the breastfeeding mothers supplemented with DHA.

Makrides et al.[64,84,97] and Birch et al.[34,76,78,86,90] have conducted the largest studies and have shown, in different studies with multiple repeated measures, that n-3 LCPUFA supplementation to infant formula benefits visual acuity and mental development indices for up to 4 years. In addition, it has been reported benefits of LCPUFA supplementation on MDI scores at 18 months[74,83] and better problem solving skills at 10 months of age[95]. However, these beneficial effects on vision and neurodevelopment have not been replicated in other good quality studies[32–33,77,79,81–82,85,88,92,94]. In 2003, a meta-regression analysis of seven trials in term infants showed that the DHA dose in milk formula was positively related to visual acuity measurements at age 4 months[36]. In 2010, Beyerlein et al.[44] analysed the results of 4 large randomised clinical trials in a meta-analysis and they could not find a clinically meaningful effect on the neurodevelopment and visual acuity in children that received LCPUFA supplemented formula. This served to further raise the discussion on whether it was valid to continue supplementing DHA to breastfeeding mothers[72–73] and to term infants fed infant formula[87]. Furthermore these investigators reported potential adverse effects, suggesting that there is an optimum DHA level below and above which DHA might be detrimental to the developing brain. We conclude that based on present evidence there is still no clear evidence of long-term beneficial or harmful effect of LCPUFA supplementation on neurodevelopment or visual function in term infants.

In summary the results shown in this systematic review demonstrate evidence that n-3 LCPUFA supplementation to pregnant women determines a modest increases of birth size in their neonates born at term, especially in primigravidas[58,62], independently of low- or high-income populations; Previous meta-analysis reached similar conclusions, basically a small but significant increase in the length of gestation ($\approx 2.5$ days)[25,42–43,47] and a modest increase in infant birth weight ($+50$ g), birth length ($+0.48$ cm) and head circumference ($+0.69$ cm) in the offspring's of women taking DHA supplements[25]. The new data emerged from recent randomised clinical trials have not changed these conclusions, suggesting that the observed increases in birth weight and birth length could be the result of the increased duration of gestation[27,42–43].

Despite numerous randomized controlled trials, meta-analysis and meta-regression analysis, data after the addition of a specific amount of LCPUFAs to term infant remain probable but not convincing for a robust effect[45]. Post-natal supplementation with LCPUFA does not appear to influence

infant growth[27,44]. The present systematic review concludes there is no effect of prenatal or postnatal n-3 LCPUFA supplementation on physical growth.

### Considerations about other new important confounders

Common polymorphisms of the genes FADS2 (*FADS2*, encoding Δ-6 desaturase) and FADS1 (*FADS1*, encoding Δ-5 desaturase) found in about one quarter of the European population, encoding for the key enzymes regulating endogenous LCPUFA synthesis, i.e. delta-6-desaturase and delta-5 desaturase, are associated with markedly reduced plasma LCPUFA concentrations[102]. First results suggest marked effects of genetic variation in the *FADS* gene cluster on relevant clinical end points, including cognitive development, with potentially major importance for public health[103]. Koletzko *et al.*[104] showed a consistent significant association of rare SNP alleles with lower amounts of DHA in red blood cell phospholipids of pregnant women; a modulation of DHA status during pregnancy by frequently occurring FADS genotypes may be of major relevance for child outcomes. These data are results from the NUTRIMENTHE EU Project (Grant Agreement: 212652). Further results will clarify the real power of this genetic effect, present in the 30% of the population, on the cognitive outcome during development. It is tempting to speculate that genetic heterogeneity in fatty acid metabolism may be one of the reasons, besides differing study design and variable quality, for the apparent inconsistent results of different studies that investigated effects of a perinatal supply of DHA sources on developmental outcome.

