Evidence Report/Technology Assessment
Number 118

Effects of Omega-3 Fatty Acids on Child and Maternal Health

Prepared for:
Agency for Healthcare Research and Quality
U.S. Department of Health and Human Services
540 Gaither Road
Rockville, MD 20850
www.ahrq.gov

Contract No. 290-02-0021

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AHRQ Publication No. 05-E025-2
August 2005

This report is based on research conducted by the University of Ottawa Evidence-based Practice Center (EPC), under contract to the Agency for Healthcare Research and Quality (AHRQ), Rockville, MD (Contract No. 290-02-0021). The findings and conclusions in this document are those of the authors, who are responsible for its contents; the findings and conclusions do not necessarily represent the views of AHRQ. Therefore, no statement in this report should be construed as an official position of AHRQ or of the U.S. Department of Health and Human Services.

Suggested Citation:
Lewin GA, Schachter HM, Yuen D, Merchant P, Mamaladze V, Tsertsvadze A, et al. Effects of Omega-3 Fatty Acids on Child and Maternal Health. Evidence Report/Technology Assessment No. 118. (Prepared by the University of Ottawa Evidence-based Practice Center, under Contract No. 290-02-0021.) AHRQ Publication No. 05-E025-2. Rockville, MD: Agency for Healthcare Research and Quality. August 2005.

Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-Based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. This report was requested and funded by the Office of Dietary Supplements, National Institutes of Health. The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions and new health care technologies. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for health care quality improvement projects throughout the Nation. The reports undergo peer review prior to their release.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers as well as the health care system as a whole by providing important information to help improve health care quality.

We welcome comments on this evidence report. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by email to **epc@ahrq.gov.**

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Acknowledgments

The authors would like to thank numerous individuals for their support of the present project: our collaborators at SC-RAND and Tufts-NEMC EPCs; Isabella Steffensen and Christine Murray for their ability to convey with words, tables and figures what it is we did and found; for their expert and timely translation of articles; Herb Woolf for responding with substance to our request of industry for evidence; Peter O'Blenis for assuring that our software would adapt and grow as quickly as the project requirements shifted and expanded; Mary Fewtrell; Sjurdur Frodi Olsen; Maria Makrides; Eileen Birch; Susan Carlson for timely responded to our requests to clarify their research; Khai Tran and Maia Miguelez, PhD for providing input on the data abstraction forms; Nancy Santesso for helping on the development of the search strategy; Raymond Daniel for helping to retrieve the hard copies of the studies, Manijeh Hamifard for helping on the data abstraction and Chantelle Garritty for coordinating the project.

Structured Abstract

Context: The likely significance of omega-3 fatty acids for child and maternal health is therefore suggested by the observations that: the human brain and retina each contain considerable omega-3 fatty acid content; the child delivered at term receives an important supply of omega-3 fatty acids especially in the third trimester of pregnancy; and, due to a shortened gestational period, the child delivered prematurely receives less exposure to omega-3 fatty acid content than does the term child. This evidence is systematically reviewed here.

Objectives: The purpose of this study was to conduct a systematic review of the scientific–medical literature to identify, appraise and synthesize the evidence of omega-3 fatty acids in child and maternal health. Evidence was sought to investigate a series of questions regarding the influence of the omega-3 fatty acid intake (supplemented during pregnancy) on the duration of gestation, incidence of preeclampsia, eclapmsia or gestational hypertension (GHT), and incidence of infants small for gestational age (SGA), as well as the association between the maternal biomarkers during pregnancy and the pregnancy outcomes outlined above. The influence of the omega-3 fatty acid intake (supplemented or breast milk) on the developmental outcomes in preterm and term infants, such was growth, neurocognitive development and visual function, were also investigated, as well as the association between the maternal, fetal or child's biomarkers and these clinical outcomes. The impact of effect modifiers was also examined, as well as the safety profile. The results will be used to inform a research agenda.

Data Sources: A comprehensive search for citations was conducted using five electronic databases (MEDLINE®, PreMEDLINE®, EMBASE, Cochrane Central Register of Controlled Trials, and CAB Health). Searches were not restricted by language of publication, publication type, or study design, except with respect to the MeSH term "dietary fats," which was limited by study design to increase its specificity. Search elements included scientific terms (with acronyms), generic and trade names relating to the exposure and its sources (e.g., eicosapentaenoic acid [EPA], fish oil), and relevant population terms (e.g., preterm, term, child development, etc). Additional published or unpublished literature was sought through manual searches of references lists of included studies and key review articles, and from the files of content experts.

Study Selection: Studies were considered relevant if they described live human populations of healthy preterm (< 37 weeks of GA), term (> 37 weeks of GA) infants or healthy pregnant women, investigated the use of any supplements (formula, diet, etc.) known to contain omega-3 fatty acids and/or human milk, and utilizing pertinent pregnancy and child developmental outcomes (e.g., growth, neurocognitive, visual). Studies examining the questions concerning the efficacy had to employ a controlled research design (i.e., RCTs), whereas, any type of design other than case-series or case-study was permitted to address the possible association between the content of biomarkers and the clinical outcomes. Three levels of screening for relevance, and two reviewers per level, were employed. Disagreements were resolved by consensus and, if necessary, third-party intervention.

Data Extraction: All data were extracted by one reviewer, then verified by a second one. Data included the characteristics of the report, study, population, intervention/exposure and comparator(s), cointerventions, discontinuations (with reasons), and outcomes (i.e., clinical, biomarkers, safety). Study quality (internal validity) and study applicability (external validity) were appraised.

Data Synthesis: Question-specific qualitative synthesis of the evidence was derived. Meta-analysis was conducted with data concerning the supplemental influence on incidence of premature deliveries, GHT, birth weight, incidence of IUGR, growth patterns (i.e., weight, length and head circumference) in term and preterm infants, neurological and cognitive development in term infants, and visual function in both term and preterm infants. One hundred and seventeen reports, describing 89 studies, were deemed relevant for the systematic review, with many studies described in more that one question.

Conclusions: Studies investigating the influence of omega-3 fatty acids on child and maternal health revealed the absence of a notable safety profile (i.e., moderate-to-severe AEs). Pregnancy outcomes were either unaffected by omega-3 fatty acid supplementation, or the results were inconclusive. Results suggested the absence of effects with respect to the impact of supplementation on the incidence of GHT, preeclampsia or eclampsia, as well as on infants being born SGA. However, regarding evaluations of the duration of gestation, some discrepancies were observed, although most of the studies failed to detect a statistically significant effect. Biomarker data failed to clarify patterns in pregnancy outcome data.

Results concerning the impact of the intake of omega-3 fatty acids on the development of infants are primarily, although not uniformly, inconclusive. The inconsistencies in study results may be attributable to numerous factors.

In addition, making clear sense of the absolute or relative effects of individual omega-3 fatty acids, or even omega-3 fatty acid combinations, on child outcomes is complicated or precluded by the following problem. Studies typically employed interventions that involved various cointerventional or background constituents (e.g., omega-6 fatty acids), yet whose metabolic interactions with the omega-3 fatty acid(s) were not taken into account in interpreting the results. The dynamic interplay among these fatty acid contents (e.g., competition for enzymes), and how this interplay may influence outcomes, may differ in important ways depending on whether DHA or olive oil is added to this combination of cointerventional or background constituents, particularly in the maternal population. This strategy prevented the isolation of the exact effects relating to the omega-3 fatty acid content. It is thus very difficult to reliably ascribe definite child outcome-related benefits, or the absence thereof, to specific omega-3 fatty acids. Biomarker data failed to clarify patterns in child outcome data.

Future research should likely consider investigating the impact of specific omega-6/omega-3 fatty acid intake ratios, in no small part to control for the possible metabolic interactions involving these types of fatty acids. To produce results that are applicable to the North American population, populations consuming high omega-6/omega-3 fatty acid intake ratios should likely be randomized into trials also exhibiting better control of confounding variables than was observed, especially in the present collection of studies of child outcomes.

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Appendixes and Evidence Tables are provided electronically at
http://www.ahrq.gov/clinic/tp/o3mchtp.htm

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Evidence Report/Technology Assessment

Number 118

Effects of Omega-3 Fatty Acids on Child and Maternal Health

Summary

Authors: Lewin GA, Schachter HM, Yuen D, Merchant P, Mamaladze V, Tsertsvadze A, et al.

Introduction

The purpose of this study was to conduct a systematic review of the scientific-medical literature to identify, appraise, and synthesize the human evidence for the effects of omega-3 fatty acids on child and maternal health. The review was requested and funded by the Office of Dietary Supplements, National Institutes of Health. It was undertaken as part of a consortium involving three Evidence-based Practice Centers (EPCs), which investigated the value of omega-3 fatty acid supplementation across eleven health/disease areas. The three EPCs are Southern California-RAND, Tufts-New England Medical Center, and the University of Ottawa. To ensure consistency of approach, the three EPCs collaborated on selected methodologic elements, including literature search strategies, rating of evidence, and data table design.

It has been posited that the accretion of omega-3 fatty acids within the maternal biological system has the potential to influence both maternal health during pregnancy and fetal health. Likewise, it has been hypothesized that their accumulation within the post-delivery child's biological system can affect its development and health. Birth weight is the most important factor affecting neonatal morbidity and mortality, and is thus an outcome worth monitoring.[1] Moreover, premature infants are at risk of injury to every organ system in the newborn period. Of greatest concern for infants who survive are the risks of developing permanent neurocognitive deficits that impact their lifelong health and functional capacity.[2-5]

Results of studies conducted on residents of the Faroe Islands[6,7] suggest that marine diets, which contain omega-3 fatty acids, increase birth weight either by prolonging pregnancy[8] or by increasing the fetal growth rate.[9,10] Additionally, it has been hypothesized that marine oils may lower risks of certain complications of pregnancy, in particular preterm delivery, intrauterine growth retardation, preeclampsia, and gestational hypertension,[11] given that some of omega-3 fatty acids' presumed mechanisms of action overlap with those of aspirin.[12-14]

Docosahexaenoic acid (DHA) and arachidonic acid (AA) have been identified as important structural components of the highly specialized membrane lipids of the human central nervous system, with phospholipids of brain gray matter containing high proportions of DHA.[15-17] DHA has also been observed to be the major long-chain polyunsaturated fatty acid (LC PUFA) in the outer segments of the retina's rods and cones.[15]

Based on observational studies, it has been shown that human milk fed infants have improved neurocognitive development compared to formula fed infants; it was hypothesized that one of the contributing factors may be the availability of long-chain derivatives of linoleic acid (LA) and alpha-linolenic acid (ALA) that is present only in human milk.[18,19] This difference in fatty acids intake is reflected in lower erythrocyte membrane phospholipid DHA in

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infants fed formula.[18] Until the recent availability of infant formula with added omega-3 LC PUFAs, standard infant formula was devoid of these fatty acids.

The likely significance of omega-3 fatty acids for child health is therefore suggested by the observations that (a) the human brain and retina each contain considerable amounts of omega-3 fatty acids; (b) the children delivered at term receive an important supply of omega-3 fatty acids, especially in the third trimester of pregnancy; and (c) due to a shortened gestational period, a child delivered prematurely receives less exposure to omega-3 fatty acids content than does the term child. Not surprisingly, the observation concerning preterm infants has afforded considerable empirical study of the impact of omega-3 fatty acids on the health of such infants.

Key Questions

The questions are organized by type of population (i.e., maternal/pregnancy versus child) and type of outcome data (i.e., clinical/pregnancy versus clinical/child-developmental).

Maternal population, pregnancy outcomes/biomarkers associations:

- What is the evidence that intake of omega-3 fatty acids influences
  - duration of gestation?
  - incidence of preeclampsia, eclampsia or gestational hypertension?
  - incidence of births of human infants small for gestational age (SGA)?

Child population, growth patterns, neurological, visual or cognitive developmental outcomes/biomarkers associations:

- What is the evidence that maternal intake of omega-3 fatty acids
  - during pregnancy influences any of the clinical outcomes in term or preterm human infants?
  - within maternal breast milk, infant formula, both and/or other sources (i.e., diet) influences any of the clinical outcomes in term or preterm human infants?
- What is the evidence that term or preterm human infants' clinical outcomes are associated with the omega-3 or omega-6/omega-3 fatty acids content of
  - maternal or fetal biomarkers during pregnancy?
  - child biomarkers?

Adverse effects:

- What is the evidence for the risk, in pregnant or breastfeeding women, term or preterm human infants, of short- and long-term adverse events related to their intake of omega-3 fatty acids during pregnancy or after birth?

Methods

A Technical Expert Panel (TEP) consisting of six members was convened to provide advisory support to the project, including refining the questions and highlighting key variables requiring consideration in the evidence synthesis.

Study Identification

Several electronic databases were searched: MEDLINE®, PreMEDLINE®, EMBASE, the Cochrane Library including the Cochrane Central Register of Controlled Trials, and CAB Health. Searches were not restricted by language of publication, publication type, or study design, except with respect to the MeSH term "dietary fats," which was limited by study design to increase its specificity. Search elements included scientific terms, with acronyms, as well as generic and trade names relating to the exposure and its sources (e.g., eicosapentaenoic acid [EPA], omega-3 fatty acids, infant formula) and relevant population terms (e.g., gestational hypertension). Reference lists of included studies, book chapters, and narrative or systematic reviews retrieved after having passed the first level of relevance screening were manually searched to identify additional unique references. Through contact with content experts, attempts were made to identify both published and unpublished studies. A final set of 2,049 unique references was identified and posted to an internet-based software system for review.

Studies were considered relevant if they described live, otherwise "healthy" human populations of any age. The generic term "child" was used to refer to infants (less than 12 months of age), toddlers, and children up to 18 years old. Excluded were studies whose biomarker data were solely obtained from aborted fetuses and which did not distinguish between data obtained from term and preterm births.

Interventional/exposure studies had to specifically investigate foods or supplements known to contain omega-3 fatty acids of any type, from any source, any serving size or dose, delivered in any fashion and for any length of time. No restrictions were placed on the types or doses of pre- or on-study cointerventions. While omega-6 fatty acids appear to play a key role in health and development, and their possible co-influence on outcomes is thus assessed in our review, studies exclusively investigating their impact on health outcomes were excluded.

If at least two randomized controlled trials (RCTs) were identified, no other types of design were required. Yet, if insufficient numbers of RCTs were retrieved, non-RCT (i.e., controlled clinical trials, without random allocation) and

observational studies (i.e., cohort, case-control, or cross-sectional studies) were included. Descriptive study designs were also excluded.

Any and all child developmental outcomes reflecting the four categories of the developmental arc were considered relevant. As markers of omega-3 fatty acids metabolism, the following fatty acids compositions or concentrations, from any source (e.g., red blood cell [RBC] membranes, plasma phospholipids) were considered relevant: EPA, DHA, AA/EPA, AA/DHA, AA/EPA+DHA.

Two initial levels of screening for relevance, and two reviewers per level, were employed (directed at bibliographic records, then full articles). A screening identified and excluded uncontrolled studies. Calibration exercises preceded each step of the screening process. The reasons for the unsuitability of excluded studies were noted according to a modified QUOROM format.[20] Disagreements were resolved by consensus and, when necessary, third-party intervention.

Data Abstraction

Following a calibration exercise involving two studies, eleven reviewers independently abstracted the contents of included studies using an electronic data abstraction form. A second reviewer then verified these data. Data abstracted included the characteristics of the report (e.g., publication status), study (e.g., sample size), population (e.g., preterm versus term status), intervention/exposure (e.g., omega-3 fatty acids types), and comparator(s), cointerventions (e.g., omega-6 fatty acids use), withdrawals and dropouts, including reasons, clinical outcomes, fatty acids content of biomarkers, and adverse events.

Data Synthesis

A summary table provided a question-specific overview of included studies' relevant data, which is presented in greater detail in evidence tables. A question-specific summary matrix described each study in terms of its quality and applicability ratings. Question-specific qualitative syntheses of the evidence were derived. Meta-analysis was performed if the following criteria were met: at least two RCTs, same population characteristics (mean age, health status, gender), same co-interventions, same intervention based on the type of omega-3 fatty acids supplemented (DHA+AA vs. DHA vs. DHA+EPA, etc.) regardless of the daily dose in the child population, same comparator based on source of placebo (e.g., olive oil, unsupplemented formula), outcomes relevant to respond to the key-questions: percentage (n) of premature deliveries, incidence of gestational hypertension (GHT), pre-eclampsia or eclampsia, incidence of IUGR or SGA infants, weight, length, and head circumference of infants (means), neurological and cognitive development measured by validated scales (e.g., Bayley's Developmental Scale score), and visual acuity or visual function of infants measured by appropriate tests (Teller's Card test, etc.).

Results

Literature Search

Of the 2,049 records entered into the initial screening for relevance, 1,579 were excluded. Of the 191 reports that made it to this level of screening, 74 were excluded. Hence, in total, 117 reports, describing 89 unique studies, were deemed relevant for the systematic review, with 20 studies each described by more than one report and three reports describing more than one unique study. There were 63 randomized controlled trials (RCTs) and 26 observational studies across all the key questions. Only one study required translation from German to English.[21] No studies were identified across all the child outcomes (i.e., growth patterns, neurocognitive development, and visual function) regarding the influence of the intake of omega-3 fatty acids from sources other than human milk, or infant formula, as well as the association between omega-3 or omega-6/omega-3 fatty acids content of fetal biomarkers and any of the clinical outcomes. Synopses of evidence are presented according to the clinical outcomes by population.

Safety Issues

Overall, omega-3 fatty acids supplementation in pregnant women, breastfeeding mothers, and preterm and term infants, was very well tolerated and did not generate any serious adverse events across the included RCTs. The safety data was reported in 21 RCTs. In pregnant women, the adverse events related to the omega-3 fatty acids intake were mild and transient, with nausea and gastrointestinal discomfort being the most commonly reported.[22,23] For both term and preterm populations, change in number of stools and flatulence were the most common adverse events related to the omega-3 supplemented formulas. However, most of the serious harms were related to the fact that the infants were premature with low birth weights, which increases the occurrence of necrotizing enterocolitis (NEC), bleeding problems, infections and respiratory failure, among others in the case of preterm infants.[24-43] None of the withdrawals were due to the interventional formula.

Pregnancy Outcomes

Duration of gestation-intake during pregnancy: Fifteen poor quality RCTs addressed this question.[11,44-51,59] Seven trials included otherwise healthy pregnant women,[52-58] the remaining eight studies included a high-risk population of pregnant

women. Ten studies did not find a significant difference between intervention groups in the duration of gestation measured as mean of gestational age at delivery.[22,23,53-58] Four poor quality studies observed that the omega-3 fatty acids group had a significantly greater duration of gestation after treatment compared with the unsupplemented group.[22,52]

Omega-3 fatty acids did not have a significant effect on the proportion of premature deliveries in ten studies.[11,23,52,55,59] Fish consumption in the background diet was used as a covariate in only one trial.[52] Other covariates used to control the results were: the compliance with the intervention,[52] current smoking status,[23,55] maternal BMI, and number of prior pregnancies.[55] The only variable that had an impact on the results was the smoking status in Smuts et al.'s study.[55] The duration of gestation was significantly longer in the high-DHA group in the nonsmokers.[55]

Meta-analysis of the incidence of premature deliveries was performed from eight RCTs that used capsules containing DHA+EPA (OR: 0.88 [95% CI: 0.62-1.25]),[11,44,49] and two trials using high DHA eggs (OR: 0.53 [95% CI: 0.13-2.29])[47,50] or control group. There is inconsistent evidence of the use of omega-3 fatty acids supplements during the second or third trimester of pregnancy to reduce the incidence of premature pregnancies in high- and low-risk populations. Nevertheless, the overall effect does not show a significant difference between study arms.

Duration of gestation-maternal biomarkers: Nothing conclusive can be drawn from four studies that assessed this association.[55,60-62]

Incidence of gestational hypertension (GHT), preeclampsia, or eclampsia-intake during pregnancy: Of eight RCTs with a quality score approaching good internal validity,[22,23,52,63,64] six trials compared the use of fish oil supplements containing DHA and EPA with placebo. The population included healthy or high-risk pregnant women (i.e., twin pregnancy).[22,23,63,64] The incidence of GHT in these populations, after the use of omega-3 fatty acids or placebo did not differ in six studies.[22,23,52,59,63] Regarding the incidence of preeclampsia (hypertension, edema, and proteinuria), six studies showed that compared with placebo, supplementation with omega-3 fatty acids did not have a significant effect.[22,23,55,59,63] Meta-analysis of the incidence of gestational hypertension from two studies revealed a nonsignificant difference between groups (OR: 1.07, CI 95%: 0.75; 1.51).[22,23] These findings were not adjusted for the potential covariates or confounders, such as background diet, grade of risk for GHT or preeclampsia in the current pregnancy, smoking status, and age.

Incidence of preeclampsia-eclampsia or gestational hypertension-maternal biomarkers: Five observational studies were identified,[21,65-68] of which four selected preeclamptic women and normal pregnant women as controls.[21,66-68] The results are very inconsistent across the studies.

Incidence of SGA infants- intake during pregnancy: Fourteen poor quality score RCTs showed that in the majority of the studies, the mean birth weight was not influenced by the intervention. None of the trials adjusted their results for the maternal background diet, which can be an important effect modifier.

Meta-analysis of the birth weight (mean) was combined in two studies that were comparable in terms of type of intervention and population (weight mean difference: -61.51, CI 95%: -256.21; 133.18) showing a nonsignificantly difference between groups.[23] The incidence of infants with IUGR showed a nonsignificant effect (OR: 1.14, CI 95%: 0.79; 1.64)[22,23,59] of supplementation during pregnancy.

Incidence of SGA infants-maternal biomarker: Six studies addressed this question.[58,60,61,69-71] de Groot et al.'s RCT found a significantly positive correlation between the maternal plasma and RBC DHA content and birth weight; however, this relationship was nonsignificant when measured at delivery.[58] Two observational studies found that the women with IUGR fetuses had a significantly lower content of LA (omega-6) in the plasma.[69,71] The content of DHA, EPA, AA, total omega-3 and omega-6 fatty acids, however, did not show a constant pattern across the studies. Two observational studies did not observe a correlation between maternal plasma biomarkers and birth weight,[61,69] consistent with the result in the RCT.[58]

Growth Pattern Outcomes

Maternal intake during pregnancy: One good quality RCT addressed this question,[54] showing no statistical difference between infants (n=590 enrolled, 341 completers) from mothers that were taking the supplementation with omega-3 and omega-6, or omega-6 fatty acids predominantly, on the weight, length, and head circumference (HC) from birth to 12 months of age.[54]

Maternal breast milk: One good quality RCT evaluating omega-3 supplementation in Norwegian mothers,[54] one poor quality RCT,[72] and two observational studies were identified.[73,74] Both RCTs showed no apparent effects of breast milk, with maternal intake of omega-3 (DHA) or omega-6 fatty acids (AA), on the growth patterns at any time point.[54,72] The single prospective cohort of Swedish mother/term infant pairs showed a positive correlation between the maternal mother's breast milk content of AA/DHA and the infant's rate of increase of HC at 1 and 3 months of age.[74] A cross-sectional

study from Africa showed that the differences in weight-for-age and weight-for-height z-scores and weight gain (g) were significantly lower in infants from Ouagadougou (low omega-3 fatty acids intake) compared with infants from Brazzaville (high omega-3 intake).[73]

Formula intake, preterm infants: Twenty RCTs of poor quality were identified,[25-32,34,75-85] of which eighteen failed to find an effect of the omega-3 supplementation in preterm formulas on the growth parameters at any time point.[25-30,32,34,75-84] The outcomes measured were the mean (SD) and gain in weight, length, and HC and the normalized z-score of weight. Two trials found that the omega-3 fatty acids supplemented group had a significantly lower weight from 6 to 18 months.[31,85] The results of the meta-analysis performed on the mean weight and length measured at 4 months, from studies that compared the use of formula supplemented with DHA+AA with control, showed that the overall effect was nonstatistically significant (weight: WMD: 0.04, CI 95%: -0.30; 0.38; length: WMD: 0.09, CI 95%: -0.62; 0.80).[28,29]

Formula intake, term infants: Eighteen good quality RCTs were identified.[35-43,86-93] The effects on the growth outcomes were nonstatistically different between study arms. Yet, some inconsistent differences were found across five trials at certain timepoints and subgroup of patients.[94-98] Meta-analysis demonstrated a nonstatistically significant overall effect of formulas containing DHA+AA compared with control formula at 4 or 12 months of age for the growth parameters (**4 months:** weight: WMD: -0.06, CI 95%: -0.45; 0.34; length: WMD: -0.33, CI 95%: -1.07; 0.40; **12 months:** weight: WMD: -0.33, CI 95%: -0.87; 0.21; length: WMD: -0.37, CI 95%: -1.26; 0.51; HC: WMD: 0.14, CI 95%: -0.83; 1.12) or DHA (**4 months:** weight: WMD: -0.12, CI 95%: -0.44; 0.20; length: WMD: -0.43, CI 95%: -1.20; 0.34; HC: WMD: 0.04, CI 95%: -0.37; 0.46. **12 months:** weight: WMD: -0.33, CI 95%: -0.87; 0.21; length: WMD: -0.71, CI 95%: -2.18; 0.76; HC: WMD: -0.04, CI 95%: -0.45; 0.38) [36,39] Only four trials adjusted the results for potential confounders, such as gender, maternal education, parental socioeconomic status and center, failing to find any change in the results.[39,41,43,88]

Child biomarkers: Five were RCTs in preterm infants,[25,28,29,76,85] and five RCTs[39,43,87,88,99] and a prospective single cohort[100] in term infants.

There is a negative correlation between weight and the plasma or RBC content of DHA, and a positive correlation between weight and the content of AA in plasma or RBC. However, not all of the studies found this association. The content of omega-6 fatty acids (AA) as a biomarker may be related to weight gain in infants. The content of DHA seems to be inversely related to weight gain, yet no significant clinical outcomes were detected.

Neurological Development Outcomes

Maternal intake during pregnancy: Helland et al. failed to find a significant difference between groups in maturity as evaluated from the EEGs, neither at day 1 of life nor at 3 months of age.[54]

Maternal breast milk: Two studies, one RCT[101] and one single prospective cohort design[102] showed that maternal breast milk may not have an influence on the neurological outcome, measured with the PDI scale of the Bayley's Index.

Formula intake, preterm infants: Six good quality RCTs were identified.[28,30,31,34,82,103] For the Bayley's PDI scale, two trials did not observe a significant difference between the supplemented and the control formula.[31,34] Meta-analysis was not possible for this outcome. Only Fewtrell et al. found that there was no difference between groups in the neurological impairment assessment at 9 and 18 months of corrected age (CA), and in the Knobloch, Passamanick, and Sherrards' Developmental Screening Inventory score.[34] There is not consistent evidence to suggest that the omega-3 fatty acids supplementation of infant formula, with or without breast milk, influences the neurological development in preterm infants.

Formula intake, term infants: Eight good quality RCTs,[36-39,42,43,104] of which seven failed to find a statistically significant difference between diet groups at different follow-ups (6 to 24 months of age) in the Bayley's PDI scale.[36-39,42,43] One trial showed a significantly better Brunet-Lézine test result in the LC PUFAs supplemented group compared with control at 4 months of age (after exclusive formula intake) but not at 24 months.[104] Meta-analysis of Bayley's PDI score showed a nonstatistically significant difference between groups using formula supplemented with DHA+AA and control (WMD: -2.80, CI 95%: -7.43; 1.82) at 12 months. [36,39,42]

Maternal biomarkers: One cross-sectional study showed that maternal DHA was negatively associated with active sleep (AS), AS:QS (quiet sleep) and sleep-wake transition, and positively associated with wakefulness (postpartum day 2).[105] The ratio of n-6:n-3 in maternal plasma was positively associated with AS, AS:QS and sleep-wake transition, and negatively associated with wakefulness (day 2), suggesting a greater CNS maturity.

Child biomarkers: Three RCTs[37,39,43] and a prospective cohort study[100] evaluated the association between the infant's plasma and RBC DHA content and the Bayley's psychomotor developmental index (PDI) score in healthy term infants. Two RCTs found a significant positive correlation between the

plasma DHA and the PDI score.[39,43] Two other studies (including the observational study), did not find a significant correlation between the PDI and the infant content of PUfatty acids in plasma or RBC.[37,100]

Visual Function Outcomes

Maternal intake during pregnancy: One RCT failed to find a significant effect of DHA supplementation during pregnancy on the retinal sensitivity (ERG) measured at birth in term infants.[51] One cross-sectional study failed to find a statistically significant difference in mean visual function values between the exclusively breastfed group and the infants who were also receiving formula.[106]

Maternal breast milk: Five studies found that the correlation between the DHA content in breast milk and visual function was not consistent with the clinical outcomes measured in breastfed term infants of mothers who were or were not taking supplements containing high DHA.[72,101,106-108]

Formula intake, preterm infants: Nine RCTs with a quality score approaching good internal validity were identified.[25,26,28,29,76,77,82,85,103] Of five studies that measured visual evoked potentials (VEP), two did not find a statistical difference between feeding groups at any time point (from 1 to 12 months).[82,103] Three studies found that compared with the unsupplemented group, infants fed with LC PUFAs-supplemented formula had a better or faster maturation of visual function, in terms of significantly shorter waves in the VEP.[25,28,77] Two studies found a significant difference between groups in the Teller's Acuity Card test.[85] Meta-analysis of the relevant visual outcomes comparing the studies by the type of omega-3 fatty acids used in the supplemented formula (DHA or DHA+AA) and control formula, and by the type of outcome (VEP and Teller's test of visual acuity) was done. For the VEP visual acuity outcomes, only two studies were combined.[25,28] O'Connor et al. found that the use of formulas with DHA+AA resulted in a better VEP measurements compared with control formula at 6 months of age yet not at 4 months.[25,28]

No significant effect of DHA-supplementation at 2, 4, 6, or 9 months of CA,[29,76] or DHA+AA supplementation at 2, 3, 4, or 6 months of CA was found in the visual acuity measured with the Teller's Card test.[25,28,29,85,103]

Formula intake, term infants: Thirteen RCTs, of average good quality (Jadad: 3.61/5) were identified,[36,37,39,41-43,88,89,91,93,109,110] of which five trials did not find a significant difference between groups in the VEP at any age.[36,39,41,43,89] Four trials found a significantly better VEP in the LC PUFAs-supplemented group compared with the control group at a number of time points, from 1.5 to 13 months of age.[37,87,91,93] The meta-analysis performed on this outcome, by LC PUFAs content of DHA alone (or with the addition of AA), versus control, showed that the studies that compared DHA supplemented formula with control formula did not have an overall significant effect at any age.[36,37,39] Conversely, in seven studies that compared the use of DHA+AA formula with placebo, there was no difference between groups at any age,[36,37,39,87,89,91,93] with the exception of four studies that found a significant difference at 12 months of age.[36,37,91,93]

One trial that evaluated behavioral visual acuity with the Teller's test,[110] found a significantly better acuity in the LC PUFAs formula group compared with the control group at 2 months of age, yet not at 4, 6, 9, or 12 months. The remaining four trials did not observe a significant difference between groups in this outcome, at any time point.[36,42,88] The meta-analysis performed on this outcome showed that, in studies comparing the use of DHA+AA with a control intervention, acuity was only significantly better in the DHA+AA group at 2 months of age,[36,37,110] but not at 4, 6, 9, or 12 months of age.

Maternal biomarkers: One study measured the association between the maternal content of biomarkers at 2 months postpartum and the visual acuity (Teller's Card Test) in term infants at 2 months of age that failed to find a significant correlation.[106]

Child biomarkers: Twenty-one studies assessed this association. Of five studies in the preterm group, three were RCTs,[25,76,77] and two were cross-sectional studies.[111,112] Of the 16 term infant studies, nine were RCTs,[37,43,72,87-89,91,93,101] and seven were observational studies.[100,106,107,111,113-115] There was no pattern of correlation between the infant's biomarkers in blood and the visual function outcomes across 21 studies that addressed this issue.

Cognitive Development Outcomes

Maternal intake during pregnancy: One RCT addressed this question.[54] There were no differences between groups in the novelty preference (Fagan Test of Infant Intelligence) at 6 and 9 months of age.[54]

Maternal breast milk: Two RCTs[54,101] and one prospective cohort[102] were identified. The study by Helland et al. was an RCT described above,[54] and Gibson et al. included mother of term infants who intended to breastfeed.[101] They were randomized to receive five increasing doses of DHA (algal oil) during the first 3 months postpartum. The mean Bayley's Mental Developmental Index (MDI) score did not differ between groups at 1 or 2 years of age (underpowered).[101]

Formula intake preterm infants: Six good quality (Jadad: 4.4/5) RCTs were identified.[28,30,31,34,76,103] Four of the five trials

did not find an effect on the Bayley's MDI score from 3 to 24 months of age.[28,31,34,116] Two studies found a significant difference between the omega-3 fatty acids group and the control group in the Fagan Test of Infant Intelligence.[28,76] O'Connor et al. found that there was no significant differences between groups in the Infant version of the MacArthur Communicative Development Inventories at 9 months CA and 14 months CA.[28] Meta-analysis was not possible given the heterogeneity across the studies for each of the different outcomes due to the intervention characteristics (meaning dose, source of omega-3 fatty acids, duration of intervention), cointerventions, different assessment tools, and timing of the outcomes measures.

Formula intake term infants: Six (of eight) good quality RCTs[36-39,42,43,92] did not find a significant difference between groups (supplemented vs. control) in the Bayley's MDI score from 6 to 18 months of age.[36-39,42,43] Birch et al. observed that the DHA+AA group had a significantly higher score compared with the control group at 18 months of age.[37]

The Knobloch, Passamanik, and Sherrards Development Screening Inventory test (9 months),[117] and the Fagan Test of Infant Intelligence (6 and 9 months)[98] did not differ between groups. The IQ (Stanford-Binet), Receptive Vocabulary (PPVT-R), Expressive Vocabulary, and Visual-Motor Index scores, as well as the Problem-Solving scores, did not differ between groups in two studies.[36,92]

A meta-analysis using the Bayley's MDI score at 12 months of age showed a nonstatistical difference between groups (DHA+AA vs. control) from three trials (WMD: -0.80, CI 95%: -3.24; 1.63).[36,39,42]

Child biomarkers: Four good quality RCTs and two single prospective cohort studies[100,118] showed inconsistent results.

Discussion

Studies investigating the influence of omega-3 fatty acids on child and maternal health revealed the absence of a notable safety profile (i.e., moderate-to-severe adverse events). Pregnancy outcomes were either unaffected by omega-3 fatty acids supplementation, or the results were inconclusive. Results suggested the absence of effects with respect to the impact of supplementation on the incidence of GHT, preeclampsia or eclampsia, as well as on infants being born SGA. However, regarding evaluations of the duration of gestation, some discrepancies were observed, although most of the studies failed to detect a statistically significant effect. Biomarker data failed to clarify patterns in pregnancy outcome data.

Results concerning the impact of the intake of omega-3 fatty acids on the development of infants are primarily, although not uniformly, inconclusive. The inconsistencies in study results may be attributable to numerous factors.

In addition, making clear sense of the absolute or relative effects of individual omega-3 fatty acids, or even omega-3 fatty acids combinations, on child outcomes is complicated or precluded by the following problem. Studies typically employed interventions that involved various cointerventional or background constituents (e.g., omega-6 fatty acids), yet whose metabolic interactions with the omega-3 fatty acids were not taken into account in interpreting the results. The dynamic interplay among these fatty acid contents (e.g., competition for enzymes), and how this interplay may influence outcomes, may differ in important ways depending on whether DHA or olive oil is added to this combination of cointerventional or background constituents, particularly in the maternal population. This strategy prevented the isolation of the exact effects relating to the omega-3 fatty acids content. It is thus very difficult to reliably ascribe definite child outcome-related benefits, or the absence thereof, to specific omega-3 fatty acids. Biomarker data failed to clarify patterns in child outcome data.

Future research should likely consider investigating the impact of specific omega-6/omega-3 fatty acids intake ratios, in no small part to control for the possible metabolic interactions involving these types of fatty acids. To produce results that are applicable to the North American population, populations consuming high omega-6/omega-3 fatty acids intake ratios should likely be randomized into trials also exhibiting better control of confounding variables than was observed, especially in the present collection of studies of child outcomes.

Availability of the Full Report

The full evidence report from which this summary was taken was prepared for the Agency for Healthcare Research and Quality (AHRQ) by the University of Ottawa Evidence-based Practice Center under Contract No. 290-02-0021. It is expected to be available in August 2005. At that time, printed copies may be obtained free of charge from the AHRQ Publications Clearinghouse by calling 800-358-9295. Requesters should ask for Evidence Report/Technology Assessment No. 118, *Effects of Omega-3 Fatty Acids on Child and Maternal Health*. In addition, Internet users will be able to access the report and this summary online through AHRQ's Web site at www.ahrq.gov.

Suggested Citation

Lewin GA, Schachter HM, Yuen D, Merchant P, Mamaladze V, Tsertsvadze A, et al. Effects of Omega-3 Fatty

Acids on Child and Maternal Health. Summary, Evidence Report/Technology Assessment No. 118. (Prepared by the University of Ottawa Evidence-based Practice Center under Contract No. 290-02-0021.) AHRQ Publication No. 05-E025-1. Rockville, MD: Agency for Healthcare Research and Quality. August 2005.

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www.ahrq.gov
AHRQ Pub. No. 05-E025-1
August 2005
ISSN 1530-440X

Chapter 1. Introduction

This evidence report by the University of Ottawa's Evidence-Based Practice Center (EPC) concerning the effects of omega-3 fatty acids on child and maternal health is one among several that address topics related to omega-3 fatty acids that were requested and funded by the Office of Dietary Supplements, National Institutes of Health (NIH), through the EPC program at the Agency for Healthcare Research and Quality (AHRQ). Three EPCs—the Tufts-New England Medical Center (Tufts-NEMC) EPC, the Southern California-RAND (SC-RAND) EPC, and the University of Ottawa EPC (UO-EPC)—each produced evidence reports. To ensure consistency of approach, the three EPCs collaborated on selected methodological elements, including literature search strategies, rating of evidence, and data table design.

The aim of these reports is to summarize the current evidence concerning the health effects of omega-3 fatty acids on the following: cardiovascular diseases, cancer, child and maternal health, eye health, gastrointestinal/renal diseases, asthma, autoimmune diseases, immune-mediated diseases, transplantation, mental health, and, neurological diseases and conditions. In addition to informing the research community and the public on the effects of omega-3 fatty acids on various health conditions, it is anticipated that the findings of the reports will also be used to help define the agenda for future research.

The focus of this report is on child and maternal health outcomes in humans. In this chapter, the metabolism, physiological functions, and sources of omega-3 fatty acids are briefly discussed. This constitutes background material, putting in context the data presented in the evidence report. As well, the description of the U.S. population intake of omega-3 fatty acids is provided in response to a general question posed within the task order. This introductory material is then complemented by a brief review of the epidemiology and descriptions of the child and maternal health issues related to this intervention. The brief review is intended as an overview, rather than a comprehensive description.

Chapter 2 describes the methods used to identify, review and synthesize the results from studies concerning omega-3 fatty acids and child and maternal health. Chapter 3 presents the findings of studies meeting eligibility criteria, with discussion points, including recommendations for future research completing the report in Chapter 4.

Metabolism and Biological Effects of Essential Fatty Acids

Dietary fat is an important source of energy for biological activities in human beings. It encompasses saturated fatty acids, which are usually solid at room temperature, and unsaturated fatty acids, which are liquid at room temperature. Unsaturated fatty acids can be further divided into monounsaturated and polyunsaturated fatty acids. Polyunsaturated fatty acids (or PUFAs) can be classified, on the basis of their chemical structure, into two groups: omega-3 (n-3) fatty acids and omega-6 (n-6) fatty acids. The omega-3 or *n-3* notation means that the first double bond in this family of PUFAs is 3 carbons from the methyl end of the molecule. The same

principle applies to the omega-6 or *n-6* notation. Despite their differences in structure, all fats contain the same amount of energy (i.e., 9 kcal/g or 37 kJ/g).

Of all fats found in food, two—alpha-linolenic acid (chemical abbreviation: ALA; 18:3 n-3) and linoleic acid (LA; 18:2 n-6)—cannot be synthesized in the human body, yet these are necessary for proper physiological functioning. These two fats are thus called "essential fatty acids." The essential fatty acids can be converted in the liver to long-chain polyunsaturated fatty acids (LC PUFAs), which have a higher number of carbon atoms and double bonds. These LC PUFAs retain the omega type (n-3 or n-6) of the parent essential fatty acids.

ALA and LA comprise the bulk of the total PUFAs consumed in a typical North American diet. Typically, LA comprises 89% of the total PUFAs consumed, while ALA comprises 9%. Smaller amounts of other PUFAs make up the remainder.[1] Both ALA and LA are present in a variety of foods. For example, LA is present in high concentrations in many commonly used oils, including safflower, sunflower, soy, and corn oil. ALA, which is consumed in smaller quantities, is present in leafy green vegetables and in some commonly used oils, including canola and soybean oil. Some novelty oils, such as flaxseed oil, contain relatively high concentrations of ALA, but these oils are not commonly found in the food supply.

The Institute of Medicine (IOM) suggests that, for adults 19 and older, an adequate intake (AI) of ALA is 1.1-1.6 grams/day, and 11-17 grams/day for LA.[2] Recommendations regarding AI differ by age and gender groups, and for special conditions such as pregnancy and lactation.

As shown in Figure 1, eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3) can act as competitors for the same metabolic pathways as arachidonic acid (AA; 20:4 n-6). In human studies, the analyses of fatty-acid compositions in both blood phospholipids and adipose tissue have shown a similar competitive relationship between omega-3 LC PUFAs and AA. General scientific agreement supports an increased consumption of omega-3 fatty acids and reduced intake of omega-6 fatty acids to promote good health. However, for omega-3 fatty acid intake, the specific quantitative recommendations vary widely among countries not only in terms of different units — ratio, grams, total energy intake — but also in quantity.[3]

Furthermore, there remain numerous questions relating to the inherent complexities concerning omega-3 and omega-6 fatty acid metabolism, in particular the relationships between the two fatty acids. For example, it remains unclear to what extent ALA is converted to EPA and DHA in humans, and to what extent the high intake of omega-6 fatty acids compromises any benefits of omega-3 fatty acid consumption. Without the resolution of these two fundamental questions, it remains difficult to study the importance of the omega-6/omega-3 fatty acid ratio.

Metabolic Pathways of Omega-3 and Omega-6 Fatty Acids

Omega-3 and omega-6 fatty acids share the same pools of enzymes and go through the same oxidation pathways while being metabolized (Figure 1). Once ingested, the parent of the omega-3 fatty acids, ALA, and the parent of the omega-6 fatty acids, LA, can be elongated and desaturated into LC PUFAs. LA is converted into gamma-linolenic acid (GLA; 18:3 n-6), an

omega-6 fatty acid that is a positional isomer of ALA. GLA, in turn, can be converted to the long-chain omega-6 fatty acid, AA, while ALA can be converted, to a lesser extent, to the long-chain omega-3 fatty acids, EPA and DHA. However, the conversion from parent fatty acids into LC PUFAs occurs slowly in humans, and conversion rates are not well understood. Because of the slow rate of conversion, and the importance of LC PUFAs to many physiological processes, humans must augment their level of LC PUFAs by consuming foods rich in these important compounds. Meat is the primary food source of AA, and fish is the primary food source of EPA.

The specific biological functions of fatty acids depend on the number and position of double bonds and the length of the acyl chain. Both EPA and AA are 20-carbon fatty acids and are precursors for the formation of prostaglandins (PGs), thromboxane (Tx), and leukotrienes (LTs)—hormone-like agents that are members of a larger family of substances called eicosanoids. Eicosanoids are localized tissue hormones that seem to be one of the fundamental regulatory classes of molecule in most higher forms of life. They do not travel in the blood, but are created in the cells to regulate a large number of processes, including the movement of calcium and other substances into and out of cells, dilation and contraction of muscles, inhibition and promotion of clotting, regulation of secretions including digestive juices and hormones, and, the control of fertility, cell division, and growth.[4]

As shown in Figure 1, the long-chain omega-6 fatty acid, AA, is the precursor of a group of eicosanoids including series-2 prostaglandins (PG_2) and series-4 leukotrienes (LT_4). The omega-3 fatty acid, EPA, is the precursor to a group of eicosanoids including series-3 prostaglandins (PG_3) and series-5 leukotrienes (LT_5). The series-2 prostaglandins and series-4 leukotrienes derived from AA are involved in intense actions (such as accelerating platelet aggregation, and enhancing vasoconstriction and the synthesis of mediators of inflammation) in response to physiological stressors. The series-3 prostaglandins and series-5 leukotrienes derived from EPA are less physiologically potent than those derived from AA. More specifically, the series-3 prostaglandins are formed at a slower rate and work to attenuate excessive series-2 prostaglandins. Thus, adequate production of the series-3 prostaglandins, which are derived from the omega-3 fatty acid, EPA, may protect against heart attack and stroke as well as certain inflammatory diseases like arthritis, lupus, and asthma.[4] In addition, animal studies have demonstrated that omega-3 LC PUFAs, such as EPA and DHA, engage in multiple cytoprotective activities that may contribute to antiarrhythmic mechanisms.[5] Arrhythmias are thought to contribute to "sudden death" in heart disease.

In addition to affecting eicosanoid production as described above, EPA also affects lipoprotein metabolism and decreases the production of other compounds—including cytokines, interleukin 1β (IL-1β), and tumor necrosis factor α (TNF-α)—which have pro-inflammatory effects. These compounds exert pro-inflammatory cellular actions that include stimulating the production of collagenase and increasing the expression of adhesion molecules necessary for leukocyte extravasation.[6] The mechanism responsible for the suppression of cytokine production by omega-3 LC PUFAs remains unknown, although suppression of eicosanoid production by omega-3 fatty acids may be involved. EPA can also be converted into the longer chain omega-3 form of docosapentaenoic acid (DPA, 22:5 n-3), and then further elongated and oxygenated into DHA. EPA and DHA are frequently referred to as VLN-3FA—very long chain n-3 fatty acids. DHA, which is thought to be important for brain development and functioning, is present in

significant amounts in a variety of food products, including fish, fish liver oils, fish eggs, and organ meats. Similarly, AA can convert into an omega-6 form of DPA.

