

Review

Effects of n-3 long chain polyunsaturated fatty acid supplementation on visual and cognitive development throughout childhood: A review of human studies

A. Eilander*, D.C. Hundscheid, S.J. Osendarp, C. Transler, P.L. Zock

Unilever Food and Health Research Institute, Unilever R&D, Vlaardingen, The Netherlands

Received 17 October 2006; received in revised form 15 January 2007; accepted 19 January 2007

Abstract

The present paper evaluates the most recent randomized controlled trials assessing the efficacy of n-3 LCPUFA supplementation (with or without n-6 LCPUFA) during pregnancy, lactation, infancy and childhood on visual and cognitive development.

Available evidence suggests a beneficial effect of maternal n-3 LCPUFA supplementation during pregnancy and lactation on cognitive development of infants and children, but not for visual development. Evidence for an effect of LCPUFA supplementation of preterm and term infants on cognitive development of infants remains inconclusive. However, supplementing term infants with daily doses of 100 mg docosahexaenoic acid plus 200 mg arachidonic acid improves visual development as measured by electrophysiological tests. Evidence for benefits of n-3 LCPUFA on cognitive development in healthy children older than 2 years of age is too limited to allow a clear conclusion. Taken together, the evidence for potential benefits of LCPUFA supplementation is promising but yet inconclusive.

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1. Introduction

There is considerable interest in the role of certain long chain polyunsaturated fatty acids (LCPUFA), in visual and cognitive development throughout childhood. The n-3 fatty acid docosahexaenoic acid (DHA) and the n-6 fatty acid arachidonic acid (AA) are the major LCPUFA in the brain [1]. DHA and AA are rapidly incorporated in the nervous tissue of retina and brain during the brain's growth spurt, which mainly takes place from the last trimester of pregnancy up to 2 years of age [1–4]. Beyond development of the central nervous system, n-3 and n-6 fatty acids may influence brain function throughout life by modifications of neuronal membrane fluidity, membrane activity-bound enzymes, number and affinity of receptors, function of neuronal membrane ionic channels, and production of neurotransmitters and brain peptides [5]. Although

DHA and AA are the major structural components of the central nervous system, there is currently no consensus whether dietary supplementation of LCPUFA has benefits for visual and cognitive development of infants. Two Cochrane reviews conclude that for preterm infants, there are no positive long-term effects (>6 months of age) of LCPUFA on visual or intellectual development, while evidence for a beneficial effect of LCPUFA supplementation on early visual development of preterm infants (<6 months of age) is inconclusive [6]. For term infants there is little evidence for a benefit of LCPUFA supplementation on visual or general development [7]. Recently, McCann and Ames [8] reviewed evidence from human and animal studies to assess whether DHA is required for development of normal brain function. They concluded that evidence is too inconsistent to conclude that infant formula should be supplemented with DHA.

Since publication of the Cochrane reviews and review by McCann and Ames, several new human intervention studies on this topic appeared in literature. Additionally,

*Corresponding author. Tel.: +31 10 4605810; fax: +31 10 4605993.
E-mail address: ans.eilander@unilever.com (A. Eilander).

new research focused on the effect of maternal n-3 LCPUFA supplementation during pregnancy and lactation on visual and cognitive function of infants. In this paper, we therefore aim to re-evaluate the currently available evidence on the effect of LCPUFA on visual and cognitive function during infancy and later childhood. We focus on human nutrition intervention studies on LCPUFA supplementation during pregnancy, lactation, infancy and childhood.

2. Methodology

2.1. Literature search

For assessment of the effects of LCPUFA supplementation on visual and cognitive development of children, we divided the literature into four topic areas:

1. Supplementation of mothers during pregnancy and lactation and effects on visual and cognitive development of their infants.
2. Supplementation of preterm infants during the first two years of life and effects on their visual and cognitive development.
3. Supplementation of term infants during the first two years of life and effects on their visual and cognitive development.
4. Supplementation of children older than 2 years of age and effects on their cognitive development.

For identification of studies, we searched the literature databases of Web of Science (Institute for Scientific Information). The search string consisted of combinations of the following terms: long chain polyunsaturated fatty acids, docosahexaenoic acid, eicosapentaenoic acid, linolenic acid, arachidonic acid, linoleic acid, omega-3 fatty acids, omega-6 fatty acids; with infants, children, preschoolers, toddlers, complementary feeding, neonates, offspring, babies; and with cognition, development, mental, learning, brain, visual acuity, neurology. In addition, lists of references in the identified publications were checked. We restricted this review to randomized controlled trials that supplemented subjects for periods of at least 4 weeks, and in which supplementation with LCPUFA was the sole variable differing between treatment and control groups. Therefore, comparisons of groups fed supplemented formula with groups fed human milk, as in some of the trials, are beyond the scope of this review and will not be discussed. When less than three controlled human trials of one of the five selected topics were available, we also searched for observational data. This was the case for supplementation of children older than 2 years. For studies in preterm and full term infants, we restricted our review to studies published after the two

Cochrane reviews on effects of LCPUFA on development [6,7].

2.2. Definitions and methods of investigation of visual and cognitive development

Two major outcomes that may be influenced by n-3 LCPUFA supplementation are visual and cognitive development.

Visual development reflects maturation of visual (cerebral) function and retinal (sensory) function. Retinal function can be assessed by electroretinography (ERG) which measures the electrical responses of sensory cells in the retina. Visual acuity is a measure of clearness of vision. Visual acuity can be measured by visual evoked potentials (VEP) which is the electrical response of the brain to a visual stimulus (electrophysiological methods) or by preferential looking techniques which are behavioral reactions to cards presenting different visual contrasts that are compared and assessed by a technician (behavioral methods). Most studies investigating the effects of LCPUFA on visual development measured grating acuity. This is the spatial threshold for resolving dark and light stripes, which can be studied by both electrophysiological and behavioral methods [9]. Stereo acuity is another measure of visual function of depth perception, assessing the minimum detectable binocular disparity [9].