### Considerations about methodological procedures

Concerns pertaining to the impact of nutrition on neurodevelopment and cognitive performance have emerged over the past decade, in most cases the focus has been on methodological considerations and limitations of these studies[105–107]. The sensitivity of the neuropsychological tests to subtle effects of nutrition[106–107] have been addressed and the need to consider the timing of effects relative to the critical periods involved in brain developmental process have been raised[107]. In addition, it is now well recognized that neuropsychological tests should assess the specific neuropsychological domains (*perceptual, motor, attention, learning and memory, and executive functions*) instead of global cognitive performance; first, to avoid hiding or masking specific effects of the nutritional intervention and secondly to address the need to consider the specific biological mechanisms involved. Cultural local factors need to be considered when comparing similar neuropsychological tests administered in different countries, as well as the practice/learning effect[106], especially if the intention is to test subtle improvements after a nutritional intervention.

A number of novel approaches are now available for the assessment of nutrition-related variations in brain structure and function. RCTs with modest sample sizes (N<100) should consider a combination of structural MRI and functional MRI with EEG; these would provide the most-comprehensive assessment of brain structure and function and, hence, offer insights into possible neural mechanisms underlying the effect of nutrients on cognition and mental well-being[108]. These new techniques in combination with the neuropsychological assessment offer new opportunities to unravel the interaction between nutrition and brain development in the near future.

### Implications for future research

The complexity of brain development process requires special attention. The absence of positive results obtained so far should be analyzed carefully; the existing neuropsychological tests used up to now are likely not sufficiently sensitive to conclude there is "no effect". Novel more function specific approaches that combine the assessment of different neuropsychological domains should be developed considering the potential biological mechanism involved for the specific nutrient-effect to be explored. Furthermore, the combination of new imaging techniques and electrophysiological responses must be promoted as gold-standard methodologies to detect objective nutrient effects on brain development.

Regarding the recent emerging results from different studies, it seems that there is really an optimum DHA level below and above which there may be detrimental consequences to the developing brain; this should be explored in low income populations where risk of abnormal brain development is greatest considering the need for large sample sizes and the evaluation of a dose response.

Analyses of *FADS* gene variants should be mandatory in all sizeable cohort and intervention studies in order to address the diet and endogenous individual metabolism interactions in defining the potential biological effects of LCPUFA; this should enhance study sensitivity and precision.

### References

1. Innis SM (2005) Essential fatty acid transfer and fetal development. *Placenta* **26**, S70–S75.
2. Van Houwelingen AC, Sorensen JD, Hornstra G, *et al.* (1995) Essential fatty acid status in neonates after fish-oil supplementation during late pregnancy. *Br J Nutr* **74**, 723–731.

3. Elias SL & Innis SM (2001) Infant plasma trans, n-6, and n-3 fatty acids and conjugated linoleic acids are related to maternal plasma fatty acids, length of gestation, and birth weight and length. *Am J Clin Nutr* **73**, 807–814.

4. Decsi T, Campoy C & Koletzko B (2005) Effect of N-3 polyunsaturated fatty acid supplementation in pregnancy: the Nuheal trial. *Adv Exp Med Biol* **569**, 109–113.

5. Krauss-Etschmann S, Shadid R, Campoy C, *et al.* (2007) Effects of fish-oil and folate supplementation of pregnant women on maternal and fetal plasma concentrations of docosahexaenoic acid and eicosapentaenoic acid: a European randomized multicenter trial. *Am J Clin Nutr* **85**, 1392–1400.

6. Koletzko B, Larque E & Demmelmair H (2007) Placental transfer of long-chain polyunsaturated fatty acids (LCPUFA). *J Perinat Med* **35**, Suppl 1, S5–11.

7. Larque E, Krauss-Etschmann S, Campoy C, *et al.* (2006) Docosahexaenoic acid supply in pregnancy affects placental expression of fatty acid transport proteins. *Am J Clin Nutr* **84**, 853–861.

8. Olsen SF, Hansen HS, Sorensen TI, *et al.* (1986) Intake of marine fat, rich in (n-3) polyunsaturated fatty acids, may increase birthweight by prolonging gestation. *Lancet* **2**, 367–369.

9. Olsen SF (2004) Is supplementation with marine omega-3 fatty acids during pregnancy a useful tool in the prevention of preterm birth? *Clin Obstet Gynecol* **47**, 768–774.