Studies have reported that omega-3 fatty acids decrease triglycerides (Tg) and very low density lipoprotein (VLDL) in hypertriglyceridemic subjects, concomitant with an increase in high density lipoprotein (HDL). However, they appear to increase or have no effect on low density lipoprotein (LDL). Omega-3 fatty acids apparently lower Tg by inhibiting VLDL and apolipoprotein B-100 synthesis, and decreasing post-prandial lipemia.[7] Omega-3 fatty acids, in conjunction with transcription factors (small proteins that bind to the regulatory domains of genes), target the genes governing cellular Tg production and those activating oxidation of excess fatty acids in the liver. Inhibition of fatty acid synthesis and increased fatty acid catabolism reduce the amount of substrate available for Tg production.[8]

As noted earlier, omega-6 fatty acids are consumed in larger quantities (> 10 times) than omega-3 fatty acids. Maintaining a sufficient intake of omega-3 fatty acids is particularly important since many of the body's physiologic properties depend upon their availability and metabolism.

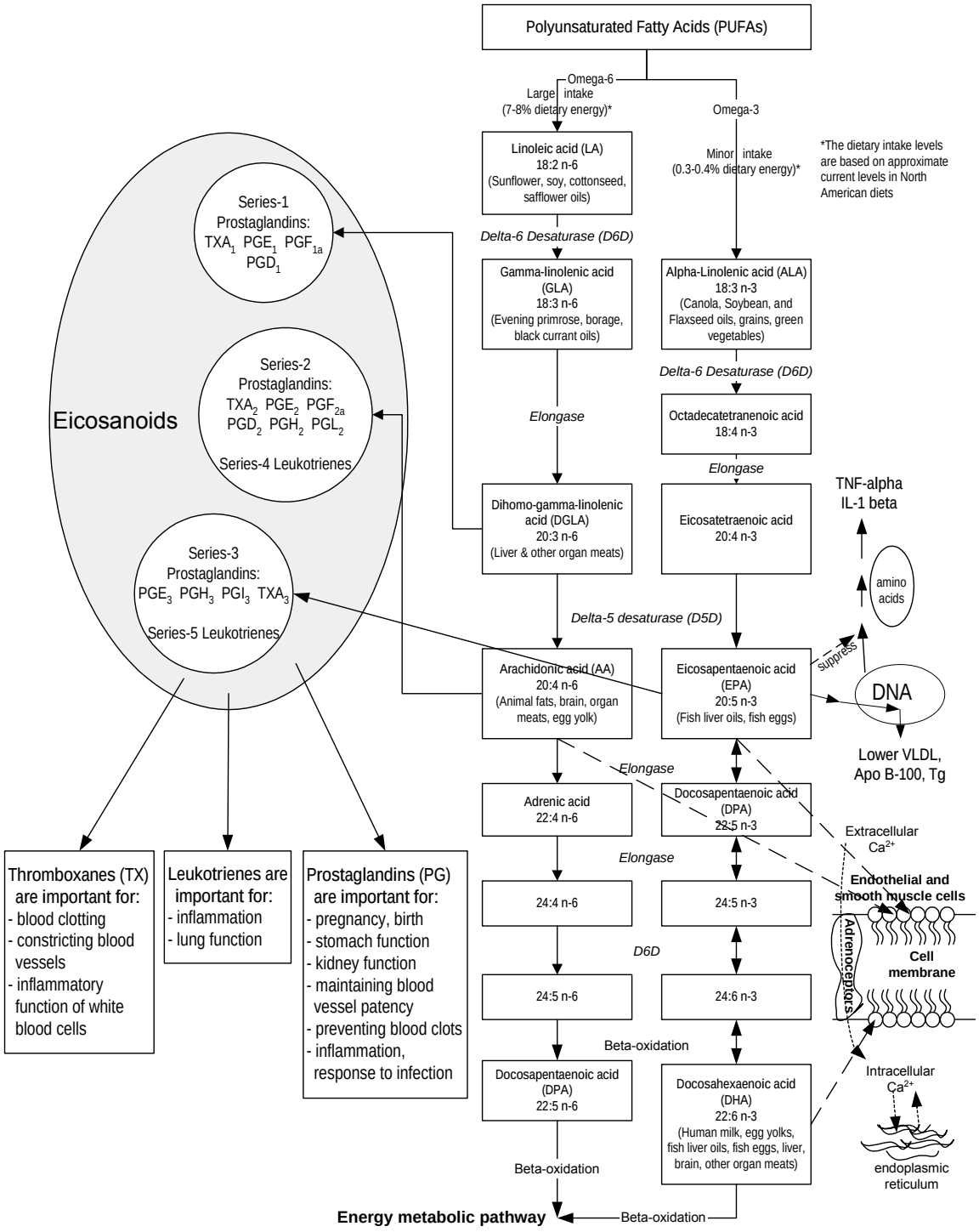

Figure 1. Classical omega-3 and omega-6 fatty acid synthesis pathways and the role of omega-3 fatty acids in regulating health/disease markers

U.S. Population Intake of Omega-3 Fatty Acids

The major source of omega-3 fatty acids is dietary intake of fish, fish oil, vegetable oils (principally canola and soybean), some nuts such as walnuts, and, dietary supplements. Two population-based surveys, the third National Health and Nutrition Examination (NHANES III) 1988-94, and the Continuing Survey of Food Intakes by Individuals 1994-98 (CSFII), are the main sources of dietary intake data for the U.S. population. NHANES III collected information on the U.S. population aged ≥2 months. Mexican Americans and non-Hispanic African-Americans, children ≤5 years old, and adults ≥ 60 years old were over-sampled to produce more precise estimates for these population groups. There were no imputations for missing 24-hour dietary recall data. A total of 29,105 participants had complete and reliable dietary recall.

The CSFII 1994-96, popularly known as the "What We Eat in America" survey, addressed the requirements of the National Nutrition Monitoring and Related Research Act of 1990 (Public Law 101-445) for continuous monitoring of the dietary status of the American population. The CSFII 1994-96 utilized an improved data-collection method for 24-hour recall known as the multiple-pass approach. Given the large variation in intake from day-to-day, multiple 24-hour recalls are considered to be best suited for most nutrition monitoring and will produce stable estimates of mean nutrient intake from groups of individuals.[9] In 1998, the Supplemental Children's Survey, a survey of food and nutrient intake by children under the age of 10 years, was conducted as a supplement to the CSFII 1994-96. The CSFII 1994-96, 1998 surveyed 20,607 people of all ages with over-sampling of low-income population (<130% of the poverty threshold). Dietary intake data from individuals of all ages were collected over 2 nonconsecutive days via two 1-day dietary recalls.

Table 1 reports the NHANES III survey mean intake ± the standard error of the mean (SEM), in addition to the median and range for each omega-3 fatty acid. Distributions of EPA, DPA, and DHA were very skewed; therefore, the means and standard errors of the means should be used and interpreted with caution. Table 2 reports the CSFII survey mean and median intakes for each omega-3 fatty acid, along with SEMs, as reported in the Dietary Reference Intakes from the Institute of Medicine.[2]

Table 1: Estimates of the mean±standard error of the mean (SEM) intake of linoleic acid (LA), alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in the US population, based on analyses of a single 24-hour dietary recall of NHANES III data

	Grams/day		% Kcal/day	
	Mean±SEM	**Median (range)[1]**	**Mean±SEM**	**Median (range)[1]**
LA (18:2 n-6)	14.1±0.2	9.9 (0 - 168)	5.79±0.05	5.30 (0 - 39.4)
ALA (18:3 n-3)	1.33±0.02	0.90 (0 - 17)	0.55±0.004	0.48 (0 - 4.98)
EPA (20:5 n-3)	0.04±0.003	0.00 (0 - 4.1)	0.02±0.001	0.00 (0 - 0.61)
DHA (22:6 n-3)	0.07±0.004	0.00 (0 - 7.8)	0.03±0.002	0.00 (0 - 2.86)

[1]The distributions are not adjusted for the over-sampling of Mexican-Americans, non-Hispanic African-Americans, children ≤5 years old, and adults ≥ 60 years old in the NHANES III dataset.

Table 2: Mean, range, median, and standard error of the mean (SEM) of usual daily intakes of linoleic acid (LA), total omega-3 fatty acids (n-3 FA), alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) in the US population, based on CSFII data (1994-1996, 1998)

	Grams/day	
	Mean±SEM	**Median±SEM**
LA (18:2 n-6)	13.0±0.1	12.0±0.1
Total n-3 FA	1.40±0.01	1.30±0.01
ALA (18:3 n-3)	1.30±0.01	1.21±0.01
EPA (20:5 n-3)	0.028	0.004
DPA (22:5 n-3)	0.013	0.005
DHA (22:6 n-3)	0.057±0.018	0.046±0.013

Dietary Sources of Omega-3 Fatty Acids

Omega-3 fatty acids can be found in many different sources of food, including fish, shellfish, some nuts, and various plant oils. Selected from the USDA website, Table 3 lists the amount of omega-3 fatty acids in some commonly consumed fish, shellfish, nuts, and edible oils, selected from the USDA website.[10]

Table 3: The omega-3 fatty acid content, in grams per 100 g food serving, of a representative sample of commonly consumed fish, shellfish, fish oils, nuts and seeds, and plant oils that contain at least 5 g omega-3 fatty acids per 100 g

Food item	EPA	DHA	ALA
Fish (Raw[a])			
Anchovy, European	0.6	0.9	-
Bass, Freshwater, Mixed Sp.	0.2	0.4	0.1
Bass, Striped	0.2	0.6	trace
Bluefish	0.2	0.5	-
Carp	0.2	0.1	0.3
Catfish, Channel	trace	0.2	0.1
Cod, Atlantic	trace	0.1	trace
Cod, Pacific	trace	0.1	trace
Eel, Mixed Sp.	trace	trace	0.4
Flounder & Sole Sp.	trace	0.1	trace
Grouper, Mixed Sp.	trace	0.2	trace
Haddock	trace	0.1	trace
Halibut, Atlantic and Pacific	trace	0.3	trace
Halibut, Greenland	0.5	0.4	trace
Herring, Atlantic	0.7	0.9	0.1
Herring, Pacific	1.0	0.7	trace
Mackerel, Atlantic	0.9	1.4	0.2
Mackerel, Pacific and Jack	0.6	0.9	trace
Mullet, Striped	0.2	0.1	trace
Ocean Perch, Atlantic	trace	0.2	trace
Pike, Northern	trace	trace	trace
Pike, Walleye	trace	0.2	trace
Pollock, Atlantic	trace	0.4	-
Pompano, Florida	0.2	0.4	-
Roughy, Orange	trace	-	trace
Salmon, Atlantic, Farmed	0.6	1.3	trace
Salmon, Atlantic, Wild	0.3	1.1	0.3
Salmon, Chinook	1.0	0.9	trace
Salmon, Chinook, Smoked[b]	0.2	0.3	-
Salmon, Chum	0.2	0.4	trace
Salmon, Coho, Farmed	0.4	0.8	trace
Salmon, Coho, Wild	0.4	0.7	0.2
Salmon, Pink	0.4	0.6	trace
Salmon, Pink, Canned[c]	0.9	0.8	trace
Salmon, Sockeye	0.6	0.7	trace
Sardine, Atlantic, Canned in Oil[d]	0.5	0.5	0.5
Seabass, Mixed Sp.	0.2	0.4	-
Seatrout, Mixed Sp.	0.2	0.2	trace
Shad, American	1.1	1.3	0.2
Shark, Mixed Sp.	0.3	0.5	trace
Snapper, Mixed Sp.	trace	0.3	trace
Swordfish	0.1	0.5	0.2
Trout, Mixed Sp.	0.2	0.5	0.2
Trout, Rainbow, Farmed	0.3	0.7	trace
Trout, Rainbow, Wild	0.2	0.4	0.1
Tuna, Fresh, Bluefin	0.3	0.9	-
Tuna, Fresh, Skipjack	trace	0.2	-
Fish, continued			
Tuna, Fresh, Yellowfin	trace	0.2	trace
Tuna, Light, Canned in Oil[e]	trace	0.1	trace
Tuna, Light, Canned in Water[e]	trace	0.2	trace
Tuna, White, Canned in Oil[e]	trace	0.2	0.2
Tuna, White, Canned in Water[e]	0.2	0.6	trace
Whitefish, Mixed Sp.	0.3	0.9	0.2
Whitefish, Mixed Sp., Smoked	trace	0.2	-
Wolffish, Atlantic	0.4	0.3	trace
Shellfish (Raw)			
Abalone, Mixed Sp.	trace	-	-
Clam, Mixed Sp.	trace	trace	trace
Crab, Blue	0.2	0.2	-
Crayfish, Mixed Sp., Farmed	trace	0.1	trace
Lobster, Northern	-	-	-
Mussel, Blue	0.2	0.3	trace
Oyster, Eastern, Farmed	0.2	0.2	trace
Oyster, Eastern, Wild	0.3	0.3	trace
Oyster, Pacific	0.4	0.3	trace
Scallop, Mixed Sp.	trace	0.1	-
Shrimp, Mixed Sp.	0.3	0.2	trace
Squid, Mixed Sp.	0.1	0.3	trace
Fish Oils			
Cod Liver Oil	6.9	11.0	0.9
Herring Oil	6.3	4.2	0.8
Menhaden Oil	13.2	8.6	1.5
Salmon Oil	13.0	18.2	1.1
Sardine Oil	10.1	10.7	1.3
Nuts and Seeds			
Butternuts, Dried	-	-	8.7
Flaxseed			18.1
Walnuts, English	-	-	9.1
Plant Oils			
Canola (Rapeseed)	-	-	9.3
Flaxseed Oil	-	-	53.3
Soybean Lecithin Oil	-	-	5.1
Soybean Oil	-	-	6.8
Walnut Oil	-	-	10.4
Wheatgerm Oil	-	-	6.9

Trace = <0.1; - = 0 or no data; Sp. = species; [a]Except as indicated; [b]Lox.; [c]Solids with bone and liquid; [d]Drained solids with bone; [e]Drained solids.

Omega-3 Fatty Acids in Child and Maternal Health

The following description is intended only as an overview of the domain of inquiry in which it has been hypothesized that omega-3 fatty acid content, which includes both their intake and their levels in specific biomarkers, plays an important role in maternal pregnancy and child health outcomes in human subjects. This account serves exclusively to introduce the pertinence of this systematic review of the empirical evidence.

Over the past 60 years, the influence of maternal nutrition on fetal growth and development has been extensively studied as part of attempts to understand the causes and consequences of protein-calorie malnutrition.[11] This field of investigation has since expanded to encompass experimental, observational and descriptive studies designed to identify the specific roles of a broad range of sources and constituents of maternal nutrition. In addition, studies have also been conducted to evaluate the impact of maternal nutrition on maternal health during pregnancy and pregnancy outcomes. The following overview will focus on the the role played by omega-3 fatty acids in modulating the duration of pregnancy, incidence of pregnancy-induced hypertension, fetal growth and development, and infant (preterm and term) neurocognitive and visual development. The mechanisms by which omega-3 fatty acids or their eicosanoid derivatives impact the observed biological outcomes may include one or more of their identified functions in modulating the cell membrane microenviroment, signaling pathways, and gene expression.[12,13]

It has been posited that the accretion of omega-3 fatty acids within, and use by, the maternal biological system has the potential to influence both maternal health during pregnancy, and fetal health. Likewise, it has been hypothesized that their accumulation within, and use by, the post-delivery child's biological system can affect their development and health. However, notwithstanding problems affecting their metabolism or availability, since EFAs must be "obtained" from "external sources" in order for their contents to accumulate and, in turn, potentially influence health, mothers and their fetuses/children require that omega-3 fatty acid content be "delivered" (i.e., via the placenta, breast milk, formula supplementation, food sources such as oily fish, or supplementation).

Birth weight is the single most important factor affecting neonatal morbidity and mortality.[14] Infants born with low birth weights (less than 2,500 grams by WHO criterion) may be the result of: 1. being constitutionally small; 2. intrauterine growth retardation (IUGR); or 3. preterm birth. In the United States, approximately 350,000 infants are born weighing less than 2,500 grams.[15]

Preterm birth is a multifactorial condition that results in significant morbidity and mortality. Premature infants are at risk of injury to every organ system in the newborn period: intraventricular hemorrhage, retinopathy of prematurity, respiratory distress syndrome, chronic lung disease, necrotizing enterocolitis, growth failure, and infections. Of greatest concern for the infants who survive are the risks of developing permanent neurocognitive deficits (i.e., cerebral palsy, hearing and vision loss, cognitive deficits) that impact on their lifelong health and functional capacity.[16-19] In addition, studies now suggest that premature infants are at higher risk for developing adult-onset chronic diseases including hypertension, cardiovascular disease, and diabetes, as a result of permanent physiologic changes induced by abnormal conditions during sensitive periods of human growth and development.[20-22] There is an hypothesis that suboptimal

n-3 and n-6 nutrititure during sensitive periods of fetal growth and development may result in permanent changes in neurocognitive and visual function and the development of adult-onset diseases such as hypertension. In the United States, preterm birth of low birth weight infants is 6%-10% of all births, which is approximately 300,000 annually.[23] In the United States, the cost of preterm births is estimated at several billion dollars annually, not including the costs of care for the associated-adult onset diseases.[24]

Without exploring too deeply what was not, in fact, eligible for synthesis in our review—because it failed to satisfy our eligibility criterion relating to research design—some evidence is introduced here merely to demonstrate that there can coexist more than one interpretation of how maternal intake of omega-3 fatty acids could influence a child outcome. Results of epidemiological studies conducted with residents of the Faroe Islands[25,26] have been taken to suggest that marine diets, which contain omega-3 fatty acids, increase birth weight either by prolonging pregnancy.[27] or increasing the fetal growth rate.[28,29] Proposed mechanisms have included: a) the delayed timing of spontaneous delivery, which results from the altered balance among the PGs involved in the initiation of labor;[27] or, b) an increased fetal growth rate, which results from enhanced placental blood flow associated with a decreased Tx/prostacyclin ratio[28] and decreased blood viscosity.[30] These observations might not be replicated in populations that regularly consume lesser amounts of omega-3 fatty acids from marine sources, however. With respect to maternal health during human pregnancy, it has been hypothesized that marine oils may lower risks of certain complications of pregnancy, in particular preterm delivery, intrauterine growth retardation, preeclampsia, and gestational hypertension.[31] Given that some of their presumed mechanisms of action overlap with those of aspirin, it was thought that omega-3 fatty acids might protect pregnant women against preeclampsia and gestational hypertension, for example.[32-34]

Essential fatty acid derived eicosanoids play important roles as biochemical mediators in normal term labor that initiate uterine contractions, cervical maturation, and rupture of membranes.[35,36] There is an elevation of omega-6 fatty acid eicosansoid series (PGE2 and PGF2alpha, LTC4, LTB4) in the maternal circulation prior to the onset of labor[37] and inhibition of their synthesis with cyclooxygenase inhibitors stops the onset of labor.[38] Women who deliver prematurely have higher erythrocyte total plasma lipid omega-6 fatty acids and lower omega-3 fatty acids compared with women who delivered at term, suggesting that an imbalance in favor of omega-6 fatty acids and their eicosanoid derivatives contribute to the premature onset of labor.[39,40] By altering the balance of omega-6 to omega-3 eicosanoids by diet supplementation with omega-3 fatty acids in human, rodent, and sheep, studies have been successful in increasing the duration of gestation.[31,41-46]

In Western societies, placental insufficiency is the major cause of IUGR, with maternal hypertension having the most profound effect.[47] Fetal adaptations that are required to compensate for poor placental function result in increased perinatal morbidity and mortality. Of greatest concern is the increased risk for permanent adverse effects on growth and development.[47-51] Epidemiologic data suggests that the fetal adaptations may be associated with an increased risk for the development of adult-onset chronic diseases including hypertension, cardiovascular disease, obesity and diabetes.[20-22] In keeping with these observations, animals studies on fetal growth restriction demonstrate metabolic, hormonal and end organ changes that

predispose the animals to the development of hypertension, cardiovascular disease and diabetes.[52-54]

Hypertension in pregnancy of varying degrees of severity (chronic hypertension, preeclampsia, eclampsia) occurs in approximately 6%8% of pregnancies and is the second leading cause of maternal death in the United States.[55] The pathophysiologic mechanisms of preeclampsia remain unclear but a consistent finding is endothelial dysfunction resulting in intense vasospasm due to increased endothelial sensitivity to pressors.[56,57] It is thought, in part, that the enhanced vasoconstriction may be caused by increased synthesis of the potent omega-6 fatty acid derived vasoconstrictor, thromboxane A_2, that is found in maternal plasma and placental tissue of preeclamptic women.[58-60] Non-pregnant hypertensive adults have been shown to have significantly lower plasma phospholipids levels of omega-3 fatty acids which results in decreased nitric oxide synthesis and increased aceylcholinesterase activity resulting in increased vascular tone.[61,62] In contrast, populations with high marine oil intake or hypertensive patients supplemented with omega-3 fatty acids had higher plasma omega-3 fatty acid levels had lower blood pressures.[62-66] Inuit women who ate a diet rich in marine foods were 2.6 times less likely to develop hypertension during pregnancy than Inuit women whose diets contained less marine foods.[67] Supplementation with omega-3 fatty acids would correct an imbalance between prostacylin and thromboxane, reduce blood viscosity, reduce endogenous pressors, or alter baroreceptor function which may help to reduce the occurrence of hypertension in pregnancy.[68-73]

Normal placental blood flow is critical for adequate delivery of nutrients to the fetus to support normal growth and development. It has been proposed that the balance of omega-3 and omega-6 derived eicosanoids may play a key role in maintaining adequate placental blood flow and delivery of nutrient substrates to support normal fetal growth and development.[74,75] Based on biochemical indices (decreased PGI_2 synthesis and increased 20:5n-6 DPA content of umbilical artery endothelium), it appears that low birth weight infants are deficient in omega-3 fatty acids.[74] In addition, observational and interventional studies have demonstrated a direct association between fetal growth and maternal intake of omega-3 fatty acids.[24,74-77]

In keeping with other nutrients, the bulk of fatty acid delivery and storage in the fetus occurs in the last trimester. Infants born prematurely have lower total body content of omega-3 LCPUFA.[78-80] Omega-3 fatty acids accumulate in fetal fat stores, liver and neural tissues. The highest quantities are found in fat stores, but the relative proportion of omega-3 LCPUFA is highest in the retina and brain.[79] It appears that the fetus is dependent on the maternal supply of omega-3 LCPUFA with levels in the umbilical plasma phosphoplipids that strongly correlate with maternal plasma phospholipids.[81-84]

The fetus is capable of converting ALA (18:3n-3) to DHA, but it remains controversial as to whether the rate of conversion is adequate to meet their needs.[85-87] Preformed DHA is preferentially transferred from the maternal circulation to the fetus, although the mechanism is unclear.[74,88,89] Maternal stores of DHA are mobilized during pregnancy for transfer to the fetus since plasma DHA (g/ml or FA%) has been shown to be decreased in multiparous versus primiparous women. This finding correlated with the lower DHA FA% in cord tissue of higher birth order newborns. Taken together, these findings suggest that the current omega-3 fatty acid intake during pregnancy in Western countries is inadequate.[90]

Parallel to the high rates of fatty acid delivery and accretion in the fetus in the third trimester, is the rapid growth and development of neural tissues which continues for the first 18 months after birth.[81,91] During this period, the accretion of DHA in the brain is about 3 times greater than the relative increase in brain weight.[92] DHA accretion in the human retinal begins in the third trimester and peaks at 36-40 weeks gestation.[93] DHA and AA have been identified as important structural components of the highly specialized membrane lipids of the human central nervous system, with phospholipids of brain gray matter containing high proportions of DHA.[94-96] DHA has also been observed to be the major LCPUFA in the outer segments of the retina's rods and cones.[94] The functional roles of DHA were first shown in animals (fetus or newborn) deprived of DHA. Investigators have reported that the depletion of DHA from the developing retina and brain leads to abnormal electroretinograms (ERGs) and decreased VEP responses, in addition to altered learning behavior (e.g., performance in maze tasks, habituation, exploratory activity in novel environments, brightness discrimination, and olfactory-based learning tasks).[97-104] There is concern with findings that suggest that these changes in function may be irreversible despite correction of DHA status after deprivation of omega-3 fatty acids during critical periods of retinal development.[105] As well, the dietary deficiency of ALA in developing animals has resulted in decreased DHA levels, with a reciprocal increase in omega-6 fatty acids, and especially DPA, observed in the retina, whole brain, isolated brain membranes, and specific brain regions.[106-108]

Animal studies have suggested the value of providing omega-3 fatty acid supplementation as well. Recent studies have shown that omega-3 fatty acids alter the metabolism of dopamine and serotonin in the brain of rodents and piglets.[109-114] Particular interest has been given to the dopaminergic system because of its role in the cognitive advances of early childhood, for example, as a modulator of attention and motivation, and in the visual pathways.[115] Other recent studies have suggested that omega-3 fatty acids regulate the expression of genes involved in cytoskeleton and membrane association, signal transduction, ion channel formation, energy metabolism, synaptic plasticity, and the retinoid X receptor in the brain.[116-119]

Supplementation with DHA in human infants have shown variable results, with improved visual acuity demonstrated in premature infants[120-123] and variable results in term infants.[124-127] In part, the variability was thought to be due to differences in study design, age and duration of intervention, method(s) of assessment. The different measures of visual function may reflect different neural processes, making the comparison of findings between studies problematic. For example, the Teller acuity card or forced choice preferential looking method evaluates an infant's tendency to gaze at a pattern and assesses not only visual acuity but also an infant's ability to respond which requires integration of motor and behavioral responses to the visual stimuli. Visual evoked potentials (VEP) directly measures the amplitude of electrical responses to visual stimuli that signal transduction from the eye to visual cortex and is not dependent on the infant's behavioral state or motor abilities.

Based on observational studies, it has been shown that human milk fed infants have improved neurocognitive development compared to formula fed infants, it was hypothesized that one of the contributing factors may be the availability of long chain derivatives of LA and ALA that is present only human milk[82,128] This difference in fatty acid intake is reflected in lower erythrocyte membrane phospholipid DHA in infants fed formula.[82] Until the recent availability infant formula with added omega-3 LCPUFA, standard infant formula was devoid of these fatty

acids. Human milk contains DHA ranging from 0.2 to 0.4 FA% and varies considerably among different populations with differences in DHA intake.[120,124] It is thought that the rate of conversion of ALA (18:3n-3) present in standard infant formula to DHA does not meet rates of accretion in the CNS that is seen in human milk fed infants.[129-131] As with the DHA intervention trials in term infants on visual acuity, the effect of DHA supplementation on neurocognitive development is also inconsistent.[127,132-134] Thevariability may, in part, be due to the use of different assessment tools.

While it could be hypothesized that the intake of omega-3 fatty acids might have a greater impact on preterm, than term, infants because the former have been exposed for a shorter period of time to what the latter likely received as significant contributors to their development, the present review was not planned to test this hypothesis. Even so, there may be considerable justification for giving omega-3 fatty acids to mothers who eventually deliver term babies as well as to these term infants post-delivery. Mothers of term infants may not exhibit uniform levels of omega-3 fatty acid content in their biomarkers, which are passed on to their children.

For example, it has recently been observed that the human milk of North American women has significantly less DHA and AA content, when compared with milk obtained from women in China, Japan, or India.[135,136] Furthermore, higher amounts of DHA in human milk have been associated with higher plasma and erythrocyte levels of DHA in breastfed infants;[137-139] and, a significant association between DHA levels in human milk and visual evoked potential (VEP) acuity was recently reported in a cross-sectional study of breastfed infants in Denmark.[140] Related observations, which are reviewed in depth here, suggest the possible importance of the intake of omega-3 fatty acids by pregnant and lactating women for the health of their offspring.

Moreover, when compared with women with lower plasma levels of AA and DHA during gestation, women with higher plasma levels gave birth to infants with higher levels of AA and DHA;[137,141,142] and, higher levels of omega-6 and omega-3 fatty acid content in biomarkers at birth were found to be associated with higher blood levels of AA and DHA in the infant for several weeks after birth.[138,140,143] Thus, individual differences in the levels of fatty acid content observed in mothers' biomarkers, which appear to be paralleled by individual differences in the levels of fatty acid content in the biomarkers obtained from their children, might ultimately be found to account for differences in child development. DHA deficiency related to low maternal intake of omega-3 fatty acids during pregnancy, for example, might adversely impact child development.

Direct measurement of tissue levels is not feasible for most tissues such as brain and retina. As such, fatty acid biomarkers are used as surrogate measures of tissue levels. How closely these biomarkers reflect tissue levels are not certain.[131,144-147] Different measurements of the fatty acid content of different lipid pools reflect either the effects of short term (hours) or long term (days to months) dietary intake of fatty acids.

The likely significance of omega-3 fatty acids for child health is therefore suggested by the observations that: a) the human brain and retina each contain considerable omega-3 fatty acid content; b) the child delivered at term receives an important supply of omega-3 fatty acids especially in the third trimester of pregnancy; and, c) due to a shortened gestational period, the child delivered prematurely receives less exposure to omega-3 fatty acid content than does the term child. Not surprisingly, the observation concerning preterm infants has afforded

considerable empirical study of the impact of omega-3 fatty acids on their health. This evidence is systematically reviewed here.

Given this overview, and the expected availability of empirical evidence, we aimed to evaluate the impact of omega-3 fatty acid content (i.e., intake; in biomarkers), from any and all sources (e.g., breast milk; formula), on the growth patterns, neurological development, visual development, and cognitive development of preterm and term children. We also planned to investigate the influence of omega-3 fatty acid content (i.e., intake; in biomarkers), from any and all sources (e.g., food; supplements), on specific pregnancy outcomes relating to offspring (i.e., preterm births; children born small for gestational age) and maternal health (i.e., preeclampsia; eclampsia; gestational hypertension). However, as pointed out in Chapter 2, not all of the relationships between the intake of omega-3 fatty acids, the fatty acid content of biomarkers, and clinical-developmental outcomes are investigated in either population (i.e., maternal; child).

It should also be pointed out that, given the likely important role played by the omega-6 fatty acids—and AA in particular—in health and development, their co-influence on clinical and developmental outcomes are investigated, where possible. Finally, safety data (i.e., adverse effects) are evaluated. For example, concerns have been raised about the safety of fish oil supplementation in infants and pregnant women include, decreased platelet aggregation, immunosuppression, growth[148,149] and environmental contaminants.[26,132,148-151] However, the clinical significance of these potential risks need to be determined.

Chapter 2. Methods

Overview

The UO-EPC's evidence report on omega-3 fatty acids in child and maternal health is based on a systematic review of the scientific-medical literature to identify, and synthesize the results from, studies addressing key questions. Together with content experts, UO-EPC staff identified specific issues integral to the review. A Technical Expert Panel (TEP) helped refine the research questions as well as highlighted key variables requiring consideration in the evidence synthesis. Evidence tables presenting key study-related characteristics were developed and are found in the Appendices. In-text summary tables were derived from the evidence tables. The methodological quality and generalizability of the included studies was appraised, and individual study results were summarized.

Key Questions Addressed In This Report

The purpose of this evidence report was to synthesize information from relevant studies to address various questions. The questions are organized by the type of population (i.e., maternal/pregnancy versus child [e.g., term versus preterm delivery]) and the type of outcome data (i.e., clinical/pregnancy versus clinical/child-developmental capacity versus biological/biomarker status versus adverse effects):

- **Maternal population, clinical/pregnancy outcomes**:
    - What is the evidence that intake of omega-3 fatty acids influences the duration of gestation in women with or without a history of a previous preterm birth (gestational duration less than 37 weeks)?
    - What is the evidence that maternal intake of omega-3 fatty acids influences the incidence of preeclampsia, eclampsia or gestational hypertension?
    - What is the evidence that maternal intake of omega-3 fatty acids influences the incidence of births of human infants small for gestational age?

- **Maternal population, biomarker data relating to clinical/pregnancy outcomes**:
    - What is the evidence that the duration of gestation in women with or without a history of a previous preterm birth is associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?
    - What is the evidence that the incidence of preeclampsia, eclampsia or gestational hypertension is associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?

- What is the evidence that the incidence of births of human infants small for gestational age is associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?

- **Child population, growth pattern outcomes**:
  - What is the evidence that maternal intake of omega-3 fatty acids during pregnancy influences growth patterns in term or preterm human infants?
  - What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences growth patterns in term or preterm human infants?
  - What is the evidence that the omega-3 fatty acid content of infant formula influences growth patterns in term or preterm human infants?
  - What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, and together with the omega-3 fatty acid content of infant formula, influences growth patterns in term or preterm human infants?
  - What is the evidence that intake of omega-3 fatty acids from sources other than maternal breast milk or infant formula supplemented with omega-3 fatty acids, by term or preterm human infants, influences growth patterns?
- **Child population, biomarker data relating to growth pattern outcomes**:
  - What is the evidence that term or preterm human infants' growth patterns are associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?
  - What is the evidence that term or preterm human infants' growth patterns are associated with the omega-3 or omega-6/omega-3 fatty acid content of fetal biomarkers during pregnancy?
  - What is the evidence that term or preterm human infants' growth patterns are associated with the omega-3 or omega-6/omega-3 fatty acid content of child biomarkers?
- **Child population, neurological development outcomes**:
  - What is the evidence that maternal intake of omega-3 fatty acids during pregnancy influences neurological development in term or preterm human infants?
  - What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences neurological development in term or preterm human infants?
  - What is the evidence that the omega-3 fatty acid content of infant formula influences neurological development in term or preterm human infants?
  - What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, and together with the omega-3 fatty acid content of infant formula, influences neurological development in term or preterm human infants?

- What is the evidence that intake of omega-3 fatty acids from sources other than maternal breast milk or infant formula supplemented with omega-3 fatty acids, by term or preterm human infants, influences neurological development?

- **Child population, biomarker data relating to neurological development outcomes**:
  - What is the evidence that term or preterm human infants' neurological development is associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?
  - What is the evidence that term or preterm human infants' neurological development is associated with the omega-3 or omega-6/omega-3 fatty acid content of fetal biomarkers?
  - What is the evidence that term or preterm human infants' neurological development is associated with the omega-3 or omega-6/omega-3 fatty acid content of child biomarkers?

- **Child population, visual function outcomes**:
  - What is the evidence that maternal intake of omega-3 fatty acids during pregnancy influences visual function in term or preterm human infants?
  - What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences visual function in term or preterm human infants?
  - What is the evidence that the omega-3 fatty acid content of infant formula influences visual function in term or preterm human infants?
  - What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, and together with the omega-3 fatty acid content of infant formula, influences visual function in term or preterm human infants?
  - What is the evidence that intake of omega-3 fatty acids from sources other than maternal breast milk or infant formula supplemented with omega-3 fatty acids, by term or preterm human infants, influences visual function?

- **Child population, biomarker data relating to visual function outcomes**:
  - What is the evidence that term or preterm human infants' visual function is associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?
  - What is the evidence that term or preterm human infants' visual function is associated with the omega-3 or omega-6/omega-3 fatty acid content of fetal biomarkers?
  - What is the evidence that term or preterm human infants' visual function is associated with the omega-3 or omega-6/omega-3 fatty acid content of child biomarkers?

- **Child population, cognitive development outcomes:**
  - What is the evidence that maternal intake of omega-3 fatty acids during pregnancy influences cognitive development in term or preterm human infants?

- What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences cognitive development in term or preterm human infants?
- What is the evidence that the omega-3 fatty acid content of infant formula influences cognitive development in term or preterm human infants?
- What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, and together with the omega-3 fatty acid content of infant formula, influences cognitive development in term or preterm human infants?
- What is the evidence that intake of omega-3 fatty acids from sources other than maternal breast milk or infant formula supplemented with omega-3 fatty acids, by term or preterm human infants, influences cognitive development?

- **Child population, biomarker data relating to cognitive development outcomes**:
  - What is the evidence that term or preterm human infants' cognitive development is associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?
  - What is the evidence that term or preterm human infants' cognitive development is associated with the omega-3 or omega-6/omega-3 fatty acid content of fetal biomarkers?
  - What is the evidence that term or preterm human infants' cognitive development is associated with the omega-3 or omega-6/omega-3 fatty acid content of child biomarkers?

- **Maternal or child population, adverse effects:**
  - What is the evidence for the risk, in pregnant women, of short and long-term adverse events related to their intake of omega-3 fatty acids?
  - What is the evidence for the risk, in breastfeeding women, of short and long-term adverse events related to their intake of omega-3 fatty acids?
  - What is the evidence for the risk, in term or preterm human infants, of short and long-term adverse events related to maternal intake of omega-3 fatty acids during pregnancy?
  - What is the evidence for the risk, in term or preterm human infants, of short and long-term adverse events related to their intake of omega-3 fatty acids after birth (e.g., maternal breast milk, infant formula supplemented with omega-3 fatty acids)?
  - What is the evidence that these adverse events, or any contraindications, are associated with the intake of specific sources (e.g., marine, plant), types (e.g., EPA, DHA, ALA) or doses of omega-3 fatty acids, including in specific populations such as diabetics?

The overarching goal was to identify and systematically review whatever evidence exists within the eligibility boundaries established for this review in consultation with our TEP and in light of the topics being addressed by SC-RAND and Tufts-NEMC EPCs. These boundaries are delineated in the Eligibility Criteria section (below). At all times, data obtained from children delivered at term and preterm (i.e., gestational duration less than 37 weeks) were evaluated separately. More details concerning the questions are provided in conjunction with the description of the Analytic Frameworks (below).

We were also guided collectively by ODS, our TEP and our UO-EPC review team content experts to examine, where data permitted, the possible influence on efficacy, association or safety evidence of the following potential effect modifiers:

- intervention/exposure length;
- timing of intervention/exposure period (e.g., beginning the 3rd day of life, for 4 months);
- type(s) of omega-3 fatty acid (e.g., ALA, EPA, DHA);
- source of the omega-3 fatty acids (e.g., marine, plant, nut), including the specific source (e.g., mackerel as an oily fish);
- total caloric/energy intake;
- delivery format (e.g., whole food servings, capsules, pourable or spreadable oils);
- dose/serving size, including the precision/control of its delivery (e.g., per-day specific, minimum, maximum or range of numbers of capsules, whole food servings or bottle-pourable litres);
- type of processing used to purify the intervention/exposure and/or to maintain the experimental blind (e.g., ethyl esterification; adding an anti-oxidant to stabilize/preserve oils; adding flavor to oils; [vacuum] deodorization);
- amount/dose of omega-6 fatty acid intake either added as a cointervention or identified as being present in the background diet, thereby establishing a specific, minimum, maximum or range of allowable or mandated on-study omega-6/omega-3 fatty acid intake;
- the identity of the manufacturer and/or certain characteristics of their product(s) (i.e., purity; presence of other potentially active agents that have not been added intentionally: e.g., methylmercury content);
- for questions relating to efficacy or association, the prestudy/baseline or on-study omega-3 or omega-6/omega-3 fatty acid content of blood lipid biomarkers;
- absolute or relative omega-3 fatty acid content of the prestudy/baseline diet;
- omega-6/omega-3 fatty acid content in the prestudy/baseline diet, with the study population's country of origin as a possible surrogate measure of the omega-6/omega-3 fatty acid content of the background diet; and,
- any study subpopulations (e.g., minority; ethnic; genetic, including diabetics).

Furthermore, where data permitted, the following factors with the potential to influence child and maternal health outcomes were also investigated:

- obstetric history (e.g., maternal age at conception and delivery; history of a previous and/or current preterm birth [length in weeks; etiology; spontaneous versus induced; history of preeclampsia, eclampsia or gestational hypertension; history of a previous birth of an infant small for gestational age);
- gynecologic history (e.g., uterine abnormalities);
- maternal general health history (e.g., medical and psychiatric), including maternal medication/treatment history (e.g., prescription and non-prescription drugs);
- breastfeeding history;
- setting (e.g., tertiary care hospital; community facility);
- other sociodemographic/economic factors (e.g., marital status, education, income, employment status);
- other maternal cointerventions (e.g., other supplement use [e.g., vitamins, minerals], psychological interventions, use of complementary/alternative [CAM] medicine/products);
- maternal illicit drug use history;
- history of domestic violence;
- maternal smoker history;
- history of maternal alcohol consumption;
- prenatal history (e.g., delivery anomalies);
- neonatal history (e.g., asphyxia; intracranial hemorrhage);
- pediatric history (e.g., medications/treatments; supplement use [e.g., vitamins, minerals]; immunizations); and,
- with respect to each child outcome in turn (e.g., cognitive development), the developmental capacity/status regarding the other child outcomes (i.e., growth patterns [e.g., weight, height and head circumference at birth]; neurological development; visual development).

Parental smoking and alcohol consumption especially during pregnancy yet also post-delivery are particularly important effect modifiers in that they have been observed to influence both child or maternal health *and* essential fatty acid status, with levels of the latter potentially affecting the former.[152]

Analytic Framework

Two analytic frameworks were developed to make explicit the review's specific links relating the populations and settings of interest (i.e., term versus preterm infants), the focal exposure or intervention (i.e., omega-3 fatty acids ingested as supplementation and/or from food sources), potential effect-modifying factors, key child and maternal health outcomes, and the possible role played by the omega-3 or omega-6/omega-3 fatty acid content of biomarkers in mediating the intake-outcome relationship. A first analytic framework (Figure 2) highlights maternal outcomes, whereas a second one focuses on child/developmental capacity outcomes (Figure 3). The possibility of adverse events (e.g., side effects) and contraindications is recognized in each framework. In short, the analytic frameworks outline the various lines of logic defining the review's research questions. But, not all linkages in each analytic framework were investigated.

One criterion established in this review is that each researchable question had to be clinically relevant. That is, irrespective of the population of interest, a question had to involve the investigation of at least one relevant clinical/pregnancy (i.e., maternal population: Figure 2) or developmental (i.e., child population: Figure 3) outcome. Likewise, to be eligible for inclusion in the review each study had to entail an investigation of at least one such outcome. Considering the purpose of the two-year task order is to afford a clinically-relevant research agenda, this decision was judged to be appropriate by both our TEP and our review team. Thus, excluded were studies whose sole focus was to examine the impact of omega-3 fatty acid interventions or exposures on the omega-3 or omega-6/omega-3 fatty acid content of biomarkers, even if the study populations met other eligibility criteria for the present review.

The questions investigating maternal/pregnancy outcomes refer to clinical events whose likelihood might be influenced by the maternal intake of omega-3 fatty acids (i.e., from supplementation and/or the diet) and/or which might be associated with specific levels of omega-3 fatty acid content (i.e., composition or concentrations) derived from any biomarker type obtained from pregnant women (e.g., red blood cells [RBCs]; plasma phospholipids) (Figure 2).

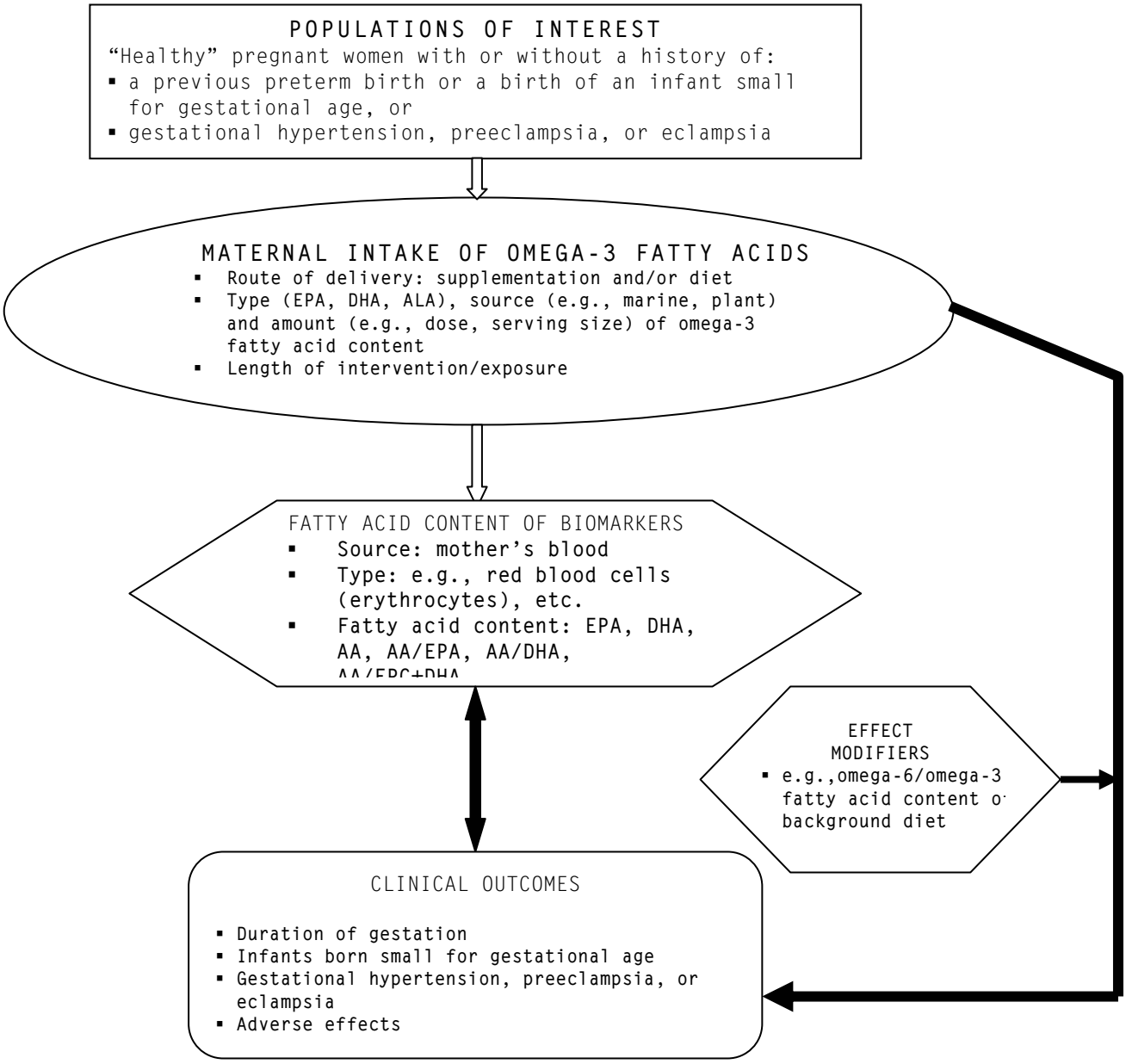

Figure 2. Analytic Framework for omega-3 fatty acids in maternal health. Populations of interest in rectangles. Exposure in oval. Outcomes in rounded rectangles. Effect modifiers in hexagons. Solid connecting arrows indicate associations and effects reviewed in this report.