The term cognition is broad and covers various high-level psychological processes, such as memory, learning, reasoning, attention and language. Cognitive development refers to the changes of the cognitive processes observed over longer periods of time (months or years) and is usually assessed in children by batteries of performance tests assessing specific cognitive abilities. Mental or cognitive development of infants and young children is multidimensional and nonlinear. It is a result of physical growth, neurological maturation, interactions with the environment and the integration of stimuli provided by immediate caregivers and broader social and economic context. Therefore, the assessment of mental development of infants requires the examination of multiple domains and multiple sources of information; the comparison of developmental milestones with standardized populations is essential. The Bayley Scales of Infant Development (BSID) is used for infants and young children and measures general development. The BSID has strong technical characteristics, because it has been standardized on a very large population. It has, however, a poor predictive validity for later intelligence [10]. More specific cognitive functions, such as gross motor skills, parent-child interaction, language comprehension, sleep-wake behavior, and attention can be assessed in infants and young children with more precise methods. However, these assessments are rarely used as they require

time-consuming observation, specific experimental settings and multiple sources of information.

In older children (>3 years), intelligence is usually assessed by sets of short tests measuring various cognitive abilities all correlated with a general factor of intelligence, such as the Kaufman Assessment Battery for Children (K-ABC) and Wechsler tests (WISC) [11].

3. Results

3.1. Maternal n-3 LCPUFA supplementation and effect on visual and cognitive development of their neonates

Six studies have been found in literature assessing the effect of maternal supplementation during pregnancy and/or lactation with n-3 LCPUFA on visual and cognitive development of their infants. The characteristics of these trials are shown in Table 1.

3.1.1. Supplementation during pregnancy

Three trials investigated maternal supplementation before birth, when the fetus is developing, and measured effects on visual and/or cognitive development after birth. One of these trials was excluded because no results on effects of DHA supplementation of mothers on cognitive development of the infants were reported [12]. Another trial by Malcolm et al. [13,14] assessed the effect of fish oil supplementation (0.2 g DHA/d) to mothers from week 15 of pregnancy to delivery, on infant retinal and visual development. No differences were found, neither in maturity of the retina at 1 week of age, nor in visual function measured by VEP to flash and pattern reversal stimuli at birth and at 10 and 26 weeks of age, between neonates born to mothers supplemented with fish oil versus those from mothers supplemented with high-oleic acid sunflower oil. A third trial by Tofail et al. [15] investigated the effect of supplementing 249 pregnant Bengalese women with either fish oil (1.2 g DHA + 1.8 g EPA/d) or soy bean oil (2.25 g LA + 0.27 g ALA/d) from the 25th week of pregnancy until delivery on cognitive outcomes of her infant. Cognitive development was measured using the BSID when infants were 10 months of age. Furthermore, measurements of infant's behavior, quality of stimulation at home (Home Observation for Measurement of Environment) and socio-economic indicators were used to control outcomes of the intervention [15]. The mean Mental Development Index (MDI) and Psychomotor Development Index (PDI) sub-scores of the BSID did not differ significantly between the two groups.

The studies by Malcolm and Tofail did not detect an effect of maternal n-3 LCPUFA supplementation during pregnancy on visual function or cognitive development in the first year of life. Explanations for this could be

that the dose and/or duration of the DHA supplementation were not sufficient. In fact, the dose used in the study by Malcolm et al. [13,14] was low (0.2 g DHA/d) and did not significantly increase the infant's DHA status [16]. Tofail et al. [15] used a high dose of DHA (1.2 g/d) for a supplementation period that was 6–10 weeks shorter than in the study by Malcolm et al. [13,14], but no biochemical parameters were measured to confirm that the DHA status of the infant increased. Cross-sectional analyses of the data of Malcolm et al. [13,14] showed that maturity of the retina at 1 week, and visual acuity at 10 and 26 weeks of age were positively associated with the DHA status of infants at birth. This indicates that the outcome measures in Malcolm et al. [13,14] were sufficiently sensitive to detect effects on retinal and visual function, while there is no such evidence for the BSID measurement in the study by Tofail et al. [15]. Because of the differences and limitations in the designs of these two studies, it is yet uncertain whether n-3 LCPUFA supplementation during pregnancy can have a beneficial effect on visual or cognitive development of the infants.

3.1.2. Supplementation during pregnancy and lactation

One study by Helland et al. [17] investigated the effect of supplementing mothers with either cod liver oil (1.2 g DHA/d) or corn oil (4.7 g linoleic acid (LA)/d) from week 17–19 of pregnancy until delivery and continued for three months during lactation. No treatment differences between the groups were found in electroencephalogram (EEG) maturity of the 2-day old neonates, EEG maturity at 3 months of age and for novelty preference at 6 and 9 months of age. When these children were 4 years of age, however, IQ estimated by the Mental Processing Composite of the K-ABC was significantly (4 points) higher in the cod liver oil group than in the control group [18]. It is unclear whether this can be ascribed to supplementation during pregnancy, during lactation or both. The dose of DHA used was effective in increasing infant' DHA status, and the Mental Processing Composite score was significantly positively correlated with infants' DHA status at 4 weeks of age [18]. No positive correlations were found between EEG maturity at 3 months and novelty preference and DHA umbilical plasma, except for EEG maturity at 2 days of age [17]. Outcomes of this study suggest that effects of DHA supplementation during pregnancy and lactation may appear later in life, when cognitive function is more mature and cognitive psychometric tests have higher discriminative power.