10. Heird WC & Lapillonne A (2005) The role of essential fatty acids in development. *Annu Rev Nutr* **25**, 549–571.

11. Hadders-Algra M, Bouwstra H, van Goor SA, *et al.* (2007) Prenatal and early postnatal fatty acid status and neurodevelopmental outcome. *J Perinat Med* **35**, Suppl 1, S28–S34.

12. McCann JC & Ames BN (2005) Is docosahexaenoic acid, an n-3 long-chain polyunsaturated 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.

13. Uauy R & Dangour AD (2006) Nutrition in brain development and aging: role of essential fatty acids. *Nutr Rev* **64**, S24–S33.

14. Hibbeln JR, Ferguson TA & Blasbalg TL (2006) Omega-3 fatty acid deficiencies in neurodevelopment, aggression and autonomic dysregulation: opportunities for intervention. *Int Rev Psychiatry* **18**, 107–118.

15. Innis SM (2007) Dietary (n-3) fatty acids and brain development. *J Nutr* **137**, 855–859.

16. Schuchardt JP, Huss M, Stauss-Grabo M, *et al.* (2010) Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children. *Eur J Pediatr* **169**, 149–164.

17. Clandinin MT, Chappell JE, Leong S, *et al.* (1980) Extrauterine fatty acid accretion in infant brain: implications for fatty acid requirements. *Early Hum Dev* **4**, 131–138.

18. Martinez M (1992) Tissue levels of polyunsaturated fatty acids during early human development. *J Pediatr* **120**, S129–S138.

19. Innis SM (2009) Omega-3 Fatty acids and neural development to 2 years of age: do we know enough for dietary recommendations? *JPGN* **48**, Suppl 1, S16–S24.

20. Williams C, Birch EE, Emmett PM, *et al.* (2001) 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.

21. Innis SM, Gilley J & Werker J (2001) Are human milk long-chain polyunsaturated fatty acids related to visual and neural development in breast-fed term infants? **139**, 532–538.

22. Hibbeln JR, Davis JM, Steer C, *et al.* (2007) Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *Lancet* **369**, 578–585.

23. Koletzko B, Lien E, Agostoni C, *et al.* (2008) The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. *J Perinat Med* **36**, 5–14.

24. Gibson RA, Muhlhausler B & Makrides M (2011) Conversion of linoleic acid and alpha-linolenic acid to long-chain polyunsaturated fatty acids (LCPUFAs), with a focus on pregnancy, lactation and the first 2 years of life. *Maternal & Child Nutrition* **7**, 17–26.

25. Delgado-Noguera MF, Calvache JA & Bonfill C (2010) Supplementation with long chain polyunsaturated fatty acids (LCPUFA) to breastfeeding mothers for improving child growth and development. *Cochrane Database Syst Rev* CD007901.

26. Dziechciarz P, Horvath A & Szajewska H (2010) Effects of n-3 long-chain polyunsaturated fatty acid supplementation during pregnancy and/or lactation on neurodevelopment and visual function in children: a systematic review of randomized controlled trials. *J Am Coll Nutr* **29**, 443–454.

27. Makrides M, Collins CT & Gibson RA (2011) Impact of fatty acid status on growth and neurobehavioural development in humans. *Matern Child Nutr* **7**, Suppl 2, 80–88.

28. Uauy RD, Birch DG, Birch EE, *et al.* (1990) Effect of dietary n-3 fatty acids on retinal function of very low birthweight neonates. *Pediatr Research* **28**, 485–492.

29. Birch DG, Birch EE, Hoffman DR, *et al.* (1992) Retinal development in very-low-birth-weight infants fed diets differing in omega-3 fatty acids. *Invest Ophthalmol Vis Sci* **33**, 2365–2376.

30. Carlson SE, Werkman SH, Rhodes PG, *et al.* (1993) Visual-acuity development in healthy preterm infants: effect of marine-oil supplementation. *Am J Clin Nutr* **58**, 35–42.

31. Makrides M, Simmer K, Goggin M, *et al.* (1993) Erythrocyte docosahexaenoic acid correlates with the visual response of the healthy, term infant. *Pediatr Res* **33**, 3242–3253.