The clinical events constitute the outcomes of interest, and include the shorter-than-term duration of gestation, the birth of an infant small for gestational age, or the maternal development of preeclampsia, eclampsia, or gestational hypertension. Otherwise "healthy" pregnant women, with or without a history of the following, constitute the study populations of interest:

- a previous preterm birth (i.e., gestational duration less than 37 weeks);
- preeclampsia, eclampsia or gestational hypertension; or,
- a previous birth of an infant small for gestational age.

The questions investigating child outcomes refer to progress along four developmental arcs, which might be influenced by the term or preterm child's intake of omega-3 fatty acids from various sources (i.e., mother via the placenta, breast milk, post-delivery formula supplementation, and/or from other food sources or supplementation) and/or which might be associated with specific levels of omega-3 fatty acid content (i.e., composition or concentrations) derived from any biomarker type (e.g., RBCs; plasma phospholipids) or source (i.e., mother; child) (Figure 3).

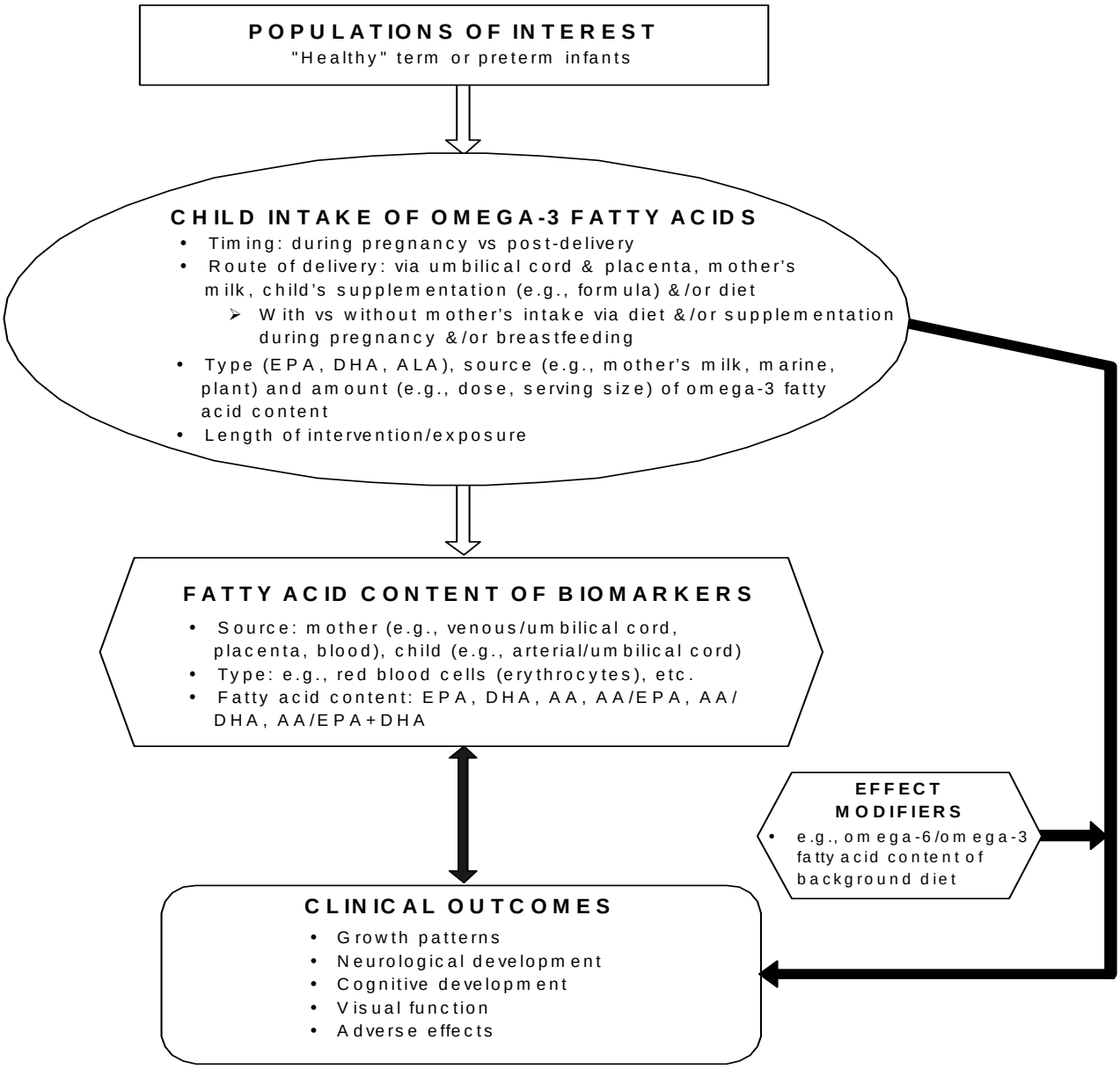

Figure 3. Analytic Framework for omega-3 fatty acids in child health. Populations of interest in rectangles. Exposure in oval. Outcomes in rounded rectangles. Effect modifiers in hexagons. Solid connecting arrows indicate associations and effects reviewed in this report.

At the time they and their breast milk serve as the child's source of omega-3 fatty acids, mothers may or may not have been receiving a supply of omega-3 fatty acids in their diet and/or from supplementation. The developmental arcs constitute the clinical-developmental outcomes of interest: growth patterns, neurological development, visual development, and cognitive development. The child populations of interest include otherwise "healthy" children delivered at term or preterm, with data from these populations investigated separately.

Overall, questions pertaining to maternal populations center on the possible preventive, or protective, value of omega-3 fatty acid content (i.e., intake and/or in biomarkers) with respect to specific pregnancy outcomes. On the other hand, questions regarding child populations concern the possible value of omega-3 fatty acid content (i.e., intake and/or in biomarkers) in facilitating (e.g., "catching up to," maintaining, or accelerating) expected or possible types or rates of development. Questions relating to adverse effects in both populations are investigated with data obtained from interventional/exposure studies meeting eligibility criteria.

The possible influence of predefined effect modifiers is evaluated in relation to each of the questions. Where data permit, question-specific sections titled "Impact of Covariates and Confounders" elucidate a) those variables (e.g., intervention/exposure; population) that were consistently observed, across reviewed studies, to influence study outcomes as well as b) those variables (e.g., caloric/energy intake; smoker status; alcohol consumption), which having been controlled for either experimentally or analytically in reviewed studies, were observed to consistently influence, or consistently fail to influence, study outcomes.

Study Identification

Search Strategy

The search strategy for this project was designed to be comprehensive and achieve the highest possible recall of relevant clinical studies. The electronic search strategy was developed by an information specialist in consultation with clinical content experts in child and maternal health. The child and maternal health search concept was combined with the core omega-3 fatty acids search strategy established in collaboration with the project librarians, biochemists, nutritionists, and clinicians from the three EPCs involved in the 2-year, Health Benefits of Omega-3 Fatty Acids task order. Consultation among these sources provided the biochemical names and abbreviations of omega-3 fatty acids, names of commercial omega-3 fatty acids products, and food sources of omega-3 fatty acids.

The following electronic databases were searched: Medline (1966 - November Week 2 2003 and updated to February Week 3 2004), Premedline (Dec 13 2003), Embase (1980 to 2003 Week 50 and updated to 2004 Week 09), the Cochrane Library including the Cochrane Central Register of Controlled Trials (3rd Quarter 2003) and CAB Health (1973-Sept 2003). All databases were searched via the Ovid interface using Search Strategy 1 (Appendix A[*]), except CAB Health, which was searched through SilverPlatter using Search Strategy 2 (Appendix A). Searches were not restricted by language of publication, publication type, or study design, except with respect to the MeSH term "dietary fats," which was limited by study design to increase its specificity. A total of 2,932 bibliographic records were downloaded, with duplicate records identified and removed using citation management software (Reference Manager®).

Reference lists of included studies, book chapters, and narrative or systematic reviews retrieved after having passed the first level of relevance screening, were manually searched to identify additional unique references. Through contact with content experts, attempts were made

[*] Appendices and Evidence tables cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/o3mchtp.htm

to identify both published and unpublished studies. On behalf of the three EPCs investigating the evidence concerning the health benefits of omega-3 fatty acids, a letter was written to industry representatives to obtain additional evidence (Appendix B*). Investigators who frequently published study reports that were included in the review were contacted to clarify which of their reports were companion documents (i.e., multiple reports referring to the same study yet where each contains some unique outcome data or unique descriptions of the methods: e.g., additional follow-up data) or duplicate documents (i.e., a report which exclusively presents data published elsewhere). These informants were also asked to provide citations or copies of reports that our searches failed to detect and to identify the study each described. Investigators who responded with clarifying information included: Drs. Eileen Birch, Susan Carlson, Maria Makrides, Sjurdur Frodi Olsen, and Mary Fewtrell. All of these supplementary efforts to identify more evidence identified an additional 18 records that were entered into the collection for review. A final set of 2,049 unique references was identified.

Eligibility Criteria

Published and unpublished studies, written in any language, were eligible for inclusion. Excluding grey literature from systematic reviews of interventions can lead to the overestimation of effect sizes.[153] Substantial bias in the results of a systematic review pertaining to a complementary/alternative medical (CAM) intervention can ensue from the exclusion of data from reports written in languages other than English.[154] AHRQ and ODS consider omega-3 fatty acids to be a CAM exposure.

To maximize their generalizability, clinical, developmental and biomarker data were required from live, otherwise "healthy" human study populations or subpopulations (e.g., genetic, minority, ethnic: e.g., diabetic) of any age. For sake of simplicity, we decided to use the generic term "child" when referring to infants (less than 12 months of age), toddlers and children up to 18 years old. Excluded were studies whose biomarker data were solely obtained from aborted fetuses because the circumstances associated with or leading to spontaneous or elective abortions (e.g., chromosomal abnormalities; non-chromosomal congenital abnormalities) could influence the fatty acid status of biomarkers in ways that would preclude an interpretation of these observations that is meaningful for the purposes of the present review. Moreover, different types of abnormal fetus may exhibit different rates of omega-3 fatty acid accumulation in tissue and/or different patterns of tissue-specific omega-3 fatty acid accumulation during gestation, resulting in the limited generalizability of the respective data.

Explicit affirmation of the health status of both the maternal and child populations, as well as the preterm/term status of the child populations, had to be provided in study reports. The concept of "child" was not predefined, and the impact on outcomes of any idiosyncratic definitions could not be evaluated *post hoc*. To allow the meaningful comparison of results from term and preterm infants, age was defined as postconceptional age. Also, if a study did not distinguish data obtained from term and preterm births, it was excluded from the review. Additional details concerning eligibility criteria (e.g., specific types of population required to

* Appendices and Evidence tables cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/o3mchtp.htm

address the research questions) have already been described with reference to the Analytic Frameworks, and are not repeated here. Excluded populations were those with peroxisomal (e.g., Zellweger's) disorders since this topic was addressed in SC-RAND's year-2 review of the evidence concerning omega-3 fatty acids in neurology.

Ideal interventional/exposure studies of newborns might be expected to enroll and expose them to sources of omega-3 fatty acids immediately post-delivery so as to have, at least in theory, the greatest possible impact on development, and to minimize confounding from earlier exposure to other sources of nutrition,. However, neither the exact or requisite timing of the onset of the intervention/exposure nor the absence of an intervention/exposure to other sources of nutrition (e.g., parenteral feeding in preterm infants) prior to study entry constituted eligibility criteria. Plans were nevertheless made to explore, where data would permit, the possible impact of these factors on outcomes.

No restrictions on the length or number of followups with respect to either study population were pre-established. Yet, given the dynamic nature of development, ideal studies of children might be thought to include multiple followups conducted at least according to expected developmental milestones specific to the four types of developmental arc of interest to the present review.

Interventional/exposure studies had to specifically investigate foods or supplements known to contain omega-3 fatty acids of any type (e.g., EPA, ALA), from any source (e.g., mother's milk, fish, walnuts, seed oil), any serving size or dose, delivered in any fashion (e.g., breastfeeding, capsules, liquid, LCPUFA-rich diet), and for any length of time. In all studies, some method had to have been employed to suggest the presence of omega-3 fatty acid content in the exposure, if not its actual amount (e.g., g/d). Studies investigating "PUFAs" or " LC PUFAs," or even types of diet one might presume would contain marine or land sources of omega-3 fatty acids (e.g., "Mediterranean diet") at minimum had to highlight at least one source of the omega-3 fatty acid content (e.g., oily fish servings). No restrictions were placed on the types or doses of pre- or on-study cointerventions (e.g., omega-6 fatty acid intake, other dietary supplements). While omega-6 fatty acids appear to play a key role in health and development, and their possible co-influence on outcomes is thus assessed in our review, studies exclusively investigating their impact on health outcomes are excluded. A table placed at the end of this report summarizes the content of the fatty acids (and other constituents) in the various types of infant formula provided as supplementation in the included studies.

Randomized controlled trials (RCTs) are the gold standard method to investigate questions of intervention efficacy or effectiveness.[155] and were sought to address the research questions. If at least two RCTs were identified, no other types of design were required. Yet, if insufficient numbers of RCT were retrieved, non-RCT (i.e., controlled clinical trials, without random allocation) and observational studies were included. Excluded from this review were descriptive study designs, however (i.e., noncomparative case series; case studies).

RCTs exhibit a greater inherent potential to deal with potentially serious biasing influences (e.g., selection bias) although a poorly designed or conducted RCT can produce results whose interpretability is no less complicated by the presence of confounding influences, for example, than observations derived from a well-constructed and conducted study employing a design with a lesser intrinsic capacity to control for these biases (e.g., non-RCT; prospective cohort study). For example, not all intervention RCTs succeed, either through an explicit experimental plan or

the process of randomization per se, to equally distribute known confounding influences (e.g., background diet; energy/caloric intake from the intervention) across their respective study groups.

That said, questions concerning the impact on child developmental outcomes of omega-3 fatty acid intake via formula supplementational alone, or formula supplementation given in addition to breast milk, could be investigated exclusively by RCT evidence. Other questions required the inclusion of observational study evidence (e.g., maternal intake of omega-3 fatty acids, and child developmental outcomes; the role of biomarkers). The observational studies included cross-sectional designs, which by virtue of the lack of temporal separation in their assessments of exposure and outcome, constitute the weakest evidence when it comes to suggesting causal relationships.

Any definition of control or comparator was permitted in the controlled studies (e.g., DHA versus olive oil placebo). However, not every control or comparator group constituted the most appropriate one. For example, with women in a study permitted to choose either to breast- or formula-feed their child, selection bias makes the analyzed comparison of the outcomes from these two groups difficult to interpret unequivocally. The breatfeeding group cannot be construed as the most appropriate control, even though some manufacturers of formula supplementation have attempted to match their fatty acid contents and other constituents to what is contained in human breast milk.

Designs potentially affording less equivocal interpretations include women, having chosen not to breastfeed their children, being randomized to receive formula supplementation either with or without omega-3 fatty acid content. These data would be eligible for inclusion in one type of meta-analysis in our review. Often, as stated earlier, these designs can also include women who exclusively chose to breastfeed their children. However, data from the breastfed children in such studies are exclusively used here as a possible reference standard, or comparison group, yet whose data are not entered into possible meta-analysis as control observations. Another type of design potentially affording less equivocal interpretations involve women, having chosen to exclusively breastfeed their children, who are then randomized to receive formula supplementation either with or without omega-3 fatty acid content. These data would be eligible for an independent meta-analysis.

The specific pregnancy outcomes were identified with reference to the Analytic Frameworks. Any and all child developmental outcomes reflecting the four categories of developmental arc were considered relevant. As markers of omega-3 fatty acid metabolism, the following fatty acid compositions or concentrations, from any source (e.g., red blood cell [RBC] membranes, plasma phospholipids), were considered relevant: EPA, DHA, AA/EPA, AA/DHA, AA/EPA+DHA. Studies exclusively evaluating the role of other biomarkers (e.g., cytokine production, eicosanoid levels), including preconditions (e.g., specific PG levels) often thought to be associated with our review-relevant clinical outcomes (i.e., the development of preeclampsia), were not included. These decisions were made with the assistance of our TEP.

Study Selection Process

The present review employed specific electronic functionality in the form of an internet-based software system, housed on a secure web site. It brings appreciable efficiencies to the systematic review process and the management of a systematic review team. Electronic yields of literature searches are posted to the system for review. Reviewers then submit all of their results of relevance screening, data appraisal or data abstraction directly to the system. The software system automatically conducts an internal comparison of multiple reviewers' responses to screening questions, to determine the eligibility/relevance of a bibliographic record or a full report. As well, the software captures responses to specific requests to abstract pre-specified data (e.g., mean age of study participants; the assessment of a study's internal validity) from pertinent reports. One large advantage associated with using this software is that review team members are able to complete their work from wherever they have internet access.

Following a calibration exercise, which involved screening five sample records using an electronic form developed and tested especially for this review (Appendix C[*]), two reviewers independently screened the title, abstract, and key words from each bibliographic record for relevance by liberally applying the eligibility criteria. A record was retained if it appeared to contain pertinent study information. If the reviewers did not agree in finding at least one unequivocal reason for excluding it, it was entered into the next phase of the review. The reasons for exclusion were noted using a modified QUOROM format (Appendix D).[156] The screening process also aimed to identify the exact child and maternal health question a record addressed, in addition to determining whether it might also or instead pertain to any of the other topics being systematically reviewed by the three EPCs in year 2 of the omega-3 fatty acids project.

Print or electronic copies of the full reports for those citations having passed level one screening were then retrieved. After completing a calibration exercise which involved evaluating five sample reports using the same eligibility criteria (Appendix C), the rest of the reports were independently assessed by two reviewers. Reports were not masked given the equivocal evidence regarding the benefits of this practice.[157] To be considered relevant at this second level of screening, all eligibility criteria had to be met. A third level of dual-review screening aimed to exclude studies whose designs were not required to investigate the research questions (see Eligibility Criteria). All the levels of evidence were reviewed and when there were at least one study to address a given question, it was included regardless of the level of evidence. However, if there were at least two RCTs addressing the question, lower level of evidence reports were excluded (see list of excluded observational trials in Appendix F).

Disagreements arising either at screening levels 2 or 3 were resolved by consensus and, if necessary, third party intervention. Excluded studies at each of these levels are noted as to the reason for their ineligibility in listings found at the end of this report.

[*] Appendices and Evidence tables cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/o3mchtp.htm

Data Abstraction

Following a calibration exercise involving two studies, 11 reviewers independently abstracted the contents of included studies using an electronic Data Abstraction form developed especially for this review (Appendix C*). A second reviewer then verified these data. Data abstracted included the characteristics of the:

- report (e.g., publication status, language of publication, year of publication);
- study (e.g., sample size; research design; number of study arms/groups, cohorts, or phases; funding source);
- population (e.g., preterm versus term status);
- intervention/exposure (e.g., omega-3 fatty acid types, sources, doses, and intervention/exposure length), and comparator(s);
- cointerventions (e.g., omega-6 fatty acid use);
- withdrawals and dropouts, including reasons;
- clinical outcomes;
- fatty acid content of biomarkers; and,
- adverse events (e.g., side effects).

Summarizing the Evidence

Overview

The evidence is presented in three ways. Evidence tables in the Appendices offer a detailed description of the included studies (e.g., study design, population characteristics, intervention/exposure characteristics [e.g., omega-3 fatty acid types and doses], cointervention [e.g., background diet]), with a study represented only once. These tables are organized by research design (Evidence Table 1: RCTs; Evidence Table 2: observational studies), with studies arranged alphabetically within each of the two table/design categories.

Question-specific summary tables embedded in the text describe each study addressing a given question in abbreviated fashion, highlighting some key characteristics, including sample size (as measure of the "weight" of the evidence and possible precision of the results), dose and type of omega-3 fatty acids, and comparators' (i.e., comparison groups') specifications. This affords a comparison of all studies addressing a given question. A study can appear in more than one summary table since it can address more than one research question. Also question-specific is each summary matrix, situating each study in terms of its study quality and its applicability.

* Appendices and Evidence tables cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/o3mchtp.htm

Study Quality

Study quality refers to the internal validity, or methodological soundness, of a study. A systematic review can be faced with great variability in the quality of its included studies. Our approach is not to use a minimal level of quality as an inclusion criterion since this precludes assessing the possible impact of study quality on study results.

A study with low quality can make it difficult to clearly and meaningfully interpret its results, that is, to unequivocally attribute a significant observed benefit exclusively to an intervention/exposure (as opposed to other factors). Since definitions, or standards, of study quality can depend on the type of research design, different constructs were selected to evaluate, from study reports, the quality of RCTs and studies employing other types of research design. After a calibration exercise involving two studies with an RCT design, two assessors independently evaluated study quality. Disagreements were resolved via forced consensus. In the case of designs other than RCTs, a single quality assessor performed the evaluations. Time did not permit their dual assessment.

Four fundamental quality constructs from two instruments were used to rate the internal validity of RCTs. These tools were chosen collectively by the three EPCs involved in the 2-year task order because they have been validated. The Jadad items[158] assess the reporting of randomization, double blinding, and, withdrawals and dropouts (Appendix C[*]). Total scores range from 0 to 5, with a score less than 3 indicating low quality. The reporting of the concealment of a trial's allocation to treatment[159] yields three grades (A = adequate; B = unclear; C = inadequate) (Appendix C).

The assessment of the quality of studies using designs other than RCTs is complicated by the dearth of validated instruments and the variety of such designs (e.g., non-randomized controlled trials; uncontrolled studies). Nevertheless, a recent systematic review by Deeks et al. identified a number of "best tools" for use with these designs.[160] Among them was a published instrument developed by Downs and Black[161] and an unpublished albeit validated instrument derived by experts in Newcastle and Ottawa (NOS).[162] The former validated both design-specific and design-neutral items.

Where case-control studies were included in the review, the validated NOS was employed. Items applicable to cross-sectional designs were taken from the Downs and Black instrument; or, if the required constructs were not operationalized in this instrument, they were developed as modifications of existing NOS items (e.g., single prospective cohort studies).(Appendix C).

It should be noted that the items defining the case-control assessment tool from the NOS were used as a whole, although specific guidelines as to which total score indicates either low or sound quality are unavailable. Likewise, no guidelines exist to mark low or sound study quality based on any subset of Downs and Black's 27-item instrument. As already asserted, an Jadad total quality score of less than 3 indicates low quality. To permit the entry of these quality data into a summary matrix, cutpoints for each type of design were set somewhat arbitrarily to establish three levels of internal validity (see Summary Matrix).

[*] Appendices and Evidence tables cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/o3mchtp.htm

It was decided by our review team that, given the limitations of space, especially in print-based study reports, and the amount of detail that would likely be required to provide all of the details we needed to fully establish that only appropriate methods had been used to extract, prepare, store and analyze lipid content, it was reasonable to appraise these methods by focusing instead on identifying extant descriptions of inappropriate methods. On occasion, the inappropriateness of methods had to be determined by reference to standard protocols.

Pilot-tested exclusively for their ease of use within the data abstraction form were questions designed to informally assess the successful control of study confounding from variables identified by content experts as potential threats to the internal validity of studies pertinent to the review. In their view, these variables required experimental or statistical control to permit an uncomplicated interpretation of study results (Appendix C[*]). The two major categories of threat in controlled designs came from having study groups vary in terms of key prestudy or baseline characteristics (e.g., background diet), or from having certain on-study changes (e.g., unexpected illness) unrelated to the exposure or intervention, occur unequally across study groups to produce confounding. Even RCTs are not immune to being influenced by these threats to internal validity.

For example, if in a placebo-controlled RCT test of the supplemental treatment efficacy of omega-3 fatty acids, only certain treatment group members' background diets changed appreciably from what was observed at baseline (e.g., decreased fish intake and thus an increased omega-6/omega-3 ratio in the background diet), at which point the two study groups' baseline diets had been deemed comparable, then this on-study inequality could influence study outcomes. Because of this change in background diet, one study group might all of a sudden be receiving a different ratio of omega-6/omega-3 fatty acid intake than what had been set in the study protocol. This would amount to a change in the planned, on-study between-group difference in omega-6/omega-3 fatty acid intake; and, it is this intake ratio which could have the greatest influence on clinical outcomes. In general, contraventions of planned on-study between-group equivalences (e.g., caloric/energy intake; background diet; current smoker status; alcohol consumption) or of planned, on-study between-group differences (e.g., amount of omega-3 fatty acid intake) related to events other than the intervention/exposure (e.g., stressors, which can alter participants' patterns of eating, smoking, and alcohol consumption), that is, in variables with the potential to affect child and maternal health outcomes (and biomarker levels), could either "mask" or incorrectly "reveal" clinical benefits of the intervention depending on the groups in which these unexpected changes occurred. Then, unless statistical adjustments are made, such a scenario will complicate the meaningful interpretation of outcomes.

These informal assessment items were modified to assess single group studies since on-study changes involving the same key variables can also complicate the interpretation of their study results. However, no quality scores were derived from the data abstractors' responses to these questions pertaining to controlled or uncontrolled studies.

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Study Applicability

As specified in the scope of work for this series of evidence reports on the health benefits of omega-3 fatty acids, the primary focus is on the US population. Given the geographical location of the UO-EPC, however, the definition of study applicability was expanded slightly to include Canada as part of a larger North American context. This study's reference point became the "typical" North American.

Also known as external validity, or generalizability, the construct of applicability refers to the degree to which a given study's sample population is sufficiently representative of the population to which one wishes to generalize its results. In the present review, two schemes operationally defined applicability (Appendix C[*]). One assessed studies involving at least one otherwise "healthy" maternal population, with the other evaluating studies involving at least one otherwise "healthy" maternal population with a known elevated risk for a particular pregnancy and/or infant outcome.

With regards to the highest level of applicability (Level I) in the first scheme, the broadest definition of the population of interest is the otherwise "healthy" North American (or similar individual), drawn from a somewhat broad socio-demographic spectrum (i.e., age, race), and who eats a diet "typical" of a broad spectrum North American population (e.g., with an estimated omega-6/omega-3 intake ratio of at least 15: see below for references). For Level I applicability in the second scheme, the broadest definition of the population of interest is the otherwise "healthy" North American (or similar individual), at known risk for a particular pregnancy and/or infant outcome perhaps because of a similar past occurrence, representing a somewhat broad socio-demographic spectrum (i.e., age, race), and eating a diet "typical" of a broad spectrum North American population (e.g., with an estimated omega-6/omega-3 intake ratio of at least 15). Together, these level I definitions represent the respective reference points, with applicability decreasing as the definition of the sample study population narrows in terms of the factors represented in the two schemes.

Operationalized ideally in this review as the omega-6/omega-3 fatty acid ratio, background diet may be an important factor in assessing both types of study population (i.e., no known risk versus known risk). Given the competitive relationship between omega-3 and omega-6 fatty acids, both for enzymes to yield key metabolites with specific effects in the human biosystem (see Chapter 1) and for positions in cell membranes from which to have these and other possible influences (e.g., clinical prevention), the absolute and relative intake of omega-3 and omega-6 fatty acids from all sources, and not just from the identified exposure, likely need to be taken into account when deciding whether populations assessed in different studies are comparable. The likelihood of biological and/or clinical effects in studies may turn out to vary depending on these absolute or relative intake values. A high background dietary omega-6/omega-3 fatty acid intake ratio—potentially reflected in a corresponding differential in these contents in cell membranes—may make it harder for omega-3 fatty acid supplementation to make a clinically meaningful difference,[163] although already having considerable omega-3 fatty acid content in the background diet and in cell membranes because of a low omega-6/omega-3 fatty acid intake ratio may make

[*] Appendices and Evidence tables cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/o3mchtp.htm

it difficult for typically small amounts of omega-3 fatty acid supplementation to make a clinically meaningful difference (see Discussion).

Irrespective of which of these hypotheses may be eventually confirmed elsewhere, the fact that national, and sometimes regional, populations can vary in terms of their diet's omega-6/omega-3 fatty acid intake ratio strongly suggests that this potential confounding influence on study outcomes needs to be represented in the applicability schemes whereby the North American value is the reference point. The typical North American diet contains an omega-6/omega-3 fatty acid intake ratio of at least 15, whereas urban India and Japan's corresponding values are 38-50 and 4, respectively.[152,164-175]

UK populations represent somewhat of a special case in that, while they can exhibit socio-demographic pictures similarly broad to the ones seen in North American study populations, their somewhat different lifestyle and background diet recommended an applicability value of "II." However, if participants were drawn from a narrower UK population, then a "III" was assigned. One assessor evaluated study applicability.

Summary Matrix

For a given research question, and where possible (e.g., more than one study addressing the question), a summary matrix situates the pertinent studies in terms of their respective study quality (internal validity) and applicability (external validity) values. The Jadad total quality score defined RCTs' internal validity in summary matrices. A three-level format was derived from the range of possible RCT quality scores (A = Jadad total score of 4 or 5; B = Jadad total score of 3; C = Jadad total score of 0, 1 or 2). Given that allocation concealment scores have in the past tended to vary less widely than Jadad total scores, allocation concealment values were entered as superscripts in the summary matrices.[163] A similar approach was taken for the studies employing other research designs. The following cutpoints were established, albeit without benefit of a validational exercise:

- case-control study (NOS): A = 9-12; B = 5-8; C = 1-4;
- (multiple-group) cross-sectional study: A = 8-11; B = 5-7; C = 1-4; and,
- single prospective cohort study (Modified NOS): A = 8-10; B = 4-7; C = 1-3.

The three-level applicability format was established by the 3 EPCs involved in the 2-year project for practical reasons, to permit the incorporation of quality scores within a summary matrix. Studies assigned an "X" (i.e., insufficient information to establish applicability) were excluded from summary matrices.

Qualitative Data Synthesis

An overarching qualitative synthesis describes the progress of each citation, then report, through the stages of the systematic review. It also highlights certain report and study design characteristics of included studies (e.g., distributions of research design by research question). Then, for each question, a separate qualitative synthesis is derived for included evidence,

organized by broad categories of research design (i.e., RCTs vs observational studies). A brief study-by-study overview typically introduces the synthesis, followed by a narrative summary of the key defining features of relevant studies (e.g., inclusion/exclusion criteria), including their populations (e.g., diagnosis-related), intervention/exposures (e.g., types of omega-3 fatty acid), cointerventions (e.g., psychotropic medication), outcomes, study quality, applicability, and results. Whether or not data can be organized according to these subheadings depends on the number of studies addressing a given question and the amount or variety of detail available in the study reports. For example, having identified too few studies per research question that exhibit significant effects for a given clinical outcome can preclude determining the impact of covariables with the potential to modify or confound study results (e.g., type or dose of omega-3 fatty acids).

Juxtaposing, in turn, all pertinent studies' parameters for a given research question has two key consequences. It allows us to identify the "gaps" in knowledge deemed crucial by content experts to understanding the clinical phenomenon (e.g., efficacy of omega-3 fatty acids). That is, data regarding possible confounders may be lacking, making it difficult to interpret study results with unfettered confidence. These gaps point to those variables requiring measurement and experimental or statistical control in future research. Second, it affords an understanding of the definition and extent of the included studies' clinical homogeneity (i.e., population, intervention, cointervention, outcome), which can then inform decisions regarding the appropriateness of meta-analysis. Where strong clinical heterogeneity is observed, it may be important to forego meta-analysis because the "population" to which any point estimate, and its measure of precision, might be extrapolated may not exist per se; it, too, is synthetic (e.g., the "average" preterm infant). Subject to scrutiny in the evaluation of cross-study clinical homogeneity is the ability of each study to control for confounding influences and yield results that can be interpreted without serious question marks. The existence of statistical heterogeneity also plays a role in the decision to do without a quantitative synthesis. Whether or not meta-analysis is considered appropriate, an attempt is made to make sense of the possible influence of covariates and confounders within the context of the qualitative synthesis.

Where eligibility criteria permit, evidence from research designs with a lesser inherent potential to control for biasing influences are used to see whether, collectively, they confirm the picture of efficacy, or association, derived from designs with a greater inherent potential to achieve this goal (e.g., RCTs: see Eligibility Criteria). For the purposes of interpreting results, greater emphasis is placed on the latter, with "greater emphasis" meaning that we assign greater interpretative, not numerical or statistical, weight to these intrinsically stronger designs. Factors other than study design also taken into account in interpreting results include study quality, the number of studies, and whether studies were sufficiently powered.

Quantitative Data Synthesis

Meta-analysis was conducted providing there was a clearly defined population to which to generalize the synthetic result (and its precision). Given its greater potential to control for possible confounding factors, only RCT evidence regarding the question of efficacy/effectiveness was considered for inclusion in meta-analysis. Details concerning certain study design requirements for entry into meta-analysis are presented in the Eligibility Criteria

section (see above), and are not repeated here. All things being equal, it was also assumed that priority in meta-analysis should be given to clinical outcomes evaluated using validated measures pertinent to the present day practice of medicine (e.g., respective Bayley's scales for neurological and cognitive development).

The inclusion criteria to conduct meta-analysis were:

1. at least two RCTs;
2. same population characteristics (mean age, health status, gender);
3. same co-interventions;
4. same intervention based on the type of omega-3 FA supplemented (DHA+AA vs. DHA vs. DHA+EPA, etc.) regardless of the daily dose in the child population;
5. same comparator based on source of placebo (e.g., olive oil, unsupplemented formula);
6. outcomes relevant to respond the key-questions: percentage (n) of premature deliveries, incidence of GHT, pre-eclampsia or eclampsia, incidence of IUGR or SGA infants, weight, length and head circumference of infants (means), neurological and cognitive development measured by validated scales (e.g., Bayley's Develomental Scale score), and visual acuity or visual function of infants measured by appropriate tests (Teller's Card test, etc.).

Insufficient numbers of study with comparable populations, interventions, intervention-comparator contrasts or outcomes precluded the conduct of a) many planned meta-analyses; b) planned subgroup analyses involving virtually all of the predefined covariables with the presumed potential to influence pertinent clinical-developmental outcomes (e.g., source, type or dose of omega-3 fatty acids); and c) planned sensitivity analyses investigating the possible impact of study quality and publication bias on clinical-developmental outcomes.

Decisions regarding statistical models and related issues such as statistical heterogeneity are provided where results of meta-analysis are reported.

Chapter 3. Results

Results of Literature Search

Regardless of its source, the progress of each bibliographic record through the stages of the systematic review is illustrated in the modified QUOROM flow chart (Appendix D*). Ideally, a record included an abstract and key words, in addition to a citation. When a citation was discovered, for example, through a manual search of a reference list, its complete bibliographic record was sought (e.g., PubMed®) and then entered into the first level of relevance screening.

Of the 2,049 records entered into the initial screening for relevance, 1,579 were excluded. Reflecting the specific eligibility criteria, the reasons for exclusion were: a. did not involve human participants (n=301); b. did not involve omega-3 FAs as an exposure/intervention (n=827); c. the purpose of the exposure/intervention was not for the assessment of child or maternal health outcomes (n=253); and, d. not a primary study (e.g., a review; n=198). All of the remaining 470 reports were then retrieved and subjected to a more detailed relevance assessment. The second relevance screening then excluded 279 reports for the following reasons: a. did not involve human participants (n=15); b. did not involve omega-3 FAs as an exposure/intervention (n=101); c. the purpose of the exposure/intervention did not concern maternal or childhood health outcomes (n=69); and, d. not a primary study (e.g., a review; n=76). There were an additional number of reports not retrieved at this level (n=18). The third relevance screening took into the account the level of evidence appropriate to answer each question. A list of excluded due to level of evidence (i.e., observational studies) studies for each topic is included in the Appendix F. Of the 191 reports that made it to this level of screening, 74 were excluded. Hence, in total, 117 reports, describing 89 unique studies, were deemed relevant for the systematic review, with 20 studies each described by more than one report and three reports describing more than one unique study.

The 20 unique studies reported by more than one report were: Agostoni et al.[176] (Agostoni et al.[177,178]), Al et al. 1995[179](Al et al.[180]), Auestad et al.,[104] (Scott et al.[104], Auestad et al.[181]), Birch et al.[182] (Birch et al.,[183] Hoffman et al.[184]), Carlson et al.[185] (Werkman et al.,[186] Carlson et al.[187-190]), Carlson et al.[191] (Carlson et al.[192]), Clandinin et al.[193] (secondary reports[194,195]), de Groot et al.,[196] (de Groot et al.[197]), Faldella et al.[198] (Faldella et al.[199]), Helland et al.,[141](Helland et al.[200]), Innis et al.[201] (Diersen-Schade et al.[202]), Jensen et al.[203] (Voigt et al.[204]), Makrides et al.[205] (secondary report[206]), O'Connor et al.[207] (secondary report[208]), Olsen et al.[209] (Olsen et al.,[210] Salvig et al.[211]), Uauy et al.[212] (Uauy et al.,[213] Hoffman et al.,[214,215] Birch et al.,[216] Uauy et al.,[217]), Vanderhoof et al.[218] (Vanderhoof et al.[219,220]), Vilbergsson et al.[221] (secondary report[222]), Willatts et al.[223] (secondary report[224]), Woltil et al.[225] (secondary report[226]).

Auestad et al.[227] that included two uniques studies as well as Birch et al.[228] Olsen et al. reported 6 unique trials.[31]

* Appendices and Evidence tables cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/o3mchtp.htm

Report and Study Design Characteristics of Included Studies

Of the 117 relevant reports describing 89 unique studies, there were 63 randomized controlled trials (RCTs) and 26 observational studies across all the key questions. As an overview, the number of included studies investigating each question are described below, distinguishing the reports by population type (maternal, preterm or term infants), by intake of omega-3 FA supplements, or by research design. Since a given study may address more than one question, some studies may be described for more than one question.

Only one study required translation from German to English.[229]

Fifteen uniques studies investigated the influence of omega-3 FAs during pregnancy on the duration of gestation.[141,196,209,230,231,231-235] All reports were RCTs since we had decided to exclude other research designs if enough well-conducted RCTs were identified. Eight RCTs evaluated the question regarding the influence of maternal intake of omega-3 FA during pregnancy on the incidence of gestational hypertension (GHT), pre-eclampsia or eclampsia,[209,230,234,236-238] whereas, 14 RCTs assessed the outcome of incidence of infants small for gestational age (SGA).[141,196,209,230-236,238]

Regarding the question of the association between the duration of gestation in women with or without a history of a previous preterm birth with the omega-3 or omega-6/omega-3 FA content of maternal biomarkers during pregnancy, four studies were identified—one RCT,[234] one case-control study,[239] one single prospective cohort study,[240] and one cross-sectional study.[241] Five observational studies addressed the question of the association between maternal biomarkers and the incidence of GHT, pre-eclampsia or eclampsia—one was a prospective cohort study[179] and four were of cross-sectional design.[229,242-244] Whereas, one RCT,[196] two case-control studies,[245,246] one single prospective cohort study[240] and two cross-sectional studies[241,247] were identified that addressed the possible association between the incidence of SGA infants and the omega-3 or omega-6/omega-3 FA content of maternal biomarkers during pregnancy.

No studies were identified across all the child outcomes (i.e., growth patterns, neurocognitive development and visual function) regarding the influence of the intake of omega-3 FA from sources other than human milk, or infant formula.

Only one RCT was identified to answer the question of maternal intake of omega-3 FA during pregnancy and its influence of the growth pattern in term and preterm infants.[141] One RCT,[248] one prospective cohort study,[249] and one cross-sectional study addressed the question of the influence of omega-3 FA content of human milk, with or without known maternal intake, on growth patterns in term infants. No studies were identified that addressed this question in the preterm population. Twenty RCTs investigated the influence of omega-3 FA content in formula, with or without human milk intake, on the growth patterns in preterm infants,[185,193,198,201,207,212,218,225,250-259] whereas, 18 RCTs were conducted in term infants.[104,182,203,205,223,227,260-270]

No studies were identified regarding the association between the omega-3 or omega-6/omega-3 FA content of maternal or fetal biomarkers during pregnancy and the growth patterns of term or preterm infants. However, a total of 12 studies addressed the question of child biomarkers, of which five RCTs included a preterm population of infants,[185,191,201,207,212] and five

RCTs[143,203,205,262,263] and one prospective single cohort study[271] included a term population of infants; the Woltil et al. study, which was deliberately described only in the preterm section of this question, selected a group of very low birth weight (VLBW) preterm and term infants.[225]

Only one RCT was identified to answer the question of maternal intake of omega-3 FA during pregnancy and its influence on the neurological development in term and preterm infants.[141] One RCT[138] and one prospective cohort study evaluated the influence of omega-3 FA content of human milk, with or without known maternal intake, on the neurological development in term infants. No studies were identified in the preterm population for this particular question. Six RCTs investigated the influence of omega-3 FA content in formula, with or without human milk intake, on the neurological development outcomes in preterm infants,[193,207,254,270,272,273] whereas, eight RCTs were conducted in term infants.[104,176,182,203,205,227,227,265]

One cross-sectional study conducted in the United States assessed the association between maternal omega-3 FA content during pregnancy and the neurological development of the infants.[274] No studies were identified to assess the association between the neurological develoment in term or preterm infants and the omega-3 or omega-6/omega-3 FA content of fetal biomarkers during pregnancy. However, four RCTs[176,182,203,205] and one prospective cohort study[271] investigated this association, but in child biomarkers.

One RCT[235] and one cross-sectional study[275] evaluated the question of maternal intake of omega-3 FA during pregnancy and its influence on the visual function in term and preterm infants. Two RCTs,[138,248] one prospective cohort[276] and one cross-sectional study[140] evaluated the influence of omega-3 FA content of human milk, with or without known maternal intake, on the visual function in term infants. No studies were identified in the preterm population for this particular question. Nine unique RCTs investigated the influence of omega-3 FA content in formula, with or without human milk intake, on visual function outcomes in preterm infants,[185,191,198,201,207,212,251,254,272] whereas, 13 RCTs were conducted in term infants.[104,182,203,205,227,262-264,266,269,270,277]

One cross-sectional study assessed the association between maternal omega-3 FA content during pregnancy and the visual function of the infants.[275] No studies were identified to assess the association between visual function in term or preterm infants and the omega-3 or omega-6/omega-3 FA content of fetal biomarkers during pregnancy. However, 21 studies investigated this association in child biomarkers. Five studies included a preterm population,[185,198,212,278,279] whereas, 16 studies included term infants. Of five studies in the preterm group, three were RCTs[185,198,212] and two were cross-sectional studies.[278,279] Of the 16 term infant studies, nine were RCTs[138,182,203,248,262-264,269,270] and seven were observational studies.[140,271,275,278,280-282]

One RCT[283] evaluated the question of maternal intake of omega-3 FA during pregnancy and its influence on the cognitive development outcomes in term and preterm infants. One RCT[138] and one single prospective cohort study[284] evaluated the influence of omega-3 FA content of human milk, with or without known maternal intake, on the cognitive development of term infants. No studies evaluated this outcome in preterm infants.

Six RCTs investigated the influence of omega-3 FA content in formula, with or without human milk intake, on the cognitive develoment in preterm infants,[185,193,207,258,272,273] while eight RCTs were conducted in term infants.[104,182,203,205,223,227,265] No studies were identified that evaluated the association between the omega-3 FA content of maternal or fetal biomarkers

during pregnancy and the cognitive development outcomes. However, six studies addressed the question of child biomarkers. Four studies were RCTs[138,182,203,205] and two were single prospective cohort studies.[271,285]

All of the RCT's were evaluated for safety data. In addition, two other RCTs, although not providing efficacy data, did provide safety data and hence were also evaluated.[286,287]

The remainder of this chapter is organized by group of outcomes (pregnancy, growth, neurological, visual and cognitive), with the evidence addressing each of the key questions related to the type of intake, where at least one study was identified. Safety data is presented last. A table describing the composition of the interventional infant formulas used across the trials was added to Appendix G*. We begin with pregnancy outcomes.

Pregnancy Outcomes

What is the Evidence That Intake of Omega-3 Fatty Acids Influences the Duration of Gestation in Women With or Without a History of a Previous Preterm Birth (Gestational Duration Less Than 37 Weeks)?

Fifteen RCTs met eligibility criteria for investigating a possible influence of maternal intake of omega-3 FA supplementation on the duration of gestation.[141,196,209,230,231,231-235] The studies were published between 1992 and 2004 (see Summary Tables 1 to 3).