3.1.3. Supplementation during lactation

Three randomized controlled trials have assessed the effect of supplementing lactating mothers with n-3 LCPUFA, on cognitive development of their children. Gibson et al. [19] investigated the effect of

Table 1
Overview of randomized controlled trials on effect of DHA supplementation in pregnant and lactating women on visual and cognitive development of infants

Reference	Location	N	Supplementation to mothers		Functional measurements: age at assessment	Significant effects of supplementation	
			Period	Dose/d		Functional	Biochemical DHA status
Malcolm et al. [13,14]	UK	63	Wk 15 to delivery	I: 200 mg DHA + 36 mg C: 400 mg oleic acid	Flash VEP: 0–5 d Flash and pattern-reversal VEP: 50, 66 wk PCA ERG: 0–7 d	None	Maternal:* Infant: none
Tofail et al. [15]	Bangladesh	249	Wk 25 to delivery	I: 1200 mg DHA + 1800 mg EPA C: 2250 mg LA + 270 mg ALA	BSID: 10 mo	None	ND
Helland et al. [17]	Norway	341	Wk 17–19 to 3 mo post partum	I: 1183 mg DHA + 803 mg EPA + 160 mg LA C: 8.3 mg DHA + 4747 mg LA	EEG: 2 d, 3 mo FT: 27, 39 wk	None	Infant:* HM:*
Helland et al. [18]	Norway	90	Wk 17–19 to 3 mo post partum	I: 1183 mg DHA + 803 mg EPA + 160 mg LA C: 8.3 mg DHA + 4747 mg LA	IQ (K-ABC): 4 yr	Positive: IQ 4.1 points higher in I versus C	Infant:* HM:* Child 4 yr: ND Infant:* HM:*
Gibson et al. [19]	Australia	52	12 wk post partum	I1: 0 g DHA I2: 0.2 g DHA I3: 0.4 g DHA I4: 0.9 g DHA I5: 1.3 g DHA	VEP: 12, 16 wk BSID: 1, 2 yr	None	Infant:* HM:*
Lauritzen et al. [20,21]	Danmark	97	16 wk post partum	I: 1.3 g DHA + EPA + DPA C: olive oil	VEP: 2, 4 mo	Positive: MPS intention scores 2.0 point higher in I versus C in girls Negative: MACI vocabulary comprehension 17 point lower in I versus C; 33 point lower in I versus C in boys; sentence complexity 3.0 point lower in I versus C in boys	Infant:* HM:*
Jensen et al. [22]	USA	160	4 mo post partum	I: 200 mg DHA C: soy + corn oil	TAC, VEP: 4, 8 mo GGM, CLAMS, CAT: 12, 30 mo BSID: 30 mo	Positive: BSID-PDI 8.4 point higher in I versus C	Infant:* HM:*

I, intervention group; C, control group; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; ALA, α -linolenic acid; DPA, docosapentaenoic acid; VEP, visual evoked potential; PCA, post conceptual age; ERG, electroretinography; BSID, Bayley Scales of Infant Development; EEG, electroencephalogram; FT, Fagan Test of Infant Intelligence; K-ABC, Kaufman Assessment Battery for Children; MPS, Means-end Problem Solving; MACDI, MacArthur Communicative Development Inventories; TAC, Teller Acuity Card procedure; GGM, Gesell Gross Motor; CLAMS, Clinical Linguistic and Auditory Milestone Scale; CAT, Clinical Adaptive Test; IQ, Intelligence Quotient; PDI, Psychomotor Development Index; ND, not determined; HM, human milk.

*Increased levels.

supplementing lactating women with one of five increasing doses of DHA (0–1.3 g/d) from algae oil for the first 12 weeks post partum on visual and cognitive development of their infants. No significant effects of DHA supplementation were found on visual acuity at 12 and 16 weeks of age and on MDI and PDI sub-scores of the BSID at 1 and 2 years of age. Nevertheless, a small but statistically significant association of infant DHA status and breast milk DHA concentration at 12 weeks of age with the MDI measured at 1 year was found. This positive correlation disappeared, however, when the children were 2 years old. No relationships with infant DHA status or breast milk DHA were observed for either the PDI or for visual acuity. In a study in Danish lactating women with low fish intake, Lauritzen et al. [20] found no significant effects of fish oil supplementation on visual acuity of infants at 2 and 4 months (1.3 g n-3 LCPUFA/d for 16 weeks) as compared to olive oil supplementation. However, in a cross-sectional analysis of the fish oil supplemented infants, a significant positive association was demonstrated between infant DHA status and visual acuity at 4 months, but not at 2 months of age. Infants were followed to investigate the effects of the supplementation on motor function at 2, 4 and 9 months, problem solving ability at the age of 9 months and linguistic development at 1 and 2 years of age [21]. Fish oil supplementation had a significant positive effect on problem solving in girls at 9 months, while no such effect was detected in boys and the total group. In contrast, fish oil supplementation resulted in a significantly negative effect on vocabulary comprehension at 1 year in the total group and in boys, and on sentence complexity in boys. However, no differences between groups were found on vocabulary tests when children were 2 years of age. Correlation of infant DHA status at 4 months with cognitive tests yielded a significant positive relationship with problem solving in girls at 9 months and a significant negative relationship with vocabulary comprehension in boys at 12 months. No significant correlations were found for any of the other outcome measures or at other ages. In the third study, Jensen et al. [22] investigated the effect of supplementing lactating mothers with either DHA from algae oil (200 mg DHA/d) or vegetable oil for 4 months on visual function and neurodevelopment of their infants. Visual function was assessed with the Teller Acuity Card procedure, and by measuring sweep and transient VEP at 4 and 8 months of age. Neurodevelopment status was evaluated by the BSID at 30 months. At 12 and 30 months of age, gross motor development (Gesell Development Inventory), language development (Clinical Linguistic and Auditory Milestone Scale), and visual-motor problem-solving (Clinical Adaptive Test) were also determined. No significant effects of intervention were observed, with the exception of significantly higher scores on the PDI sub-score of the BSID in 30

months old infants of mother supplemented with DHA compared to controls. No significant correlations were found between infant DHA status and any of the functional outcome measures.