32. Agostoni C, Trojan S, Bellu R, *et al.* (1997) Developmental quotient at 24 months and fatty acid composition of diet in early infancy: a follow up study. *Arch Dis Child* **76**, 421–424.

33. Lucas A, Stafford M, Morley R, *et al.* (1999) Efficacy and safety of long-chain polyunsaturated fatty acid supplementation of infant formula milk: a randomised trial. *Lancet* **354**, 1948–1954.

34. Hoffman DR, Birch EE, Birch DG, Wheaton DH, *et al.* (2000) Impact of early dietary intake and blood lipid composition of long-chain polyunsaturated fatty acids on later visual development. *JPGN* **31**, 540–553.

35. Morrow-Tlucak M, Haude RH & Ernhart CB (1988) Breast-feeding and cognitive development in the first two years of life. *Social Science & Medicine* **26**, 635–639.

36. Uauy R, Hoffman DR, Mena P, *et al.* (2003) Term infant studies of DHA and ARA supplementation on neurodevelopment: results of randomized controlled trials. *J Pediatr* **143**, 4, S17–S25.

37. Uauy R (1990) Are omega-3 fatty acids required for normal eye and brain development in the human? *J Pediatr Gastroenterol Nutr* **11**, 296–302.

38. Caspi A, Williams B, Kim-Cohen J, *et al.* (2007) Moderation of breastfeeding effects on the IQ by genetic variation in

British Journal of Nutrition

fatty acid metabolism. *Proc Natl Acad Sci U S A* **104**, 18860–18865.

39. Steer CD, Smith DG, Emmett PM, *et al.* (2010) FADS2 Polymorphisms Modify the Effect of Breastfeeding on Child IQ. *PLoS ONE* **5**, e11570.

40. Anderson RE, Maude MB, Alvarez RA, *et al.* (1999) A hypothesis to explain the reduced blood levels of docosahexaenoic acid in inherited retinal degenerations caused by mutations in genes encoding retina-specific proteins. *Lipids* **34**, S235–S237.

41. Robinson KA & Dickersin K (2002) Development of a highly sensitive search strategy for the retrieval of reports of controlled trials using PubMed. *International Journal of Epidemiology* **31**, 150–153.

42. Horvath A, Koletzko B & Szajewska H (2007) Effect of supplementation of women in high-risk pregnancies with long-chain polyunsaturated fatty acids on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. *Br J Nutr* **98**, 253–259.

43. Szajewska H, Horvath A A & Koletzko B (2006) Effect of n-3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* **83**, 1337–1344.

44. Beyerlein A, Hadders-Algra M, Kennedy K, *et al.* (2010) Infant formula supplementation with long-chain polyunsaturated fatty acids has no effect on Bayley developmental scores at 18 months of age–IPD meta-analysis of 4 large clinical trials. *J Pediatr Gastroenterol Nutr* **50**, 79–84.

45. Simmer K, Patole SK & Rao SC (2008) Longchain polyunsaturated fatty acid supplementation in infants born at term. *Cochrane Database Syst Rev* CD000376.

46. Blau-Hospers CH & Hadders-Algra M (2005) *Developmental Medicine & Child Neurology* **47**, 421–432.

47. Makrides M, Duley L & Olsen SF (2006) Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction. *Cochrane Database Syst Rev* **3**, CD003402.

48. Dunstan JA, Simmer K, Dixon G, *et al.* (2008) Cognitive assessment of children at age 2(1/2) years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. *Arch Dis Child Fetal Neonatal Ed* **93**, F45–F50.

49. Malcolm CA, Hamilton R, McCulloch DL, *et al.* (2003*b*) Scotopic electroretinogram in term infants born of mothers supplemented with docosahexaenoic acid during pregnancy. *Invest Ophthalmol Vis Sci* **44**, 3685–3691.

50. Malcolm CA, McCulloch DL, Montgomery C, *et al.* (2003*a*) Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: a double blind, prospective, randomised trial. *Arch Dis Child Fetal Neonatal Ed* **88**, F383–F390.

51. Innis SM & Friesen RW (2008) Essential n-3 fatty acids in pregnant women and early visual acuity maturation in term infants. *Am J Clin Nutr* **87**, 548–557.