Overview of relevant studies

Olsen et al. investigated the effect of n-3 LCPUFA supplementation given as fish oil in 533 women with singleton pregnancies in their 30th week of pregnancy (mean age=29 [18-44] years, smokers [31.2%], primiparae [59%]) on pregnancy duration.[209] The women were randomally assigned to one of three three diet regimens: daily intake of four 1 g capsules of fish oil (Pikasol) containing EPA (32 % by weight [wt%]) and DHA (23wt%) corresponding to 2.7 g omega-3 FA daily intake; four 1 g capsules of olive oil daily each containing oleic acid (72wt%) and LA (12wt%); or, no supplement.[209] (Summary Table 1)

Bulstra-Ramakers et al. investigated the effect of dietary supplementation with EPA on the incidence rate of premature deliveries and GHT in 68 pregnant women (68 completed the study) with or without a previous history of prematurity or GHT.[238] The intervention consisted of EPA capsules (each containing a mixture of 0.25 g EPA and DHA) in a daily dose of 3 g of EPA (four capsules three times per day). The placebo capsules, which were similar to the EPA capsules in appearance, smell, and taste, contained coconut oil. The interventions started between 12 and 14 weeks of GA.[238] (Summary Table 1)

* Appendices and Evidence tables cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/o3mchtp.htm

Onwude et al. conducted an intention-to-treat (ITT) RCT to evaluate the effect of omega-3 FA (EPA/DHA) on the occurrence of proteinuric and nonproteinuric gestational hypertension (GHT) and asymmetrical intrauterine growth retardation (IUGR) in 233 pregnant women (232 completed the study; age range=16–40 years; mean gestational age (GA) at study entry=24 [18–32] weeks) at high-risk for developing these disorders.[233] GA was a secondary outcome measure for this study. The participants study were categorized as being multigravida, a history of one or more small babies (n=68), history of proteinuric or nonproteinuric GHT (n=76), history of unexplained stillbirth (n=16), and primigravida with abnormal uterine arcuate artery Doppler blood flow at 24 weeks GA (n=72). Participants were randomized to receive either 2.7 g MaxEpa daily containing 180 mg EPA and 120 mg DHA per capsule or matching air-filled capsules. The women were instructed to take nine capsules each day until the 38th week of pregnancy.[233] (Summary Table 1)

Summary Table 1: Omega-3 fatty acid influences on the duration of gestation in women with or without a history of a previous preterm birth

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)			
Olsen, 1992, Denmark: NR parallel RCT[209]	2.7g n-3 FAs fish oil (n=266)	Olive oil (n=136)/ pb (n=131)	S↑ GA in fish oil grp++	Jadad total: 2 [Grade: C]; Schulz: Inadequate	III
Bulstra-Ramakers, 1994, Netherlands: 27 wks parallel RCT[238]	n-3 FA-enriched capsules: EPA 3 g/d DHA NR (n=32)	Control capsules: coconut oil (n=31)	NS in % premature deliveries	Jadad total: 5 [Grade: A]; Schulz: Adequate	III
Onwude, 1995, UK: NR parallel RCT[233]	DHA+EPA (1620mg EPA+1080mg DHA) (n=113)	pb (n=119)	NS in GA NS in % premature deliveries	Jadad total: 5 [Grade: A]; Schulz: Adequate	II

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significantly different; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; FAs = fatty acids; GA = gestational age; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); +p<.05 or significant with 95% confidence interval; ++p<.01; +++p<.001; ++++p<.0001; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower

Olsen et al.,[230] in six multicenter RCTs including 19 hospitals, examined the preventative (prophylactic) and therapeutic effects of dietary n-3 FAs on pre-term delivery, IUGR and GHT in women with an increased risk for these clinical outcomes. Four prophylactic trials enrolled women after 16 weeks of GA with an uncomplicated pregnancy who had experienced previous pre-term delivery (n=232), IUGR (n=280), or GHT (n=386) and women who were currently pregnant with twins (n=579).

The two therapeutic trials enrolled women with threatening preeclampsia (n=79) or suspected IUGR (n=63). Participants were randomly assigned to receive fish oil (Pikasol: EPA [32wt%] and DHA [23wt%]) or olive oil in identical-looking capsules from approximately 20 weeks (prophylactic trials) or 33 weeks (therapeutic trials) until delivery. Treatment with fish oil corresponded to 1.3 g EPA and 0.9 g DHA daily intake for the prophylactic group and 2.9 g/d EPA and 2.1 g/d DHA for the therapeutic group. (Summary Table 2 to 3)

Summary Table 2: Omega-3 fatty acid influences on the duration of gestation in women with or without a history of a previous preterm birth

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)			
Olsen, 2000a, multicenter: 20 wks parallel RCT[230]	Earl-PD: Pikasol (fish oil) 0.9g DHA, 1.3g EPA capsules (n=110)	Olive oil capsules (n=122)	(ITT) S↑ GA in fish oil gp^{+} S↓ % Premature delivery in fish oil gp^{+}	Jadad total: 2 [Grade: C]; Schulz: Adequate	III
Olsen, 2000b, multicenter: 20 wks parallel RCT[230]	Earl-IUGR: Pikasol (fish oil) 0.9g DHA, 1.3g EPA capsules (n=141)	Olive oil capsules (n=139)	(ITT) S↑ GA in fish oil gp^{+}	Jadad total: 2 [Grade: C]; Schulz: Adequate	III
Olsen, 2000c, multicenter: 20 wks parallel RCT[230]	Earl-PIH: Pikasol (fish oil) 0.9g DHA, 1.3g EPA capsules (n=184)	Olive oil capsules (n=202)	(ITT) NS in GA	Jadad total: 2 [Grade: C]; Schulz: Adequate	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significantly different; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; FAs = fatty acids; GA = gestational age; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower; Earl-PD = pregnant women with antecedent of premature delivery; Earl-IUGR = pregnant women with antecedent of IUGR; Earl-PIH = pregnant women with antecedent of gestational hypertention in past pregnancies

Summary Table 3: Omega-3 fatty acid influences on the duration of gestation in women with or without a history of a previous preterm birth

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Internal validity	Applicability
Olsen, 2000d, multicenter: 20 wks parallel RCT[230]	Twins trial: Pikasol (fish oil) 0.9g DHA, 1.3g EPA capsules (n=289)	Olive oil capsules (n=290)	(ITT) NS in GA	Jadad total: 2 [Grade: C]; Schulz: Adequate	III
Olsen, 2000e, multicenter: 33 wks parallel RCT[230]	Threat-PE: Pikasol (fish oil) 2.1g DHA, 2.9g EPA capsules (n=44)	Olive oil capsules (n=35)	(ITT) NS in GA	Jadad total: 2 [Grade: C]; Schulz: Adequate	III
Olsen, 2000f, multicenter: 33 wks parallel RCT[230]	Susp-IUGR: Pikasol (fish oil) 2.1g DHA, 2.9g EPA capsules (n=36)	Olive oil capsules (n=27)	(ITT) S↑ GA in fish oil gp^{+}	Jadad total: 2 [Grade: C]; Schulz: Adequate	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significantly different; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; FAs = fatty acids; GA = gestational age; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower; Threat-PE = pregnant women with threatening preeclampsia; Susp-IUGR = pregnant women with suspected IUGR

Helland et al. randomly assigned 590 (341 completers) healthy, nulli- or primiparous women in weeks 17 to 19 of pregnancy to receive either 10 mL/day of cod liver oil (containing 1,183 mg DHA, 112 mg EPA and 27.5 mg AA) or 10 mL/day of corn oil (containing only 8.3 mg DHA) until delivery.[141] The study evaluated GA as a primary outcome.[141] (Summary Table 4)

Smuts et al. randomized 347 women in their third trimester of pregnancy (350 pregnancies; three women got pregnant twice during the study), who were supplied with DHA-enriched eggs (mean of 133±15 mg of DHA per egg) or ordinary eggs (mean of 33±11 mg of DHA per egg), and assessed GA and birth weight as primary outcomes (291 completed the study).[234] The study also assessed the risk of preeclampsia/eclampsia. The mean number of consumed eggs was 6.8±4.6 per week for the group consuming high-DHA eggs and 7.7±5.6 for the group consuming ordinary eggs.[234] (Summary Table 4)

The second Smuts et al. study monitored the safety of consuming high-DHA hen eggs compared with ordinary eggs with respect to pregnancy outcomes as well as infant anthropometric parameters.[232] Fifty-two, mostly African-American women, in their third trimester of pregnancy were randomized to the two diet groups: 25 to the regular-egg group (mean daily DHA intake was 35.1±13.2 mg) and 27 to the high-DHA egg group (mean daily DHA intake was 183.9±71.4 mg). Another 21 pregnant women were not randomized and were

not given supplementary eggs (low-egg intake group with a mean daily DHA intake 10.8±4.0 mg).[232] (Summary Table 4).

Summary Table 4: Omega-3 fatty acid influence on the duration of gestation in women with or without a history of a previous preterm birth

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
	Group 1 (n)/Group 4 (n)	Group 2 (n)/ Group 3 (n)				
Helland, 2001, Norway: 8 mo parallel RCT[141]	CGA 1183 mg/d DHA + 803 mg/d EPA + 27.5 mg/d AA (n=301)	COG pb 8.3mg/d DHA (n=289)	NS in GA	n/a	Jadad total: 4 [Grade: A]; Schulz: Unclear	III
Smuts, 2003, US: 13 wk parallel RCT[232]	High-DHA eggs (183.9 mg/d DHA) (n=18)	Regular-DHA eggs (35.1 mg/d DHA) (n=19)	NS in GA High-DHA eggs ↓ PTDR than control (no p-value)	n/a	Jadad total: 2 [Grade: C]; Schulz: Unclear	II
Smuts, 2003, US: 13 wk parallel RCT[234]	High-DHA eggs (133 mg/d DHA) (n=176)	Regular-DHA eggs (33 11mg/d DHA) (n=174)	S↑ in GA in High-DHA vs Regular-DHA[+] NS in PTDR	S (+) correlation between infant RBC DHA & GA[+] NS correlation between maternal RBC DHA & GA	Jadad total: 3 [Grade: B]; Schulz: Inadequate	II

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3 fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significantly different; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; FAs = fatty acids; CGA = cod liver oil group; COG = corn oil group; GA = gestational age; PTDR = preterm delivery rate; RBC = red blood cells; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); [+]p<.05 or significant with 95% confidence interval; [++]p<.01; [+++]p<.001; [++++]p<.0001; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower

Malcolm et al.[235] investigated the duration of gestation in healthy pregnant women (ages 17-36 years) that received fish oil capsule supplements from a mean of 15.4 wk gestation until delivery (Marinol D40, 100 mg DHA per capsule) compared with sunflower oil capsules.[235] (Summary Table 5)

Dunstan et al. examined the effect of fish oil supplementation on maternal and neonatal FA status.[231] The study also investigated if the fish oil supplementation to the diet of pregnant women had any effect on the duration of pregnancy and the size of their infants at birth (birth weight, length, and head circumference [HC]). The study recruited 98 healthy non-smoking pregnant women (83 completed the study); 58% of the women had a known history of allergic rhinitis and 40% had a history of asthma. Participants were randomly assigned to receive their

usual diet supplemented with either 4 g/day fish oil (1.1 g EPA and 2.2 g DHA per day) or olive oil capsules, from GA of 20 weeks until delivery.[231] (Summary Table 5)

de Groot et al. conducted a double-blind RCT in 79 pregnant women (58 completed the study) who were randomly assigned to receive at least 25 g/day of either an ALA-enriched, high-LA margarine (experimental group) or a high-LA margarine without ALA (control group), from week 14 of pregnancy until delivery. Subjects in the experimental group consumed 9.02 g LA and 2.82 g ALA daily, whereas, women in the control group received 10.94 g LA and 0.03 g ALA daily. One of the outcomes evaluated was the GA of the infant.[196] (Summary Table 5)

Summary Table 5: Omega-3 fatty acid influence on the duration of gestation in women with or without a history of a previous preterm birth

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Notable clinical-biomarker correlations	Internal validity	Applicability
Malcolm , 2003, Denmark: 15 wks parallel RCT[235]	Fish oil (DHA 100 mg) capsules (n=50)	pb (n=50)	NS in GA	NS correlation umbilical cord DHA & GA	Jadad total: 3 [Grade: B]; Schulz: Unclear	II
Dunstan, 2004, Australia: 19 wk parallel RCT[231]	LCPUFA (2.2 g/d DHA + 1.1 g/d EPA) (n=40)	pb (n=43)	NS in GA	NS correlation between infant RBC DHA, EPA, AA & GA	Jadad total: 3 [Grade: B]; Schulz: Unclear	III
de Groot, 2004, Netherlands: 24 wk parallel RCT[196]	LCPUFA (9.02 g/d LA+2.82 g/d ALA) (n=40)	pb (10.94 g/d LA+0.03 g/d ALA) (n=39)	NS in GA	n/a	Jadad total: 3 [Grade: B]; Schulz: Unclear	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significantly different; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; FAs = fatty acids; GA = gestational age; RBC = red blood cells; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower; LA = linoleic acid; ALA = alpha-linolenic acid

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. Only the study of Olsen et al. had more than two study groups.209 Countries where the studies were conducted included the United States,232,234 the United Kingdom,233,235 The Netherlands,196,238 Australia,231 Denmark209 and Norway.141 One multicenter study involved six trials conducted in 19 centers in Denmark, Scotland, Sweden, England, The Netherlands, Norway, Belgium and Russia.230

Both of the studies by Smuts et al.[232,234] were financially supported by Market Biosciences Boulder Corporation (former Omega Teach Inc.), Boulder, Colorado. The study by Onwude et al.[233] was sponsored by Yorkshire Region Locally Organized Research, Glaxo (Leeds) and Seven Seas (Hull). Olsen et al.'s studies[230] were funded by Conserted Action and PECO programmes of European Comission and the Danish National Research Foundation. The study of de Groot et al.[196] was supported by Unilever Research and Development (Vlaardingen, Netherlands). Dunstan et al.'s[231] was funded by the NH and MRC and Raine Medical Research Foundation, Australia. The other study by Olsen et al.[209] was supported by the Danish Medical Research Council, Sygekassernes Helsefond, Weman's Legat and Michaelsen Fonden. The study by Helland et al.[141] was financed by Peter Moller, Avd. Orkla ASA and "Aktieselskabet Freia Chocoladefabriks Medicinske Fond." Malcolm et al. was supported by the Chief Scientist's Office, Scottish Office Health Department.[235] Finally, Bulstra-Ramakers et al. failed to provide this information.[238]

Population characteristics. There was a total number of 3,686 pregnant women enrolled across the fifteen trials. The sample size varied from as low as 37[232] to 590[141] women. However, Helland et al. analysed only the patients who completed the study (n=341 of 590, 57%).[288] The mean age-range of study participants across the eight studies was 19.9 (SD=4.1) years to 32.9 (SD=14.6) years. Participants in both of the Smuts et al. trials[232,234] tended to be younger (mean age range for high-DHA egg group=19.9 [SD=4.1] to 21.7 years; mean age range for placebo group=21.6 [SD=4.2] years to 24.8 [SD=7.8] years) than the participants in the rest of the studies (mean age range for treatment groups=27.6 [SD=3.2] years, and 32.9 [SD=14.6] years for the placebo groups).[141,196,209,230,231,233] Two trials did not provide this information.[235,238]

A thorough description of both inclusion and exclusion criteria were given in all trials. Information about racial/ethnic backgrounds were given in three of the 15 studies.[196,232,234] Study participants in two trials were predominantly of African-American descent, comprising 79% and 73% of participants in the ordinary egg groups, and 83% and 73% of participants in the high-DHA egg groups, respectively.[232,234] Only White participants were recruited in the Groot et al. study.[196]

The exact duration of maternal dietary intervention during pregnancy and/or breastfeeding was reported in all but two studies,[209,233] and ranged from 5 weeks[230] to 8 months.[141] In most of the studies, LCPUFA supplementation was prescribed in the second trimester of pregnancy.[141,196,231,233,235,238] In three studies, PUFA supplementation was administered from the third trimester until delivery.[209,232,234] There was no study where participants were randomized from the first trimester. In one of the studies of Olsen et al., four prophylactic groups of pregnant women were randomized from gestational week 20, whereas, in the therapeutic trials women were randomized around gestational week 33.[230] Detailed information about the duration of the

LCPUFA supplementation is provided in the Evidence Tables (Appendix E*). Maternal social status, defined as years of education, was determined in two studies.[141,196]

Information regarding maternal smoking history and/or smoking during pregnancy was provided in eleven studies.[141,196,209,230,233,234] Alcohol consumption at 14 weeks of pregnancy was reported in one study.[196]

In the majority of RCTs, there was no evidence that randomization failed to produce comparable groups in terms of previous obstetric history, socioeconomic status, dietary intake of fish, smoking habits, alcohol intake, body mass index and GA.[141,209,231,234,235,289] Onwude et al. showed that significantly more women were current smokers at enrollment in the treatment group than in the placebo group.[233] Smuts et al. reported that women assigned to consume ordinary eggs were significantly older than those in the high-DHA egg group.[232] Olsen et al. reported that in women with suspected IUGR, those in the placebo group had significantly higher GA after randomization.[230]

Intervention/exposure characteristics. Across the 15 studies, the sources of omega-3 LCPUFA were identified as being either from natural feeding sources, such as eggs, fish and margarines, or from manufactured medical supplementations, such as capsules containing fish oil. Eggs as a source of omega-3 FA were used in two studies[232,234] and margarine, containing different amounts of LA and ALA, was used in one study.[196]

Gelatin capsules containing a fish oil were utilized in 11 studies. In most of the studies, LCPUFA supplementation was prescribed in the second trimester of pregnancy.[141,196,231,233,235,238] In three studies, PUFA supplementation was administered from the third trimester until delivery.[209,232,234] There were no studies where participants were randomized from the first trimester. In one of the studies of Olsen et al., four prophylactic groups of pregnant women were randomized from gestational week 20, whereas, in the therapeutic trials women were randomized around gestational week 33.[230]

Detailed information about the duration of the LCPUFA supplementation[209,230,231,233,235,238] is provided in the Evidence Tables (Appendix E). Helland et al. failed to report the manner in which study participants received their oil supplementation;[141] however, the investigators were the only ones to identify the exact sources of dietary FAs (i.e., cod liver oil and corn oil as the placebo). The daily amount of omega-3 LCPUFA intake, as well as the start and duration of intake, varied across the studies.

Pregnant women in the Bulstra-Ramakers et al. study received four capsules containing 0.25 mg EPA or placebo (coconut oil) three times daily. The EPA capsules contained a mixture of 3 g EPA and DHA. Both capsules were similar in appearance, smell and taste.[238]

The two Smuts et al. studies[232,234] used similar regimens of FA supplementation for the high-DHA eggs and the ordinary egg groups. Daily DHA intake was reported to be 183.9 (SD=71.4) mg in the high-DHA diet and 35.1 (SD=13.2) mg for placebo in the one study[232] and 133 (SD=15) mg and 33 (SD=11) mg, respectively, in the other Smut et al. study.[234] Women in both

* Appendices and Evidence tables cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/o3mchtp.htm

studies were randomized to the different dietary groups in their third trimester of pregnancy (24 to 28 weeks), for a mean duration of supplementation of approximately 13 weeks.[232,234]

de Groot et al. randomized a sample of women to receive margarine containing different amounts of LA and ALA from week 14 of GA until delivery.[196] The experimental group received 9.02 g LA and 2.82 g ALA per day, whereas, the control group received 10.94 g LA and 0.03 g ALA daily.[196]

Pregnant women in the Onwude et al. study were randomized to receive either fish oil or placebo.[233] Women were allocated to treatment groups at a very wide range of GA, ranging from 18 to 32 weeks (mean of 24 weeks). Hence, the time of exposure to the intervention was not equal for the study participants. Women in this study were instructed to take nine capsules daily, each containing either 180 mg EPA and 120 mg DHA (treatment group), or air (placebo group); timing of the intake of the nine capsules was left to the participants.[233]

The patients in the Olsen et al. study received fish oil (Pikasol containing EPA [32wt%] and DHA [23wt%]) or olive oil as placebo (oleic acid [72wt%] and ALA [12wt%]), provided in 1 g identical-looking gelatine capsules, but which were not identical in taste.[230] In the four prophylactic trials, four capsules of either oil were given per day, while in the two therapeutic trials, nine capsules were given per day. In the prophylactic trials women were randomized around gestational week 20, whereas, in the therapeutic trials women were randomized around gestational week 33. The same sources of intervention with the same regimen were used in the other study of Olsen et al.[209]

The pregnant women in the study of Malcolm et al. received two fish oil capsules, rich in DHA (Marinol D40, 100 mg DHA per capsule, R.P. Scherer Ltd, Swindon, UK) per day or identical sunflower oil placebo capsules without DHA or ALA.[235] Maternal diet, including fish intake, was assessed by interview at 15 and 28 weeks of pregnancy and delivery.[235]

The 98 women with a history of rhinitis or asthma in the Dunstan et al. study were randomized to receive either 4 g/day of fish oil or olive oil in capsules, as a supplement to their usual diet from 20 weeks gestation until delivery, when supplementation was ceased.[231] Women in the fish oil group consumed about 1.1 g EPA and 2.2 g DHA daily. All capsules contained α-tocopherol (3-4 mg/g oil) as an antioxidant.[231]

Helland et al. randomly assigned 590 study participants to either a treatment group (10 mL cod liver oil/day; Peter Moller, Avd Orkla, Oslo, Norway) or a placebo group (10 mL corn oil/day).[141] Women in the cod liver oil group consumed 1,183 mg DHA, 803 mg EPA and 27.5 mg AA daily compared with 8.3 mg DHA in the placebo group. Randomization started at 17 to 19 weeks of gestation and supplementation continued until approximately 3 months after delivery, for a total of approximately 8 months of exposure.[141]

Dietary intake information was not well documented in all studies. There was no clear data to suggest that all eight studies were equally able to eliminate the possible confounding influence of having unequal amounts of calories (i.e., as energy) provided to their different study groups. Information about caloric balance of food intake among the study groups was reported in only one RCT.[141] The daily energy intake (expressed as MJ/day) of participants in the Helland et al. study was similar among the two diet groups and varied from 8.2 (SD=2.0) MJ/day at week 18 of pregnancy to 8.7 (SD=2.3) MJ/day at week 35 of pregnancy.[141]

None of the study investigators made an effort to deodorize the LCPUFA supplementation. In the study by Smuts et al., attempts were made to maintain blinding by conducting their own sensory test with clinic nurses who were blinded to the egg source. All of the nurses felt that the omega-3-fortified eggs looked and tasted like the non-enriched eggs.[232]

Attempts to optimize and assess the compliance of the study participants were made in twelve trials.[141,196,209,230,232,233,235] In all of these studies, women were asked to fill a food-frequency questionnaire indicating the exact amount of assigned dietary supplement consumed, followed by conversion of this information into dietary intake using either a computer program[196] or simple percentage calculations.[209,230,233] Smuts et al. utilized phone interviews with the women since few participants were compliant with the request to keep written records of their food intake.[232]

The manufacturer of the omega-3 intervention was reported in seven trials.[141,196,209,231,232,235] Purity data on the exposures used were not provided in any of the 15 studies. In five of seven studies that evaluated the FA content of biomarkers, appropriate methods to extract, prepare, store or analyze lipids were described.[196,231,232,234] Helland et al. gave little information about the details of blood FA composition analysis.[141] None of the trials reported details as to whether, or how, the presence of methylmercury was tested or eliminated from the omega-3 FA exposure when fish oil was the source.[31,41,290]

Cointervention characteristics. Three studies reported the use and/or LCPUFA content of additional vitamin and mineral supplements taken by the pregnant participants.[141,231,234] Smuts et al. reported that prenatal vitamin use in ordinary and high-DHA groups was 83.2% and 84.6%, respectively.[234] Helland et al. reported that the amount of fat-soluble vitamins was identical between the two oil groups i.e., 117 µg/mL vitamin A; 1 µg/mL vitamin D; and, 1.4 µg/mL of dl-ά-tocopherol.[141] Dunstan et al. used ά-tocopherol as an antioxidant to stabilize omega-3 FAs.[231] No studies reported the prestudy medication use by either pregnant or breastfeeding mothers. On-study antihypertensive therapy to treat GHT was used in one of the Olsen et al. studies.[230]

Outcome characteristics. Fourteen studies addressed the question of whether or not omega-3 FA supplementation affects the duration of gestation (gestational age as mean±SD). Preterm delivery rate was assessed in 11 trials.[41,230,232,234,291,292] However, three more studies reported the number of premature deliveries excluded from the analysis (reported as dropouts).[288,290,293] The use of ultrasound in the second trimester of pregnancy to determine GA was reported in four studies.[209,230,233,234] If the ultrasound measurement was not available, the length of gestation was estimated from the date of last normal menstrual period.[209,230] In seven studies, preterm delivery was defined as delivery at an estimated GA of less than 37 weeks.[230,234]

Study quality and applicability. The 15 RCTs received a mean Jadad total quality score of 2.8, approaching a good internal validity (Summary Matrix 1). Two trials received a score of 5,[233,238] the trial of Helland et al. received a score of 4,[141] four trials received a score of 3,[196,231,234,235] and eight reports received a score of 2.[209,230,232]

Randomization method was not clearly reported in four trials,[290,293-295] eight trials were not double-blinded,[31,41,296] while double-bliniding method was not reported across five trials.[288,290,293-295] Reasons for dropouts were not reported across eight trials.[31,41,294]

Summary Matrix 1: Study quality and applicability of evidence for the influence of LCPUFA on the duration of gestation

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author Onwude[A]	Year 1995	n 233	Author Smuts[I] Malcolm[U]	Year 2003 2003	n 350 100	Author Sumts[U]	Year 2003	n 73
	III	Author Bulstra-Ramakers[A] Helland[U]	Year 1994 2001	n 68 590	Author Dunstan[U] De Groot[U]	Year 2004 2004	n 98 79	Author Olsen[I] Olsen[A]	Year 1992 2000	n 533 see below[*]

n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment;[I] = Inadequate;
[*]Olsen 2000 6 trials: a) n=232; b)=280; c)n=579; d) n=386;e) n=79; f) n=63

Qualitative synthesis of individual study results

Ten studies evaluating the influence of LCPUFA supplementation on the duration of gestation, did not find any beneficial effect of omega-3 FAs over their comparators.[141,196,230-235] Conversely, the other four studies found that dietary modifications by LCPUFA significantly prolonged the duration of gestation.[209,230] However, the population characteristics, as well as the interventions, were different across these studies. The preterm delivery trial of Olsen et al. found a significantly increased mean duration of gestation in the treatment group (fish oil) of mothers with a preterm delivery in a previous pregnancy compared with mothers in the placebo group.[230] Preterm delivery rate was not affected by omega-3 FA supplementation during pregnancy and was not statistically different in randomized groups in ten trials that evaluated this outcome.[31,209,233,234,238] Smuts et al. on the other hand, observed that 5.6% of women in the high-DHA group had a premature delivery compared with 25% in the control group (no statistical significance was reported).[232]

Dunstan et al. did not find any statistically significant relationship between GA and neonatal RBC DHA, EPA, and AA content.[231] Contrary to these findings, Smuts et al.[234] observed a statistically significant positive correlation between infant RBC DHA content at delivery and GA in the treatment group, whereas, maternal RBC phospholipid DHA content at the time of delivery was not significantly correlated with GA in either the treatment or placebo groups.[231] Malcolm et al. measured umbilical cord plasma DHA levels in infants of supplemented mothers and observed that the duration of gestation was significantly greater in infants in the upper quartile for cord blood DHA compared with infants in the lower quartile. However, gestational length did not differ based on quartiles of umbilical cord RBC DHA.[235]

Quantitative synthesis

Meta-analysis was performed for incidence of premature deliveries, given that represents the most clinically relevant. Eleven of 15 trials reported this particular outcome. Eight of ten compared the use of DHA+EPA capsules intake with olive oil (control group).31,41,291 Olsen et al. 2000 reported the pooled data of six different RCTs, including pregnant women with

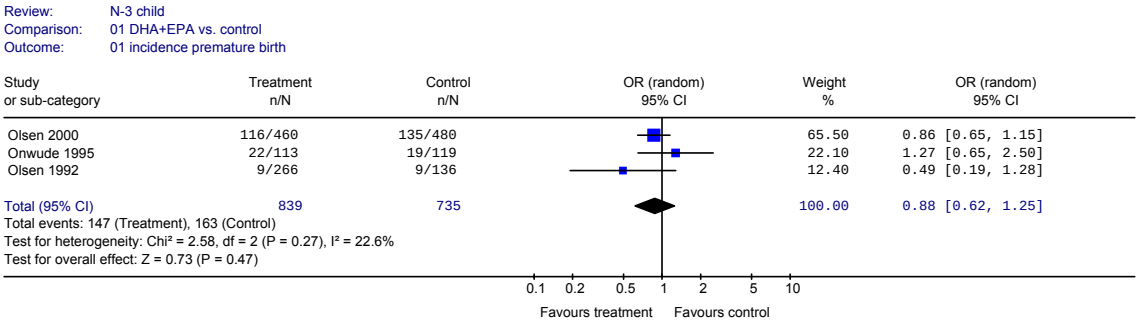
different risk for prematurity.31 Five of eight trials provided the intervention from the second trimester (week 22) until delivery,31,291 and three trials from week 30-33 until delivery (3rd Trimester).31,41 Subgroup analysis (by risk of prematurity) was not possible for this outcome given the lack of individual data from Olsen et al. 2000.31

Two studies by Smuts et al. comparing the use of eggs with high DHA content (mean 133 mg DHA per egg)[296] or 12 high-DHA hen eggs (135 mg DHA/egg)[294] with ordinary eggs (low DHA content: 18-33 mg DHA/egg)[294,296] from the second trimester to delivery reported the incidence of premature delivery as an outcome.

Two other studies compared the use of EPA alone[292] or DHA+AA (from cod oil)[288] with control, yet pooling was not possible due to the difference in omega-3 FA content.

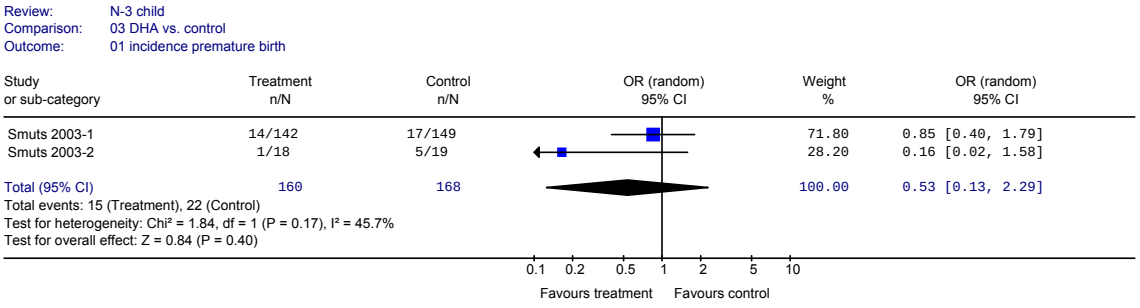
Meta-analyses for incidence of prematurity were performed by using a random effect model for odds ratio.

Figure 1. Meta-analysis of studies comparing intake of DHA+EPA vs. control



From eight RCTs, the incidence of premature deliveries did not differ significantly between groups, OR: 0.88 (95% CI: 0.62-1.25), p=0.47.

Figure 2. Meta-analysis of studies comparing intake of DHA vs. control.
Smuts et al 2003-1[296] and Smuts et al. 2003-2.[294]



From two RCTs,[294,296] the incidence of premature deliveries did not differ significantly between groups, OR: 0.53 (95% CI: 0.13-2.29), p=0.40.

Impact of covariates and confounders

Olsen et al. adjusted the duration of gestation for fish consumption, as well as for compliance to the oil supplementation.[209] Differences between groups in the average duration of gestation were significantly correlated with increasing fish consumption, with the mean length of gestation highest in the fish-oil group and lowest in the olive-oil group. The difference between fish oil and olive oil was nonstatistically significant between compliers and noncompliers.[209]

Helland et al. adjusted the duration of gestation for the concentration of DHA in umbilical plasma phospholipids and reported that neonates with high concentration of DHA in umbilical plasma phospholipids (upper quartile) had longer gestational length than neonates with low concentration.[141]

Onwude et al. stratified the results by use of tobacco, failing to observe a difference between groups.[233]

Smuts et al. adjusted the results by smoking status, maternal BMI and number of prior pregnancies.[234] The duration of gestation was significantly longer in the high-DHA egg group in the nonsmoking women, and when adjusted by maternal BMI and parity.[234]

The power analysis was reported in nine trials,[31,288,292,296] while the intention-to –treat analysis approach was reported in six trials from the same author.[31]

What is the Evidence That Maternal Intake of Omega-3 Fatty Acids Influences the Incidence of Preeclampsia, Eclampsia or Gestational Hypertension?

Eight unique studies met the eligibility criteria for investigating the effect of dietary supplementation of omega-3 FAs on the incidence of GHT, preeclampsia, or eclampsia, in pregnant women. All eight studies were parallel RCTs published between 1992 and 2003. Olsen et al.[230] reported two unique trials relevant to this question—the "Twins trial" (twins in the current pregnancy) and "Earl-PIH" trial (women who had GHT in an earlier pregnancy). Of the eight RCTs, seven were double-blind.[209,230,233,234,236,238] Of these, one trial was partially double-blind.[236] The overview of five trials was summarized in the question of duration of gestation (see key question: Duration of Gestation.). (Summary Tables 6-7)

Overview of relevant studies

D'Almeida et al. evaluated the effect of dietary supplementation with fish oil in preventing preeclampsia in pregnant primiparous and multiparous women with GA of less than 4 months.[236]

The study participants (n=150; age range: 14–40 years) were randomized to receive eight capsules per day of either a mixture of evening primrose oil and fish oil (containing gamma-linolenic acid [GLA] 37 mg, EPA [18 mg] and DHA [10 mg]) or magnesium oxide (2 tablets/2 x 500 mg/day) or placebo (olive oil), for 6 months. The main study outcome was the cumulative incidence rate of preeclampsia (complete triad of hypertension, edema, and proteinuria). Other

study outcomes were individual cumulative incidence rates of GHT, edema, and proteinuria.[236] (Summary Table 6)

The trial of Laivuori et al. investigated the influence of dietary supplementation with fish oil on the urinary excretion of antiaggregatory prostacyclin (PGI2) and proaggregatory thromboxane (TXA2) metabolites in women with preeclampsia. Of 18 women enrolled, 12 completed the study (mean age: 31 [range 23-40] years; parous: 50%; mean GA: 33 [range 26-37] weeks).[237] Changes in clinical signs of preeclampsia such as blood pressure (BP), proteinuria, and edema were also examined. Participants were randomized to receive 10 capsules per day of either Preglandin (containing 375 mg LA and 45 mg GLA), MaxEPA (containing 180 mg EPA, 120 mg DHA and 680 mg of other fish oils) or placebo (containing 500 mg maize oil and 500 mg olive oil).[237] (Summary Table 6)

Summary Table 6: Influence of maternal intake of omega-3 fatty acids on the incidence of preeclampsia, eclampsia or gestational hypertension

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)			
D'Almeida, 1992, Angola: 24 wk parallel RCT[236]	n-3 FA-enriched capsules: fish & primrose oil EPA 0.15 g/d DHA 0.08 g/d (n=50)	Mg^{2+} oxide capsules: 1 g/d (n=50)/ olive oil capsules: (n=50)	Rate of GHT ↑ in grps 1-3 vs. grp 2 (p = NR) Rate of preeclampsia/eclampsia ↑ in grp 3 vs. grps 1-2^{+++}	Jadad total: 2 [Grade: C]; Schulz: Inadequate	III
Laivuori, 1993, Finland: 8 wk parallel RCT[237]	n-3 FA-enriched capsules: fish oil EPA 1.80 g/d DHA 1.20 g/d (n=5)	Primrose oil capsules: LA 3.75 g/d GLA 0.45 g/d (n=7)/ maize-olive oil capsules: 10 g/d (n=6)	NS BP, proteinuria, & rate of edema (grp 1 vs. grps 2-3)	Jadad total: 2 [Grade: C]; Schulz: Adequate	III
Bulstra-Ramakers, 1995, Netherlands 27 wks parallel RCT[238]	n-3 FA-enriched capsules: EPA 3 g/d DHA NR (n=32)	Control capsules: coconut oil (n=31)	NS rate of GHT (grp 1 vs. grp 2)	Jadad total: 5 [Grade: A]; Schulz: Adequate	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; GLA = gamma-linolenic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significantly different; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; RBC = red blood cells; PL = phospholipid; CPG = choline phosphoglycerides; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; GHT = gestational hypertension; BP = blood pressure; GHT = gestational hypertension

Summary Table 7: Influence of maternal intake of omega-3 fatty acids on the incidence of preeclampsia, eclampsia or GHT

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Internal validity	Applicability
Onwude, 1995, UK: 14 wks parallel RCT[233]	n-3 FA-enriched capsules: fish oil EPA 1.62 g/d DHA 1.08 g/d (n=113)	Control capsules: air-filled (n=119)	NS rate of GHT (grp 1 vs. grp 2)	Jadad total: 5 [Grade: A]; Schulz: Adequate	II
Olsen, 1992, Denmark: NR parallel RCT[209]	n-3 FA-enriched capsules: fish oil EPA 1.30 g/d DHA 0.90 g/d (n=266)	Control capsules: olive oil 4 g/d LA 12% (n=136)/ placebo capsules: no oil (n=131)	NS in BP or rates of GHT & preeclampsia (grp 1 vs. grps 2-3) NS in BP (grp 1 vs. grps 2-3)	Jadad total: 2 [Grade: C]; Schulz: Inadequate	III
Olsen, 2000, multicenter* 20 wks parallel RCT[230]	Twins trial: n-3 FA-enriched capsules: fish oil EPA 1.30 g/d DHA 0.90 g/d (n=289)	Control capsules: olive oil 4 g/d LA 12% (n=290)	(ITT) NS in rates of GHT & preeclampsia (grp 1 vs. grp 2) NS BP (grp 1 vs. grp 2)	Jadad total: 2 [Grade: C]; Schulz: Adequate	III
Olsen, 2000, multicenter* 20 wks parallel RCT[230]	Earl-PIH: n-3 FA-enriched capsules: fish oil EPA 1.30 g/d DHA 0.90 g/d (n=184)	Control capsules: olive oil 4 g/d LA 12% (n=202)	(ITT) NS in rates of GHT & preeclampsia (grp 1 vs. grp 2) NS in BP (grp 1 vs. grp 2)	Jadad total: 2 [Grade: C]; Schulz: Adequate	III
Smuts, 2003, US: 16 wks parallel RCT[234]	n-3 FA-enriched eggs: DHA 0.23 g/d (n=142)	Control regular eggs: DHA 0.056 g/d (n=149)	NS in rates preeclampsia (grp 1 vs. grp 2)	Jadad total: 3 [Grade: B]; Schulz: Inadequate	II

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; GLA = gamma-linolenic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significantly different; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; GHT = gestational hypertension; BP = blood pressure; * Denmark, Scotland, Sweden, UK, Italy, Netherlands, Norway, Russia, Belgium

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. Of the eight RCTs, seven were double-blinded studies[209,230,233,234,236,238] of which, one was partially double-blind.[236] For one study,[237] it was not clear whether the study authors used a single or double-blind design. Authors of all eight trials reported inclusion criteria. Of the eight trials, two trials failed to report their exclusion criteria.[236,237] Three trials[209,236,237] had three arms and the remaining five trials[230,233,234,238] had two arms. All arms in the three-arm trials were randomized.

The studies were conducted in the following countries: the Republic of Angola,[236] Finland,[237] the Netherlands,[230,238] England,[230,233] Denmark,[209,230]Norway,[230] Russia,[230] and, the U.S.[234] All but two studies[237,238] reported their funding source. These included: Efamol, Ltd;[236] Yorkshire Region Locally Organized Research, GLAXO (Leeds) and Seven Seas (Hull);[233] Danish Medical Research, Sygekassernes Helsefond, Weiman's Legat and Michaelsen Fonden;[209] Concerted Action and PECO programmes of the European Commission and the Danish National Research Foundation;[230] and, Martek Biosciences Boulder Corporation (formerly OmegaTech, Inc).[234]

Population characteristics. The total number of enrolled pregnant women across the included studies was 2,335 and ranged from 18[237] to 579[230] participants.

In general, participants included in most of the trials were healthy, with uncomplicated pregnancies. Patients in the Laivuori et al. trial were diagnosed with preeclampsia (GHT and protein in urine >0.5 g/d).[237] The study sample in another trial consisted of healthy women with previous history of anemia (27%), sickle-cell disease (34%), malaria (67%), or GHT (21%).[236] Four trials[230,233,236,238] included pregnant women who had a history of GHT. In three trials,[230,233,238] a previous episode of GHT was defined by a diastolic BP ≥90 mm Hg[233,238] or >100 mm Hg.[230] The proportion of women with a previous history of GHT in the four trials ranged from 21%[236] to 100%[230] of participants. The between-arm proportions of women with a previous history of GHT were not similar in the study of Bulstra-Ramakers et al. (75% vs 48.4%).[238] In another trial, the distribution of women with a previous history of GHT between the two randomized arms was more balanced (31.8% vs. 33.6%).[233]

The age of the study participants was not reported in one study.[238] In the remaining studies, the age ranged from 14 [236] to 40 years.[233,236,237] The approximate mean age values across the trials[209,230,233,234,237] ranged from 26.5[233] to 31.0 years,[237] and were similarly distributed across the treatment groups.

The women's baseline mean diastolic BP across the trials[209,230,233,234] ranged from 64[234] to 74 mm Hg.[230] In these trials, the mean values of diastolic BP were similar across the randomized arms. The baseline mean (arm-specific) systolic BP was reported only in two studies,[209,234] and ranged from 111[234] to 124 mm Hg.[209] In both trials, the randomized arms had similar mean values of systolic BP.

All trials reported the GA of the study participants at enrollment, randomization and start of intervention. The women's GA at enrollment and randomization across the trials, ranged from 16[209,236] to 37 weeks.[237] The range of GA was reported in four trials.[233,234,237,238] The arm-specific mean GA (SD) was reported in five studies,[230,233,234,237] which was distributed evenly across the randomized arms. Three trials included only parous women (those with previous live births).[230,233,238]

The proportion of parous women across the remaining trials ranged from 48.5%[230] to 67.8%[209] of participants and were similar across the study arms. Five studies reported on maternal tobacco smoking.[209,230,233,234] The proportion of tobacco smokers ranged from 22%[230]to 32%[209,230,233] of participants. In three trials,[209,230,234] the arm-specific distributions of smokers were more or less comparable. However, in two other trials,[230,233] the proportions of smokers across the randomized arms were not as similar—in the Onwude et al. trial,[233] 42% of participants in the fish oil arm were smokers compared with 32% in the placebo arm; in the

Olsen et al. "Earl-PIH" trial, 19.1% of participants in the fish oil arm were smokers compared with 24.2% in the placebo arm.[230]

The trials excluded subjects who had diabetes,[230,233,234,238] systemic lupus erythematosus,[234,238] chronic hypertension,[233,234] placental abruption,[209,230,233] asthma,[233] severe fetal malformation,[230] drug and/or alcohol abuse,[230] regular intake of fish oil,[196,209,230,231] allergy to fish oil,[209] chronic illness (cardiovascular, cancer, renal, psychiatric, or neurological disorder) and a serious infectious disease (hepatitis).[234] Regular users of prostaglandin inhibitors were also excluded.[209]

Intervention/exposure characteristics. In all but one study,[234] the experimental intervention was dietary supplementation with omega-3 FA-enriched capsules. In the study by Smuts et al.,[234] women were assigned to receive omega-3-enriched eggs. The daily number of assigned capsules across the trials varied from 4[209,230] to 12.[238] Five trials[209,230,236,237] reported fish oil as a primary source of omega-3 FAs (i.e., ALA, LA EPA, DHA). The experimental intervention in most of the trials consisted of the combined supplementation with DHA and EPA.[209,230,233,236-238] The enriched eggs in the trial of Smuts et al. provided DHA only.[234] In three trials,[209,230] the relative contents of DHA and EPA in each experimental capsule were 23% and 32%, respectively. In two trials,[233,237] each experimental capsule contained 120 mg and 180 mg of DHA and EPA, respectively. In two other trials,[209,230] the absolute amounts of DHA and EPA were 225 mg and 325 mg per experimental capsule, respectively. In one trial, each capsule contained 250 mg of EPA.[238]

The daily dose of DHA and EPA differed across the studies. The range of daily DHA intake was 0.08 g[236] to 1.20 g[237] and 0.15 g[236] to 3.00 g[238] for EPA. Three trials had a control arm with standard intervention such as magnesium oxide tablets (37 mg GLA),[236] preglandin capsules (45 mg GLA)[237] or olive oil[209], besides the experimental and placebo arms. In seven trials, intervention in the control/placebo arms consisted of capsules with an identical appearance and taste as the experimental capsules. In these trials, placebo capsules contained olive oil,[209,230,236,237] maize oil,[237] coconut oil,[238] or no oil.[233] In the trial conducted by Smuts et al., omega-3-enriched eggs contained a mean of 33 [range 22-51] mg of DHA.[234]

Information about patient compliance (numbers of partially- or non-compliant participants and/or reasons for non-compliance) were reported in six trials.[209,230,233,234,238] The type of analysis performed (i.e., ITT) were reported in three trials.[230,233] All three studies used ITT analyses. Two trials[236,237] did not report any information on the rates and/or reasons of compliance.

The manufacturers of the omega-3 FA-enriched supplemental products in the eight studies were: Efamol Research Institute and Efamol, Ltd (England);[236] Orion OY (Finland);[237] Lube Ltd. (Denmark);[209,230] and, OmegaTech, Inc. (Bouldwer, CO)/Gold Circle Farms (U.S.).[234] Two trials did not report the names of manufacturers who provided the omega-3 FA-enriched capsules.[233,238] The trials had varying lengths of intervention (in weeks) i.e, 24,[230,236] 27,[238] 1 to 8,[237] 14 to 16[233,234] and 9.[209]

Cointervention characteristics. Olsen et al.'s "Earl-PIH" and "Twins" trials allowed 2 mg tocopherol/mL in the fish oil capsules only.[230] Only two studies assessed the background diet of participants during the study.[209,236] Olsen et al. used a simple food-frequency questionnaire, reporting the amount of fish consumed before the trial: the low-fish intake group (at most one

fish snack per month) to high fish intake (at least four fish meals per month). More than 50% of the women (n=327) were in the middle category of fish intake.[209] D'Almeida et al. measured background diet with a 24-hour dietary recall questionnaire.[236]

Outcome characteristics. The incidence (or recurrence) rate of GHT was the primary outcome investigated in six trials.[209,230,233,236,238] The definition of GHT varied slightly across the trials. Most trials defined GHT as diastolic BP above 90 mm Hg.[209,230,233,238] These definitions were based on the number of measurements taken and the time-interval between measurements. One trial[236] defined GHT as a rise in diastolic BP of >15 mm Hg, whereas, another study[238] defined it as a rise in diastolic BP of >25 mm Hg. D'Almeida et al., defined GHT as a rise in systolic BP >30 mm Hg and/or a rise in diastolic BP >15 mm Hg.[236] Since one of the trials of Olsen et al.[230] included only pregnant females with a previous history of GHT (BP >100 mm Hg), the outcome of interest was the recurrence (not incidence) rate of GHT (BP >90 mm Hg). Note that, in this trial, the definitions for the previous/prevalent and incident GHT, differed.