In the three randomized controlled trials summarized above, DHA supplementation during 4 months increased the DHA content of human milk and that of infant blood DHA status in a dose-dependent way. However, none of the three trials found significant effects of DHA supplementation on any of the indicators of visual development. It could be speculated that supplementation during 3–4 months is too short to have a beneficial effect on visual development and should even start during pregnancy. However, it may also be that DHA supplementation during lactation has no relevant effect on visual development.

For cognitive development, the effects of DHA supplementation during lactation are inconsistent. The study by Gibson et al. [19] with only 52 children was considered too small to detect any effect. Jensen et al. [22] and Lauritzen et al. [21] had adequate power and found mixed effects of DHA supplementation at 9–30 months of age. No effects of the interventions were observed on motor development before the age of 12 months, but Jensen et al. [22] showed a large positive effect of DHA supplementation on psychomotor development at 30 months. This finding indicates that effects of supplementation on motor development may appear later in life. No positive effects of DHA supplementation were shown on any of the measures of general intelligence in the three studies, except for problem solving ability in girls at 9 months in the study by Lauritzen et al. [21]. Effects of DHA supplementation on language development were not consistent between the studies by Lauritzen et al. [21] and Jensen et al. [22]. This could be explained by use of different test methodologies, the higher dose of DHA and higher sensitivity of mothers with low fish intake to fish oil supplementation in the study by Lauritzen et al. [21]. There is no good explanation why some positive effects on cognitive development were only found in girls and why negative but transient effects on language development were more pronounced in boys.

In conclusion, DHA supplementation during lactation seems not effective for visual development of breastfed infants, but there is some evidence for a beneficial effect on psychomotor and cognitive development of the infant. An important question is whether these positive effects would be sustainable in later childhood.

3.2. Effect of LCPUFA supplementation on visual and cognitive development in preterm infants

Preterm born infants have significantly lower LCPUFA concentrations than infants born after a full term

Table 2
Overview of randomized controlled trials on effect of LCPUFA supplementation and cognition in premature infants

Reference	Location	N	Subjects	Supplementation		Functional measurements: age at assessment after term	Significant effects of supplementation	
				Period	Dose (% of total fatty acids)		Function	Biochemical DHA status
Fewtrell et al. [23]	UK	204	Infants born <35 wk and ≤2000 g	Birth to 9 mo after term	F1: 0.1% EPA + 0.5% DHA + 0.9% GLA F2: no LCPUFA	KPS: 9 mo NI: 9, 18 mo BSID: 18 mo	Positive in subgroup analysis: F1 boys 5.7 point higher BSID-MDI score versus F2 boys	ND
Clandinin et al. [25]	Australia	179	Infants born ≤35 wk	Birth to 92 wk PMA (12 mo after term)	F1: 0.32% DHA-algae + 0.64% AA F2: 0.32% DHA-algae + 0.10% EPA + 0.64% AA F3: no LCPUFA	BSID: 18 mo	Positive: F1 and F2 had 6 and 10 point higher BSID-MDI scores versus F3 F1 and F2 had 6 point higher BSID-PDI scores than F3	ND
Fang et al. [26]	Taiwan	27	Infants born 32–37 wk. Age at enrollment >32 wk and >2000 g body weight	Birth to 6 mo	F1: 0.05% DHA + 0.10% AA F2: no LCPUFA	VEP: 4, 6 mo L&HH: 4, 6 mo BSID: 6, 12 mo	Positive: F1 had significantly higher scores on BSID-MDI (4.4 and 8.2 points) and BSID-PDI (6.8 and 11.3 points) compared to F2 at 6 and 12 mo respectively	ND

PMA, postmenstrual age; F1–3, formula group; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; GLA, γ -linoleic acid; LCPUFA, long chain polyunsaturated fatty acids; AA, arachidonic acid; KPS, Knobloch, Passamick, and Sherrards' Developmental Screening Inventory; NI, neurologic impairment diagnosed by a pediatrician; BSID, Bayley Scales of Infant Development; VEP, visual evoked potential; L&HH, Lea grating acuity and Hiding Heidi low contrast "FACE" cards; MDI, Mental Development Index; PDI, Psychomotor Development Index; ND, not determined.

pregnancy, because preterm infants miss the major period in utero of DHA and AA accretion in the brain, which is during the third trimester of gestation [4]. Simmer and Patole [6] concluded in a Cochrane review of nine studies published between 1990 and 2002, that there were no positive long-term effects (>6 months of age) of LCPUFA supplementation on visual or intellectual development in preterm infants. Evidence for a beneficial effect of LCPUFA on early visual development (<6 months of age) was inconclusive [6]. Since that review, two large randomized controlled trials and one smaller trial assessing the effect of LCPUFA supplemented formula on visual and general development of preterm infants were published (Table 2). Fewtrell et al. [23] investigated the effects of supplementing 204 preterm infants of tuna oil in combination with borage oil rich in γ -linoleic acid (GLA) (0.5 g DHA and 0.9 g GLA/100 g fat) on mental and psychomotor development and neurological status at 9 and 18 months after term. GLA is a precursor of AA; it is readily converted to AA in human body [23]. No significant differences in MDI and PDI sub-scores of the BSID were found between the groups. However, pre-planned subgroup analysis showed that LCPUFA supplemented boys, but not girls, had significantly higher mental development scores at 18 months after term than controls. Authors suggested that boys may be more sensitive to the effects of suboptimal early nutrition on neurodevelopment [24]. In another recent study, Clandinin et al. [25] found significantly higher MDI and PDI sub-scores of the BSID at 18 months after term in 179 preterm infants who received LCPUFA supplemented formula (0.32 g DHA from algae or fish oil plus 0.67 g AA from fungi oil/100 g fat), compared to the control group. Subgroup analyses to assess effects in boys or girls were not conducted.