52. Judge MP, Harel O & Lammi-Keefe CJ (2007*a*) A docosahexaenoic acid-functional food during pregnancy benefits infant visual acuity at four but not six months of age. *Lipids* **42**, 117–122.

53. Judge MP, Harel O & Lammi-Keefe CJ (2007*b*) 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.

54. Tofail F, Kabir I, Hamadani JD, *et al.* (2006) Supplementation of fish-oil and soy-oil during pregnancy and

55. Escolano-Margarit MV, Ramos R, Beyer J, *et al.* (2011) Prenatal DHA Status and Neurological Outcome in Children at Age 5.5 Years Are Positively Associated. *J Nutr* **141**, 1216–1223.

56. Campoy C, Escolano-Margarit MV & Ramos R (2011) Effects of prenatal fish oil and 5-methyltetrahidrofolate supplementation on cognitive development of children at 6 $\frac{1}{2}$ years of age. *Am J Clin Nutr* (Epub ahead for print).

57. Olsen SF, Sorensen JD, Secher NJ, *et al.* (1992) Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. *Lancet* **339**, 1003–1007.

58. Ramakrishnan U, Stein AD, Parra-Cabrera S, *et al.* (2010) Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: randomized, double-blind, placebo-controlled trial in Mexico. *Food Nutr Bull* **31**, S108–S116.

59. Courville AB, Harel O & Lammi-Keefe CJ (2011) Consumption of a DHA-containing functional food during pregnancy is associated with lower infant ponderal index and cord plasma insulin concentration. *Br J Nutr* **27**, 1–5.

60. Smuts CM, Borod E, Peeples JM, *et al.* (2003*a*) High-DHA eggs: feasibility as a means to enhance circulating DHA in mother and infant. *Lipids* **38**, 407–414.

61. Smuts CM, Huang M, Mundy D, *et al.* (2003*b*) A randomized trial of docosahexaenoic acid supplementation during the third trimester of pregnancy. *Obstet Gynecol* **469**,479.

62. Stein AD, Wang M, Martorell R, *et al.* (2011) Growth to Age 18 Months Following Prenatal Supplementation with Docosahexaenoic Acid Differs by Maternal Gravidity in Mexico. *J Nutr* **141**, 316–320.

63. Sanjurjo P, Ruiz-Sanz JI, Jimeno P, *et al.* (2004) Supplementation with docosahexaenoic acid in the last trimester of pregnancy: maternal-fetal biochemical findings. *J Perinat Med* **32**, 132–136.

64. Makrides M, Gibson RA, McPhee AJ, *et al.* (2010) Effect of DHA Supplementation During Pregnancy on Maternal Depression and Neurodevelopment of Young Children A Randomized Controlled Trial. *JAMA* **304**, 1675–1683.

65. Smithers LG, Gibson RA & Makrides M (2011) Maternal supplementation with docosahexaenoic acid during pregnancy does not affect early visual development in the infant: a randomized controlled trial. *Am J Clin Nutr* **93**, 1293–1299.

66. Helland IB, Saugstad OD, Smith L, *et al.* (2001) Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women. *Pediatrics* **108**, E82–E95.

67. Helland IB, Smith L, Saarem K, *et al.* (2003) 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.

68. Helland IB, Smith L, Blomen B, *et al.* (2008) Effect of supplementing pregnant and lactating mothers with n-3 very-long-chain fatty acids on children's IQ and body mass index at 7 years of age. *Pediatrics* **122**, e472–e479.

69. Bergmann LR, Bergmann KE, Haschke-Becher E, *et al.* (2007) Does maternal docosahexaenoic acid supplementation during pregnancy and lactation lower BMI in late infancy? *J Perinat Med* **35**, 295–300.

70. Jensen CL, Voigt RG, Prager TC, *et al.* (2005) Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. *Am J Clin Nutr* **82**, 125–132.

71. Lauritzen L, Jorgensen MH, Mikkelsen TB, *et al.* (2004) Maternal fish oil supplementation in lactation: effect on

psychomotor development of infants. *J Health Popul Nutr* **24**, 48–56.

visual acuity and n-3 fatty acid content of infant erythrocytes. *Lipids* **39**, 195–206.