Five trials investigated the incidence of preeclampsia.[209,230,234,236] Of these, four trials reported the definition of incident preeclampsia.[209,230,236] D'Almeida et al. defined preeclampsia as the simultaneous occurrence of the clinical triad: GHT, proteinuria, and edema.[236] However, in the remaining three trials,[209,230] the definition was restricted to GHT accompanied only by proteinuria (proteins >0.3 g/L). Only D'Almeida et al.[236] investigated the incidence of eclampsia which was defined by the simultaneous presence of GHT and two convulsive episodes.

Systolic and/or diastolic BP (measured in mm Hg), as the outcome of interest was assessed in four studies.[209,230,237] The cumulative incidence rates of proteinuria and edema were explored in two trials.[236,237]

Study quality and applicability. The eight RCTs received a mean Jadad total quality score of 2.9, approaching a good internal validity (Summary Matrix 2). The trials conducted by Bulstra-Ramakers et al. and Onwude et al. received a score of 5,[233,238] Smuts et al. received a score of 3,[234] and the remaining five reports received a score of 2.[209,230,236,237] All reported an adequate randomization method. Six trials were not double-blinded,[31,41,296-298] and five trials failed to report the reasons for dropouts.[31,41,297,298]

Summary Matrix 2: Study quality and applicability of evidence for the effect of LCPUFA supplementation on the incidence of gestational hypertension, preeclampsia and eclampsia

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author	Year	n	Author	Year	n	Author	Year	n
		Onwude[A]	1995	233	Smuts[I]	2003	350			
	III	Author	Year	n	Author	Year	n	Author	Year	n
		Bulstra-Ramakers[A]	1994	68				D'Almeida[I]	1992	150
								Laivuori[A]	1993	18
								Olsen[A]	2000	579*
								Olsen[A]	2000	386**
								Olsen[I]	1992	533

n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment; [I]Inadequate
*"Earl-PIH" trial; **"Twins" trial

Qualitative synthesis of individual study results

Six trials investigating the effect of omega-3 FA-dietary supplementation on the incidence rate of GHT[209,230,233,237,238] showed a nonstaistically significant difference between-groups in the incidence of GHT. In contrast, D'Almeida et al. observed that women randomized to receive the diet enriched with magnesium oxide had lower incidence rates of GHT compared with those participants in the omega-3 FA-supplemented and placebo groups (4% vs 18% and 26%, respectively; p-value NR).[236]

Three trials demonstrated an effect of omega-3 FA-dietary supplementation in reducing risk of preeclampsia.[209,230,234] The mean number of women who had developed preeclampsia in all study arms was 15 (range from five to 28 women). Although the proportion of women developing preeclampsia tended to be lower in the experimental/omega-3 FA-supplemented arms,[209,230,234] the statistical power of these trials was too low to detect these differences. Only one trial[236] was able to show that women in the fish oil arm had a lower rate of preeclampsia than those in the placebo and magnesium oxide groups. In the D'Almeida et al. study, none of the women in the fish oil and magnesium oxide groups developed severe eclampsia compared with 3/50 (2.1%) patients in the placebo group.[236]

The findings of Laivuori et al. suggested that dietary supplementation with fish oil did not have any effects on BP, proteinuria, and edema in women with preeclampsia in 12 of 18 women enrolled.[237] Findings from trials that measured BP during the follow up,[209,230] suggested that dietary supplementation with omega-3 FAs did not affect the BP of the women i.e., randomized groups had similar BP (systolic and diastolic) readings at follow up.

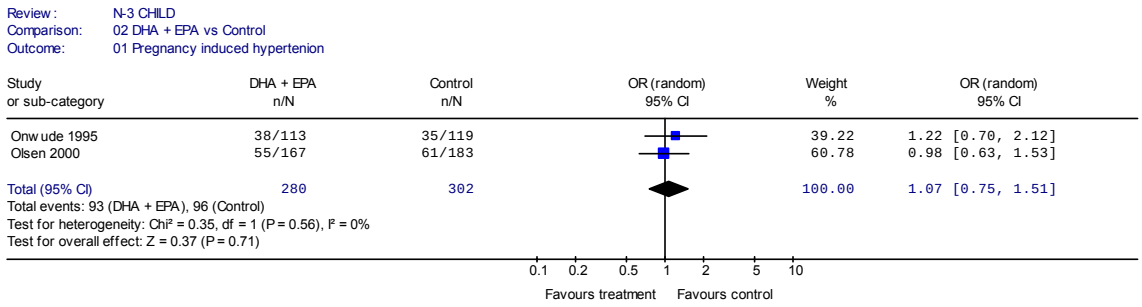
The cumulative incidence rates of proteinuria and edema were measured in two studies.[236,237] D'Almeida et al. found similar incidence rates of proteinuria in the randomized groups. However, women in the placebo group had a significantly higher rate of edema than those in fish oil/primrose oil and magnesium oxide groups (58% vs 26% and 24%, respectively).[236] Three studies reported data on dropouts and withdrawals with different detail.[233,234,238] The number of non-completers across the trials ranged from 1[233] to 57.[234]

Quantitative synthesis

In total, seven studies were identified by our search that reported on incidence of pre-eclampsia or GHT. After examining the studies for source of oil and duration of supplementation, five studies[209,230,233,236,237] were initially considered for meta-analysis.

Upon further examination, three studies[209,236,237] were excluded. Lavuiori et al. did not report quantitative outcome data.[237] D'Almeida et al. included a population with unique comorbities in a developing-world population.[236] Olsen et al. was carried out in a healthy population (i.e., women not at high risk of pre-eclampsia/GHT).[209] Thus, two studies[230,233] reporting on the incidence of GHT were available for meta-analysis.

Figure 3. Gestational hypertension incidence. Meta-analysis was performed using a random-effects model for odds ratios (n/N = number of patients with GHT/total sample in each arm).



In two studies,[230,233], the overall size of the effect was nonstatistically significant between the DHA+EPA and the control groups in the incidence of GHT (OR: 1.07, CI 95%: 0.75; 1.51).

Impact of covariates and confounders

None of the included studies reported the use of multivariable techniques such as logistic or Cox regression modeling in order to adjust for the effects of dietary supplementation on the dichotomous outcomes (GHT, preeclampsia/eclampsia). Most of the studies reported having used a Chi-square or Fisher's test. In one study,[238] the randomized groups were not balanced with respect to the important prognostic/predictive factor such as a history of previous GHT (i.e., 75% vs 48.4%). The trial conducted by D'Almeida et al.[236] did not report the arm-specific proportions of women with a previous history of GHT. It is not clear whether the study authors adjusted the effect of interest for any between-group differences with respect to the proportion of women with GHT.

The power calculation was reported in four trials,[31,292,296] while the intention-to treat analysis approach was reported in two trials.[31]

What is the Evidence that Maternal Intake of Omega-3 Fatty Acids Influences the Incidence of Births of Human Infants Small for Gestational Age?

Fourteen unique studies were identified to answer this question. The studies were parallel RCTs, published between 1994 and 2004. Olsen et al.[230] reported four unique trials: "Earl-PD" (women with history of premature delivery); "Earl-IUGR" (women who had IUGR in an earlier pregnancy); "Twins trial" (twins in the current pregnancy); and, "Susp-IUGR" (women suspected of having IUGR <10th percentile [PC] by ultrasonography in the current pregnancy). All the trials were already summarized above, therefore we only included the summary tables (see above key questions: Duration of Gestation. and Preeclampsia, Eclampsia or Gestational Hypertension) (Summary Table 8-10)

Overview of Relevant Studies

Summary Table 8: Maternal intake of omega-3 fatty acids and the incidence of births of human infants small for gestational age

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Internal validity	Applicability
D'Almeida, 1992, Angola: 24 wk parallel RCT[236]	n-3 FA-enriched capsules: fish & primrose oil EPA 0.15 g/d DHA 0.08 g/d (n=50)	Mg^{2+} oxide capsules: 1 g/d (n=50)/ olive oil capsules: (n=50)	% <2,000 g at birth: pb 3.3% vs. n-3: 1.3% vs. Mg^{2+}: 4.7% (no p-value)	Jadad total: 2 [Grade: C]; Schulz: Inadequate	III
Olsen, 1992, Denmark: NR parallel RCT[209]	2.7g n-3 FAs fish oil (n=266)	NR olive oil (n=136)/ pb (n=131)	NS birth wt	Jadad total: 2 [Grade: C]; Schulz: Inadequate	III
Bulstra-Ramakers, 1994, Netherlands 27 wks parallel RCT[238]	n-3 FA-enriched capsules: EPA 3 g/d (n=32)	Control capsules: coconut oil (n=31)	NS in IUGR recurrence rate (grp 1 vs. grp 2)	Jadad total: 5 [Grade: A]; Schulz: Adequate	III
Onwude, 1995, UK: 14 wks parallel RCT[233]	n-3 FA-enriched capsules: EPA 1.62 g/d DHA 1.08 g/d (n=113)	Control capsules: air-filled (n=119)	NS in birth wt & IUGR recurrence rate (grp 1 vs. grp 2)	Jadad total: 5 [Grade: A]; Schulz: Adequate	II
Olsen, 2000a, multicenter: 20 wks parallel RCT[230]	Earl-PD: Pikasol (fish oil) 0.9g DHA, 1.3g EPA capsules (n=110)	Olive oil capsules (n=122)	(ITT) S↑ birth wt in fish oil NS % IUGR	Jadad total: 2 [Grade: C]; Schulz: Adequate	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; $^{+}p<.05$ or significant with 95% confidence interval; $^{++}p<.01$; $^{+++}p<.001$; $^{++++}p<.0001$; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; GA = gestational age; IUGR = intrauterine growth retardation; FA = fatty acids; * Scotland, Sweden, UK, Italy, Netherlands, Norway, Russia, Belgium

Summary Table 9: Maternal intake of omega-3 fatty acids and the incidence of births of human infants small for gestational age

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Study groups[1] Group 2 (n)/ Group 3 (n)	Notable clinical effects	Internal validity	Applicability
Olsen, 2000b, multicenter* 20 wks parallel RCT[230]	Earl-IUGR trial: n-3 FA-enriched capsules: fish oil EPA 1.30 g/d DHA 0.90 g/d (n=141)	Control capsules: olive oil (n=139)	(ITT) S↑ birth wt in olive oil NS % IUGR	Jadad total: 2 [Grade: C]; Schulz: Adequate	III
Olsen, 2000c, multicenter*: 20 wks parallel RCT[230]	Twins trial: n-3 FA-enriched capsules: fish oil EPA 1.30 g/d DHA 0.90 g/d (n=289)	Control capsules: olive oil (n=290)	(ITT) NS in birth wt & % IUGR	Jadad total: 2 [Grade: C]; Schulz: Adequate	III
Olsen, 2000d, multicenter*: 33 wks parallel RCT[230]	Susp-IUGR trial: n-3 FA-enriched capsules: fish oil EPA 2.9 g/d DHA 2.1 g/d (n=36)	Control capsules: olive oil (n=27)	(ITT) NS in birth wt & % IUGR	Jadad total: 2 [Grade: C]; Schulz: Adequate	III
Helland, 2001, Norway: 23 wks parallel RCT[141]	Cod liver oil: 10 mL/d EPA 0.80 g/d DHA 1.18 g/d (n=175)	Corn oil: 10 mL/d (n=166)	NS in birth wt, birth length, & HC (grp 1 vs. grp 2)	Jadad total: 4 [Grade: A]; Schulz: Unclear	III
Malcolm , 2003, Denmark: 15 wks Parallel RCT[235]	Fish oil (DHA 100 g) capsules (n=50)	pb n=50)	NS in birth wt, length & HC	Jadad total: 3 [Grade: B]; Schulz: Unclear	II

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; $^{+}p<.05$ or significant with 95% confidence interval; $^{++}p<.01$; $^{+++}p<.001$; $^{++++}p<.0001$; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; GA = gestational age; IUGR = intrauterine growth retardation; FA = fatty acids; HC = head circumference; * Scotland, Sweden, UK, Italy, Netherlands, Norway, Russia, Belgium

Summary Table 10: Maternal intake of omega-3 fatty acids and the incidence of births of human infants small for gestational age

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Notable clinical-biomarker Correlations[2,3]	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)				
Smuts, 2003, US: 16 wks parallel RCT[232]	n-3 FA-enriched eggs: DHA 0.23 g/d (n=18)	Control regular eggs (n=19)/ non-randomized low eggs grp (n=16)	Wt, length, & HC at birth ↑ in grp 1 vs. grp 2 (p-value: NR) rate of PD & LBW ↓ in grp 1 vs. grp 2 (p-value: NR)	n/a	Jadad total: 2 [Grade: C]; Schulz: Unclear	II
Smuts, 2003, US: 16 wks parallel RCT[234]	n-3 FA-enriched eggs: DHA 0.23 g/d (n=142)	Control regular eggs (n=149)	NS in birth wt, birth length, HC, NS rate of LBW	n/a	Jadad total: 3 [Grade: B]; Schulz: Inadequate	II
de Groot, 2004, Netherlands: 26 wks parallel RCT[196]	n-3 FA-enriched margarine: 25 g/d ALA 2.82 g/d LA 9.02 g/d (n=29)	Control margarine: 25 g/d ALA 0.03 g/d LA 10.94 g/d (n=29)	Birth wt S ↑ in ALA+LA vs. LA[+]	S (+) correlation maternal plasma & RBC DHA & birth wt S +correlation DHA intake & bith wt	Jadad total: 3 [Grade: B]; Schulz: Unclear	III
Dunstan, 2004, Australia, UK: 20 wks parallel RCT[231]	n-3 FA-enriched capsules: fish oil EPA 1.10 g/d DHA 2.20 g/d (n=40)	Control capsules: olive oil (n=43)	NS in length, wt, & HC at birth	n/a	Jadad total: 3 [Grade: B]; Schulz: Unclear	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; RBC = red blood cells; $^{+}p<.05$ or significant with 95% confidence interval; $^{++}p<.01$; $^{+++}p<.001$; $^{++++}p<.0001$; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; GA = gestational age; IUGR = intrauterine growth retardation; FA = fatty acids; PD = pre-term delivery (GA < 37 wks); LBW = low birth weight; HC = head circumference

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. All but one study236 were double-blind parallel RCTs. The study by D'Almeida et al. was partially blinded.236 All the included studies were published in English scientific journals. Eleven trials had two arms; two studies included a third study group.209,236 The trials had been conducted in the following countries: South Africa,236 Denmark,209 The Netherlands,196,238 England,233,235 Norway,141 the U.S.,232,234 Australia and England.231 Olsen et al. conducted the three hospital-based trials in Denmark, Scotland, Sweden, England, Italy, The Netherlands, Norway, Russia, and Belgium.230 All but one study238 reported their funding sources: Enfamol Ltd.,236 Danish Medical Research Council, Sygekassernes Helsefond,

Weiman's Legat & Michaelsen Fonden,209 Yorkshire Region Locally Organized Research, GLAXO (Leeds) and Seven Seas (Hull);233 Concerted Action and PECO programmes of the European Commission and the Danish National Research Foundation;230 Peter Moller Grants, Avd. Orkla ASA and "Aktieselskabet Chocololadefabrils Medicinske Fond;141 Scottish Office Health Department;235 Martek Biosciences Boulder Corporation (formerly OmegaTech, Inc.);232,234 Unilever Research and Development (Vlaardingen, Netherlands);196 and, NH & MRC and Raine Medical Research Foundation (Australia).231

Population characteristics. The total number of enrolled pregnant women across the 10 trials was 3,404 and ranged from 60[235] to 590[141] participants. Helland et al. had a high rate of dropouts, leaving 341 women in the final analysis (57%).[288]

The age distribution of participants was reported in all but two trials.[235,238] The age of women across these studies ranged from 14[236] to 40 years.[233] Smuts et al. studied the youngest population of women with about 50% of participants aged between 16 and 21 years.[234] Whereas, in the study by Bulstra-Ramakers et al., more than 50% of the women were between 20 and 29 years old.[233] The age distribution across the study arms was not statistically different. However, in the Smuts et al. study,[232] the experimental arm (omega-3 enriched eggs) consisted of significantly younger women than in the control arm ($p < 0.05$).[232]

All but one study[236] reported both inclusion and exclusion criteria. The 13 trials can be categorized into two groups—those trials investigating the effect of omega-3 dietary supplementation in pregnant women at risk of IUGR, due to a previous history of IUGR, twin pregnancy or history of premature delivery,[230,233,238] and those trials that included only healthy pregnant women.[141,196,209,231,232,232,234-236]

The definition of a previous history of IUGR varied across the first group of studies. For example, Bulstra-Ramakers et al.[238] defined IUGR as birth weight <10th PC, Onwude et al.[233] defined it as birth weight <3rd PC, and Olsen et al.[230] as a birth weight <5th PC.

In the second group of studies, women were relatively healthy except in the Dunstan et al. study,[231] who reported that 40% and 58% of the women had asthma and allergic rhinitis, respectively. The second group of trials studied multiparous, as well as nulliparous women. The corresponding data on parity were reported in five of the 9 trials.[141,196,209,231,234] The proportion of multiparous women across the studies ranged from 43%[234] to 60%[196] and with the exception of Smuts et al.'s study (42% vs 32%), were evenly distributed between the study arms.[141,196,231]

The trials excluded women with diabetes,[230,233-235,238] gestational diabetes,[232] systemic lupus erythematosus,[234,238] chronic hypertension,[196,233,234] GHT,[232,235] placental abruption,[209,230,233,235] asthma,[233] severe fetal malformation,[141,230] drug/alcohol abuse,[230] regular intake of fish oil,[196,209,230,231] chronic illness (cardiovascular, cancer, renal, psychiatric, or neurological disorder),[196,232,234] preeclampsia,[232,235] serious infectious disease (hepatitis),[141,234] serious bleeding episodes,[209,235] allergy to fish[209,235] or use of prostaglandin inhibitors.[209,235] Smuts et al. excluded women who had more than four pregnancies.[232] Enrollment in one trial was restricted to non-smoking women.[231] Malcolm et al also excluded twin pregnancies.[235]

Only three trials reported the racial composition of the study population.[196,232,234] In two trials,[232,234] the majority of women were Black (81.0 and 73.2%, respectively). The third trial

included only White women.[196] There was no statistically different racial distribution between the study arms among these trials.

Ten studies reported on maternal tobacco smoking.[141,196,209,230,231,233,234] The "Earl-IUGR" study by Olsen et al.[230] had the highest prevalence of smokers (about 50%). In contrast, the lowest prevalence of smokers (about 19%) was in the study by Helland et al.[141] In these trials, the arm-specific distributions of smokers were similar. In their trial, Dunstan et al. included only non-smokers.[231]

All trials reported the GA of the study participants at enrollment/intervention. In five trials,[141,232-234,238] GA of women at the start of intervention ranged from 12 weeks[238] to 32 weeks.[233] For the remaining six trials, the lowest reported value of GA at intervention start was 16 weeks. The between-arm distribution of GA after randomization was reported as not different between-arms in nine trials.[209,230,232-235]

Only three trials reported on alcohol use,[196,231,234] and in all of them, the distribution of alcohol users was similar between the randomized arms. The years of maternal education was reported in only two trials.[141,196]

Intervention/exposure characteristics. In all 14 trials, the experimental intervention was the supplementation of the women's usual diet with omega-3 FA-enriched products. In 10 trials,[209,230,231,233,235,236,238] the omega-3 FA supplementation was provided in capsules. The number of assigned capsules given to the women in these trials ranged from 4[230] to 12 per day.[238] In two trials,[232,234] women received omega-3 FA-enriched eggs. In 10 studies, the primary source of omega-3 FA supplementation was fish oil.[141,209,230,231,233,235,236] In de Groot et al., the source of omega-3 FA supplementation was margarine.[196] The experimental intervention in the majority of the trials consisted of the combined supplementation of DHA and EPA.[141,209,230,231,233,238] Participants in the de Groot et al. trial received dietary supplementation with ALA and LA.[196] The supplementation provided to participants in the two Smuts et al. trials was eggs enriched with only DHA.[232,234] D'Almeida et al. used a mixture of evening primrose oil (GLA) and fish oil (DHA+EPA).[236]

The absolute amount of DHA ranged from 120[233] to 135 mg per capsule (or per egg).[232,234] The study-defined daily dose (in grams) of DHA and EPA varied across the trials. The daily dose of DHA ranged from 0.20 g[232] to 2.20 g.[231] Whereas, the daily dose of EPA ranged from 0.80 g[141] to 3.0 g.[238] In the study by de Groot et al., the daily doses of ALA and LA were 2.8 g and 9 g, respectively.[196]

In most of the studies, intervention for the control group consisted of capsules,[230,233,238] eggs,[232,234] or margarine[196], with similar appearance and/or taste as those for the experimental intervention. The participants in the control arms received olive oil,[209,230,231] coconut oil,[238] or corn oil.[141] Onwude et al.'s control group received airfilled capsules.[233]

The duration of the intervention was, in general, until delivery. The manufacturers of the omega-3 FA-enriched supplemental products were reported in 12 studies: R P Scherer Ltd. (UK);[235] Enfamol Ltd.;[236] Lube Ltd. (Denmark);[209,230] Peter Moller, Avd Orkla ASA (Norway);[141] OmegaTech, Inc. (Bouldwer, CO)/Gold Circle Farms (U.S.);[232,234] Unilever Research and Development (Vlaardingen, Netherlands);[196] and, Ocean Nutrition (Nova Scotia, Canada).[231] Two trials did not report the names of the manufacturers.[233,238]

The data on compliance (numbers of non-compliant participants and reasons for non-compliance) and type of analysis performed (i.e., ITT) were reported in six trials.[209,230,233] Five studies used ITT analyses.[230,233] The numbers of non-compliant participants were reported in five studies.[141,196,232,234,238] Dunstan et al. did not report well-documented compliance-related data.[231]

Cointervention characteristics. Six trials allowed 2 to 4 mg tocopherol/mL in the fish oil capsules.[209,230,231] de Groot et al.'s margarines also contained vitamins (0.04%).[196] In the Helland et al. study,[141] the amount of fat-soluble vitamins was identical in the two oils provided to partipants (i.e., 117µg/mL of vitamin A, 1 µg/mL of vitamin D, and 1.4 mg/mL of tocopherol).

Five studies assessed the background diet of participants during the study.[141,209,232,235,236] The studies used either a food-frequency questionnaire or a 24 hour recall questionnaire.[236]

Outcome characteristics. Of the 14 studies, three looked at the recurrence rate (i.e., percentage, relative risk, or odds ratio) of IUGR.[230,233,238] Olsen et al., in the "Earl-IUGR" trial, evaluated the incidence of IUGR (not recurrence).[230] Twelve trials measured and compared mean birth weight values (in grams) between the randomized arms, adjusted for GA and sex.[141,196,209,230-235] The rate of birth (i.e., percentage) of infants weighing <2,500 grams (LBW) was looked at in seven trials.[230,232,234,236,238] The infants' birth length and HC (in cm) between the randomized groups were compared in five trials.[141,231,232,234,235]

Study quality and applicability. The 14 RCTs received a mean Jadad total quality score of 2.85, with an average poor internal validity (Summary Matrix 3). The trials conducted by Bulstra-Ramakers et al. and Onwude et al. received a score of 5,[233,238] Helland et al. received a score of 4,[141] four trials received a score of 3,[196,231,234,235] seven reports received a score of 2.[209,230,236,294] Four trials failed to report the randomization method,[290,293-295] seven trials were not double-blinded,[31,41,296,297] while Smuts et al. did not provide the method of double-blinding.[294] Seven trials did not report the reasons for dropouts.[31,41,294,297]

Summary Matrix 3: Study quality and applicability of the evidence for the effect of LCPUFA supplementation on the incidence of infants small for gestational age

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author Onwude[A] Helland[U]	Year 1995 2001	n 233 590	Author Smuts[I] Malcolm[U]	Year 2003 2003	n 250 100	Author Smuts[U]	Year 2003	n 73
	III	Author Bulstra-Ramakers[A]	Year 1994	n 68	Author Dunstan[U] de Groot[U]	Year 2004 2004	n 98 79	Author Olsen[I] D'Almeida[I] Olsen[A] Olsen[A] Olsen[A] Olsen[A]	Year 1992 1992 2000 2000 2000 2000	n 533 150 232* 280** 579*** 63^

n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment; [I] Inadequate
*"Earl-PD" trial; **"Earl-IUGR" trial; ***"Twins" trial;^"Susp-IUGR" trial

Qualitative synthesis of individual study results

The three studies investigating the effect of omega-3 FA dietary supplementation on pregnant women with a previous history of IUGR, concluded that the randomized groups did not differ with respect to the recurrence of IUGR (birth weight < 3rd and 10th PC adjusted for GA).[230,233,238]

The between-group difference in the mean birth weight was not significantly different in eight of 12 studies.[141,209,230,231,233-235] However, in three trials, the mean birth weight was significantly higher in the omega-3 FA-supplemented group compared with the group without supplementation.[196,230,232] In contrast, the "Earl-IUGR" trial found a significantly higher mean birth weight in the olive oil group compared with the fish oil group.[230]

Regarding birth length, three studies did not find a statistical difference between study arms.[141,231,235] On the other hand, in the Smuts et al. trial, infants in the high-DHA egg group had a significantly higher birth length compared with those in the ordinary egg group.[234] HC at birth was similar in both groups across four trials.[141,231,234,235]

Results of five trials showed that omega-3 FA supplementation did not influence the incidence rate of LBW infants from pregnant women with or without a history of previous IUGR.[230,234,238] In the trial conducted by Smuts et al., no LBW infants were born to women receiving omega-3 FA supplementation, and the incidence rate of LBW infants born to women in the control arm was 26%.[232] In D'Almeida et al., the percentage of infants born weighing <2,000 g was noticeable lower in the omega-3 FA supplemented group compared with the other two groups (placebo: 3.3%, magnesium: 4.7%, fish oil+primrose oil: 1.4%); however, no p-value was reported.

Only one study evaluated the association between maternal biomarkers with this clinical outcome.[196] de Groot et al. found a positive correlation between maternal plasma and RBC DHA and birth weight, when controlled for birth order. This difference was nonsignificant at delivery. There was also a statistically positive correlation between the total estimated DHA intake and birth weight. However, this study provided ALA and LA as supplementation.[196]

Seven studies reported data on dropouts/withdrawals, albeit with different detail.[141,196,209,231,234,235,238] The most frequent reasons for study drop-out were: discomfort in consuming fish oil or margarine; lack of compliance; refusal to participate because it was time consuming; morning sickness; and/or, nausea. The number of non-completers across the trials ranged from 1[233] to 57.[234]

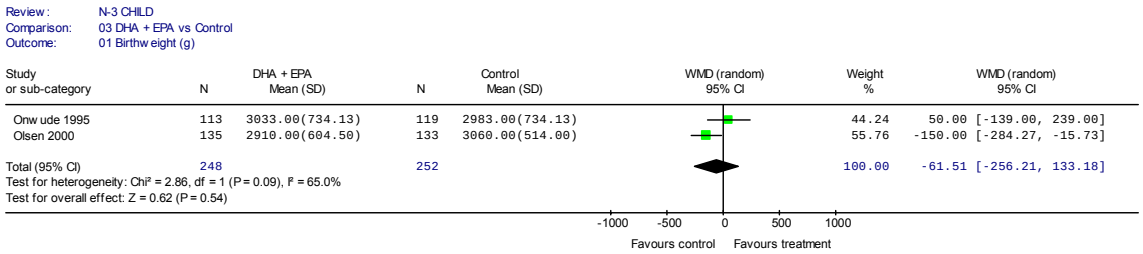
Quantitative synthesis

After examining the studies for source of oil and duration of supplementation, seven trials[209,230,231,233,238] were initially considered for meta-analysis. For Olsen et al. data from only three of six trials was considered (DHA+EPA vs. control): prophylactic EARL-IUGR trial, therapeutic Susp-IUGR trial, and prophylactic Twins trial.[230] Olsen et al.[209] and Dunstan et al.[231] were carried in a healthy population (i.e. women without previous history of high risk pregnancy). Thus five trials[230,233,238] were considered for meta-analysis.

For the birth weight outcome, data from the Susp-IUGR trial[230] could not be included since it was reported as birth weight adjusted for GA, unlike the other studies. Bustra-Ramakers et al.[238] did not report birth weight. Thus three trials[230,233] were available for meta-analysis.

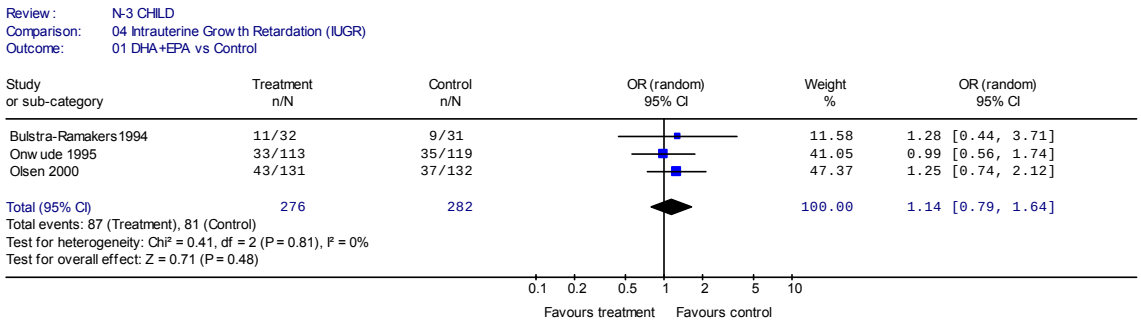
For the intra-uterine growth retardation (IUGR) outcome, the therapeutic trial Susp-IUGR[230] could not be included since it did not report IUGR outcomes. Thus four trials[230,233,238] were available for meta-analysis.

Figure 4. Birth weight (grams). Meta-analysis was performed using the random effects weighted mean difference. For the Onwude et al. study'[233] the standard deviations in the two study groups were not reported, however, a 95% confidence interval for the difference in means was reported. We assumed the standard deviations were the same in both groups, and computed the standard deviation from the confidence interval.



In two studies,[230,233] the overall size of the effect in the mean birth weight did not reach statistical significance (weight mean difference: -61.51, CI 95%: -256.21; 133.18).

Figure 5. Incidence of intra-uterine growth retardation (IUGR). Meta-analysis was performed using a random-effects model for odds ratios.



In three studies,[230,233,238] the overall size of the effect on the incidence of IUGR between DHA+EPA and control groups was nonstatistically significant (OR: 1.14, CI 95%: 0.79; 1.64).

Impact of covariates and confounders

The observed between-group differences in birth weight in three studies,[209,230,232] were adjusted for potential effect modifiers (i.e., duration of pregnancy, infant's gender, placental weight, maternal age, other characteristics).

Linear regression analysis revealed that the duration of pregnancy was an important predictor (potential confounder) of birth weight.[230] The higher birth weight observed in the experimental group compared with the control group was partially due to the effect of duration of pregnancy, which was not evenly distributed between the randomized groups. Once this difference was accounted for, by adjusting for duration of pregnancy, the earlier observed difference in birth weight was attenuated.[230] In another study,[232] using ANOVA, it was found that birth order was an important predictor of birth weight and length. Smuts et al. used a multiple linear regression to account for effect modifiers by adjusting the effects of interest for race, the number of prior pregnancies, previous premature deliveries, smoking, maternal body mass index (BMI), age, alcohol use, and maternal RBC-DHA levels.[234]

In the study of Smuts et al., women randomized to receive the diet supplemented with omega-3 FAs (DHA-enriched eggs) were substantially younger compared with those women receiving the diet without this supplementation (regular eggs) (mean age: 19.9 vs 24.8 year, $p < 0.05$).[232] The authors did not report any attempt to adjust for the effect of age.

In de Groot et al.,[196] the observed difference in birth weight was adjusted for the duration of pregnancy. In their "Susp-IUGR" trial, Olsen et al. found that the mean birth weight adjusted for GA at delivery did not differ between the two randomized groups.[230]

The analysis revealed that the effect estimates for birth weight, length, and HC were strongly influenced by maternal BMI, race, smoking status, and number of pregnancies. The adjustment for the above-mentioned covariates attenuated the earlier observed crude differences in birth weight, length, and HC. In de Groot et al., duration of pregnancy was an influential covariate for the association between the allocation to the experimental intervention and birth weight.[196]

In two studies,[232,238] the randomized groups were not balanced with respect to the important prognostic/predictive factors such as GHT[238] and age.[232]

None of the studies adjusted the outcomes results for the maternal background diet.

The power calculation was reported in seven trials,[31,288,292,296] while the intention-to-treat analysis approach was reported in four trials.[31]

Pregnancy Outcomes in Light of Biomarker Data

What is the Evidence That the Duration of Gestation in Women With or Without a History of a Previous Preterm Birth is Associated With the Omega-3 or Omega-6/Omega-3 Fatty Acid Content of Maternal Biomarkers During Pregnancy?

Four studies were identified that answered this question.[234,239-241] Smuts et al.'s RCT[234] was described above; hence, only the three observational studies will be presented in this section.[239-241] The observational studies were published between 1997 to 2001 in English scientific journals. The study by Reece et al.[239] was a case-control study, whereas, Elias and Innis was a single prospective cohort study[240] and Rump et al. was a cross-sectional study.[241] (Summary Table 11)

Overview of relevant study characteristics and results

Reece et al. compared blood LCPUFA content of 37 mother-infant pairs with preterm delivery (mean GA 34 weeks) with a group of 34 control full-term mother-infant pairs (mean GA 40 weeks).[239] The study was conducted in the U.S. and was supported by the Colorado Agricultural Experiment Station. The study included a sample of preterm and term cases based on the duration of gestation.[239] "Preterm delivery" (n=37) was defined as GA of less than 37 weeks, whereas, "term delivery" (n=34) was defined as GA of 37 or more weeks. The patients were excluded if they had a recognized cause of preterm birth (i.e., uterine abnormality, intrauterine infection, substance abuse, multiple gestation, pregnancy-onset hypertension). Exclusions for controls included recognized medical problems, multiple gestations, multiple parity, GHT, and substance abuse.[239] Participants were enrolled at 18 weeks of GA and followed until delivery.[239]

In preterm cases, the maternal blood samples were obtained at delivery, while the control women were sampled at 34 weeks of GA and at delivery.[239]

The cases were well-matched with the controls in terms of marital status (50% married), race (82% white), financial support (80% public), pre-pregnancy body mass index, maternal infection detected (70% none), type of labor and maternal age.[239] Both populations significantly differed in the duration of gestation (mean GA: 40.2 [SD=0.2] weeks vs 33.9 [SD=0.6] weeks), birth weight, length and HC (preterm infants had significantly lower growth parameters at birth than term infants).[239]

Reece et al. found that the RBC FA content (% total) of LA (omega-6), AA, and DHA was significantly higher in the preterm cases compared with the controls at 34 weeks GA and at term.[239] The percent total EPA in RBC in controls at term was significantly higher than both preterm deliveries and 34-week controls. The maternal RBC omega-3/omega-6 ratio content was significantly higher in control term deliveries compared with preterm cases. The maternal plasma percent total LA (omega-6) was significantly increased in the 34-week control and preterm groups compared with the term control group. The plasma percent total LA, AA, EPA was significantly higher in preterm cases compared with term controls. The plasma AA content was increased in 70% of preterm cases compared with control cases at term.[239]

Elias and Innis determined the association between length of gestation and the maternal plasma concentration of AA and DHA in a cohort of pregnant women (n=84) at 35 weeks of GA.[240] The study was conducted in Canada and was supported by the Molly Towell Perinatal Research Foundation and the National Science and Engineering Research Council of Canada.[240] The cohort included 60 women at 22 to 24 weeks of GA that were recruited from predelivery registration records and were followed until delivery. An additional 24 pregnant women were

recruited from a low-risk delivery unit in Canada. Women with a history of surgical or medical problems that could influence the lipid metabolism or fetal growth were excluded from the study. These included women with more than one fetus, hyperemesis, psychological or social problems, illicit drug or alcohol use, cardiac or renal disease, diabetes, epilepsy, respiratory or rheumatoid conditions, cholestasis, high cholesterol or triglycerides before pregnancy, HIV infection, hepatitis, or tuberculosis.[240]

The study measured the maternal intake, during pregnancy, of the different FAs through a food-frequency questionnaire designed to collect data on amounts and sources of fat, methods of food preparation, brand names and places of food purchase.[240]

The outcome measures were the maternal blood content of omega-3 and/or omega-6 FA during pregnancy and its relationship with the duration of gestation, as well as the infant FA blood content.[240]

Ellis and Innis did not find a significant association between the maternal plasma content of omega-3 and omega-6 FA and the duration of gestation, except for the maternal plasma triglyceride (TGL) AA content that was positively related to the length of gestation. However, this uncontrolled study did not provide the details regarding this association, as well as the fact that all the pregnancies reached term.[240]

Rump et al. was a cross-sectional study that included a sample of healthy pregnant woman and their term infants.[241] It was conducted in the Netherlands and supported by a Hospital, and Nutricia Research. The blood samples were taken at 16 weeks and after delivery.[241]

The cohort was separated by weight for gestational age groups, SGA (PC $<10^{th}$), AGA (PC $>10^{th}$ and $<90^{th}$) and LGA (PC $>90^{th}$). The groups were comaparable in terms of maternal characteristics like age, height, weight, parity, smoking status, and mode of delivery.[241]

There was no correlation between the maternal content of PUFA and the birth weight.[241]

Summary Table 11: Association between duration of gestation in women with or without a history of a previous preterm birth and the the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy (Observational studies)

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Study groups[1] Group 2 (n)/ Group 3 (n)	Notable associations[2,3]	Internal validity	Applicability
Reece, 1997, US: Case-control study[239]	Preterm births (n=37)	Term deliveries controls (n=34)	Maternal RBC LA, AA, DHA S ↑ in preterm vs. 34-wk control^{+} & term^{+++} Maternal RBC EPA S ↑in term controls vs. both preterm & 34-wk control^{++} Maternal RBC & plasma n-3/n-6 ratio was S↑ in term controls vs. preterm^{++} NS Maternal RBC n-3/n6 between preterm & 34-wk control Maternal plasma LA S ↑ in preterm & 34-wk control vs. term control^{+} Maternal plasma LA, AA, EPA S↑ in preterm vs. term controls^{+}	Quality score: 4 [Grade C]	III
Elias, 2001, Canada: Single prospective cohort[240]	Healthy pregnant women (n=84)	n/a	Umbilical cord plasma TGL & CE AA S (+) associated with GA^{++} NS association between other maternal n-3 or n-6 BMK & GA Maternal plasma TGL AA S (+) correlated to GA^{++}	Quality score: 6 [Grade B]	III
Rump, 2001, Netherlands: Cross-sectional[241]	Healthy pregnant women-term infants (n=627)	n/a	NS correlation between maternal plasma FA at 11 (8) wk GA & at delivery & GA	Quality score: 9 [Grade A]	III

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; LA = linoleic acid; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; N/A = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; BMK = biomarker; RBC = red blood cells; PL = phospholipid; CE = cholesteryl ester; TGL = triacylglycerol; GA = gestational age/duration of gestation; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ↑ = increase; ↓ = decrease/reduction

Study quality and applicability. Although they employed different research designs, all the studies were assigned a level III for applicability, and together they received a mean quality score of 6.3.

Summary Matrix 4: Association between duration of gestation in women with or without a history of a previous preterm birth and the the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author	Year	n	Author	Year	n	Author	Year	n
	III	Author Rump	Year 2001	n 627	Author Elias	Year 2001	n 84	Author Reece	Year 1997	n 71
n = number of allocated/selected participants										

What is the Evidence That the Incidence of Preeclampsia, Eclampsia or Gestational Hypertension is Associated With the Omega-3 or Omega-6/Omega-3 Fatty Acid Content of Maternal Biomarkers During Pregnancy?

Five observational studies were identified that addressed this question.[179,229,242-244] The studies were published between 1991 and 1999. The trials were included if they selected both preeclamptic and normal pregnant women, and blood samples were drawn before delivery. Three studies used blood samples taken after delivery and hence were excluded from the review.[299-301] (Summary Table 12, 13)

Four studies had a cross-sectional design,[229,242-244] whereas, one was a nested case-control study derived from a prospective cohort.[179]

Overview of relevant studies

Wang et al. assessed the association between the plasma levels of omega-6 FA (LA, AA) and omega-3 FA (ALA, EPA, DHA) in a sample of American nonpregnant, normal pregnant and preeclamptic patients (n=30).[242] (Summary Table 12)

Craig-Schmidt et al. evaluated the LCPUFA composition of plasma phospholipid in a small sample of American healthy pregnant women compared with women with GHT, preeclampsia and chronic hypertension (n=36).[243] (Summary Table 12)

Al et al.'s sample of Dutch healthy pregnant women were compared with pregnant women with GHT in a nested case-control study. The study assessed the plasma FA content during pregnancy in both groups (n=208).[179] (Summary Table 12)

Summary Table 12: Association between omega-3 or omega-6/omega-3 fatty acid content of biomarkers during pregnancy and incidence of preeclampsia, eclampsia or GHT

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable associations[2,3]	Internal validity	Applicability
Wang, 1991, US: Cross sectional study[242]	Preeclampsia (n=9)/	normal pregnant pts(n=11)/ nonpregnant women volunteers (n=10)	Total PUFA, LA (n-6), ALA (n-3) & EPA plasma of normal pregnant women was S > preeclamptic pts[+] NS between groups plasma AA & DHA S > EPA & DHA in normal pregnant women vs. nonpregnant[++]	Quality score: 5 [Grade B]	III
Craig-Schmidt, 1994, US: Cross-sectional study[243]	preeclampsia (n=10)/ normal pregnancy (n=10)	GHT (n=10)/ CHT (n=6)	NS among groups in plasma saturated, monosaturated & PUFAs NS in n-6 or n-3 FA between normal pregnancies & GHT, preeclamsia or CHT CHT S ↑ AA in plasma PL vs. other groups NS in plasma PL EPA among the groups NS in AA/EPA ratio & n-6/n-3 ratio	Quality score: 2 [Grade C]	III
Al, 1995, Netherlands: nested case-control study[179]	GHT women (n=52)	Healthy pregnant controls (n=156)	NS in absolute FA composition (mg/L) of maternal plasma PL (before 16, at 22 & 32 wks GA) Severe GHT women (n=17) mean GA & mean birth wt of their babies S ↓ than mild GHT During gestation & after delivery NS in maternal FA composition of the severe GHT vs. mild GHT	Quality score: 11 [Grade A]	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; grp = group; wk = week(s); mo = month; PL = phospholipid; CPG = choline phosphoglycerides; EPG = ethanolamine phosphoglycerides; [+]p<.05 or significant with 95% confidence interval; [++]p<.01; [+++]p<.001; [++++]p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; GHT = gestational hypertension; PL = phospholipids; CHT = chronic hypertension

Hofmann et al. evaluated the LCPUFA composition of maternal blood in a small sample of German pregnant women with preeclampsia compared with healthy controls (n=30).[229] (Summary Table 13)

Shouk et al. compared the LCPUFA plasma content in Egyptian women (mean age 29 [SD=8.2] years, range: 20-40 years) with severe preeclampsia with healthy pregnant subjects during the third trimester.[244] (Summary Table 13)

Summary Table 13: Association between omega-3 or omega-6/omega-3 fatty acid content of biomarkers during pregnancy and incidence of preeclampsia, eclampsia or GHT

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable associations[2,3]	Internal validity	Applicability
Hofmann, 1998, Germany: Cross-sectional study[229]	Preeclampsia (n=14)	Healthy pregnant controls (n=16)	Total FA in plasma TGL during pregnancy were S > in preeclamptic group vs. control^{+++} NS between groups in AA plasma TGL during pregnancy LA (n-6) & DHA (n-3) content in plasma TGL were S ↓ in preeclamptic pts vs. controls^{+} NS between groups LA & AA (n-6) in plasma PL DHA plasma PL content was S ↓ in preeclamptic women^{++}	Quality score: 6 [Grade B]	III
Shouk, 1999, Egypt: Cross-sectional study[244]	severe preeclampsia in 3rd trimester (n=25)	healthy pregnant controls (n=20)	AA in plasma was S > in preeclamptic women vs. control^{+++} NS between groups LA & ALA (n-3) content	Quality score: 7 [Grade B]	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; PL = phospholipid; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; TGL = triglycerides

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. Of the five observational studies that met eligibility criteria, two studies were conducted in the U.S.,[242,243] one was conducted in The Netherlands[179], one in Germany[229] and one in Egypt.[244] Two studies compared the outcomes in more than two groups,[242,243] whereas, three studies involved only three arms.[179,229,244]

Most studies were published in scientific journals in English, but one required translation from German.[229] The funding source was reported in two of five studies. Wang et al. was supported by a pharmaceutical industry (Glaxo, Inc.),[242] whereas, Al et al. was funded by Nutricia BV, Zoetermeer, The Netherlands.[179]

Population characteristics. There were 349 subjects included across the studies. The sample sizes ranged from 30 to 208 patients. Three studies reported the inclusion and exclusion criteria.[179,229,244]

Wang et al. selected three groups of women between 20 and 40 years, normal pregnant patients (n=11), preeclamptic patients (n=9) and nonpregnant female volunteers as controls. All were at term.[242] Craig-Schmidt et al. included nulliparous pregnant women (mean age: 21 [SD=6] years).[243] The study groups were composed of women with normal pregnancy (n=10), GHT (n=10), preeclampsia (n=10), and chronic hypertension (n=6).[243]

Al et al. selected, from the prospective cohort of healthy pregnant women (GA <16 wks), a group of women with GHT and matched them with a group of healthy pregnant patients.[179] Hofmann et al.[229] and Shouk et al.[244] compared a group of women with preeclampsia with a healthy pregnant control group, although Shouk et al.'s patients had a severe preeclamsia in the third trimester.