Fang et al. [26] showed in a small study of 27 preterm infants a significant beneficial effect of LCPUFA supplemented formula containing 0.05 g DHA and 0.10 g AA/100 g fat on the PDI and MDI sub-scores of the BSID at 6 and 12 months of age compared to the controls. The source of DHA and AA was not reported. No differences were found in the different measures of visual development at 4 and 6 months of age. Formula was supplemented from birth to 6 months. Infants were less premature (born at 30–37 weeks of gestation) and had a higher birth weight (nearly 2000 g) than infants in the studies by Fewtrell and Clandinin (born <35 weeks of gestation, birth weight <2000 g). The dosage of LCPUFA was low and duration of the intervention was short compared to the other two studies. Other limitations of the study were the small sample size and a complete description of the composition of the formula was lacking. Therefore, these outcomes should be interpreted with care.

Simmer and Patole [6] reviewed five studies that assessed the effect of LCPUFA supplemented formula on cognitive development of preterm infants as measured by BSID and Fagan Test. These studies had mixed results. Five studies assessed cognitive development only when infants were 12 months after term or older. Two of these five studies [27,28] measured also effects of LCPUFA supplementation at 18 and 24 months after term using BSID, but no significant effects on cognitive development of infants were found. However, the duration of supplementation in these studies, 1 [27] and 6 [28] months, was much shorter than the 12 months in the study by Clandinin et al. [25].

Taken together, there are indications for a beneficial effect of LCPUFA supplementation on cognitive development of preterm infants. The three studies mentioned above showed positive effects, but evidence is as yet inconclusive. It may be that effects of LCPUFA supplementation are larger when the duration of supplementation is continued until the age of 12 months after term; or that the effect can be better detected at older ages, when cognitive tests are more sensitive and reliable.

3.3. Effect of LCPUFA supplementation on visual and cognitive development in full term infants

Simmer [7] concluded in a Cochrane review that there was little evidence for a benefit of LCPUFA supplementation on visual or general neural development in the first 36 months of age. This conclusion was based on systematic review of nine randomized controlled trials of term infants fed infant formulas with or without LCPUFA. However, they concluded that supplementation of LCPUFA appeared to be beneficial on information processing during the first year of life [7]. SanGiovanni et al. [29] performed a meta-analysis of five of these nine studies and assessed the effect of DHA supplementation on visual acuity in term infants. A significant better visual acuity at 2 months of age (0.32 ± 0.09 octaves better acuity in DHA supplemented groups) was found when visual acuity was measured using behavioral tests, but not when measured with electrophysiological tests. At older ages up to 12 months, no significant effects could be detected at all. In a meta-regression analysis of 14 intervention studies, Uauy et al. [30] demonstrated a significant positive association ($r^2 = 0.35$) between the DHA provided by the supplement and higher visual acuity at 4 months of age. These data suggest that when increasing the DHA content of formula with 10% of total fatty acids (28 mg DHA/d), the visual acuity response improves with -0.067 log of the minimum angle of resolution (logMAR) When taking into account also the hypothetical DHA converted from ALA (1%, 5% or 10%) and EPA (18%) plus DHA content of the formula (DHA

Table 3
Overview of randomized controlled trials on effect of LCPUFA supplementation and cognition in full term infants

Reference	Location	N	Subjects	Supplementation		Cognitive measurements: age of assessment	Significant effects of supplementation	
				Period	Dose (% of total fatty acids)		Functional	Biochemical DHA status
Auestad et al. [31]	USA	404	Healthy infants gestational age 37–42 wk	Birth to 1 yr	F1: 0.45% AA + 0.14% DHA F2: 0.46% AA + 0.13 % DHA F3: no LCPUFA	TAC: 2, 4, 6, 12 mo FT: 6, 9 mo BSID: 6, 12 mo MACDI: 9, 14 mo IBQ: 6, 12 mo	None	Infant.*
Birch et al. [37]	USA	65	Healthy infants gestational age 37–40 wk; HM up to 6 wk	From 6–52 wk of age	F1: 0.72% AA + 0.36% DHA	VEP: 6, 17, 26, 52 wk	Positive: VEP at 17 wk –0.11 logMAR ^a , at 26 wk –0.15 log–MAR and at 52 wk –0.16 log–MAR better in F1 versus F2 SA was at 17 wk 0.5 log–sec ^b better in F1 versus F2	Infant.*
Auestad et al. [33]	USA	157	Healthy infants followed up at 39 mo	Birth to 1 yr	F1: 0.45% AA + 0.12% DHA F2: 0.23% DHA F3: no LCPUFA	SBIS: 39 mo PPVTR: 39 mo MLU: 39 mo BVMi: 39 mo TAC: 39 mo	None	Infant: ND Child 39 mo: none
Bouwstra et al. [34,35]	Netherlands	472	Healthy infants gestational age 37–42 wk	Birth to 2 mo	F1: 0.39% AA + 0.23% DHA + 0.18% GLA F2: no LCPUFA	QGM: 3 mo H: 18 mo BSID: 18 mo	Positive on QGM: Significant less mildly abnormal general movements in F1 (31%) versus F2 (19%)	ND
Hoffman et al. [40]	USA	61	Healthy infants, HM up to 4/6 mo	From 4/6 to 12 mo of age	F1: 0.72% AA + 0.36% DHA F2: no LCPUFA	VEP: 4/6, 12 mo SA: 4/6, 12 mo	Positive: VEP 0.1 log–MAR better in F1 versus F2	Infant.*