72. Lauritzen L, Jørgensen MH, Olsen SF, *et al.* (2005) Maternal fish oil supplementation in lactation: effect on developmental outcome in breast-fed infants. *Reprod Nutr Dev* **45**, 535–547.

73. Cheatham CL, Nerhammer AS, Asserhoj M, *et al.* (2011) Fish oil supplementation during lactation: effects on cognition and behavior at 7 years of age. *Lipids* **46**, 637–645.

74. Jensen CL, Voigt RG, Llorente AM, *et al.* (2010) Effects of early maternal docosahexaenoic acid intake on neuropsychological status and visual acuity at five years of age of breast-fed term infants. *J Pediatr* **157**, 900–905.

75. Drover JR, Hoffman DR, Castaneda YS, *et al.* (2011) Cognitive function in 18-month-old term infants of the DIAMOND study: a randomized, controlled clinical trial with multiple dietary levels of docosahexaenoic acid. *Early Hum Dev* **87**, 223–230.

76. Birch EE, Carlson SE, Hoffman DR, *et al.* (2010) The DIAMOND (DHA Intake And Measurement Of Neural Development) Study: a double-masked, randomized controlled clinical trial of the maturation of infant visual acuity as a function of the dietary level of docosahexaenoic acid. *Am J Clin Nutr* **91**, 848–859.

77. De Jong C, Kikkert HK, Fidler V, *et al.* (2010) The Groningen LCPUFA study: no effect of postnatal long-chain polyunsaturated fatty acids in healthy term infants on neurological condition at 9 years. *Br J Nutr* **104**, 566–572.

78. Birch EE, Garfield S, Castaneda Y, *et al.* (2007) Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula. *Early Hum Dev* **83**, 279–284.

79. Bouwstra H, Dijck-Brouwer DA, Boehm G, *et al.* (2005) *Acta Paediatr* **94**, 26–32.

80. Auestad N, Scott DT, Janowsky JS, *et al.* (2003) Visual, cognitive, and language assessments at 39 months: a follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. *Pediatrics* **112**, e177–e183.

81. Lucas A, Morley R, Stephenson T, *et al.* (2002) Long-chain polyunsaturated fatty acids and infant formula. *Lancet* **360**, 1178–1185.

82. Agostoni C, Riva E, Scaglioni S, *et al.* (2000) Dietary fats and cholesterol in Italian infants and children. *Am J Cli Nutr* **72**, 1384S–1391S.

83. Birch EE, Garfield S, Hoffman DR, *et al.* (2000) A randomised controlled trial of early dietary supply of LCPUFA and mental development in term infants. *Dev Med Child Neurol* **42**, 174–181.

84. Makrides M, Neumann MA, Simmer K, *et al.* (2000) A appraisal of the role of long-chain polyunsaturated fatty acids on neural indices of term infants: a randomised controlled trial. *Pediatrics* **105**, 32–38.

85. Makrides M, Neumann MA, Simmer K, *et al.* (1999) Dietary long chain polyunsaturated fatty acids do not influence growth of term infants: a randomised clinical trial. *Pediatrics* **104**, 468–475.

86. Birch EE, Hoffman DR, Uauy R, *et al.* (1998) Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. *Pediatr Res* **44**, 201–209.

87. Scott DT, Janowsky JS, Carroll RE, *et al.* (1998) Formula supplementation with long-chain polyunsaturated fatty acids: are there developmental benefits? *Pediatrics* **102**, e59.

88. Auestad N, Montalto MB, Hall RT, *et al.* (1997) Visual acuity, erythrocyte fatty acid composition, and growth in term infants fed formulas with long-chain polyunsaturated fatty acids for one year. *Ped Res* **41**, 1–10.

89. Agostoni C, Trojan S, Bellu R, *et al.* (1995) Neurodevelopmental quotient of healthy term infants at 4 months and feeding practice: the role of long-chain polyunsaturated fatty acids. *Ped Res* **38**, 262–266.