Shouk et al. did not provide a definition for preeclampsia.[244] In general, preeclampsia was defined as as BP greater than 140/90 mm Hg measured on two occasions, 6 hours apart starting from the 20th week of GA. Proteinuria was defined as greater than 300 mg urinary protein per 24 h; preeclampsia was the combination of hypertension and proteinuria with or without edema.[179,229,242,243]

Wang et al.[242] and Craig-Schmidt et al.[243] failed to provide information about the between-group difference in terms of population characteristics (i.e., maternal age, GA, parity, education, smoking status, etc.) at baseline or before the study. Al et al. did not find a significant difference between groups in maternal age, number of nulliparous women, percentage of smoking women, or number of infants small for gestational age (SGA) at term.[179] There was a significant difference between groups in diastolic BP at entry (GHT higher than control), maximum diastolic BP (GHT >control), GA at delivery (GHT < control), birth weight (GHT < control), and APGAR score at 5 min (GHT < control).[179] Control of selection bias was achieved by measuring the FA content of pregnant women (at 16 weeks GA) who decided not to participate in the trial.[179]

Hofmann et al.'s study groups were well-matched for maternal age, BMI, GA, serum creatinine, blood glucose and hematocrit. Blood pressure was significantly higher in the preeclamptic women.[229] Similarly, Shouk et al.'s patients were well-matched for age, parity and GA.[244]

Regarding the medications and/or treatments allowed before study entry, Wang et al.[242] and Hofmann et al.'s[229] preeclamptic women did not receive aspirin. The rest of the studies did not report the use of medication in their patients.

Hofmann et al. and Shouk et al. included patients without other comorbid conditions.[229,244] The remainig three studies did not provide this information.[179,242,243]

Intervention/exposure characteristics. Groups in the study by Al et al. did not differ in their nutrient intake during pregnancy.[179] None of the identified studies described the nature of the nutritional intake, including the use of supplements or any other substance that could alter the lipid content in maternal blood biomarkers.

Outcome characteristics. All studies examined the omega-3 and omega-6 FA content in plasma of maternal blood from preeclamptic women compared with healthy controls.

Study quality and applicability. The total quality score across the studies was 6.2, however the applicability level was III.

Summary Matrix 5: Association between omega-3 or omega-6/omega-3 fatty acid content of biomarkers during pregnancy and incidence of preeclampsia, eclampsia or GHT

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author	Year	n	Author	Year	n	Author	Year	n
	III	Author	Year	n	Author	Year	n	Author	Year	n
		Al	1995	208	Wang	1991	30	Craig-Schmidt	1994	36
					Hofmann	1998	30			
					Shouk	1999	45			
n = number of allocated/selected participants										

Qualitative synthesis of individual study results

Wang et al. found that the total PUFA, LA (omega-6), ALA (omega-3) and EPA content in plasma (mg/L, mean) of normal pregnant women was significantly higher than in the preeclamptic patients.[242] There was a nonsignificant difference between groups in the content of AA and DHA in plasma. However, there was a significantly higher content of EPA and DHA in normal pregnant women compared with nonpregnant.[242]

Craig-Schmidt et al. did not observe a significant difference between groups in saturated, monosaturated and PUFAs, or in the content of omega-6 or omega-3 FA (mg/L and % of total FA) between women with normal pregnancies and women with GHT, preeclamsia or chronic hypertension.[243] The women with chronic hypertension had a significantly greater AA in plasma phospholipid compared with the other three groups. There was a nonsignificant difference in plasma phospholipid EPA concentrations among the groups, as well as in the AA/EPA ratio or omega-6/omega-3 ratio at baseline.[243]

During pregnancy (before 16, at 22 and 32 weeks GA) no significant differences in the absolute FA composition (mg/L and % total FA) of maternal plasma phospholipid were observed between groups in the Al et al. study.[179] After delivery, however, the amount of ALA (omega-3) was significantly lower in the GHT women compared with women who had normal pregnancies. After correction for differences in GA between groups, significantly higher levels of DHA were observed in umbilical plasma of the GHT compared with controls.[179] When the GHT women were stratified by severity of hypertension, patients with severe GHT (diatolic BP >105 mmHg) (n=17), 12 of which had proteinuria, had a mean GA and mean infant birth weight that were significantly lower than those in the group with mild GHT (diastolic BP <105 mmHg). During gestation and after delivery, no significant differences were observed in the maternal FA composition of women with severe GHT compared with those with mild GHT.[179]

Hofmann et al. found that the total amount of FA in plasma triglycerides during pregnancy were significantly higher in the preeclamptic group compared with the healthy control group. The difference disappeared on the 5th day after delivery.[229] The AA content in plasma triglycerides did not differ between groups during pregnancy. On the other hand, the LA (omega-6) and DHA (omega-3) content in this blood fraction were significantly lower in the preeclamptic women compared with the controls. The LA and AA (omega-6) concentration in plasma phospholipid were not significantly different between groups, however, the DHA plasma phospholipid content was significantly lower in preeclamptic women.[229]

Shouk et al. observed that the AA in plasma (mcg/L) was significantly higher in preeclamptic women. LA and ALA (omega-3) content did not differ between groups.[244]

What is the Evidence That the Incidence of Births of Human Infants Small for Gestational Age is Associated With the Omega-3 or Omega-6/Omega-3 Fatty Acid Content of Maternal Biomarkers During Pregnancy?

Five observational studies were identified that addressed the possible association between the incidence of SGA infants and the omega-3 or omega-6/omega-3 FA content of maternal biomarkers during pregnancy.[240,241,245-247] Two were cross-sectional studies,[241,247] two were case-control studies[245,246] and one was a single prospective cohort.[240] Studies were published between 1991 and 2002. (Summary Table 14, 15)

Overview of relevant studies

Vilbergsson et al. assessed the association between LCPUFAs of pregnant women considered to be at an increased risk for IUGR and the incidence of SGA deliveries.[247] Investigators recruited 28 eligible women at week 33 or 34 of pregnancy who were considered as high risk for SGA delivery after thorough evaluation using a special risk scoring system, ultrasonographic measurements of fetuses' growth parameters, nonstress test, and biophysical profile following regular monitoring. Twenty pregnant women with no risk factors were enrolled into the study as a control group.[247] (Summary Table 14)

Matorras et al., in a case-control intrapartum study, analyzed the relationship between maternal plasma LCPUFAs and IUGR in an apparently well-nourished population of pregnant women in the second stage of labor.[245]

The study population consisted of 23 women in labor whose infants had prenatally-suspected IUGR and were at term delivery and 34 newborn control cases who whose size were appropriate for gestational age (AGA).[245] (Summary Table 14)

Summary Table 14: Incidence of births of SGA human infants and the association with the omega-3 or omega-6/omega-3 FA content of maternal biomarkers during pregnancy

Author, Year, Location: Design	Study groups[1]		Notable associations[2,3]	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)			
Vilbergson, 1991, Sweden: Cross-sectional[247]	SGA grp (n=13)	Term AGA (control) (n=20)	S↓ maternal plasma DHA & AA in SGA grp than in ctrl at 34 weeks GA & at delivery[+]	Quality score: 7 [Grade B]	III
Matorras, 1994, Spain: Case-control[245]	IUGR grp (n=23)	AGA (control) (n=34)	S↑ maternal plasma EPA in IUGR grp than in ctrl at delivery[++] NS in maternal plasma DHA & AA at delivery	Quality score: 9 [Grade A]	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; AGA = appropriate for gestational age; IUGR = intrauterine growth restriction; GA = gestational age; ct = control group; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; BW = birth weight; Fas = fatty acids; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; grp = group; wk = week(s); mo = month; [+]p<.05 or significant with 95% confidence interval; [++]p<.01; [+++]p<.001; [++++]p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; SGA = small for gestational age; AGA = adequate for gestational age; IUGR = intrauterine growth retardation

Elias and Innis determined the association between birth weight and length and the maternal plasma concentration of AA and DHA in a cohort of Canadian pregnant women (n=84) at 35 weeks of GA.[240] (Summary Table 15)

Rump et al., in a cross-sectional study, evaluated the relationship between the incidence of term SGA births and observed changes in maternal plasma LCPUFA composition during pregnancy.[241] The study population consisted of 81 SGA infants and 505 AGA infants. Maternal plasma FA analysis was performed at study entry (≤16 weeks GA), at delivery, and in cord plasma at birth. (Summary Table 15)

Cetin et al.,[246] in a case-control study, determined maternal FAs profiles in utero in 11 AGA and in 10 IUGR fetuses from 19 to 39 weeks of gestation and studied the relationship between maternal plasma LCPUFA status and the incidence of SGA. (Summary Table 15)

Summary Table 15: Incidence of births of SGA human infants and the association with the omega-3 or omega-6/omega-3 FA content of maternal biomarkers during pregnancy

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable associations[2,3]	Internal validity	Applicability
Elias, 2001, Canada: Single prospective cohort[240]	Healthy pregnant women (n=84)	n/a	Maternal plasma TGL AA, S (+) correlated to infant birth wt & length^{++}	Quality score: 6 [Grade B]	III
Rump, 2001, Netherlands: Cross-sectional[241]	Healthy pregnant women-term infants (n=627)	n/a	NS relation between maternal plasma FA at 11 (8) wk GA & at delivery & infants BW	Quality score: 9 [Grade A]	III
Cetin, 2002, Italy: Case-control[246]	IUGR grp (n=10)	AGA (control) (n=11)	S↑ maternal plasma EPA in IUGR grp than in pb at ≈28.2(8.0) wk GA^{+} NS in maternal plasma DHA & AA at ≈28.2 (8.0) wk GA	Quality score: 5 [Grade B]	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; GA = gestational age; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; BW = birth weight; TGL = triacylglycerol; FAs = fatty acids; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; grp = group; wk = week(s); mo = month; wt = weight; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; SGA = small for gestational age; LGA = large for gestational age

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. The studies were conducted in different countries, including one from Canada,[240] and one each from Sweden,[247] Spain,[245] Italy[246] and The Netherlands.[241] Four studies reported their funding sources and these included a professional society, university and foundation,[240,247] and government.[240,245,246]

Population characteristics. Four studies selected a small number of participants, ranging from 21[246] to 84.[240] Only Rump et al. studied a large sample of infants (n=81 SGA, n=505 AGA, n=41 LGA).[241]

Four studies presented clearly-defined inclusion and exclusion criteria[240,241,245,247] and one study exclusively described exclusion criteria.[246] Vilbergsson et al. included only singleton pregnancies and made an effort to equally distribute subjects to groups by age, parity, and dietary intake; maternal diabetes was an exclusion criterion.[247] Matorras et al. included term SGA infants with no malformations and chromosomal abnormalities, delivered from a singleton pregnancy, with an accordance between GA (determined by last menstrual period and early ultrasound) and pediatric evaluation using the Dubowitz test.[245] Elias and Innis included healthy pregnant women (GA 22-24 weeks), whereas, women with medical or surgical problems that could influence lipid metabolism were not eligible.[240] In the study of Rump et al., selection criteria for inclusion/exclusion were GA <16 weeks at entry, diastolic BP <90 mmHg and no

signs of cardiovascular, neurologic, renal, or metabolic disorders at the time of recruitment.[241] Cetin et al. set the following exclusion criteria for both normal and IUGR pregnancies: subsequent development of gestational diabetes or GHT; abnormal fetus caryotype; or, malformation at birth.[246]

The mean GA was reported in all of the five studies. The mean GA for the entire SGA group of infants ranged from 36[247] to 40.6 weeks.[241] Statistically significant differences in GA between the SGA/IUGR and AGA groups were reported in two studies.[246,247] In the remaining three studies, the SGA/IUGR cohort and AGA controls were of similar age at birth.[241,245,246]

Definition of IUGR and/or SGA was given in four studies.[241,245-247] Cetin et al.[246] and Matorras et al.[245] established IUGR by performing ultrasonographic examination measuring fetal biparietal diameter and/or abdominal circumference, which had to be under the 10th PC of reference values for fetuses of a similar age. In the study of Cetin et al., growth retardation was confirmed at birth if the neonatal weight was below the 10th PC according to standards for birth and weight and GA.[246] Rump et al.[241] classified infants as SGA if their birth weight was ≤10th PC of reference values, whereas Vilbergsson et al.[247] defined SGA as an infant birth weight two standard deviations below the mean when compared with a standard growth chart.

No authors explicitly stated the racial/ethnic background of the study participants, yet it is likely that Caucasian/Europeans were represented as a majority in all of these studies.

Information regarding maternal smoking history and/or smoking during pregnancy was available in two studies and even though there was a higher proportion of smokers in the SGA/IUGR group than in control group, the difference did not reach statistical significance.[241,245] Vilbergsson et al reported that the control group contained no smokers and in the group at risk for IUGR, there were no differences between smokers and nonsmokers with respect to clinical characteristics or FAs results.[247] Alcohol consumption during pregnancy was not reported in any of the five studies.

None of the studies reported the use of medication and/or supplements before study entry or any comorbid conditions in newborn babies. Maternal characteristics such as parity, and age, height, weight at study entry, were similar between study groups in three studies.[241,246,247] However, in the study of Matorras et al.,[245] IUGR mothers had lower height, pregestational weight and weight increase during pregnancy than mothers in the control group.

Only one study reported the mean maternal energy intake during pregnancy, which was similar between control and IUGR groups.[245] The same study evaluated socioeconomic levels of study population and reported that twice as many women with IUGR pregnancies belonged to low socioeconomic strata. The description of lipid extraction and biochemical analysis was adequate in all but one study.[247]

Outcome characteristics. The main outcome evaluated in these observational studies was incidence of births of SGA infants and its relation to either the absolute or relative amount of maternal plasma FA concentrations during pregnancy. Information regarding the timing of the maternal plasma LCPUFA analysis was reported in all but one study.[247]

In the study of Vilbergsson et al., maternal blood samples were drawn in the 34th and 37th week of pregnancy, at delivery, and at 4 days postpartum. This study measured the plasma content in phospholipids (lecitin) of LCPUFA (mol %).[247] Cetin et al. reported that maternal sample

collection and analysis were done at 28.2±8.0 weeks GA in the AGA control group and at 28.6±4.3 weeks GA in IUGR group. The plasma PUFA were measured in mcg/ml and % weight of total FA.[246] In the study of Rump et al., maternal venous blood samples were collected at 11±3 weeks GA. The plasma FA were measured in % weight of total FA.[241] Matorras et al. obtained maternal blood samples during the second stage of labor. The plasma FA were measured in % weight of total FA [245] The correlation between maternal plasma FA composition and the main outcomes was calculated using Pearson's correlation coefficient, following the standard criteria of applicability,[245] linear regression analysis,[246] and simple and multiple regression models.[241,247] Elias and Innis assessed the association between maternal plasma PUFA and the birth weight and length of infants. The plasma FA were measured in % weight of total FA.[240]

Study quality and applicability. Although they employed different research designs, all the studies were assigned a level of applicability of III and together, received a mean quality score of 7.2.

Summary Matrix 6: Incidence of births of SGA human infants and the association with the omega-3 or omega-6/omega-3 FA content of maternal biomarkers during pregnancy

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author	Year	n	Author	Year	n	Author	Year	n
	III	Author	Year	n	Author	Year	n	Author	Year	n
		Matorras	1994	69	Vilbergson	1991	33			
		Rump	2001	627	Elias	2001	84			
					Cetin	2002	21			
n = number of allocated/selected participants										

Qualitative synthesis of individual study results

Vibergsson et al.[247] found that in a subgroup of SGA participants, maternal plasma DHA and AA concentrations were significantly lower than those in a control group at 34 weeks GA as well as at delivery. The study results of both Matorras et al.[245] and Cetin et al.[246] were similar. In the Spanish case-control study, Matorras et al. revealed that maternal plasma EPA concentrations expressed in percentage values of total amount of plasma FAs, were significantly increased in IUGR mothers compared with controls at delivery.[245] Conversely, there were no differences in percentage values nor in absolute values in the other FAs analyzed in newborn infants.[245] Cetin et al. observed significantly higher maternal plasma EPA in the IUGR group compared with the normal control group in the third trimester of pregnancy.[246]

Rump et al. found that observed changes in maternal plasma LCPUFA concentrations (% wt FA) were related to the size of the infants.[241] Significantly bigger decreases in plasma concentrations of AA and DHA were noted in mothers of AGA control infants compared with mothers of the SGA group, whereas, the largest reduction in the fraction of linoleic acid was found in the mothers of SGA infants. No cross-sectional association was found between

maternal FA concentrations and infant size at birth at study entry or at delivery, as well as between maternal plasma FA concentrations and the total duration of gestation.

Elias and Innis observed that the maternal plasma TGL AA, but not phospholipid or cholesteryl ester AA, was positively related to infant birth weight and length ($p<0.01$). No other correlations were found between maternal plasma omega-3 or omega-6 FAs and these variables.[240]

Growth Pattern Outcomes

What is the Evidence That Maternal Intake of Omega-3 Fatty Acids During Pregnancy Influences Growth Patterns in Term or Preterm Human Infants?

One RCT, published in 2002, was identified to answer this question.[141] Helland et al.[141,200] had two publications related to the same study population, yet this review will refer only to the earlier one.[141] (Summary Table 16)

Overview of relevant study characteristics and results

Helland et al.,[141] has been described in detail in the Pregnancy Outcomes section. A summary and the results relating to the current question are discussed here.

Helland et al. assessed the gestational length, birth weight, and neurologic and cognitive outcomes in a sample of infants born of healthy pregnant women. Participants were randomized to receive either cod liver oil (1,183 mg/10 mL DHA, 803 mg EPA, 27.5 mg AA) or corn oil (LA and ALA) from week 18 of pregnancy to 3 months post delivery.[141]

The participants (n=590 enrolled) were included if they were healthy, with single pregnancies, between 19 and 35 years of age, and intended to breastfeed their infant. They should not have taken any supplements of omega-3 FA earlier in the pregnancy. The exclusion criteria were premature births, birth asphyxia, infections, and anomalies in the infants that required special attention.[141] Infant growth patterns (i.e., weight, length and HC) were measured at birth, 6 weeks and 3, 6, 9 and 12 months. Helland et al. had a high rate of dropouts, leaving 341 women in the final analysis (57%).[288]

Summary Table 16: Omega-3 fatty acids and its influence on growth patterns in infants after intake during pregnancy and breastfeeding

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Internal validity	Applicability
Helland, 2001, Norway: 34 wks parallel RCT[141]	Cod liver oil (DHA+AA+EPA) (n=301 mothers; n=175 infants)	Corn oil (LA+ALA) (n=289 mothers; n=166 infants)	NS between groups in weight, length & head circumference at any point	Jadad total: 4 [Grade: A]; Schulz: Unclear	III

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; N/A = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; RBC = red blood cells; PL = phospholipid; $^{+}$p<.05 or significant with 95% confidence interval; ++p<.01; +++p<.001; ++++p<.0001; ↑ = increase; ↓ = decrease/reduction

The groups did not differ significantly in weight, length and HC at any time point during the study.[141]

No correlation was found between these parameters and infant plasma biomarkers.

Study quality and applicability. The Jadad total quality score was 4 (did not report double-blinding method) and the allocation concealment was unclear in the report. The applicability level was III.

Summary Matrix 7: Omega-3 fatty acids and its influence on growth patterns in infants after intake during pregnancy and breastfeeding

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author	Year	n	Author	Year	n	Author	Year	n
	III	Author	Year	n	Author	Year	n	Author	Year	n
		Helland[U]	2001	590						

n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment

What is the Evidence That the Omega-3 Fatty Acid Content of Maternal Breast Milk, With or Without Known Maternal Intake of Omega-3 Fatty Acids, Influences Growth Patterns in Term or Preterm Human Infants?

One RCT and two observational studies published between 1999 and 2003 met eligibility criteria regarding the influence of maternal milk intake on growth patterns.[248,249,302] Jensen et al. was a double-blind RCT,[248] Xiang et al. was a single prospective cohort study[249] and Rocquelin et al. was a cross-sectional study.[302] Helland et al.'s RCT (see above and Summary Table 16)

also addressed this question since the mothers of the infants included in the study breastfed their infants while taking PUFA supplementation.[141]

Overview of relevant study characteristics and results

Jensen et al. investigated the effect of DHA supplementation in lactating women on the visual function and growth of their infants.[248] Mothers were assigned randomily to receive 200 to 250 mg DHA per day as either algal DHA (n=42), refined high-DHA fish oil (n=42) or placebo (n=42), for 120 days after delivery. Infant characteristics, as well as maternal characteristics, were not described in this abstract.[248] The study showed no differences between the three diet groups in the weight, length or HC of the infants at 120 and 240 days.[248]

Xiang et al. evaluated the growth patterns in a random sample of healthy mother-term infant pairs (n=19) at 1 and 3 months of age. The infants were exclusively breastfed during the study period.[249] Rocquelin et al. investigated the role of human milk LCPUFAs in term infant growth in two African suburban random samples of nursing mothers and their 5 month old infants.[302]

Xiang et al. was conducted in Sweden and was supported by the Wenner-Gren Centre Foundation.[249] Rocquelin et al. was conducted in in The Congo and Burkina Faso (Africa), and supported partly by the Institut National de la Recherceh Agronomique.[302]

Xiang et al. did not report the inclusion and exclusion criteria, yet described the included sample as mother-infant pairs without acute or chronic conditions. The infants were exclusively breastfed during the 3 months of the study. The mothers registered the total intake of food and fluid, and a 3-day dietary record was obtained; however, the LCPUFA content was not measured. The maternal milk FA composition was measured at each visit.[249]

Rocquelin et al. conducted a survey in two random samples of nursing mothers and their 5-month old infants born at term—102 participants in Congo and 101 in Burkina Faso.[302] The report failed to describe the inclusion and exclusion criteria. The dietary habits of the mothers was established using a Food-frequency questionnaire. The outcomes measured were the growth patterns (weight and height from birth to 5 months of age).[302] The maternal age, height, BMI, and maternal occupation did not differ significantly between both locations, however, maternal education was significantly superior in participants in Congo compared with those in Burkina Faso. The characteristics of the participants' homes (i.e., electricity, refrigerator, private water supply, private toilets, radio set, TV set) were significantly different between cities.[302]

The feeding practices of the mothers were measured in each location. None of the infants were exclusively breastfed. All the infants in Burkina Faso were receiving extra fluids (e.g. water or juice) compared with 51% of Congo infants. However, the Burkina Faso infants had a significantly higher proportion of predominance of breast feeding and exclusion of solid foods. The LCPUFA content in breast milk and foods given to the infants were measured at both sites. The breast milk fat content was slightly lower in mothers in Congo. The content of omega-6 FA in the human milk of women in Burkina Faso was significantly higher than in Congo, yet it provided significantly lower (half) concentrations of omega-3 FA. Consequently, the LA omega-6/ALA omega-3 ratio and the LC omega-6/LC omega-3 ratio were 4.3 and 4.5 times higher, respectively, in Burkina Faso than in Congo.

The fat and PUFA concentrations in flours fed as gruels were predominantly from corn and millet. In Burkina Faso, infants also received commercial infant formula (Cerelac) containing LA (800 mg/100g), ALA (29 mg/100g) (i.e., LA/ALA=28.0). In Congo, the FA content was LA 1,080 mg/100g, ALA 73 g/100g (i.e., LA/ALA=14.8).[302]

Summary Table 17: Omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences growth patterns in term or preterm human infants

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Study groups[1] Group 2 (n)/ Group 3 (n)	Notable clinical effects	Internal validity	Applicability
Jensen, 1999, US: 120 d parallel RCT[248]	DHA algal (n=42)	High DHA fish oil (n=42)/ pb (n=42)	NS in wt, length & HC at 4-8 mo	Not assessed	X
Xiang, 2000, Sweden: Single prospective cohort[249]	Mother-breastfed term infants (n=19)	n/a	LA, ALA in maternal milk S↑ during 3 mo DHA in maternal milk S↓ during 3 mo AA/DHA in maternal milk S correlated with infants' rate ↑HC at 1 & 3 mo^{++} AA/DHA in maternal milk S correlated with infants' brain wt gain at 1 & 3 mo^{++}	Quality score: 5 [Grade B]	III
Rocquelin, 2003, The Congo & Burkina Faso: Cross-sectional study[302]	Mother-breastfed term infants Congo (n=102)	Mother-breastfed term infants Burkina Faso (n=101)	S↓ wt-for-age & wt-for height z-scores & wt gain (g) in Burkina Faso than in Congo^{++++} NS birth wt, age, weight gain of predominantly breastfed to complementary fed infants in Burkina Faso	Quality score: 5 [Grade B]	III

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; N/A = not applicable; grp = group; wk = week(s); mo = month; wt = weight; $^{+}$p<.05 or significant with 95% confidence interval; ++p<.01; +++p<.001; ++++p<.0001; ↑ = increase; ↓ = decrease/reduction; HC = head circumference;

In the Xiang et al. study, the LC PUFAs fraction (13.5% of total FA) in human milk (LA and ALA) increased significantly during the 3 months of lactation, whereas, DHA decreased significantly but not the EPA maternal milk content.[249] The ratio of AA to DHA in the mother's milk correlated positively with the infants' rate of increase of HC at 1 month and 3 months of age, as well as with the gain in estimated brain weight at 1 and 3 months of age. No relations were found between HC or estimated brain weight and LA, ALA, AA or DHA content in human milk.[249]

Infants in Rocquelin et al.'s study did not differ in gender, percentage of LBW (<2,500 g), birth weight or length, between the two sites.[302] However, the infants in Congo were significantly younger than in Burkina Faso. The weight-for-age and weight-for height z-scores and weight gain (in grams) were significantly lower in infants in Burkina Faso than in those in Congo.

When comparing the anthropometric data (birth weight, age, weight gain) of predominantly breastfed to complementary fed infants in Burkina Faso, no differences between groups were detected. Since both populations were extremely different, the analysis of the relationship between the FA content in breast milk and anthropometric data between cities was excluded from the review.[302]

Study quality and applicability. Jensen et al. was not assessed by Jadad scale give that it was an abstract.[248] Both observational studies had a mean total quality score of 5, and a level of applicability of III.[249,302]

Summary Matrix 8: Omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences growth patterns in term or preterm human infants

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author	Year	n	Author	Year	n	Author	Year	n
	III	Author	Year	n	Author Xiang Rocquelin	Year 2000 2003	n 19 203	Author	Year	n

n = number of allocated/selected participants; [U] = unclear allocation concealment

What is the Evidence That the Omega-3 Fatty Acid Content of Infant Formula Influences Growth Patterns in Term or Preterm Human Infants?

What is the Evidence That the Omega-3 Fatty Acid Content of Maternal Breast Milk, With or Without Known Maternal Intake of Omega-3 Fatty Acids, and Together With the Omega-3 Fatty Acid Content of Infant Formula, Influences Growth Patterns in Term or Preterm Human Infants?

Infant Formula Intake—Preterm Infants.

Twenty double-blinded RCTs met eligibility criteria for investigating a possible effectiveness of omega-3 fatty acid content of infant formula on growth patterns in preterm infants. Studies were published between 1987 and 2004. (Summary Tables 18–21)

Overview of relevant studies

All of the included studies assessed the effect of omega-3 FA content of infant formula on growth patterns in preterm human infants. One study evaluated the effect of maternal breastfeeding together with the intake of omega-3 FA supplemented formula on growth patterns

in preterm infants, as well as the effect of omega-3 FA content of infant formula on growth parameters.[253] With the exception of the three Carlson et al. studies,[185,191,250] as well as the studies of Clandinin et al.,[193] Groh-Wargo et al.[256] and Fewtrell et al.,[258] all studies included a non-randomized group of breastfed infants that served as a reference standard.

Carlson et al. conducted a study involving 61 preterm infants (<1500 g) with no major congenital abnormalities and major medical conditions.[250] The infants were randomized to receive either preterm control formula (Similac Special care, or Enfamil Premature) or fish oil supplemented infant preterm formula for 4 weeks. (Summary Table 18)

In another study by Carlson et al., 79 preterm, premature infants weighed less than 1400 g were randomly assigned to receive either control or marine oil-enriched preterm infant formulas (DHA [0.2wt%], EPA [0.3wt%]), followed by term placebo and experimental formulas (DHA [0.2wt%], EPA [0.3wt%]) for up to 57 weeks postconceptional age (PCA).[185] (Summary Table 18)

Koletzko et al. compared LCPUFA supplemented preterm formula containing DHA (0.3wt%), EPA (0.03wt%) and AA (0.05wt%) with a control formula in a small study involving 19 preterm babies with a weight less than 1850 g.[251] Infants were followed for a period of 21 days of full enteral feeding.[251] (Summary Table 18)

Uauy et al. randomized 60 preterm infants with a birth weight of 1,000 g to 1,500 g and no major neonatal morbidity by the tenth day of life, to receive one of three formulas for 6 months.[212] The feeding formulas differed only in the amounts and sources of LCPUFAs—two control formulas contained no added LCPUFAs and had different amount of 18:2 n-3 and 18:2 n-6 FAs, whereas, the experimental formula contained additional LCPUFAs derived from marine oil (DHA [0.35wt%], EPA [0.65wt%] and AA [0.1wt%]). (Summary Table 18)

Carlson et al. enrolled 59 preterm infants with or without bronchopulmonary dysplasia and randomly assigned them to receive standard preterm formula, which contained linolenic acid as 2.5% of total FA (Similac Special Care) or a formula that provided n-3 LCPUFAs from marine oil (DHA [0.2wt%] and EPA [0.06wt%]) but did not differ otherwise from the standard formula.[191] Randomization took place between 3 and 5 days of life and formula intake continued for up to 2 months PCA.[191] (Summary Table 18)

Faldella et al. recruited 46 preterm infants less than 33 weeks GA with no neurological, visual, acoustic, or gastrointestinal illnesses and randomly assigned them to a formula for preterm infants enriched with marine oil derived LCPUFAs (Preaptamil with Milupan) containing DHA (0.3wt%), EPA (0.05wt%), and AA (0.44wt%) or a traditional formula for preterm infants.[198] Feeding regimens continued up to 52 weeks of PCA.[198] (Summary Table 18)

Summary Table 18: Omega-3 fatty acids and growth parameters of preterm infants

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)				
Carlson, 1987 US: 4 wk parallel RCT[250]	MaxEPA preterm formula (n=30)	Preterm formula (n=31)	NS in Δ wt at 4 wks	n/a	Jadad total: 2 [Grade: C]; Schulz: Unclear	II
Carlson, 1992 US: up to 57wk PCA parallel RCT[185]	marine oil (DHA+AA) formula (n=31*)	Control formula (n=34*)	S↓ wt, L, HC in marine oil at 40, 48, 57, 68, 79, 93 wks PCA[+]	wt & L z-scores correlated + with plasma & RBC AA at 2,4,5,6,9, 12 mo HC correlated + plasma & RBC AA at 2, 4 mo	Jadad total: 4 [Grade: A]; Schulz: Adequate	II
Uauy, 1992 US: 6 mo parallel RCT[212]	Soy/ marine oil formula (n=22)/ HM (n=10)	Soy oil formula (n=18)/ corn oil formula (n=20)	NS in wt, L, HC, TST, SST at 3, 9, 17, 26 wks	S correlation (-) between RBC AA at 57 wk & length z score at 57 wks PCA	Jadad total: 2 [Grade: C]; Schulz: Unclear	II
Koletzko, 1994 Germany: 3 wk parallel RCT[251]	Egg lipids + primrose oil formula (DHA+EPA) (n=9)	Control formula (n=10)/ HM (n=8)	NS in wt, L, HC at 3 wks	n/a	Jadad total: 2 [Grade: C]; Schulz: Unclear	III
Carlson, 1996, US: 5 mo parallel RCT[191]	Marine oil (DHA +EPA) formula (n=26)	Control formula (n=33)	S↓ wt, L, HC in LCPUFA at 6[+], 9[++] mo PT	S (-) correlation between wt-for-L & RBC PE DHA at 5 mo S (+) correlation between L & RBC PC AA at 5 mo	Jadad total: 3 [Grade: B]; Schulz: Unclear	II
Faldella, 1996 Italy: up to 52 wk PCA parallel RCT[198]	DHA+EPA formula (n=23)	Control formula (n=26)/ HM (n=17)	NS in Δ wt, ΔL, ΔHC at 52 wks PCA	n/a	Jadad total: 1 [Grade: C]; Schulz: Unclear	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; FAs = fatty acids; * = completed study; PCA = postconceptional age; ITT = intention to treat study; HM = human milk group; wt = weight; L = length; HC = head circumference; Δ = change; RBC = red blood cells; [+]p<.05 or significant with 95% confidence interval; [++]p<.01; [+++]p<.001; [++++]p<.0001; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower; PE: phosphatidyl ethanolamine; PC: phosphatidyl choline; TST = triceps skinfold thickness; SST = subscapular skinfold thickness

Vanderhoof et al. conducted a double-blinded RCT of two formula-fed groups and a parallel reference group of breastfed infants. Medically-stable preterm infants with a birth weight ranging from 750 g to 2000 g were assigned to receive either control preterm formula (Preemie

SMA®) or LCP-supplemented Preemie SMA (DHA [0.35wt%], AA [0.5wt%]) for up to 48 weeks PCA.[218] (Summary Table 19)

Lapillone et al. evaluated 33 preterm infants appropriate for GA who were randomized to receive either standard preterm formula from inclusion to 40 weeks term corrected age (CA), then a standard term formula until 4 months CA, or preterm formula enriched with the fish oil containing DHA (0.37wt%) and EPA (0.05wt%) until 40 weeks CA and then a term formula supplemented with a fish oil containing DHA (0.45wt%) and EPA (0.09wt%) until 4 months CA.[252] A reference group of 10 breastfed infants was also recruited for the trial.[109] (Summary Table 19)

Martinez et al. assessed 40 preterm infants (VLBW) who received in a double-blinded fashion either LCPUFA supplemented or control formula for 30 days. A group of 18 breastfed infants served as reference standard. The outcomes were the weight, length and head circumference at 30 days.[120] (Summary Table 19)

Woltil et al. conducted a double-blind RCT where preterm newborn babies were allocated to receive two experimental formulas supplemented with evening primrose oil and either a single (DHA [0.20wt%] and EPA [0.17wt%]; n=13) or double dosage (DHA [0.43wt%] and EPA [0.34wt%]; n=16) of purified fish oil, and three control formulas containing different amount of protein and ribonucleotides.[225] Dietary intake took place for 6 weeks. Thirty-three infants received their mother's own milk.[225] (Summary Table 19)

Ghebremeskel et al. randomized healthy preterm infants with no congenital malformations and metabolic disorders into four feeding groups: (1) breast milk and LCP-enriched formula (0.85±0.25wt% DHA); (2) breast milk and standard formula (0.55±0.25wt% DHA); (3) LCP-supplemented formula (0.30wt% DHA); or, (4) exclusively standard formula.[253] Mean duration of an intervention was 11 weeks with a range of 7 to 15 weeks. Twenty exclusively breastfed infants formed a standard reference group. (Summary Table 19)

Summary Table 19: Omega-3 fatty acids and growth parameters of preterm infants

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
Vanderhoof, 1997, US: Up to 48 wk PCA parallel RCT[218]	Microbial fermentation (DHA+AA) formula (n=77)	Control formula (n=78)/ HM (n=133)	S↑ wt, L, HC, MAC in LCP & control than in HM at 40 wk PCA[+] NS in L, HC at 48 wks PCA S↑ L, MAC in LCP than in HM at 48 wks PCA[+] NS in wt, L, HC at 92 wks PCA	n/a	Jadad total: 4 [Grade: A]; Schulz: Adequate	I
Lapillonne, 1997, France: 4 mo CA parallel RCT[252]	DHA+ EPA formula (n=11)	Control formula (n=12)/ HM (n=10)	NS in GP at 4 mo CA	n/a	Not assessed	X
Martinez, 1999, Brazil 30 d parallel RCT[259]	Egg-lipid + primrose oil (formula (n=20)	Control formula (n=20)/ HM (n=18)	NS in wt, L, HC at 30 d	n/a	Jadad total: 1 [Grade: C]; Schulz: Unclear	III
Woltil, 1999, Netherlands 6 wks parallel RCT[225]	High-DHA formula (n=16)/ HM (n=33)	Low-DHA formula (n=13) pb-1 (n=13)/ pb-2 (n=37)/ pb-3 (n=31)	NS in Δ wt, ΔL, & ΔHC between LCP-1, LCP-2 & pb at 1 mo S↑ Δ wt, ΔL, Δ brain wt, ΔHC in pb-1 than in pb-2 & pb-3 at 1mo[+]	S (+) correlation between Δwt, ΔL, ΔHC & plasma & RBC DHA at 1mo	Jadad total: 1 [Grade: C]; Schulz: Unclear	III
Ghebremeskel1999, UK: 11 wk parallel RCT[253]	Egg-lipid+ primrose oil (DHA+AA) +HM (n=12)/ control formula (n=8)	LCP formula (n=7)/ control formula+HM/ (n=14)/ HM (n=20)	NS in wt, L, HC at ≈11 wk among 5 grps	n/a	Jadad total: 2 [Grade: C]; Schulz: Unclear	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; [†] = mg/kg/day; pb = placebo; grp = group; wk = week(s); mo = month; FAs = fatty acids; PCA = post conceptional age; CA = corrected age; HM = human milk group; wt = weight; L = length; HC = head circumference; MAC = mid arm circumference; Δ = change; GP = growth parameters; RBC = red blood cells; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); [+]p<.05 or significant with 95% confidence interval; [++]p<.01; [+++]p<.001; [++++]p<.0001; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower

Bougle et al. conducted a small efficacy study involving healthy, AGA premature infants of less than 34 weeks postmenstrual age, who were randomized into two groups within the first 2 days of enteral feeding—LCP-supplemented (DHA [0.6wt%], EPA [0.1wt%] and AA [0.1wt%]; n=14) or control formula without any LCPUFA supplementation (n=11).[254] The end of the study occurred at the expected date of delivery, after babies were fed for at least 1 month on the study diet.[254] (Summary Table 20)

Field et al. conducted a double-blind RCT in which 44 medically-stable preterm newborn babies were allocated to receive either preterm formula (Preemie SMA) or the same formula manufactured to contain LCPUFAs (DHA [0.35wt%] and AA [0.49wt%]).[303] Feeding of formulas began before day eight of postnatal life and continued until day 42. Seventeen exclusively breastfed infants were included as a reference group.[303] (Summary Table 20)

O'Connor et al. randomized 283 preterm infants of less than 33 weeks GA without any congenital abnormalities to one of three formula groups received in-hospital: (1) control; (2) treatment formula with supplemental LCPUFAs derived from fish/fungal oils (0.27±±0.04 g/100g DHA, 0.08±0.01 g/100g EPA, and 0.43±0.02 g/100g AA); or (3) treatment formula with supplemental LCPUFAs derived from egg-triglycerides/fish oils (0.24±0.01 g/100g DHA and 0.41±0.0 g/100g AA).[207] After discharge, infants received postdischarge formulas with the same content of AA, but reduced amount of DHA (0.16±0.01 g/100g DHA in fish/fungal oil group and 0.15±0.02 g/100g DHA in egg-triglycerides/fish oil group). The intervention lasted up to 12 months PCA. (Summary Table 20)

Fewtrell et al. recruited 195 preterm infants with no congenital malformations and randomized them to receive either preterm infant formula without additional LCPUFA (Prematil, Milupa) or a supplemented formula (Prematil with Milupan) containing DHA (0.17wt%) and EPA (0.04wt%) from egg lipids.[273] All infants were fed and followed for up to 9 months PCA. A group of 88 breastfed infants formed a reference group.[273] (Summary Table 20)

Clandinin et al., in a double-blind multicenter RCT, randomized LBW infants to one of three feeding groups: (1) control (n=119); (2) LCP-1 (17mg/100kcal DHA and 34mg/100kcal AA, derived from single cell oils, n=112); or, (3) LCP-2 (17mg/100kcal DHA derived from fish oil and 34mg/100kcal AA, derived from single cell oils, n=130).[193] Each group included three formula types: preterm, postdischarge, and term, which investigators chose based on infant needs. Formulas were the infant's sole diet until 57 weeks PCA.[193] (Summary Table 20)

Summary Table 20: Omega-3 fatty acids and growth parameters of preterm infants

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Study groups[1] Group 2 (n)/ Group 3 (n)	Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
Bougle, 1999, France: 1 mo parallel RCT[254]	LCP formula (n=14)	Control formula (n=11)/ HM (n=15)	S↑ Δ wt in LCP than in HM at 1 mo[+] NS in wt, L, HC, ΔL, & Δ HC at 1 mo[+]	n/a	Jadad total: 3 [Grade: B]; Schulz: Unclear	III
Field, 2000 Canada: 5.5 wk parallel RCT[303]	LCP formula (n=15)	Control formula (n=12)/ HM (n=17)	S↓ Δ wt in HM than in LCP & pb at 28 d[+] NS in L, HC at 35 d[+]	n/a	Jadad total: 1 [Grade: C]; Schulz: Unclear	II
O'Connor, 2001 US, UK, Chile: 12 mo CA parallel RCT[207]	DHA+AA (Fish/ fungal oil) formula (n=140)	DHA+AA (Egg-TG/ fish oil) formula (n=143)/ control formula (n=144)	(ITT) NS Δ wt, ΔL, Δ HC at 8 wk, 4 mo, 12 mo CA	S (+) correlation rate wt gain & RBC PE AA at 28 d wt & L S correlated RBC PE AA at 28 d	Jadad total: 3 [Grade: B]; Schulz: Unclear	I
Fewtrell, 2002 UK: 9 mo CA parallel RCT[273]	LCPUFA formula (n=95)	Control formula (n=100)/ HM (n=88)	(ITT) S↓ wt, L in LCPUFA than in pb at 9 , 18 mo CA[+] NS in HC at 9, 18 mo CA	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	II
Clandinin 2002 Canada: 57 wk PMA parallel RCT[193]	DHA+AA (SCO) (n=112)	DHA+AA (fish oil) (n=130)/ control formula (n=119)	NS in GP at 40, 57 wks PMA S↑ wt in DHA+AA (SCO) than in control at 66-118 wks PMA[+] S↑ L in DHA+AA (SCO) than in other 2 formulas at 79, 92 wks PMA[+]	n/a	Not assessed	X

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; FAs = fatty acids; HM = human milk group;wt = weight; L = length; HC = head circumference; AC = arm circumference; Δ = change; RBC = red blood cells; PE = phosphatidyl ethanolamine; PC = phosphatidylcholine; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); [+]p<.05 or significant with 95% confidence interval; [++]p<.01; [+++]p<.001; [++++]p<.0001; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower; PMA = postmenstrual age; GP = growth patterns;CA = corrected age; SCO = single cell oil; TG = tryglicerids

Innis et al. conducted a double-blind, multicenter study of 194 healthy premature, VLBW (846 g-1560 g) infants who were randomized to receive either preterm formula with no DHA or AA (control, n=62), DHA (0.15wt% ; n=66) or DHA (0.14wt%) and AA (0.27wt%) (n=66)

derived from single cell triglycerides, for at least 28 days and then fed term formula with no LCPUFA supplementations for up to 57 weeks postmenopausal age.[201] Ninety breastfed infants served as a reference.[201] (Summary Table 21)

Groh-Wargo et al. evaluated the effect of feeding formula supplemented with DHA (0.42wt%) and AA (0.26wt%) derived from fish/fungal oils (LCP-1 group, n=18) or DHA (0.26wt%) and AA (0.26wt%) derived from egg phospholipid/fish oil (LCP-2 group, n=18) on growth parameters of preterm infants at 12 months of CA compared with infants fed unsupplemented formula (control group, n=21).[256] Randomization of infants took place within 72 hours of first enteral feeding. [256] (Summary Table 21)

Koletzko et al. randomized 30 preterm infants with a stable medical condition and birth weight of less than 1800 g to receive either preterm control formula (n=15) or LCP-supplemented formula (DHA [0.57wt%], EPA [0.13wt%] and AA [0.1wt%]; n=15) within 3 days of established full enteral feeding to 28 days post partum.[257] Nineteen breastfed infants formed a reference group.[257] (Summary Table 21)

Fewtrell et al. randomly assigned preterm infants with a birth weight less than 2000 g and GA less than 35 weeks to unsupplemented (control group, n=116) or LCPUFA-supplemented formula (treatment group; DHA [0.5wt%], EPA [0.1wt%] and AA [0.04wt%]; n=122) until 9 months PCA.[258] (Summary Table 21)

Summary Table 21: Omega-3 fatty acids and growth parameters of preterm infants

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)				
Innis, 2002, US, Canada: 28 d multicenter parallel RCT[201]	DHA+AA formula (n=66)	DHA formula (n=66)/ control formula (n=62)	S↑ Δ wt in DHA+AA than in control at 40 wks PMA^{++} S↑ wt, L, wt-to-L in DHA+AA than in DHA at 48 wks PMA^{++} S↑ wt, wt-to-L in DHA+AA than in control at 48 wk PMA^{++} NS in HC at 48, 57 wk PMA	S (+) correlation between Δ wt & RBC PE AA at 8 wks S (+) correlation between wt, L & RBC PE AA at 8 wks	Jadad total: 3 [Grade: B]; Schulz: Unclear	I
Groh-Wargo, 2002, Canada, US: 12 mo CA parallel RCT[256]	LCP-1 (n=18)	LCP-2 (n=18)/ control formula (n=21)	NS in GP at 12 mo CA	n/a	Not assessed	X
Koletzko, 2003 Germany: 28 days parallel RCT[257]	LCP formula (n=15)	Control formula (n=15)/ HM (n=19)	NS wt, L, HC at 28 d	n/a	Jadad total: 3 [Grade: B]; Schulz: Unclear	III
Fewtrell, 2004 UK: 9 mo CA parallel RCT[258]	LCPUFA formula (n=122)	Control formula (n=116)	(ITT) S↑ Δ wt, ΔL in LCPUFA than in control at 9 mo CA^{+} NS in HC at 9 mo CA NS in PG at 18 mo CA	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	II

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; FAs = fatty acids; ITT = intention to treat; HM = human milk group; wt = weight; L = length; HC = head circumference; GP = growth parameters; PMA = post menstrual age; PT = post term; CA = corrected age; Δ = change; RBC = red blood cells; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. All studies were parallel RCTs with at least two groups, although the study of Ghebren et al. involved five feeding groups.[253]

The inclusion/exclusion criteria were described by 11 of 20 studies.[198,201,207,212,218,250,251,253,257,259,303] Only inclusion criteria were reported in four studies[225,254,258,273] and only exclusion criteria were reported in two studies.[185,191] Three studies failed to report either inclusion or exclusion criteria.[193,252,256] Three studies defined maternal substance abuse (cocaine and alcohol) history as exclusion criteria.[191,207,218] The definition of a preterm infant (<37 weeks GA) was described in eight studies,[198,251,253,254,258,259,273,303] although included preterm infants in these studies were at different GAs. Koletzko et al.[251] and Fewtrell et al.[273] included infants less than 37 weeks GA, whereas, Field et al.[303] evaluated infants born at less than 36 weeks GA, Fewtrell et al.[258] at less than 35 weeks GA, Bougle et al.[254] at less than 34 weeks GA, and Faldella et al.[198] and Ghebremeskel et al.[253] at less than 33 weeks GA. Eight studies were typically small, with a mean of 30 participants (range 19–41).[251-254,256,257,259,303] The study duration ranged from 3 weeks to 12 months.