Ben et al. [36]	China	121	Infants without congenital abnormalities	Birth until 6 mo	F1: 0.18% AA +0.18% DHA F2: no LCPUFA	BSID: 3, 6 mo	None	Infant.*
Hoffman et al. [41]	USA	51	Healthy infants HM up to 6 mo	From 6–12 mo of age	I: 83 mg DHA enriched egg yolk C: no DHA	VEP: 6, 9, 12 mo SA: 6, 12 mo	Positive: VEP at 9 mo –0.14 log-MAR and VEP at 12 mo –0.16 log-MAR better in F1 versus F2	Infant.*
Ünay et al. [39]	Turkey	80	Healthy infants	Birth to 16 wk	F1: 0.5% DHA F2: no DHA	BAEP: 1, 16 wk	Positive: More rapidly maturation of auditory brainstem at 16 wk in F1 than F2	Infant.*
Birch et al. [38]	USA	71	Healthy infants gestational age 37–40 wk	Birth until 1 yr	F1: 0.72% AA + 0.36% DHA F2: no LCPUFA	VEP: 6, 17, 39, 52 wk SA: 17, 39, 52 wk	Positive: VEP –0.12 log-MAR better in F1 at 17, 39 and 52 wk than F2 SA was 0.46 logsec better in F1 at 17 wk than to F2	Infant.*

HM, human milk; F1–3, formula group; AA, arachidonic acid; DHA, docosahexaenoic acid; GLA, γ -linoleic acid; LCPUFA, long chain polyunsaturated fatty acids; TAC, Teller Acuity Cards; FT, Fagan Test of Infant Intelligence; BSID, Bayley Scales of Infant Development; MACDI, MacArthur Communicative Development Inventories; IBQ, Infant Behavior Questionnaire; VEP, visual evoked potential; SA, stereoacuity; SBIS, Stanford Binet Intelligence Scale; PPVT-R, Peabody Picture Vocabulary Test Revised, MLU, Mean Length of Utterance; BVMI, Beery Visual-Motor Index; QGM, Quality of General Movements; H, Hempel; BAEP, Auditory Brainstem Evoked Potential; ND, not determined.

*Increased levels.
^aLog-MAR corresponds to the log of the minimum angle of resolution.
^bLog-sec, log seconds expresses look duration.

equivalents), then the association was stronger ($r^2 = 0.53–0.68$) and improvements in visual acuity response were higher. Increasing formula with 28 mg DHA equivalents would result in an improvement of visual acuity response of $-0.252 \log \text{MAR}$ [30].

After the review by Simmer in 2001 [7], there were nine new randomized controlled trials published on the effect of LCPUFA supplementation in full term infants on visual and cognitive development (Table 3). Four of these nine studies have also recently been reviewed by McCann and Ames [8]. Auestad et al. [31] found no beneficial effects on visual acuity and mental development in the first 14 months of life after supplementation with infant formula with 0.14% DHA and 0.45% AA from egg-derived triglyceride or from a combination of fish and fungal oil for 12 months. The authors applied multiple measures of general development, including the Fagan Test assessing general information processing and the BSID. Furthermore, they measured language development using MacArthur Communicative Development Inventories and overall behavior and well-being of children by the Infant Behavior Questionnaire and Behavior Rating sub-score of the BSID. In the follow-up of an earlier supplementation study [32], Auestad et al. [33] also observed no significant effects of infant formula on visual acuity, visual-motor function, IQ and language assessments when children were 39 months of age [32]. These children were initially fed formula with either 0.14% DHA and 0.45% AA from egg-derived phospholipids, or with 0.23% DHA only from fish oil, or control formula without added LCPUFA during their first year of life. Bouwstra et al. assessed the effect of infant formula supplemented with 0.23% DHA, 0.39% AA, and 0.18% GLA during the first two months of life on neurodevelopment as reflected by general movements [34] and the Hempel test and BSID [35]. The source of LCPUFA was a combination of egg-yolk, tuna and fungal oil which resembled the composition of LCPUFA in human milk. LCPUFA supplementation reduced the occurrence of mildly abnormal general movements of infants at 3 months of age compared to control formula [34]. However, no significant effects were observed on neurodevelopment when infants were 18 months of age [35]. Similarly, Ben et al. [36] found no significant effects of supplementing Chinese infants with formula containing 0.18% DHA and 0.18% AA (source unknown) for a period of 6 months on MDI and PDI sub-scores of the BSID when infants were 3 and 6 months of age. In contrast, two trials by Birch et al. [37,38] demonstrated significant improvement in visual acuity and visual stereo-acuity throughout the first year of life in LCPUFA supplemented infants compared to controls. Infants received formula supplemented with 0.36% DHA and 0.72% AA from single-cell oil or control formula for 12 months. Also, Ünay et al. [39] showed a significantly more rapid maturation of the

auditory brainstem response as measured by changes in auditory evoked potentials in infants who were supplemented with 0.5% DHA from unknown source during the first 16 weeks of life compared to controls [39].

Two studies conducted by Hoffman et al. assessed the effects of formula supplemented with 0.36% DHA plus 0.72% AA from single-cell oil [40] or baby food with egg yolk enriched with 83 mg DHA versus non-enriched egg-yolk [41] on visual function in infants. Supplementation occurred only during the complementary feeding period from 4–6 months until 12 months of age following exclusive breastfeeding. Both studies showed that visual acuity at age 9 and 12 months, but not visual stereo-acuity, was significantly more mature in LCPUFA supplemented infants. The studies by Hoffman et al. suggest that DHA supplementation may be of benefit during the period of weaning when infants receive complementary foods next to breast-feeding or infant formula, as these foods may be low in n-3 LCPUFA.

Data from four studies by the groups of Birch and Hoffman, all showing positive effects of LCPUFA supplementation on visual development, were combined by Morale et al. [42] to assess the effect of duration of supplementation. This analysis demonstrated that longer duration of LCPUFA supplementation by either human milk or formula was associated with higher improvement in visual acuity at 52 weeks of age, i.e. $-0.002 \log \text{MAR}$ improvement per week of LCPUFA supply.