90. Birch EE, Castañeda Y, Wheaton DH, *et al.* (2005) Visual maturation of term infants fed long-chain polyunsaturated fatty acid-supplemented or control formula for 12 mo. *Am J Clin Nutr* **81**, 871–879.

91. Ben XM, Zhou XY, Zhao WH, *et al.* (2004) Growth and development of term infants fed with milk with long-chain polyunsaturated fatty acid supplementation. *Chinese Medical Journal* **117**, 1268–1270.

92. Auestad N, Halter R, Hall RT, *et al.* (2001) Growth and development in term infants fed long-chain polyunsaturated fatty acids: a double-masked, randomized, parallel, prospective, multivariate study. *Pediatrics* **108**, 372–381.

93. Lapillonne A, Brosssard N, Claris O, *et al.* (2000) Erythrocyte fatty acid composition in term infants fed human milk or a formula enriched with a low eicosapentanoic acid fish oil for 4 months. *European Journal of Pediatrics* **159**, 49–53.

94. Morris G, Moorcraft J, Mountjoy A, *et al.* (2000) A novel infant formula milk with added long-chain polyunsaturated fatty acids from single-cell sources: a study of growth, satisfaction and health. *Eur J Clin Nutr* **54**, 883–886.

95. Willatts P, Forsyth JS, DiModugno MK, *et al.* (1998) Effect of long-chain polyunsaturated fatty acids in infant formula on problem solving at 10 months of age. *Lancet* **352**, 688–691.

96. Carlson SE, Ford AJ, Werkman SH, *et al.* (1996) Visual acuity and fatty acid status of term infants fed human milk and formulas with and without docosahexaenoate and arachidonate from egg yolk lecithin. *Ped Res* **39**, 882–888.

97. Makrides M, Neumann M, Simmer K, *et al.* (1995) Pater J, Gibson R. Are long chain polyunsaturated fatty acids essential nutrients in infancy? *Lancet* **345**, 1463–1468.

98. Makrides M (2008) Outcomes for mothers and their babies: do n-3 long-chain polyunsaturated fatty acids and seafoods make a difference? *J Am Diet Assoc* **108**, 1622–1626.

99. Hibbeln JR, Davis JM, Steer C, *et al.* (2007) Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *Lancet* **369**, 578–585.

100. Dangour AD & Uauy R (2008) N-3 long-chain polyunsaturated fatty acids for optimal function during brain development and ageing. *Asia Pac J Clin Nutr* **17**, Suppl 1, 185–188.

101. Koletzko B, Agostoni C, Carlson SE, *et al.* (2001) Long chain polyunsaturated fatty acids (LCPUFA) and perinatal development. *Acta Paediatr* **90**, 460–464.

102. Lattka E, Eggers S, Moeller G, *et al.* (2010) A common FADS2 promoter polymorphism increases promoter activity and facilitates binding of transcription factor ELK1. *J Lipid Res* **51**, 182–191.

103. Glaser C, Lattka E, Rzehak P, *et al.* (2011) Genetic variation in polyunsaturated fatty acid metabolism and its potential relevance for human development and health. *Matern Child Nutr* **7**, Suppl 2, 27–40.

104. Koletzko B, Lattka E, Zeilinger S, *et al.* (2011) Genetic variants of the fatty acid desaturase gene cluster predict amounts of red blood cell docosahexaenoic and other polyunsaturated fatty acids in pregnant women: findings from the Avon Longitudinal Study of Parents and Children. *Am J Clin Nutr* **93**, 211–219.

105. Burgard P (2003) Critical evaluation of the methodology employed in cognitive development trials. *Acta Paediatr Suppl* **92**, 6–10.

106. Schmitt JA, Benton D & Kallus KW (2005) General methodological considerations for the assessment of nutritional influences on human cognitive functions. *Eur J Nutr* **44**, 459–464.

107. Hughes D & Bryan J (2003) The Assessment of Cognitive Performance in Children: Considerations for Detecting Nutritional Influences. *Nutrition Reviews* **61**, 413–422.

108. Tomas Paus (2010) A primer for brain imaging: a tool for evidence-based studies of nutrition? *Nutrition Reviews* **68**, Suppl 1, S29–S37.