The trials were conducted in various countries, with five undertaken in the U.S.,[183,185,191,212,218,250] three in the U.K.[253,258,273] and Canada,[193,201,303] two in France[252,254] and Germany,[251,257] one in Italy,[198] one in Brazil,[259] and one in The Netherlands.[225] One multicenter study was conducted in three countries—the U.S., U.K and Chile.[207] Groh-Wargo et al. failed to indicate the country where their study was undertaken.[256]

The study of Carlson et al.[250] was supported by Ross laboratories, Columbus, OH. Another Carlson et al. study was sponsored by Ross Laboratories, Columbus, OH, and the National Eye Institute.[185] Koletzko et al. received a grant from Deutsche Forschungsgemeinschaft, Bonn, Germany and Milupa AG, Friedrichsdorf, Germany.[251] Uauy et al.'s study was financially supported by the National Institute of Health.[212] The Carlson et al. study[191] was funded by the National Eye Institute, the National Institute of Child Health and Human Development, and Ross Products Division, Abbott Laboratories, Columbus, OH. Vanderhoof et al.'s study was supported by Wyeth Nutritionals International, Philadelphia, Pennsylvania, U.S.A.[218] Martinez et al. was funded by the Brazilian Research Council and Milupa GmbH.[259] Woltil et al.'s study was supported by grants from Friesland Nutrition, Leeuwarden, The Netherlands.[225] The study of Ghebremeskel et al. was financed by The Christopher H.R. Reeves Charitable Trust and Milupa Plc.[253] Field et al.'s study was supported by grants from the Natural Sciences and Engineering Research Council of Canada and the Medical Research Council of Canada, as well as Wyeth-Ayerst Research.[303] Fewtrell et al.'s study was funded by Numico Research BV (Wageningen, The Netherlands).[273] Clandinin et al.'s[193] and Innis et al.'s[201] studies were financed by grants from Mead Johnson & Company. The Groh-Wargo et al. study was supported by Abott Laboratoris, Columbus and GCRC NIH.[256] Koletzko et al.'s study was sponsored by Deutsche Forschungsgemeinschaft, Bonn, Germany, Nestec S.A., Vevey, Switzerland and Nestle Alete GmbH, Munchen, Germany.[257] Fewtrell et al.'s study was supported by grant from H.J. Heinz Company, Ltd, Hayes, Middlesex, U.K.[258] Four trials did not provide information concerning their funding source.[198,207,252,254]

In general, six studies[193,201,218,225,250,258] were granted only by pharmaceutical companies, six studies[185,191,251,256,257,303] by both pharmaceutical and governmental fundings, two studies[212,273] by only governmental sources, and one study[253] partly by private and pharmaceutical sources.

Pre-study sample size calculation to reach statistical significance and power was performed in seven studies.[191,201,207,212,218,258,273]

Population characteristics. A total of 2,650 preterm infants were enrolled across 20 RCTs. The total number of infants that completed the trials could not be calculated since six of the studies failed to report these data, providing only the number of infants who entered the trial.[193,218,225,252,256,259]

Eligibility criteria varied broadly across studies. Most importantly, body mass of recruited preterm infants, GA, and age at study enrollment, differed substantially from trial to trial. Some investigators randomized very small preterm infants (i.e., weighing less than 1,400 g to 1,500 g),[185,201,212,250,259] whereas, other authors broadened their criteria to include preterm infants with a birth weight ranging from 2,000 g to 2,500 g.[218,225,258] Six studies failed to report predefined eligibility criteria regarding infant's weight.[191,198,253,254,256,303]

The gender distribution of randomized infants was reported in ten studies.[191,207,212,218,225,257-259,273,304] In eight of these studies, male infants constituted the majority of participants, although the gender ratio of infants among different diet groups were evenly distributed in all of these studies.

The racial/ethnic background of study participants were described in only four trials.[191,207,212,218] In two studies,[191,212] the majority of infants were Black, accounting for 60% and 83% of study population, respectively. In the two other trials,[207,218] White infants comprised the majority of study participants accounting for 58% and 70% of participants, respectively.

Different variables were used to demonstrate family sociodemographic status in the studies (e.g., maternal education, social class, professional qualification, home inventory score, maternal WAIS-R raw vocabulary score). Maternal social status was determined in two studies,[258,273] whereas, information about maternal education or maternal professional qualification was given in three trials.[207,258,273] O'Connor et al. measured and compared the quality and quantity of stimulation and support available to a child in the home environment in different groups by means of a HOME inventory score. Maternal intelligence was assessed by administering a WAIS-R raw vocabulary score.[207] There were no differences in sociodemographic variables among the study groups of randomized infants in all of these studies with the exception of HOME inventory scores, which were better in the control group than in both treatment groups.[207] Mothers of infants in the reference breastfed group had a more prestigious social score and attained a higher level of professional qualification compared with mothers of formula-fed infants.[273]

Only one study reported on maternal smoking during pregnancy and postnatal smoking in the home.[207]

Intervention/exposure characteristics. Only one of 20 reviewed studies reported the exact amount of supplementary LCPUFAs consumed per day by the preterm infants.[225] Woltil et al.[225] assigned preterm infants to two LCPUFA-supplemented feeding groups with different anounts of DHA—group LCP-1 consumed 23.3±9.9 mg/kg/day DHA, whereas group LCP-2 consumed

13.3 to 41.8 mg/kg/day DHA. The rest of the studies failed to indicate daily consumption of omega-3 FAs by feeding infants. In all studies, formula-fed infants received preterm formula and depending on the ultimate interest of the research project, went to either post-discharge or term formula. In fourteen studies, the effect of only preterm formulas with supplemented LCPUFAs on growth indices of preterm infants were assessed.[191,193,198,212,218,225,250,251,253,254,257,259,273,303] In six studies, infants continued to receive a formula designed for term infants (with or without LCP supplementation) according to their original assignments, and their effect on each child's growth was further estimated.[185,201,207,252,256,258]

Preterm infants were eligible to enter the study after they attained full enteral feeding without intravenous support. The minimum amount of formula-intake required in order to be considered fully enteral-fed differed across the trials. In two studies infants became eligible to enroll when they received at least 130 mL/kg/day of a preterm formula.[251,257] Carlson et al. allowed preterm infants to enroll in the study after they had reached intakes of nutrient-enriched formula of at least 60 kcal/kg/day,[250] whereas, Carlson et al. established criteria for enrollment of more than 110 kcal/kg/day.[185] Enteral feeding of at least 70 to 120 kcal/kg/day was required in the trial of Uauy et al.;[212] Carlson et al. required an intake of at least 100 kcal/kg/day.[191] Vanderhoof et al. specified an intake of 145 mL/kg/day,[218] whereas, Woltil et al. required an intake of 80 kcal/kg/day.[225] In the Martinez et al. study, an intake of 112 kcal/kg/day was indicated[259] and Innis et al. specified an intake of at least 90 kcal/kg/day.[201] Eight studies failed to report a minimum daily food or caloric intake required for preterm infants.[198,207,253,254,256,258,273,303]

Only two studies reported as part of the protocol that the volume of formula consumed, i.e., calculated as the difference in the volume of formula in the bottle at the start and end of the feed, was recorded.[185,225] Daily intake of formula did not differ in the three feeding groups of Woltil et al. (171 [SD=21] mL/kg vs 172 [SD=17] mL/kg vs 176 [SD=17] mL/kg).[225] In a study of Carlson et al.,[185] all except one infant consumed at least 720 g of formula per day through 79 weeks PCA. Duration of formula feeding ranged from 3 weeks[251] to 12 months CA.[207,256]

The sources of omega-3 FA intervention varied across the RCTs. Three trials described the source of LCPUFA supplementation as purely fish oil.[225,250,252] The specific type of fish from which fish oil exposures were derived was described in only one study.[258] O'Connor et al.[207] and Groh-Wargo et al.[256] used a treatment formula with omega-3 FAs derived from fish and fungal oils, whereas, Fewtrell et al.[258] supplemented a treatment formula with a combination of DHA derived from fish oil and AA derived from borage oil. The remaining studies employed either single cell sources of FAs,[193,201,218,303] marine oils,[185,191,212] egg phospholipids with primrose oil,[253,259,273] or a combination of egg triglyceride and fish oil sources.[207,256,257] The sources of supplemental LC PUFAs were not reported in three trials.[198,251,254]

The type of omega-3 FA employed in four studies included a combination of DHA and EPA;[185,191,225,252] DHA alone was used in one trial.[201] Supplementation of formulas with omega-6 FA AA was reported in 12 studies.[193,198,201,207,213,218,253,254,256,258,273,303]

Seven studies failed to report the name of feeding formulas, although all of them indicated the manufacturers of the product.[185,193,212,225,252,254,256] The brands of formulas employed in the rest of the studies were: Enfamil Premature (Mead Johnson Nutritionals, Evansville, Ind);[201,250] Similac Special Care (Ross Laboratories, Columbus, OH);[191,207,250] Prematil with Mipupan

(Milupa, AG, Friedrichsdorf, Germany);[198,251,253,259,273] SMA "Preemie" (Wyeth-Ayerst Laboratories, Randor, Philadelphia, PA);[218,250,303] Alprem (Nestle, Vevey, Switzerland);[257] OsterPrem with LCPUFA (Heinz Co, Ltd, Hayes, Middlesex, U.K.);[258] NeoSure (Ross Product Division, Columbus, OH, USA);[207] and, Farley's PreCare with LCPUFA (Heinz Co, Ltd, Hayes, Middlesex, U.K.) were used as a term formulas after hospital discharge. Five studies indicated the manufacturer of at least one omega-3 FA product used in their study.[201,212,218,250,303] In three of these trials supplemented LCPUFAs were manufactured and supplied by Market Biosciences Corporation (Columbia, MD, USA),[201,218,303] whereas, in two other studies omega-3 FAs were produced by MaxEPA, R.P. Scherer, Troy, MI[250] and Zapata-Haynie Co., Reedville, Va.[212] Only one study reported on the purity of their omega-3 FA exposure.[225]

Formula was the only source of alimentation in 14 studies and no solid foods were introduced during the entire trial period.[185,191,193,198,201,218,225,250,251,253,254,257,259,303] Only one study reported the time of introduction of solid foods—Uauy et al.[212] permitted cereals, fruit juices, or fruits at 4 months of CA in both study groups. Fewtrell et al.,[273] Groh-Wargo et al.,[256] Fewtrell et al.,[258] and O'Connor et al.[207] did not report of any solid food introduction to infants even though their study durations were up to 9 and 12 months CA.

Information about caloric balance of feeding formulas was reported in eight RCTs.[185,191,201,212,225,254,259,273] Nutritional and energy intake were similar between randomized groups throughout the study period in the majority of trials. However, Carlson et al. reported that the mean energy intake from formula was not affected by dietary assignment or gender at 48 and 57 weeks PCA; however, at 68 weeks PCA, infants consuming the marine oil-supplemented formula had significantly higher energy intake from formula compared with infants fed standard formula.[185]

Only three RCTs[212,258,273] mentioned that study treatment formulas were indistinguishable in appearance and odor. Uauy et al. also reported that supplemental marine oil was winterized and stabilized.[212]

Cointervention characteristics. Six studies reported the content of vitamin and mineral supplements of feeding formulas or multivitamin preparations taken by preterm infants.[191,207,212,251,253,303] All of these formulas or oral vitamin supplements provided alpha-tocopherol ranging from 4.5 mg/day[303] to 15 mg/day.[253] Ghebremeskel et al. used a formula also supplemented with 0.22 μmol/100 mL vitamin A.[253] The formula used by O'Connor et al.[207] was supplemented with 0.60 mg/L vitamin A and 0.50 mg/L beta-carotene, and Field et al. added 1200 U/day vitamin D to their infant formulas.[303]

Due to the physical immaturity of LBW preterm infants, many of the newborns required pre- or on-study medical cointerventions, such as oxygen supply, mechanical ventilation, intravenous nutrition, blood or blood product transfusion, and corticosteroid treatment. The most frequently reported cointervention was oxygen supply or mechanical ventilation and measurements were provided in four studies.[185,191,212,258] Carlson et al.[191] allowed a significant subgroup of patients (n=23) who continued to require supplemental oxygen for 28 days and had lung changes on X-ray characteristic of bronchopulmonary dysplasia, to remain in the study. Two studies reported use of blood or blood products.[212,303] Uauy et al. described that only five preterm infants required blood transfusion after random assignment, and all transfusions were given at least 2 weeks before blood sampling.[212]

In the study of Field et al., two infants received an intravenous bolus of albumin on day 2 of life.[303] Some investigators set strict inclusion criteria for infants requiring additional medical treatment. Vanderhoof et al., for example, excluded preterm infants with consistent requirements for oxygen at 36 weeks PCA and administration of more than a 5-day course of corticosteroids.[218] In studies of Koletzko et al.[251] and Koletzko et al.,[257] infants requiring artificial ventilation or an oxygen supply with FiO_2 >0.3 at the time of enrollment were excluded. Uauy et al. reported that no infants had used a ventilator after day 5 or for more than 3 days.[212] None of the participants received corticosteroids, red blood cells and plasma transfusions or intravenous lipid emulsions beyond day 8 of life in the Field et al. trial.[303] However, none of these studies reported how many newborn babies received cointerventional measures below the set limit.

Outcome characteristics. Of 20 trials, 12 assessed the growth parameters as primary outcomes[123,150,305-314] while the remaining eight trials evaluated them as a secondary outcome or part of the safety profile. Thirteen included RCTs employed infants' weight, length, and HC as main outcome measures for growth.[185,191,193,201,212,218,251,253,254,257,259,273,303] Two trials (abstracts) did not specify the growth indices evaluated, rather they described changes in growth parameters.[252,256] Carlson et al. evaluated only weight gain from birth to 4 weeks of study period in two randomized dietary groups.[250] The rate of gain in weight, length and HC were assessed in five studies.[198,207,225,254,258] Triceps skinfold thickness and subscapular skinfold thickness were measured in two RCTs.[212,259] Another study evaluated mid-arm circumference,[218] another measured weight-to-length ratio,[201] and one study used estimated brain weight gain in preterm infants as one of the growth outcomes.[225]

Study quality and applicability. Seventeen (of twenty) RCTs received a mean Jadad total quality score of 2.64, indicating a poor internal validity (Summary Matrix 9). Three abstracts were not quality assessed.[306,311,313] The trials conducted by Fewtrell et al. received a score of 5,[258,273] Carlson et al. and Vanderhoof et al. received a score of 4,[185,218] five trials received a score of 3,[191,201,207,254,257] four reports received a score of 2,[212,250,251,253]and four received a score of 1.[198,225,259,303] Eleven trials failed to report the method of randomization,[123,305,309,312,314-320] while one study reported an inappropriate method of randomization.[308] Seven trials were unblinded,[309,310,315-318,320] seven trials failed to report the double-blinding method,[123,150,305,308,312,314,319] and six trials did not report the reasons for dropouts.[305,307-309,317,320]

Summary Matrix 9: Omega-3 fatty acids and growth parameters of preterm infants

		Study Quality								
		A			B			C		
Applicability	I	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Vanderhoof[A]	1997	288	O'Connor[U]	2001	470			
					Innis[U]	2002	194			
	II	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Carlson[A]	1992	79	Carlson[U]	1996	36	Carlson[U]	1987	61
		Fewtrell[A]	2002	283				Uauy[U]	1992	81
		Fewtrell[A]	2004	238				Ghbremeskel[U]	1999	61
								Field[U]	2000	44
	III	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
					Koletzko[U]	2003	49	Koletzko[U]	1994	27
					Bougle[U]	1999	40	Faldella[U]	1996	66
								Woltil[U]	1999	143
								Martinez[U]	1999	40
n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment										

Qualitative synthesis of individual study results

The most frequently investigated outcomes across the reviewed studies were infant weight, length, and HC. Weight and/or weight gain was evaluated in all trials, and infant's length and/or length gain was evaluated in all but one[250] trial. The majority of the studies did not find any statistically significant difference between randomized groups regarding these two parameters at different time points. Carlson et al.,[250] who randomized preterm infants to receive either preterm control formula or MaxEPA supplemented infant preterm formula for 4 weeks, did not find any better weight and length gain in the treatment group. Similar results were obtained at 3 weeks in the study of Koletzko et al.,[251] at 3, 9, 17, and 26 weeks in the study of Uauy et al.,[212] at 52 weeks PCA in the Faldella et al. study,[198] at 92 weeks PCA in the Vanderhoof et al. study,[218] at 4 months of CA according to Lapillonne et al.,[252] a mean of 11 weeks according to Ghebremeskel et al.,[253] at 1 month in three studies.,[225,254,259], at 8 weeks, 4, and 12 months CA in the study of O'Connor et al.,[207] at 40 and 57 weeks postmenstrual ages in Clandinin et al.,[193] at 12 months CA according to Groh-Wargo et al.,[256] and at 28 days of age in the study by Koletzko et al.[257]

Three studies revealed statistically significant weight and length gain in LCPUFA-supplemented diet groups compared with placebo.[193,201,258]

Clandinin et al. randomized LBW infants to one of three feeding groups: (1) control LCP-1 (DHA [17mg/100kcal] and AA [34mg/100kcal] derived from single cell oils); (2) LCP-2 (DHA [17mg/100kcal] derived from fish oil); or, (3) AA (34mg/100kcal, derived from single cell oils).[193] The study found a significantly higher weight in the LCP-1 group of infants compared with infants in in the placebo group at 66 weeks to 118 weeks postmenstrual ages. In addition, infants in the LCP-1 group were significantly longer than infants in the LCP-2 or placebo groups at 79 to 92 weeks postmenstrual ages.[193]

Innis et al., who randomly assigned VLBW (846g-1560g) infants to receive either preterm control formula (no DHA or AA), preterm formula containing only DHA (0.15wt%; DHA group) or DHA+AA formula (DHA [0.14wt%] and AA [0.27wt%]; DHA+AA group), found significantly higher body weight, length and weight-to-length ratio in infants in the DHA+AA

group compared with those in the DHA formula group, and significantly higher body weight and weight-to-length ratio in DHA+AA group compared with those in the control group at 48 weeks postmenstrual age.[201] Moreover, infants fed the DHA+AA formula gained weight significantly faster during premature formula feeding than infants fed the control formula. The rate of weight gain of infants fed the formula with DHA was not different from that of infants fed the control formula or the formula with DHA+AA.[201]

The study of Fewtrell et al. involving preterm infants with a birth weight less than 2,000 g and GA less than 35 weeks, found a significantly greater increase in weight and length of infants in the LCPUFA-supplemented formula group (DHA [0.5wt%], EPA [0.1wt%], and AA [0.04wt%]) compared with infants fed unsupplemented control formula at 9 months CA.[258]

Contrary to these findings, three trials revealed statistically significant weight and length gain in infants in the placebo group compared with the LCPUFA-supplemented group, suggesting that omega-3 LCPUFA can have a negative effect on growth of very-low-birth infants.[185,191,273]

The trial of Carlson et al.[185] that compared growth parameters in preterm, premature infants weighed less than 1400 g fed marine oil-enriched preterm infant formula with infants in a placebo group, found that by 40 weeks and continuing throughout infancy (i.e., up to 93 weeks PCA), infants supplemented with marine oil had significantly lower normalized weight, length, HC and weight-to-length ratio than those receiving standard formula.[185]

Carlson et al. randomly assigned preterm infants with or without bronchopulmonary dysplasia to receive standard preterm formula, or a formula that provided n-3 LCPUFAs from marine oil (DHA [0.2wt%] and EPA [0.06wt%]).[191] The investigators reported that n-3 LCPUFA-supplemented infants weighed significantly less than placebo group babies both at 6 and 9 months post term and had significantly lower weight-to-length ratios at 2, 6, 9, and 12 months post term.[191]

Fewtrell et al observed that at 9 and 18 months CA, treatment formula infants were significantly lighter and shorter than control group babies. This weight difference was present in both boys and girls, and it remained significant at 18 months after adjusting for parental smoking, social class, and level of maternal education. [273]

In the three studies where a weight and length gain benefit was observed in LCPUFA supplemented formula fed infants,[193,201,258] investigators used experimental formulas containing AA. Conversely, in trials that showed a decrease in weight,[185,191,273] length,[185,191,273] and HC[185,191] in infants fed LCPUFA-supplemented formula, the formula did not contain AA. It can be assumed that the growth benefit in preterm infants might be attributed to supplemented AA, and omega-3 FAs negatively affect infant weight gain.

Infant HC and/or HC gain was evaluated in all but two trials.[193,250] Most of the studies did not find any statistically significant difference between randomized groups regarding this parameter at different time point. Only two studies[185,191] reported a significantly lower HC in the omega-3 FA supplemented group compared with the placebo group at 40 to 93 weeks PCA[185] and at 6 and 9 months post term.[191] None of the studies revealed any benefit of LCPUFA supplementation regarding the HC gain of premature infants.

Other growth outcomes assessed were triceps skinfold thickness, subscapular skinfold thickness, and mid-arm circumference. Uauy et al. did not find any statistically significant

difference in triceps skinfold thickness and subscapular skinfold thickness among the randomized study groups at 3, 9, 17, and 26 weeks.[212] Martinez et al. had the same result at 30 days.[259] Vanderhoof et al. did not find a statistically significant difference in mid-arm circumference between study groups, although this parameter was significantly lower in the breastfed group.[218]

Carlson et al. found that the weight and length z-scores were positively correlated with the plasma and RBC AA content at 2, 4, 5, 6, 9, and 12 months of age. However, HC was positively correlated at 2 and 4 months of age only.[185] Uauy et al. found that the length z-score at 57 weeks of PCA was negatively correlated with the RBC AA at 57 weeks PCA.[212]

The other Carlson et al. study reported a negative correlation between the weight-for-length z-score and the RBC PE DHA at 5 months of age, whereas, there was a positive correlation between length and RBC PC AA at 5 months.[191] Innis et al. observed a positive correlation between the rate of weight gain and the RBC PE AA at 28 days (end of feeding), as well as the weight and length.[201] Woltil et al. found a significantly positive correlation between the weight, length and HC gain and the plasma and RBC DHA content at 1 month of age.[225]

Finally, O'Connor et al. found a significantly postive correlation between the rate of weight gain, weight (mean) and length (mean) and the RBC AA at 1 month of age.[207]

Quantitative synthesis

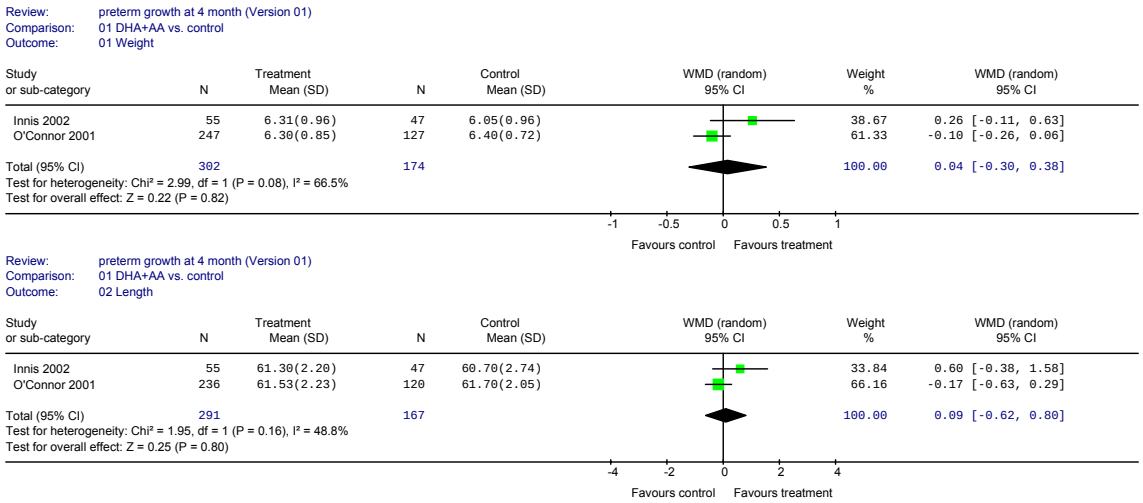
The outcomes considered for meta-analysis for growth development were weight, height and HC at 4 and 12 months. These end-points were selected given that the intervention with supplemented formula was exclusively administered until 4 months and 12 months (as a longterm followup measure), yet with the possible confounder factor of the background diet. Outcome results were available for more than one study at six different end-points in time: CA 4, 6, 9, 11, 12, and 18 months in 19 studies.

At 4 months CA, outcomes were available for four studies.[185,191,201,207] Carlson et al.[185] provided growth z-scores with standard deviation in a figure, and reported absolute growth data (by sex) in a table but without any measure of variability. In another report by Carlson et al.[191] supplementation only continued to 2 months CA. Innis et al.[201] did not report HC data on the grounds that results were not found to be statistically significant. We were thus able to combine weight and length results from Innis et al. and O'Connor et al.[201,207] Both trials assessed these outcomes as primary outcomes.

At 12 months CA, outcomes were available for three studies.[185,191,207] Supplementation in Carlson et al.[191] only continued until 2 months CA.

Supplementation in Carlson et al.[185] continued only until 9 months CA. In addition, Carlson et al.[185] provided growth z-scores with SD in a figure, and absolute growth data (by sex and without any measure of variability) in a table. We were thus unable to combine any results**.**

Figure 6. Child pre-term growth 4 months DHA+AA vs. control. Meta-analysis was performed using the random effects weighted mean difference.



The mean weight difference (WMD) in weight (kg) and length (cm) at 4 months (DHA+AA vs. control) in two studies[201,207] were nonstatistically significant. For weight: WMD: 0.04, CI 95%: -0.30; 0.38. For length: WMD: 0.09, CI 95%: -0.62; 0.80.

Impact of covariates and confounders

In the majority of the RCTs there was no evidence that randomization failed to produce comparable groups with the exception of scores on the HOME Inventory.[268] In the study of O'Connor et al.,[268] HOME Inventory scores were higher (better) in infants weighing less than 1,250 g randomized to the control group than those randomized to the fish/fungal oil group. HOME Inventory scores were lower in infants in the more than 1,250 g birth weight stratum randomized to the egg-TG/fish oil group compared with scores in the control and fish/fungal oil groups.[268]

Carlson et al. used a multiple regression analysis to control for potential effect modifiers such as maternal height, marine oil supplementation, and birth order.[185] Length achieved at 12 months of age was positively associated with maternal height, but negatively associated with marine oil supplementation. Weight was negatively associated with both birth and marine oil supplementation.[185]

Fewtrell et al. controlled the growth changes for covariates like gender, center, parental smoking, social class and level of maternal education.[273]

Differences in weight and length at 18 months post-term remained after adjusting for parental smoking, social class and level of maternal education.[273] There were no differences in HC between groups. The growth differences were greater in one center than the other, however, there was no interaction between center and growth patterns.[273] O'Connor et al. observed that

the females in the DHA+AA (egg-TG/fish) group had a greater mean HC gain from day 1 to term CA compared with the females in the other groups.[207]

The power calculation was reported in eight trials,[123,307,310,312,315,316,321,322] while the intention-to-treat analysis approach was reported in only three studies.[310,321,322]

Infant Formula Intake—Term Infants

Eighteen double-blinded RCTs met eligibility criteria for addressing the question relating to the possible effectiveness of formula intake enriched with omega-3 FA on growth patterns in term infants.[104,182,203,205,223,227,260-270]

Auestad et al. included two unique trials in one report.[227] The studies were published between 1992 and 2004. (Summary Tables 22–24)

Overview of relevant studies

Ponder et al. conducted a small efficacy study involving 25 full-term, healthy infants who were randomized to receive either soy-based (Similac with Iron 20 ready-to-feed) or corn oil-based (Similac with Iron 20 powder) formulas for 8 weeks.[260] None of the formulas contained either DHA, EPA, or AA supplementations and their FA composition differed primarily in the percentage of ALA (omega-3) and ratio of LA (omega-6)/ALA (omega-3) The outcomes were the mean weight, length and HC at 3 days, 4 and 8 weeks of age.[260] (Summary Table 22)

Decsi et al. randomly assigned 22 term infants to receive either conventional infant formula (Pre-Aptamil, placebo group) or the same formula enriched with egg lipids and evening primrose oil (Pre-Aptamil with Milupan, LCP-F group).[261] All infants were fed ad libitum throughout the study but investigators failed to report the duration of interventions in both groups. The outcomes were the change in weight, length and HC at 4 months.[261] (Summary Table 22)

Makrides et al. compared fish oil and evening primrose oil derived LCPUFA-supplemented formula with placebo formula in a double-blinded RCT involving 89 healthy full-term infants.[262] Infants were fed for 30 weeks and growth parameters were measured and compared at 6, 16, and 30 weeks.[262] (Summary Table 22)

Jensen et al. randomly assigned 80 healthy term infants to receive one of four formulas as his/her sole source of nutrition from birth to 120 days of age.[203] LA comprised 15.6% to 17.6% of the total FAs of all formulas. The ALA content was 0.4%, 1%, 1.7%, and 3.2% of total FAs, and LA/ALA ratios were 44, 18.2, 9.7, and 4.8, respectively.

The outcomes assessed were the growth patterns at 4 and 8 months of age and the correlation with infant biomarkers.[203] (Summary Table 22)

Innis et al. conducted a 3-month multicenter RCT at seven different centres in the U.S. and Canada involving 139 term infants who were randomized to receive one of two cow milk-protein based formulas (Mead Johnson Nutritionals), which differed only in FA composition and blend (18.0% LA, 1.9% ALA, with LA/ALA ratio of 9.5:1 vs 34.2% LA, 4.7% ALA, with an LA/ALA ratio of 7.3:1).[263] Neither formula had any detectable DHA, EPA, or AA.[263] (Summary Table 22)

Auestad et al. randomized 134 term, healthy infants to receive one of three formulas from less than 7 days of age to 12 months.[104] The feeding formulas differed only in the amounts and sources of LCPUFAs: (1) the control formula contained no added LCP FAs; (2) formula containing AA (0.43wt%) and DHA (0.12wt%) from egg yolk phospholipids; and, (3) formula providing DHA (0.2wt%) from a high-DHA, low-EPA tuna fish oil with a ratio of DHA to EPA of 4:1.[104](Summary Table 22)

Summary Table 22: Omega-3 fatty acids and growth parameters of term infants

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)				
Ponder, 1992, US: 8 wk parallel RCT[260]	Soy oil formula (n=11*)	Corn oil formula (n=14*)/ HM (n=18*)	NS in wt, L, HC at 3d, 4wk, 8wk	n/a	Jadad total: 1 [Grade: C]; Schulz: Unclear	II
Decsi, 1995, Hungary: parallel RCT[261]	DHA+EPA +AA formula (n=10)	Control formula (n=12)	NS in Δ wt, ΔL, ΔHC at 4 mo	n/a	Jadad total: 1 [Grade: C]; Schulz: Unclear	III
Makrides, 1995, Australia: 30 wk parallel RCT[262]	DHA+EPA +AA fish oil formula (n=13*)	Control formula (n=19*)/ HM (n=47*)	NS in wt, L, HC at 6, 16, 30 wks	NS correlation of RBC LCPUFA & GP	Jadad total: 2 [Grade: C]; Schulz: Unclear	II
Jensen, 1997, US: 120 d parallel RCT[203]	F1 (LA/ALA 44) (n=20)/ F4 (LA/ALA 4.8) (n=20)	F2 (LA/ALA 18.2) (n=20)/ F3 (LA/ALA 9.7) (n=20)	S↓ wt in F4 than in F1 at 4 mo[+] NS in L, HC, TST, & SST at 4 & 8 mo	S (+) correlation between W at 4 mo & plasma AA at 120d NS correlations between wt & plasma n-3 at 4 mo	Jadad total: 2 [Grade: C]; Schulz: Unclear	II
Innis, 1997, US, Canada: 3 mo MLT parallel RCT[263]	LA/ALA 9.5 (n=69)	LA/ALA 7.3 (n=70)/ HM (n=99)	NS in wt, L, & HC at 3 mo	NS correlations between GP & plasma & RBC AA	Jadad total: 2 [Grade: C]; Schulz: Unclear	I
Auestad, 1997, US: 12 mo parallel[104]	DHA+AA (n=46*)/ HM (n=63*)	DHA (n=43*)/ control formula (n=45*)	NS in wt, L, HC at 12 mo	n/a	Jadad total: 3 [Grade: B]; Schulz: Unclear	I

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; FAs = fatty acids; * = number of participants who completed study; HM = human milk group; BW = birth weight; BL = birth length; wt= weight; L = length; HC = head circumference; Δ = change; RBC = red blood cells; GP = growth parameters; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); $^{+}p<.05$ or significant with 95% confidence interval; $^{++}p<.01$; $^{+++}p<.001$; $^{++++}p<.0001$; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower; HM = human milk; GP = growth parameters; TST = triceps skinfold thickness; SST = subscapular skinfold thickness

Jorgensen et al. included 39 formula-fed infants randomized to receive one of the three formulas for at least 3 months: (1) formula with DHA (0.3wt%) and EPA (0.4wt%) derived from fish oil (DHAF group); (2) formula with DHA (0.3wt%) and EPA (0.4wt%) derived from fish oil, and GLA (0.5wt%) derived from borage oil (DHAGF group); and,(3) control formula with no supplemented LCPUFA. The outcomes were the growth patterns at 1, 2, and 4 months of age.[264] (Summary Table 23)

Birch et al. enrolled 79 exclusively formula-fed infants and randomized them to receive one of the three formulas from birth to 17 weeks of age. Study diets were Enfamil with iron (control group), Enfamil with iron supplemented with DHA (0.35wt%, DHA group), and Enfamil with iron supplemented with DHA (0.36wt%) and AA (0.72wt%).[182] Treatment formulas contained single cell oils, specifically DHASCO® and ARASCO® (Market Biosciences, Columbia, MD). An exclusively breastfed reference group included 29 infants.[182] (Summary Table 23)

Willatts et al. randomized English term infants to receive LCPUFA (DHA 0.15-0.25 g/10 g fat + AA 0.30- 0.40 g/100 g fat) supplemented formula or standard formula during 4 months.[223] The outcome evaluated was the growth patterns at 3 months of age.[223](Summary Table 23)

Makrides et al. conducted a double-blinded RCT of three formula-fed groups and a parallel reference group of breastfed infants.[205] The study formulas contained (1) DHA (0.34wt%) and AA (0.34wt%) from egg phospholipid (DHA+AA group, n=28): (2) DHA (0.35wt%) and EPA (0.10wt%) derived from tuna fish oil (DHA group, n=27), and (3) placebo formula (n=28) with no LCPUFA supplementation. Formulas were given to the infants for 12 months. A reference group of 33 breastfed infants was also recruited for the trial.[205] (Summary Table 23)

Lucas et al. evaluated the effect of feeding formula supplemented with DHA (0.32wt%), EPA (0.01wt%) and AA (0.30wt%) derived from purified egg phospholipid (LCPUFA group, n=154) compared with unsupplemented formula (control group, n=155) on growth parameters of infants at 18 months of age.[265] Randomization of infants took place during the first week after delivery. One hundred and thirty-eight breastfed infants also were recruited as a reference group.[265] (Summary Table 23)

Makrides et al. conducted a double-blind RCT of newborn babies allocated to receive formula with an LA/ALA of either 10:1 (16.9/1.7, n=36) or 5:1 (16.3/3.3, n=37) from near birth to 34 weeks of age.[266] Increased ALA was attained by replacing soy oil with low-erucic acid canola oil. A parallel group of 103 breastfed infants was also recruited.[266] (Summary Table 23)

Summary Table 23: Omega-3 fatty acids and growth parameters of term infants

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)				
Jorgensen 1997, Denmark: 3 mo parallel RCT[264]	DHA+GLA formula (n=12)/ HM (n=17)	DHA formula (n=14)/ Control formula (n=11)	NS in wt, L, HC, GV at 1, 2, & 4 mo	n/a	Jadad total: 2 [Grade: C]; Schulz: unclear	III
Birch, 1998, US: 17 wk parallel RCT[182]	DHA+AA (n=26)/ HM (n=29)	DHA (n=26)/ control formula (n=26)	NS in wt, L, HC, TST, SST at 17wk	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	I
Willatts, 1998, UK: 4 mo parallel RCT[223]	DHA + AA formula (n=20)	Control formula (n=20)	NS wt, L, HC at 3 mo	n/a	Jadad total: 3 [Grade: B]; Schulz: Unclear	II
Makrides, 1999, Australia: 12 mo parallel RCT[205]	DHA+AA formula (n=28)/ HM (n=63)	DHA formula (n=27)/ control formula (n=28)	NS in wt, L, HC at 6, 16, 34 wk, 12 mo & 24 mo	S (-) correlation of plasma DHA at 16 wks & wt at 12 mo & 24 mo	Jadad total: 5 [Grade: A]; Schulz: Adequate	III
Lucas, 1999, UK: 6 mo parallel RCT[265]	LCPUFA formula (n=154)	control formula (n=155)/ HM (n=138)	NS in wt, L, HC, MAC, SST at 6, 9, 18 mo (ITT)	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	II
Makrides, 2000, Australia: 34 wk parallel RCT[266]	LA/ALA 10 formula (n=36)	LA/ALA 5 formula (n=37)/ HM (n=103)	NS in Δ wt, ΔL, Δ HC between 10:1-F & 5:1-F at 6, 16, 34 wks S↑ wt at 6 wks & L at 16 wks in 5:1 F^{+}	n/a	Jadad total: 5 [Grade: A];Schulz: Adequate	II

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; FAs = fatty acids; HM = human milk group; wt = weight; L = length; HC = head circumference; RBC = red blood cells; GV = growth velocity; PC = phosphatidylcholine; PE = phosphatidylethanolamine; TST = triceps skinfold thickness; SST = subscapular skinfold thicknessITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower

Lapillone et al.'s group of 24 infants were randomly assigned to received a placebo or a LCPUFA-enriched formula (DHA [0.31wt%], EPA [0.08wt%] and AA [0.03wt%] derived from high DHA/low EPA fish oil, ROPUFA® "30" n-3 INF oil, Roche, Basel, Switzerland) from the

third day of life until 4 months of age.[267] A non randomized group of 13 breastfed infants was also included.[267] (Summary Table 24)

Morris et al. randomized 140 healthy, full-term infants to receive either standard formula milk with no LCPUFA supplements (control group) or milk with added DHA (0.2wt%) and AA (0.4wt%) (trial group).[268] Participants remained on these formulas for 12 weeks. Anthropometric measurements were taken at recruitment, 6 weeks, 3 months, 6 months, and 1 year.[268] (Summary Table 24)

Auestad et al.'s first trial compared the visual function of healthy term infants exclusively fed (1) formula with either DHA (0.14wt%) and AA (0.45wt%) derived from egg triglycerides, (2) formula with DHA (0.13wt%), EPA (<0.04wt%) and AA (0.46wt%), derived from fish and fungal oils, or (3) formula with no LCPUFAs (control group), from less than 9 days to 12 months.[227] (Summary Table 24)

Auestad et al.'s second trial included a sample of healthy term infants who were exclusively breastfed for 3 months and then weaned to formula.[227] Infants were randomized to receive a control formula and a DHA +AA supplemented formula derived from egg-triglycerides within 11 days of birth and exclusively breastfed for 3 months. Study formulas were not provided nor fed until after 3 months of exclusive breastfeeding.[227] (Summary Table 24)

Birch et al. evaluated the effect of feeding DHA+AA supplemented formula (Enfamil with iron containing DHA [0.36 wt%] and AA [0.72 wt%], derived from single-cell oils, n=32) or unsupplemented formula (control formula, Enfamil with iron, n=33) from week 7 of life to 52 weeks of age, on growth parameters measured at 6, 17, 26, and 52 weeks of age.[269] (Summary Table 24)

Hoffman et al. evaluated the effect of feeding previously breastfed infants with DHA+AA supplemented (DHA 0.36 wt%, AA 0.72 wt%) or unsupplemented formula from 4 to 6 months of age (after weaned from breastfeeding) to 12 months of age on growth patterns at 4, 6, 9 and 12 months of age.[270] (Summary Table 24)

Summary Table 24: Omega-3 fatty acids and growth parameters of term infants

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Internal validity	Applicability
Lapillonne, 2000, France: 4 mo parallel RCT[267]	DHA(fish oil)-low EPA formula (n=12)	Control formula (n=12)/ HM (n=13)	S↑ HC in control than in LCPUFA & HM at 4mo^{+} NS in wt, L, at 2, 4 mo	Jadad total: 1 [Grade: C]; Schulz: Unclear	III
Morris, 2000, UK: 12 wk parallel RCT[268]	DHA-TGL formula (n=54*)	Control formula (n=55*)	S↑ SST in DHA at 6 wk & 3 mo^{+} NS at 6 mo & 12 mo NS in wt, L, HC, MAC, TST at 6 wk, 12 wk, 6 mo, 12 mo	Jadad total: 3 [Grade: B]; Schulz: Unclear	II
Auestad, 2001a, US: 12 mo parallel RCT[227]	DHA+ AA (egg-TG) formula (n=80)	DHA+ AA (fish/fungal) formula (n=82)/ control formula (n=77)	NS in wt, L, HC at 1, 2, 4, 6, 9, & 12 mo S↑ wt gain in males in DHA+AA (egg) at 4 mo	Jadad total: 5 [Grade: A]; Schulz: Adequate	I
Auestad, 2001b, US: 1 y, parallel RCT[227]	DHA + AA formula + HM (n=83)	Control formula + HM (n=82)	NS in wt, L, HC at 1, 2, 4, 6, 9, & 12 mo or in wt, L, HC gain	Jadad total: 5 [Grade: A]; Schulz: Adequate	I
Birch, 2002, US: 46 wk parallel RCT[269]	LCP formula (n=32)	Control formula (n=33)	NS in wt, L, HC, TST & SST at 0,6,17,26 & 52 wks	Jadad total: 5 [Grade: A]; Schulz: Adequate	II
Hoffman, 2003 US: 7 mo Parallel RCT[270]	DHA+AA formula (n=30)	Control formula (n=31)	NS in wt, L, HC, wt-for-L at 4,6,9 & 12 mo	Jadad total: 3 [Grade:B]; Schulz: Adequate	II

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; FAs = fatty acids; * = number of participants who completed study; HM = human milk group; W = weight; L = length; HC = head circumference; MAC = mid-arm circumference; SST = sum of skinfold thickness; TST = triceps skinfold thickness; SST = subscapular skinfold thickness; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. All studies were parallel RCTs with at least two groups. All the studies evaluated the effect of omega-3 FA supplementations on infant growth. Auestad et al. also evaluated the effect of maternal breastfeeding together with omega-3 FA supplemented formula intake in term infants on growth pattern.[227] Eleven of 18 studies also included a non-randomized group of breastfed infants that served as a reference standard.[104,182,205,227,260,262-267]

The trials were conducted in various countries, with eight undertaken in the U.S.,[104,182,203,227,260,269,270] three in Australia,[205,262,266] three in the U.K.[223,265,268] and one each in Denmark,[264], France,[267] and Hungary.[261] The only multicenter study was conducted in the U.S. and Canada by Innis et al.[263] Ponder et al.'s study was supported by Ross Laboratories, Columbus, OH.[260] Decsi et al.'s study was sponsored by Deutsche Forschungsgemeinschaft, Bonn, Germany and Milupa Austria, Puch, Austria.[261] Makrides et al. received grants from Channel 7 Children's Medical Research Foundation, Nestle Australia, Scotia Pharmaceuticals, U.K. and the Flinders Medical Center Research Foundation.[262] Jensen et al.'s study was financially supported by the U.S. Department of Agriculture, Agricultural Research Service, Mead-Johnson Nutritional Group, The Foundation Fighting Blindness, Research to Prevent Blindness, Inc. and Retina Research Foundation.[203] The Innis et al. study was funded by Mead Johnson Research Center, Evansville, IN.[263] Auestad et al.'s study was supported by Ross Products Division, Abbott Laboratories.[104] Makrides et al.'s was supported by Wyeth Nutritionals International, USA the Australian National Health and Medical Research Council, and the MS McLeod Research Trust.[266] The study by Birch et al. was financed by the National Institutes of Health and Mead Johnson Nutritionals Research, Evansville, IN.[182] The second Makrides et al. study was funded by Nestec Ltd, Swirzerland and the Australian National Health and Medical Research Council.[205] Jorgensen et al.'s study was supported by grants from the Food Technology Research and Development Program (FOTEK), BASF Health and Nutrition, Denmark, and the Swedish Medical Research Council.[264] Lucas et al.'s was funded by Nestec Ltd (Switzerland).[265] Both of Auestad et al.'s trials were supported by Ross Products Division, Abott Laboratoris, Columbus, OH.[227] The Lappilonne et al. study was supported by Bledina sa., Villefranche, France.[267] Birch et al. and Hoffman et al. were supported by the NIH.[269,270] Only one trial conducted in U.K. did not provide information concerning its funding source.[268] Willatts et al. was supported by Milupa Ltd.[223]

In general, eight studies were funded by grants only from pharmaceutical companies,[223,227,260,263,265,265,267] seven studies were funded by both pharmaceutical and governmental agencies,[104,182,203,205,261,262,264] two trials were funded by governmental sources alone,[269,270] and one study was funded partly by private, pharmaceutical and governmental sources.[266]

The pre-study sample size calculation to reach statistical significance and power was done in nine studies.[182,205,223,227,262,265,269,270]

Population characteristics. The total number of enrolled children across the 18 RCTs was not possible to calculate because two investigators[104,260] failed to report this data providing only the number of infants who finished the study. The sample sizes ranged from 22[261] to 447.[265]

The percentage of male randomized infants was reported in five studies[205,264,265,269,323] and ranged from 42% to 64% of the infant cohort. The male/female ratio was reported in six studies.[104,182,203,262,266,268] The gender ratio of infants among different diet groups was evenly distributed in all of these studies.