Four out of five trials mentioned above showed beneficial effects of LCPUFA on visual development. All these studies used higher doses of LCPUFA compared to the study that did not find a benefit. Also, all four studies measured visual acuity by the more sensitive electrophysiological tests [9]. This is consistent with findings from earlier studies reviewed by Simmer [7], of which Makrides et al. [43] and Birch et al. [44] found significant effects on VEP using formula with 0.36% DHA, while Auestad et al. [45] found no effects using a formula with 0.12% DHA. Also, no major effects of LCPUFA supplementation were found when visual acuity was assessed with behavioral methods in the studies reviewed by Simmer [7]. However, these findings are in contrast with earlier findings of the meta-analysis by SanGiovanni et al. [29], who found no significant effects of DHA supplementation on visual acuity measured with behavioral tests only.

Taken together, above studies suggest that LCPUFA supplementation to formula in high dose of 0.36% DHA plus 0.72% AA and prolonged supplementation up to 12 months benefits visual development of infants. These high dosages of LCPUFA compare with a daily intake of approximately 100 mg DHA plus 200 mg AA; based on a 4% lipid content of formula, a 92.5% fatty acid content in milk total lipids, and a volume intake of 750 mL/d as proposed by Uauy et al. [30].

In agreement with the conclusions by Simmer based on results of earlier studies [7], most of the more recent studies on LCPUFA supplementation in term infants failed to demonstrate effects on various aspects of cognitive development. Studies seemed to have sufficient power to detect relevant effects. Studies by Auestad et al. [31,33] used a broad assessment of various tests that have been conducted at standardized circumstances, which are expected to be sufficiently sensitive to detect effects. Possible explanations for not finding effects are that doses in these studies were relatively low (<0.23% DHA), or that effects of LCPUFA supplementation appear at older ages, or that LCPUFA supplementation does not materially affect cognitive development. Future studies are needed to assess the effect of LCPUFA supplementation on cognitive development at high dose (100 mg DHA plus 200 mg AA) and prolonged duration (preferably 12 months); conditions under which significant effects on visual development were found.

3.4. n-3 fatty acid status and cognitive performance in older children

There are no randomized controlled trials published in literature investigating the potential effects of n-3 fatty acids supplementation after the age of 2 years on cognitive performance in children. We found one prospective Dutch cohort study by Bakker et al. [46] assessing the relationship of DHA status at birth and 7 years of age with cognitive performance (K-ABC) measured when children were 7 years old. No significant relationships were found. Zhang et al. [47] studied the association between PUFA intake and psychosocial and cognitive performance of children aged 6–16 years in a cross-sectional analysis on data from the Third National Health and Nutrition Survey in the USA. Higher intake of total n-3 and n-6 PUFA (10 g/day increase) was associated with better performance on the digit span test, but not with performance on block design, arithmetic, and reading comprehension tests. These data suggest that higher PUFA intake could be beneficial for children's working memory. However, no data were available on dietary intakes of n-3 and n-6 fatty acids separately.

In contrast to the limited data in healthy children, there is more evidence for a beneficial effect of PUFA supplementation in children with diagnosed mental disorders and children with phenylketonuria (PKU). Three [48–50] out of five [48–52] double blind randomized controlled trials showed promising results of supplementation with a combination of n-3 and n-6 fatty acids on diminishing some behavioral symptoms in school-aged children with attention-deficit hyperactivity disorder (ADHD). Supplementation with n-3 fatty acids alone (DHA, EPA) seemed not effective [51,52]. These

studies mostly failed to show any improvement of attention or cognitive performance in controlled conditions. Only one of the five studies demonstrated significant improvements of PUFA supplementation on spelling and reading using tests that could also be applied in healthy children [50]. However, outcomes of this study cannot be extrapolated to healthy children as significant diminishing of symptoms of hyperactivity and inattention are likely the underlying factors of improvement on spelling and reading. Children with PKU consume diets restricted in animal foods and therefore have a low intake of AA and DHA resulting in a poor LCPUFA status which may negatively influence their development [53]. A randomized controlled and an uncontrolled trial in children aged 1–12 years with PKU have demonstrated that supplementation with n-3 fatty acids (10–15 mg DHA/kg bodyweight) improved their visual function as measured by VEP [54,55]. Although these children were diagnosed for PKU, the studies suggest that visual function can be affected by DHA supplementation in children older than 2 years of age with a poor LCPUFA status.

In conclusion, there are no trial data and only one prospective cohort study on the effect of n-3 fatty acid supplementation on cognitive performance in healthy children older than 2 years of age. However, it remains conceivable that n-3 fatty acid supplementation could be beneficial in these children, in particular when intake of n-3 fatty acids is low and nutritional status is poor. This should be addressed in future randomized controlled trials.

4. Discussion and conclusion

We reviewed the available data from randomized controlled trials that investigated effects on visual and cognitive development of n-3 fatty acid supplementation to pregnant and lactating women, infants and children. With the exception of studies in term infants, the total number of randomized controlled trials on this topic is still very limited, especially for supplementation in older children. It is difficult to directly compare studies, as they assessed visual and cognitive development by many different outcome measures, and also had multiple differences in design that could influence the outcomes and interpretation. In particular, studies differed in the type and dose of fatty acids supplied to the intervention and control groups, duration of supplementation, age of subjects at cognitive assessment, and methodology used for assessment of cognitive outcomes. Thus, there is a large heterogeneity among studies. Nevertheless, we can conclude from these studies that:

- for supplementing *pregnant and/or lactating women* with DHA, there is currently no supporting evidence for a beneficial effect on visual development, but

there is suggestive evidence for a beneficial effect of supplementation during pregnancy and lactation or lactation only on mental development and on longer-term cognition;

- for supplementing *preterm infants* with DHA and AA, evidence for benefits on visual development at <6 months of age remains inconclusive, while there are indications from two studies for a beneficial effect of supplementation in early in life on cognitive development at >12 months of age;
- for supplementing *term infants*, with LCPUFA in high doses (100 mg DHA and 200 mg AA per day), there is consistent evidence for a beneficial effect on visual development during the first year of life, while there is hardly such evidence for beneficial effects on cognitive development;
- for supplementing *healthy children older than two years of age* with DHA, there is no evidence for beneficial effect on cognitive performance.

Although the totality of the evidence is still inconclusive, there are promising indications that supplementation with DHA and other n-3 fatty acids, either or not in combination with n-6 fatty acids, during pregnancy, lactation and infancy may benefit for visual and cognitive development early in life.

As the incorporation of DHA and AA in the developing brain is particularly high in the prenatal period [3,4], supplementation during pregnancy would theoretically be expected to have the largest impact on visual and cognitive development of infants. However, the evidence from supplementation studies in pregnant women is currently too limited to support this notion. The benefit of DHA supplementation on cognition in older children has not been investigated yet, but the potential for beneficial effects is expected to be smaller than in younger infants or the fetus, as brain development and the potential incorporation of DHA becomes slower after the age of 2 years [56]. However, it remains possible that an increased supply of DHA to the brain in this period results in changes in fatty acid composition of brain tissue or that DHA has other metabolic effects that could affect brain function [5]. Obviously, such effects are very difficult or impossible to measure in humans.

When all intervention studies are taken together, most positive effects of the LCPUFA supplementation on cognitive development were detected by general tests of intelligence, such as the BSID. Motor function was less frequently improved by LCPUFA supplementation and language development may even be negatively affected.

The difficulty of measuring mental development during infancy may partly explain why studies have yielded conflicting results. The central nervous system of infants is developing and therefore all the various cognitive skills have not been differentiated yet. There-

fore, it is hard to reliably measure cognitive development at young ages. Methodologies should be both sensitive and both general, assessing a broad spectrum of cognitive functions to detect effects during early life. Measurements should take place in a standardized way, preferably at the same age and under the exact same conditions, which requires a laboratory setting. Alternatively, the effect of supplementation may be measured when children are older (≥ 3 years of age), when the brain is more differentiated and different test can be applied that assess more specific aspects of cognition. Also, new methodologies such as brain imaging techniques could help in future trials to detect subtle effects on brain structure and function [57].

The available data do not allow formulation of specific recommendations for optimal dosages, types, forms and ratio of n-6 and n-3 fatty acids for optimal development. Optimal brain development during infancy and childhood and maintenance of brain function throughout life requires an adequate and balanced supply of the EFA, LA and α -linolenic acid (ALA) in the diet [5,58]. It is generally assumed that LA and ALA will at least partly be converted into DHA and AA, respectively. However, it is presently unclear whether conversion of dietary EFA is under all circumstances sufficient to meet optimal DHA and AA requirements. A limited number of studies in human adults show that conversion of ALA to EPA and DHA synthesis is only marginal [59–62], with an overall conversion rate of at most some 4% [63]. For infants, conversion rates could be higher to cover the needs for brain development [30], but quantitative data on ALA conversion in infants and children are lacking. However, a few studies demonstrated that supplementing preterm infants with ALA [64] improved retinal function in early life, suggesting that ALA is converted to such an extent that DHA content of the retina can be influenced.

A relatively high ratio of n-6 to n-3 fatty acids in diet could theoretically impair the conversion of ALA to DHA in vivo [65], as ALA and LA compete for the same desaturation enzymes needed for the conversion of ALA to EPA and DHA and for conversion of LA to AA. Studies on LCPUFA supplementation and visual and cognitive development in children investigated effects of n-3 fatty acids alone or n-3 fatty acids in combination with n-6 fatty acids, but there are no studies specifically investigating the role of n-6 fatty acids alone on development and function of the central nervous system. The rationale for adding AA or other n-6 fatty acids to n-3 fatty acid supplementation is to prevent a decline in AA status [66] and prevent infants from possible growth faltering [67]. Although evidence from human studies is lacking, it is believed that sufficient supply of n-3 fatty acids is more important for visual and cognitive development and function. Therefore, it would be of value to investigate how mental development in infants and children may be

affected by using different types of n-3 fatty acids and at different n-6:n-3 fatty acid ratio's in the diet.

More recently, research has focused on the bioavailability of DHA from diet to the brain. Animal studies have suggested that DHA in phospholipid form would be more effective in crossing blood-brain barrier compared to DHA in triglyceride form [68,69]. In the intervention studies reviewed DHA was mostly delivered in the triglyceride form or a combination of both. The studies by Auestad et al. [31,33] have investigated different forms of DHA and did not show beneficial effects on cognitive development, but dosages may have been too low.

In conclusion, there is still limited and inconsistent evidence that supplementing mothers, infants or children with longer chain n-3 fatty acids (possibly with additional n-6 fatty acids) can improve visual and cognitive development of infant or child. However, there are promising indications for effects of supplementing pregnant and lactating mothers with n-3 LCPUFA on cognitive development of their children that warrant further studies in these and other target groups.

Future studies should specifically address the dose, type, form and ratio of n-6 and n-3 fatty acids and duration of supplementation required for optimal visual and cognitive development. These studies should be designed with high enough statistical power to detect small but relevant effects on visual and cognitive function by using standardized, sensitive test methodologies for measuring visual function and specific cognitive abilities, and measure effects over a longer period of child development. New techniques for investigating nutritional influences on the developing brain structures, such as brain imaging techniques, should also be explored.

Acknowledgments

We are grateful to G. Hornstra, Nutrisearch, Gronsveld, The Netherlands for providing critical comments on the manuscript.

Contributions: AE and DCH searched the literature. AE analyzed the literature and wrote the manuscript. CT contributed to interpretation of the literature. DCH, CT, SJO and PLZ critically reviewed and edited the manuscript.

Disclosure: All authors are employees of Unilever. Unilever markets food products, some of which are enriched with omega-3 fatty acids.

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