In five studies most of the participants were White, accounting for 75% to 93% of the study population.[104,182,227,269,270] Only one trial reported that Black infants comprised the majority of the study participants.[203] Auestad et al.'s racial distribution of infants among the groups was not equal, for example, the breastfed group included significantly more White infants than the

placebo group and the treatment groups.[104] In two studies, participants were only White.[205,266] No information about the ethic/racial background of participants was provided in the remaining trials.

The inclusion and exclusion criteria were described in twelve studies.[104,182,205,227,262-264,266,267,269,270] Only inclusion criteria were reported in one study.[203] Four studies failed to report either inclusion or exclusion criteria.[223,260,261,268] Lapillonne et al. defined maternal cocaine and alcohol abuse history as exclusion criteria.[267]

The definition of a term infant (at least 37 weeks GA) was described in 11 studies [104,223,227,260,262,263,265,266,269,270] The study duration ranged from 8 weeks to 24 months, with a mean interventional length of 27.5 weeks (range 8–52 weeks). Only one trial did not report the length of dietary intervention.[261]

Different variables were used to demonstrate the family socioconomic status across the studies (i.e., maternal education, paternal education, social score, social status of income earner, marital status). Maternal social status was determined in seven studies,[205,227,262,265,268,270] whereas, information about maternal and/or paternal education and/or maternal marital status was given in six trials.[182,205,227,265,269] Makrides et al. assessed parental education scores, as well as parental social scores in two randomized study groups.[266]

There were no differences in sociodemographic variables among the study groups of randomized infants in all of these studies. Mothers of infants in the reference breastfed group had a more prestigious social score, and attained a higher level of education compared with mothers of formula-fed infants.[205,227,262] Hoffman et al. found that maternal education was better in the LC PUFA supplemented group at baseline.[270] There was missing data about maternal smoking history before and during pregnancy in eleven studies.[104,182,203,223,262-265,267,269,270] In studies that reported information about maternal smoking history, there was a tendency for less maternal smoking during pregnancy and/or lactation among the mothers of breastfed infants compared with formula-fed groups.[205,227,266]

Intervention/exposure characteristics. Only four studies reported as part of the protocol that the volume of formula consumed, calculated as the difference in the volume of formula in the bottle at the start and end of the feed, was recorded.[203,261,265,268] Nonetheless, most of the authors failed to report the daily amount of formulas consumed by infants in the different feeding groups. Only Decsi et al. reported that daily formula intakes were between 120 mL/kg and 150 mL/kg and did not differ between the feeding groups.[261]

The duration of formula intake was not reported only in the study of Decsi et al.[261] In the remaining trials, the formula intake duration ranged from 8 weeks[260] to 12 months.[104,205,227]

The sources of omega-3 FA intervention varied across the 18 RCTs. Six trials described the source as fish oil.[104,205,227,264,267] Makrides et al. supplemented a standard formula with a combination of DHA derived from fish oil and AA derived from primrose oil.[262] The specific type of fish from which the fish oil exposures were derived were described in two studies.[104,205] The remaining studies employed either single cell sources of FA,[182,268-270] solely vegetable sources of FA,[203,223,260,261,263,266] egg phospholipids,[196,265] or at least one of the feeding formulas containing FA from vegetable or egg sources.[104,205,227,264] Decsi et al. used a formula enriched with both egg lipids and primrose oil to achieve a higher levels of omega-3 and omega-6 FA.[261]

In 10 studies, the type of omega-3 FA employed included a combination of DHA and EPA.[205,223,227,261,262,264,265,267,270] In four trials DHA was used alone;[104,182,268,269] ALA was used alone also in four trials.[203,260,263,266] Supplementation of formulas with the omega-6 FA AA was reported in seven trials.[104,182,205,227,265,269]

Nine studies failed to report the name of the intervention formulas.[104,205,227,264-268] In the rest of the studies, the brands of the employed formulas were: Enfamil (Mead Johnson Nutritionals, Evansville, Ind);[182,203,263,269,270] Similac with iron (Ross Laboratories, Columbus, OH);[260] Aptamil (Milupa Ltd.);[223] and Pre-Aptamil (Puch/Salzburg, Austria).[261] Eight trials indicated the manufacturer of at least one omega-3 FA product used in their study.[182,223,227,265,267,269,270] None of the studies reported on the purity of their omega-3 FA exposure.

Study infants were placed on the study formulas within the first week of life in most of the studies.[104,182,205,223,260,261,265-268] Study formulas were started within the first month of life in four studies,[203,227,263,264] from the beginning of week 7 in the Birch et al. study,[269] 1 month after delivery in the study of Jorgensen et al.,[264] and in the second Auestad et al. trial, infants received the formula after 3 months of being exclusively breastfed.[227] One trial failed to report information on the exact time of participants' enrollment into the study.[262] Hoffman et al.' infants were breastfed for at least 4 to 6 months and then were randomized to the study formulas until 12 months of age.[270]

Formula was the only source of alimentation in three studies and no solid foods were introduced during the entire trial period.[203,260,263] Innis et al. specified that an infant would be withdrawn from the study if more than 10% of dietary energy came from sources other than assigned formula for 5 days or more.[263] Decsi et al. permitted fruit juices at 2 months of age and solid food beginning at 3 months of age in both study groups.[261] In eight studies, introduction of solid foods was permitted after 4 months of age.[104,205,227,262,266,267,270] Both Birch et al. trials did not permit the introduction of any solid food until 17 weeks of age.[182,269] Lucas et al. reported that the mean age of first introduction of any solid food did not differ between those fed LCPUFA and those fed control formula.[265] Two trials failed to report if any solid food was permitted at all.[223,268]

Information about caloric balance of feeding formulas was reported in seven RCTs.[104,182,227,265,269,270] Only Auestad et al. mentioned that the study formulas were indistinguishable in appearance and odor.[227]

Cointervention characteristics. Three studies reported the content of vitamin and mineral supplements of feeding formulas and oils taken by pregnant or lactating women.[182,261,264] In Ponder et al., no vitamins or mineral supplementations were given to the infants fed formula, whereas, breastfed infants received routine vitamin D supplementation.[260] Only Jorgensen et al. reported about the use of preventive measures such as microencapsulation of fish and borage oils and addition of corn starch to avoid oxidation and to allow homogenization with the formula powder.[264] Toxicology studies for supplemented oils were done only in one study.[182]

Outcome characteristics. Nine (of 20) trials evaluated the growth parameters as primary outcomes,[120,151,324-329] while the remaining 11 trials assessed these outcomes as secondary outcomes. All included RCTs employed the weight, length, and HC of infants as main outcome measures for growth. The rate of gains in weight, length and HC were assessed in three studies.[261,264,266] Triceps skinfold thickness was measured in five RCTs[182,203,223,268,269]

Subscapular skinfold thickness was assessed in five studies.[182,203,265,268,269] Two studies evaluated mid-arm circumference as one of the growth outcomes.[265,268]

Study quality and applicability. The 18 RCTs received a mean Jadad total quality score of 3.2, with good internal validity (Summary Matrix 10). Seven trials received a score of 5,[182,205,265,266,269,329] four received a score of 3,[104,223,268,270] four reports received a score of 2,[203,262-264] and three received a score of 1.[260,261,267] Seven trials failed to report the randomization method,[125,324,325,328,330-332] nine were unblinded,[125,324-327,330,332-334] two failed to report the method of double-blinidng,[328,331] and five trials did not describe the reasons for dropouts.[324,326,330-332]

Summary Matrix 10: Omega-3 fatty acids and growth parameters of term infants

		Study Quality								
		A			B			C		
Applicability	I	**Author**	**Year**	**n**	**Author**	**Year**	**N**	**Author**	**Year**	**n**
		Auestad[A]	2001	239	Auestad[U]	1997	274	Innis[U]	1997	238
		Auestad[A]	2001	165						
	II	**Author**	**Year**	**n**	**Author**	**Year**	**N**	**Author**	**Year**	**n**
		Lucas[A]	1999	447	Birch[U]	1999	79	Ponder[U]	1992	43
		Makrides[A]	2000	176	Willatts[U]	1998	40	Makrides[U]	1995	89
		Birch[A]	2002	65	Morris[U]	2000	140	Jensen[U]	1997	80
					Hoffman[A]	2003	68			
	III	**Author**	**Year**	**n**	**Author**	**Year**	**N**	**Author**	**Year**	**n**
		Makrides[A]	1999	146				Decsi[U]	1995	22
								Jorgensen[U]	1998	39
								Lapillone[U]	2000	24

n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment; [I] Inadequate

Qualitative synthesis of individual study results

The most frequently investigated outcomes across the included studies were infant weight, length, and HC, expressed as mean (SD), normalized z-score or gain over time.

Most of the studies failed to find a statistical difference between groups in the growth patterns at any time point. However, some differences were detected in five trials.

Only the study of Lapillonne et al. found that infants' HC at 4 months in the placebo group was significantly larger than that in both breast and treatment formula groups.[267]

Infant length and weight were not statistically different among the three feeding groups.[267] Makrides et al., who compared growth parameters among three randomized groups, did not find statistically significant differences in weight, length, or HC at any age up to 2 years, even after adjusting for gender, GA, and postnatal age at assessment.[205] When growth parameters were compared between the two treatment formula and the breastfed infant groups, investigators found that breastfed babies were significantly shorter and lighter than infants in the DHA+AA and DHA+EPA groups at 34 weeks and 12 months of age. These differences did not reach statistical significance at 2 years of age.[205]

Decsi et al., who randomized two groups of infants to receive either placebo or treatment formula, did not find a statistically significant difference between the groups regarding gain of weight, length, or HC at 4 months of age.[261]

The Makrides et at. study, which compared growth parameters in two randomized groups receiving placebo and treatment formulas, did not find any statistically significant difference in weight, length, or HC at 30 weeks of age.[262]

Three out of four RCTs describing the use of ALA as a source of omega-3 FAs, failed to find any significant difference in growth parameters among the randomized groups receiving either placebo or treatment formula(s). The weight, length, and HC of infants were similar at 4 and 8 weeks of age in the study of Ponder et al.,[260] at 6, 16, and 34 weeks of age in the study of Makrides et al.[266] and at 3 months of age in the Innis et al. study.[263] Only one trial showed significantly lower weight at 120 days of age in the group of infants receiving the highest ALA intake, or the lowest LA/ALA ratio (LA [15.6wt%] and ALA [3.2wt%]), compared with the group receiving the lowest ALA, or highest LA/ALA ratio (LA [17.6wt%] and ALA [0.4wt%]).[203] These results were obtained after adjusting for differences in birth weight, gender, and ethnicity. In Makrides et al.'s study, where newborn babies were randomized to receive formula with an LA:ALA of either 10:1 or 5:1, there were no significant differences in weight, length, and HC gain between the two groups, although breastfed infants had significantly lower weight and length gain at 16 and 34 weeks of age than infants in the two formula fed groups.[266]

Other growth outcomes assessed were triceps skinfold thickness, subscapular skinfold thickness, and mid-arm circumference. Five studies did not find a significant difference between groups in triceps skinfold thickness and subscapular skinfold thickness among the randomized study groups at any time point.[182,203,223,265,269] Morris et al. randomized infants to receive either standard formula or the treatment formula with added DHA and found that subscapular skinfold thickness at 6 weeks and 3 months of age was significantly higher in the control group compared with the trial group, althought these differences were not evident at 6 months or at 1 year of age.[268]

Four studies measured the correlation between the plasma or RBC PUFAs and growth outcomes.[203,205,262,263] Two studies did not find a significant correlation between the omega-3 FA in plasma or RBC and the weight.[203,262] However, Jensen et al. observed a significant positive correlation between weight at 4 months and the plasma AA content at the same time.[203] Innis et al., on the contrary, did not find a correlation between growth patterns and the plasma and RBC AA content in term infants.[263]

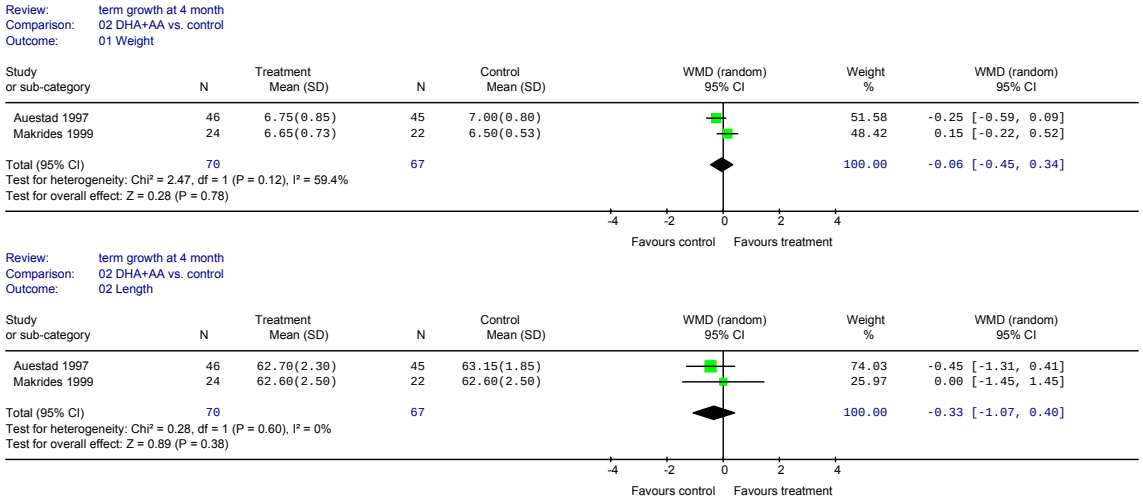
Makrides et al. found a significantly negative correlation between plasma DHA at 16 weeks and weight at 12 and 24 months of age.[205]

Quantitative synthesis

At 4 months of age, growth pattern outcomes were noted in 13 studies. However, only four studies included treatment groups of both DHA+AA and placebo.[104,182,205,227] For Auestad et al.'s first study,[227] data on weight, length, and HC could not be extracted; although partially reported in the text for statistically significant differences, the sample sizes were not given, and the weight gains were reported in grams/day. The figure provided growth data by sex at different follow-up times, but no sample sizes were indicated. For the Birch et al. 1998 study,[182] standardized weight and length were reported in a boxplot figure using z-scores, thus it was not

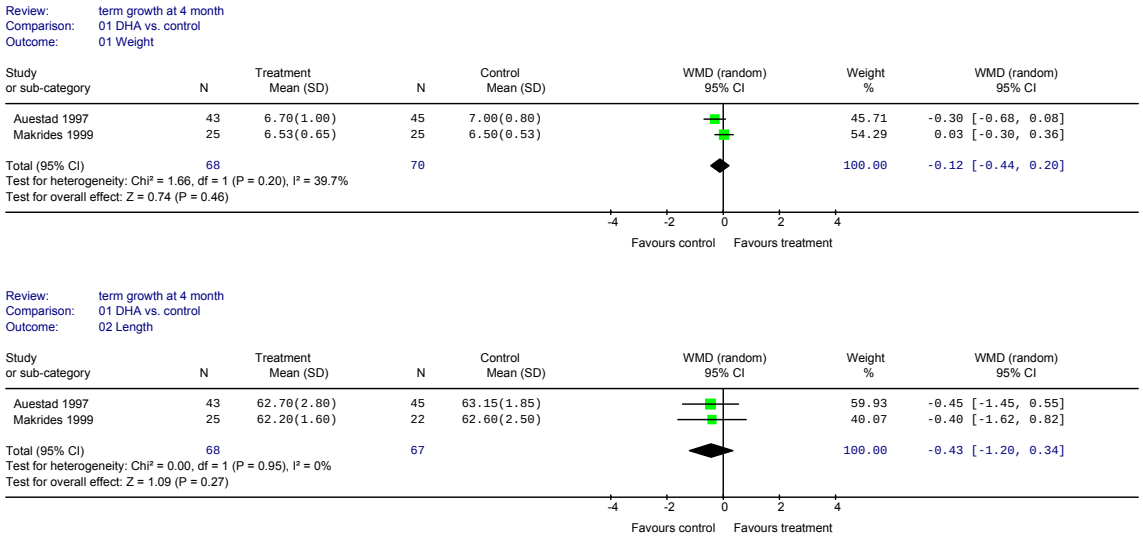
possible to obtain unstandardized growth measures. This left only two studies for meta-analysis.[104,205] Both trials assessed the growth parameters as primary outcomes.

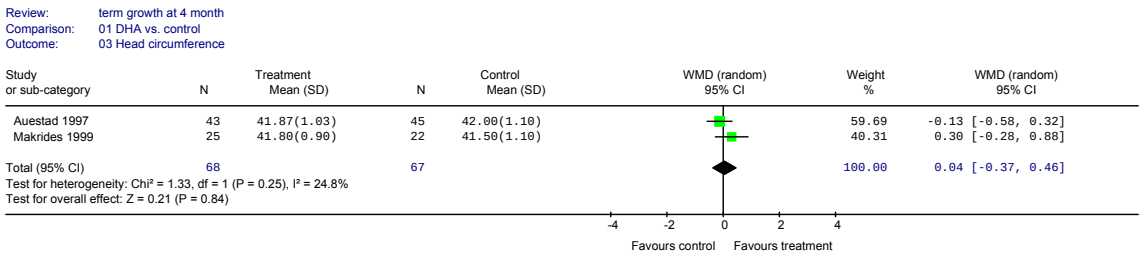
Figure 7. Child term growth 4 months DHA+AA vs. control. Meta-analysis was performed using the random effects weighted mean difference.



The WMD in the weight (kg) and length (cm) (DHA+AA vs. control) in two studies[104,205] was nonstatistically significant at 4 months. For weight: WMD: -0.06, CI 95%: -0.45; 0.34. For length: WMD: -0.33, CI 95%: -1.07; 0.40.

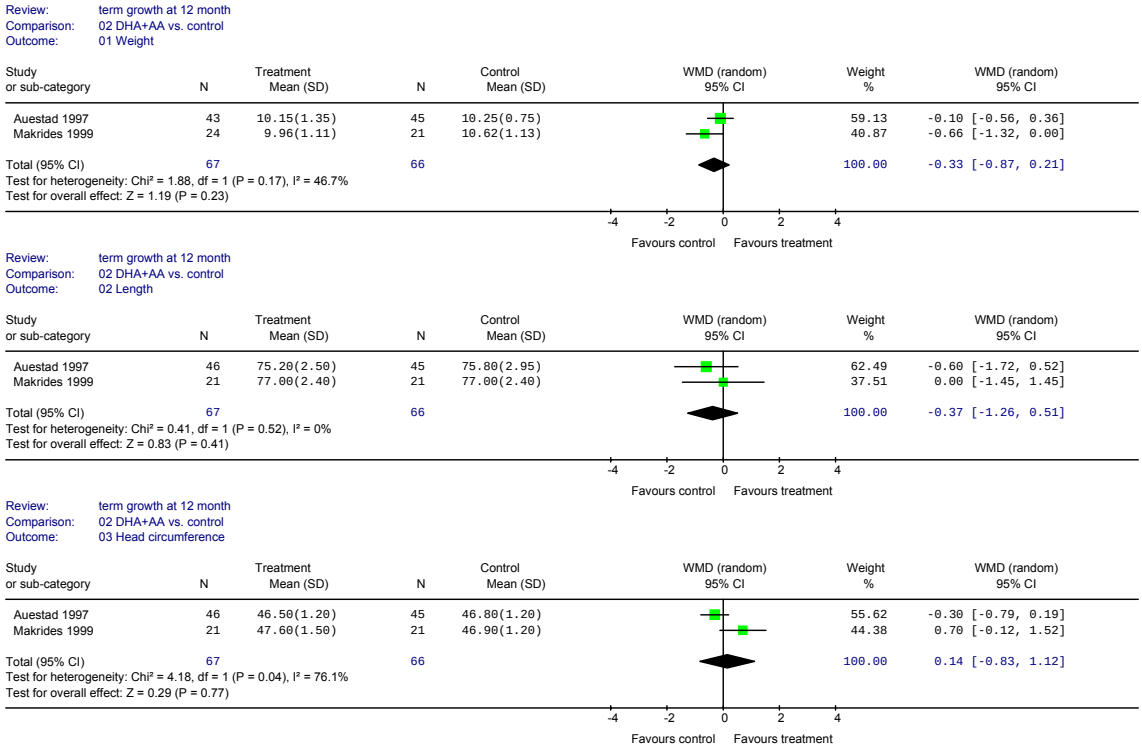
Figure 8. Meta-analysis: Child term growth 4 months DHA vs. control. Meta-analysis was performed using the random effects weighted mean difference.





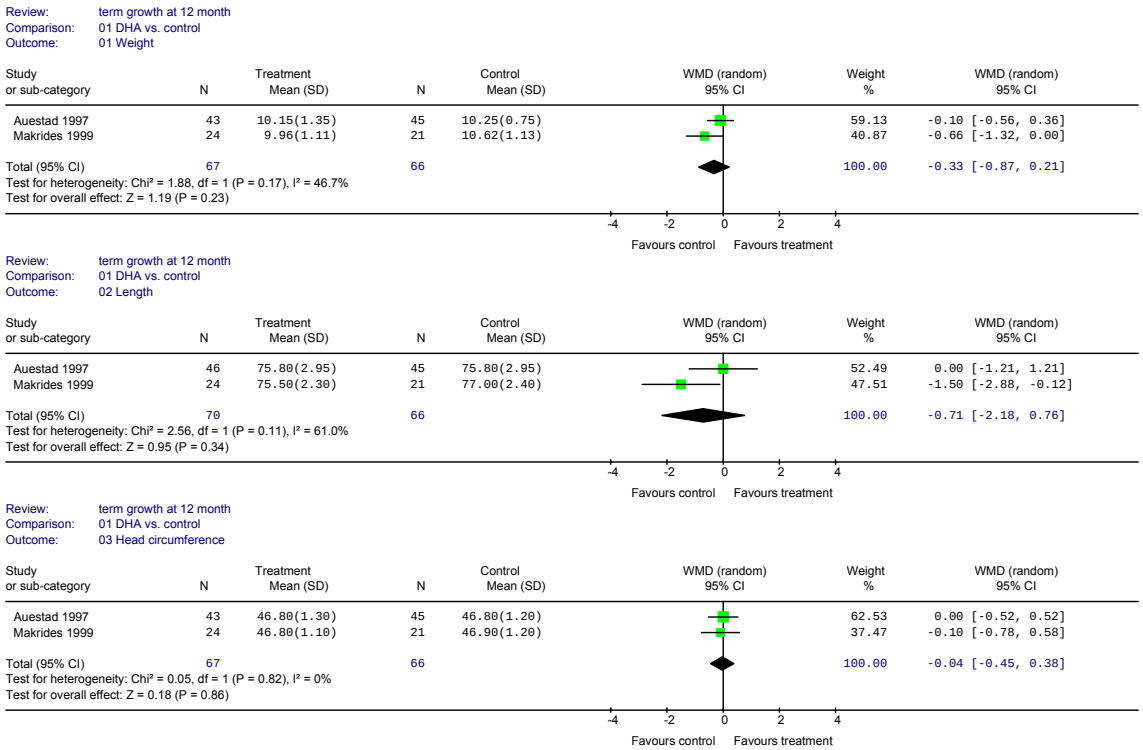
The WMD in the weight (kg), length (cm) and HC (cm) (DHA vs. control) in two studies[104,205] was nonstatistically significant at 4 months. For weight: WMD: -0.12, CI 95%: -0.44; 0.20. For length: WMD: -0.43, CI 95%: -1.20; 0.34. For HC: WMD: 0.04, CI 95%: -0.37; 0.46.

Figure 9. Meta-analysis: Child term growth 12 months DHA+AA vs. control. Meta-analysis was performed using the random effects weighted mean difference



The WMD in the weight (kg), length (cm) and HC (cm) (DHA+AA vs. control) in two studies[104,205] was nonstatistically significant at 12 months. For weight: WMD: -0.33, CI 95%: -0.87; 0.21. For length: WMD: -0.37, CI 95%: -1.26; 0.51. For HC: WMD: 0.14, CI 95%: -0.83; 1.12.

Figure 10. Meta-analysis: Child term growth 12 months DHA vs. control. Meta-analysis was performed using the random effects weighted mean difference.



The WMD in the weight (kg), length (cm) and HC (cm) (DHA vs. control) in two studies[104,205] was nonstatistically significant at 12 months. For weight: WMD: -0.33, CI 95%: -0.87; 0.21. For length: WMD: -0.71, CI 95%: -2.18; 0.76. For HC: WMD: -0.04, CI 95%: -0.45; 0.38.

Impact of covariates and confounders

In most of the RCTs there was no evidence that randomization failed to produce comparable groups, with the exception of HC.[268] In the study of Morris et al., two randomized groups had similar characteristics at recruitment, except for a small difference in mean HC which just reached statistical significance.[268] In the study of Jorgensen et al., within the formula groups there was a borderline statistical difference in birth weight in favor of the group supplemented with only DHA.[264] Jorgensen et al.[264] and Auestad et al.[227] also reported that maternal age of infants assigned to breast milk was significantly higher than that in the randomized formula-fed groups. In the study of Auestad et al., infants in the breastfed group also had a higher GA, a smaller percentage of mothers having no postsecondary education, and a smaller prevalence of smoking exposures both in utero and in the houshold.[227]

Four studies controlled the growth outcomes for potential confounders such as gender, maternal education, center, and socioeconomic status.[203,205,263,266] No differences were found after adjusting for these covariates.

The power calculation was reported in eleven trials,[120,124,132,151,325,329,331,333-335] while the intention-to-treat analysis approach was reported in only one study.[132]

Growth Pattern Outcomes in Light of Biomarker Data

What is the Evidence That Term or Preterm Human Infants' Growth Patterns Are Associated With the Omega-3 or Omega-6/Omega-3 Fatty Acid Content of Child Biomarkers?

A total of 12 studies were identified to address this question. The results of six RCTs of preterm infants were described previously in this section (see key question: Growth Patterns-Preterm Infant Formula Intake),[185,191,201,207,212,225] as well as the results of four RCTs that included a term population of infants (see key question: Growth Patterns-Term Infant Formula Intake).[203,205,262,263] Therefore, two studies will be addressed here—the RCT of Guesnet et al.[143] and the prospective single cohort study of Innis et al.[271] The studies were published in 1999 and 2001, respectively. A summary of the study characteristics and outcomes relating to the current question are described in this section. (Summary Table 25)

Overview of relevant study characteristics and results

Guesnet et al. assessed growth patterns and their correlation with the plasma and RBC PUFA content after the use of three different formulas. Healthy term infants (n=68) were randomized to receive one of three formulas, supplemented with either DHA and EPA (high dose), DHA and EPA (low dose) or unsupplemented, for 6 weeks.[143] It also included a group of infants who where breastfed, yet were nonrandomized. The formulas were provided by Gallia 1 (Bledina-sa, Groupe Danone, Villefranche-sur-saone, France).[143]

This study was conducted in France and supported by the Bledina-sa, Groupe Danon Paris, French Ministry of Cooperation in Mauritius and the University of Mauritius.

Blood samples were collected from umbilical cord at birth and venipuncture at 6 weeks of age. There was no difference between groups in the growth paramenters at 6 weeks of age.[143]

Innis et al. selected a cohort of 83 term infants who were exclusively breastfed, with birth weights ranging from 2,500 g to 4,500 g.[271] The objective of the study was to measure the infant RBC DHA content and its association with visual, neuro or cognitive development.[271] Infants were enrolled within 2 weeks of age and to be eligible, their mothers were required to intend to exclusively breastfeed their infant without providing infant formula or cow's milk for at least 3 months and without introducing solid foods for at least the first 4 months after birth. The infants were excluded if they had evidence of metabolic or physical abnormality, or if their mothers had substance abuse, metabolic or physiologic problems, or communicable diseases.[271]

Only one mother reported taking FA supplements with LA and DHA. The maternal diet was not reported or controlled. Only five mothers reported being smokers during the study. Infant measures of weight, length and HC were correlated with the RBC DHA and AA content at birth, 2, 4, 6, 9 and 12 months of age.[271]

Multiple linear regression analysis was used to determine the impact of the FA variables on the outcomes. The analysis controlled statistically for the duration of breastfeeding, maternal education, family income, gender, maternal smoking, birth order and birth weight, length and HC.[271]

Summary Table 25: Association between growth patterns and biomarker content in infants

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable associations[2,3]	Internal validity	Applicability
Guesnet, 1999, France: 6 wk parallel RCT[143]	High-EPA (n=23)/ HM (n=15)	Low-EPA (n=24)/ pb (n=21)	S (-) correlation between Δ L & plasma & RBC EPA at birth	Jadad total: 2 [Grade: C]; Schulz: Unclear	III
Innis, 2001, Canada: prospective single cohort[271]	Term breastfed infants (n=83)	n/a	RBC CPG DHA^{++} & EPG DHA^{+} negative correlation with infant weight (6 mo); no correlation at 12 mo; no correlation of blood AA & growth patterns at any age	Quality score: 8 [Grade A]	III

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; N/A = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; RBC = red blood cells; PL = phospholipid; CPG = choline phosphoglycerides; EPG = ethanolamine phosphoglycerides; Δ = change; L = length; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ↑ = increase; ↓ = decrease/reduction; HM = human milk; L = length

Guesnet et al. observed a negative correlation between postnatal gains in length and the EPA concentration at birth in total plasma PL and in RBC PE.

Innis et al. found that the RBC choline phosphoglyceride (CPG) DHA and the ethanolamine phosphoglycerides (EPG) DHA, but not the plasma DHA, were significantly inversely related to infant weight at 6 months of age, but not at 12 months. There was no significant relation between infant blood lipid concentrations of AA and growth at any age.[271]

Study quality and applicability. Guesnet et al. had a Jadad's total score of 2 (did not report method of randomization and was unblinded) and an unclear allocation concealment.143 Innis et al. had a quality score of 8 and a level of applicability of III.271 (Summary Matrix 11)

Summary Matrix 11: Association between growth patterns and biomarker content in infants

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	N	Author	Year	n
	II	Author	Year	n	Author	Year	N	Author	Year	n
	III	Author Innis	Year 2001	n 83	Author	Year	N	Author Guesnet^{U}	Year 1999	n 68

n = number of allocated/selected participants; ^{U} = unclear allocation concealment

Neurological Development Outcomes

What is the Evidence That Maternal Intake of Omega-3 Fatty Acids During Pregnancy Influences Neurological Development in Term or Preterm Human Infants?

One RCT, published in 2001, was identified to answer this question.[141] Helland et al. had two publications related to the same study population.[141,200] (Summary Table 26)

Overview of relevant study characteristics and results

Helland et al.,[141] has been described in detail in the Pregnancy Outcomes and Growth Pattern Outcomes sections (see key questions: Duration of Gestation, Infants Small for Gestational Age, and Maternal Intake/Growth Patterns). A summary and the results relating to the current question are discussed here.

Helland et al. assessed the gestational length, birth weight, and neurologic and cognitive outcomes in a sample of 590 healthy pregnant women. They were randomized to receive 10 ml of cod liver oil (1,183 mg DHA, 803 mg EPA) or corn oil (LA and ALA) from week 18 of pregnancy to 3 months post delivery.[141] They should not have taken any supplements of omega-3 FA earlier during the pregnancy. The exclusion criteria were premature births, birth asphyxia, infections, and anomalies in the infants that required special attention.[141] The neurological outcomes assessed was the electroencephalogram (EEG) recordings of the included infants to evaluate brain maturity. EEGs were performed at 1 day of life and repeated at 3 months of age.[141] Helland et al. had a high rate of dropouts, leaving 341 women in the final analysis (57%).[288]

Summary Table 26: Influence of omega-3 fatty acids intake during pregnancy on neurological development of their infants

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Internal validity	Applicability
Helland, 2001, Norway: 34 wks parallel RCT[141]	Cod liver oil DHA+EPA (n=301)	Corn oil LA+ALA (n=289)	NS EEGs scores between groups (3 mo)	Jadad total: 4 [Grade: A]; Schulz: Unclear	III

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; N/A = not applicable; grp = group; wk = week(s); mo = month; RBC = red blood cells; PL = phospholipid; [+]p<.05 or significant with 95% confidence interval; ++p<.01; +++p<.001; ++++p<.0001; ↑ = increase; ↓ = decrease/reduction; EEG = electroencephalogram

There were no differences between groups in maturity as evaluated from the EEGs, neither at day 1 of life nor at 3 months of age.[141]

Between neonates with mature (score 1; n=70) and immature EEG scores (score 2 and 3; n=51), there were significant differences in umbilical plasma phospholipid levels of EPA, DPA and DHA at the 2nd day of life. At 3 months, there were no significant differences in plasma phospholipid levels between those with mature and immature EEGs.[141]

Study quality and applicability. Helland et al. received a Jadad total quality score of 4 (did not report method of double-blinding), indicating good internal validity. However, the allocation concealment was unclear. The applicability was scored with III, since the Norwegian population has a significantly higher intake of LCPUFA from marine sources compared to the Nort American population.

Summary Matrix 12: Influence of omega-3 fatty acids intake during pregnancy on neurological development of their infants

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	N	Author	Year	n
	II	Author	Year	n	Author	Year	N	Author	Year	n
	III	Author Helland[U]	Year 2001	n 590	Author	Year	N	Author	Year	n
n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment										

What is the Evidence That the Omega-3 Fatty Acid Content of Maternal Breast Milk, With or Without Known Maternal Intake of Omega-3 Fatty Acids, Influences Neurological Development in Term or Preterm Human Infants?

One RCT and one single prospective cohort study were identified.[138,284] They were published between 1997 and 2001. (Summary Table 27)

Overview of relevant study characteristics and results

Gibson et al. was a double-blind RCT that investigated the effect of maternal intake of omega-3 FAs on breastfed infant's neurological and visual function outcomes in Australia.[138] This study included mothers of term infants (>37 weeks of GA) who intended to breast feed for at least 12 weeks (n=52, means age: 30 [SD=4] years). These mothers were randomized to receive one of five doses of a DHA-rich algal oil (0, 0.2, 0.4, 0.9, 1.3 g DHA/day; DHASCO, Market Biosciences, MD, U.S.) between day 5 and week 12 postpartum. The oil contained 43% DHA, 1% omega-6 PUFA, 38% saturates and 18% monosaturates. Infants who were exclusively breastfed for 12 weeks were assessed. Infants (n=20) were healthy, appropriate weight for GA, and had Apgar scores greater than 7 at 5 minutes.[138]

Infant's visual function was assessed using visual evoked potentials (VEP) (logMAR) at 12 and 16 weeks of life.[138] Global development (Bayley's Scales of Infant development) was assessed at 1 and 2 years of age. From Bayley Scales of Infant Development, the psychomotor

developmental index (PDI) was derived. PDI assesses the control of gross and fine muscle groups, including walking, running, jumping, comprehension, use of writing implements, and imitation of hand movements. Mothers were from middle class families and completed year 12 of education. The five groups were compared in terms of maternal age, maternal BMI, GA, infant gender, birth weight, birth length, birth HC, Apgar score, siblings, maternal social score, smoking, education, home stimulation, and length of breast feeding, at baseline. There was a predominance of boys in the group that received the highest dose of DHA.[138]

Agostoni et al. evaluated the neurodevelopmental indices at 1 year of age in a single prospective cohort of term infants (n=44; 54.5% males) who were exclusively breastfed for at least 3 months in Italy.[284] The children received breast milk for at least 3 months, after which weaning foods were introduced to all infants. Infants underwent clinical examination at 0, 1, 3, 6, 9 and 12 months of age.[284]

The mother's milk lipid composition was determined at each time-point. The day before, the control pooled milk was collected from all feedings over 24 hours. There was a progressive reduction of the number of breastfed infants to n=29 at 6 months, n=17 at 9 months and n=10 at 1 year.[284]

Summary Table 27: Omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences neurological development in term or preterm human infants

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Internal validity	Applicability
Gibson, 1997, Australia: 12 wk parallel RCT[138]	1.3 g/d DHA (n=8)/ 0.2 g/d DHA (n=10)	0.9 g/d DHA (n=10)/ 0.4 g/d DHA (n=12)/ pb (n=12)	NS in PDI at 12 mo and 24 mo No correlation of sociodemographics & PDI at 1 y Positive correlation between level of education of partner & PDI^{+}	Jadad total: 3 [Grade: B]; Schulz: Unclear	II
Agostoni, 2001, Italy: Single prospective cohort[284]	Term breastfed infants at 1 y-old (n=44)	n/a	NS correlation between Bayley's PDI & length of BF NS correlation between Bayley's PDI & milk FA content	Quality score: 8 [Grade A]	III

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; N/A = not applicable; grp = group; wk = week(s); mo = month; RBC = red blood cells; PL = phospholipid; PDI = psychomotor developmental index; $^{+}$p<.05 or significant with 95% confidence interval; ++p<.01; +++p<.001; ++++p<.0001; ↑ = increase; ↓ = decrease/reduction; BF = breast feeding; PDI = Bayley's psychomotor scale

The mean PDI score was similar in infants between dietary groups at 1 and 2 years of age in Gibson et al. study.[138] There were no associations with any sociodemographic variables at 1 year. The only association at 2 years of age was between PDI and the level of education of the partner (r^2=0.10; adjusted r^2=0.08, p<0.05).[138]

In Agostoni et al., the mean PDI in 1-year old infants, was 92 (SD=11.3).[284] After correcting for potential confounders such us parity and mother's characteristics (i.e., age, education, smoking habits), breast feeding for 6 months or longer was not significantly correlated to the mean PDI result compared with subjects breastfed for 3 to 6 months (n=15).[284] Associations between PDI and milk fat content and composition were measured with a multiple regression analysis. There was no correlation between PDI and the milk fat content at any time-point.[284]

Study quality and applicability. Gibson et al. obtained a Jadad total quality score of 3 (did not report methods of randomization and double-blinding), indicating sound internal validity.[336] However, the allocation concealment was unclear. The applicability level was II for Gibson et al. and III for Agostoni et al.[337]

Summary Matrix 13: Omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences neurological development in term or preterm human infants

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	N	Author	Year	n
	II	Author	Year	n	Author	Year	N	Author	Year	n
					Gibson[U]	1997	52			
	III	Author	Year	n	Author	Year	N	Author	Year	n
		Agostoni	2001	44						
n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment										

What is the Evidence That the Omega-3 Fatty Acid Content of Infant Formula Influences Neurological Development in Term or Preterm Human Infants?

What is the Evidence That the Omega-3 Fatty Acid Content of Maternal Breast Milk, With or Without Known Maternal Intake of Omega-3 Fatty Acids, and Together With the Omega-3 Fatty Acid Content of Infant Formula, Influences Neurological Development in Term or Preterm Human Infants?

Infant Formula Intake—Preterm Infants

Six RCTs, published between 1999 and 2004, met eligibility criteria.[193,207,254,258,272,273] Five trials were summarized in the Growth Pattern Outcomes section (see key question: Growth Patterns-Preterm Infants Formula Intake).[310,311,319,321,322] (Summary Table 28)

Overview of relevant studies

van Wezel-Meijler et al. studied the influence of supplemented formula with DHA and AA on brain maturation in preterm infants and investigated parameters of functional brain development, including cognitive development.[272] (Summary Table 36)

Summary Table 28: Omega-3 fatty acids and its influence on neurological development in preterm infants

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)			
Bougle, 1999, France: 30 d parallel RCT[254]	AA+EPA+ DHA formula (n=14)	LA (n-6)+ ALA (n-3) formula (n=11)/ HM (n=15)	NS LAEP between d 0 & 30d S↑ Δ motor NCT (m/s) in DHA/EPA/AA supplemented formula & HM from d0-30^{+} NS Δ sensory (m/s) test	Jadad total: 3 [Grade: B]; Schulz: Unclear	III
O'Connor, 2001, US, UK, Chile: 12 mo parallel RCT[207]	DHA+AA (fish/fungal) (n=140)/ HM (n=43)	DHA+AA (egg-TG/fish) (n=143)/ Control formula (n=144)	(ITT) S↑ PDI score in <1250 g birth wt fed AA+DHA (egg-TG/fish) than control infants^{++} NS score control or AA+DHA (fish/fungal) groups	Jadad total: 3 [Grade: B]; Schulz: Unclear	I
van Wezel-Meijler, 2002, The Netherlands: 6 mo, parallel RCT[272]	AA+DHA preterm formula (n=22)	Control formula (n=20)	S↑ PDI score unsupplemented group vs. supplemented formula at 3, 6, 12 & 24 mo^{+}	Jadad total: 5 [Grade: A]; Schulz: Adequate	III
Fewtrell, 2002, UK: 33 d parallel RCT[273]	AA+DHA+EPA preterm formula (n=95)	Control formula (n=100)/ HM (n=88)	(ITT) NS PDI score between formula gps at 18 mo S ↑ PDI in the HM group vs. both formula gps NS between formula gps in KPSDSI at 9 mo; HM S ↑ quotient vs. formulas	Jadad total: 5 [Grade: A]; Schulz: Adequate	II
Clandinin, 2002, Canada: 92 wks parallel RCT[193]	DAS (DHA+AA from SCO) (n=72)/ HM (n=105)	DAF (DHA from fish oils+AA from SCO) (n=90)/ Control formula (n=83)	S↑ PDI score formula gps (DAS, DAF) vs. control gp	Not assessed	X
Fewtrell, 2004, UK: 9 mo parallel RCT[258]	GLA+ DHA formula (n=122)	Control formula (n=116)	(ITT) NS formula groups in Bayley's PDI scores at 18 mo	Jadad total: 5 [Grade: A]; Schulz: Adequate	II

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acidLength = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; grp = group; wk = week(s); mo = month; wt = weight; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; NCT = nerve conduction test; LAEP = latency auditory evoked potentials; SCO = single-cell oil; HM = human milk; TG = triglycerides

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. Six parallel RCTs involving preterm infants were identified to address these questions.193,207,254,258,272,273 Five of the studies were published in English scientific journals, while one was published as an abstract.193 Bougle et al.'s study was conducted in France,254 both Fewtrell et al.'s studies were conducted in the U.K.,258,273 the van Wezel-Meijler et al. study was located in the Netherlands,272 Clandinin et al.'s study was conducted in Canada,193 and the O'Connor et al. study took place in the United States, United Kingdom and Chile.207

Three studies involved three study arms comparing the use of supplemented and unsupplemented infant formula with the addition of a fourth reference standard group (i.e., human milk).[193,207,254] Two RCTs compared only two study groups (i.e., formula with or without LCPUFA),[258,272] whereas, another study also included a group using human milk as a reference standard.[273]

van Wezel-Meijler et al.[272] and Fewtrell et al. (2002)[273] were supported by a private source (Numico Research). Clandinin et al. was funded by Mead Johnson & Company (pharmaceutical-nutritional company),[193] whereas, Fewtrell et al. (2004)[258] was supported by H.J. Heinz Company (food company). O'Connor et al. and Bougle et al. did not report their funding source.[207,254]

Population characteristics. There were 1,228 preterm infants enrolled across the included studies that were randomized to receive the supplemented or control formulas. The sample sizes ranged from 25 to 470 participants. The mean age of the infants at randomization was not significantly different between study groups across five RCTs.207,254,258,272,273 Clandinin et al. did not report the age of their infants.193 The GA of the preterm infants was below 37 weeks across five studies,207,254,258,272,273 except for Clandinin et al. that also included VLBW term infants.193 The between-group difference in GA was not significant across the studies.

In four studies, the proportion of male participants did not differ significantly between randomized groups,[207,258,272,273] although two studies did not mention this information in their report.[193,254] The range of males varied between 35%[272] to 56%.[207]

O'Connor et al. was the only one to describe the racial composition of their participants, which was predominantly White.[207] The rest of the studies failed to provide the race and/or ethnicity of their subjects.

Other variables like birth weight, proportion of SGA infants, percentage from multiple pregnancies, and Apgar score at birth, were nonstatistically different between groups in O'Connor et al.[207] van Wezel-Meijler et al. matched their population by birth weight and proportion of SGA at baseline.[272] Infants in both of Fewtrell et al.'s studies were well matched by birth weight and length, proportion of SGA, proportion from multiple pregnancies, and delivery by C-section at baseline.[258,273]

Three of six studies analyzed the between-group difference of maternal covariates. O'Connor et al. matched their study groups by maternal age, education, smoking status during pregnancy and in the home, prenatal care, the HOME inventory score and maternal intelligence measured with WAIS-R Raw vocabulary score.[207] The HOME Inventory Score was statistically different depending of the birth weight group—in infants <1,250 g, the control group had a

higher score than infants in the AA+DHA (fish/fungal) group and in infants >1,250 g, the control group had a higher score than the AA+DHA (egg-TG/fish) group. Finally, the infants with a birth weight higher than 1,250 g in the AA+DHA (fish/fungal) group had a higher score than those in the AA+ DHA (egg-TG/fish) group.[207]

The inclusion criteria were described in every included study, however, exclusion criteria were not reported in two studies.[193,273]

The studies included mostly healthy preterm infants with a defined range of weight drawn from neonatal intensive care units (NICU). Bougle et al. included healthy preterm infants (<34 weeks GA) free of respiratory, metabolic or neurological disease.[254] O'Connor et al. selected preterm infants (<33 weeks GA) with a birth weight ranging from 750 g to 1,805 g, including singleton and twin births as well as SGA subjects, that could initiate enteral feeding by the 28th day of life.[207] van Wezel-Meijler et al. included premature infants (<34 weeks GA) with birth weight of <1,750 g, normal neurological examination throughout the neonatal period, normal repeated brain ultrasound or showing minor abnormalities such as isolated subependymal haemorrhage and subventricle, with no ventricular dilation, transient periventricular echodensities, without evolution into cysts or any combination of previous findings.[272] Infants in the Fewtrell et al. (2002) trial had a GA below 37 weeks and a birth weight of <1,750 g, were free of congenital malformations known to affect neurodevelopment, and whose mothers decided not to breastfeed at 10 days of age.[273] Fewtrell et al.'s (2004) preterm infants (GA <35 weeks) with birth weight ≤2,000 g received at least one of their enteral feeds as formula milk during their hospital stay.[258] On the other hand, Clandinin et al. included VLBW term and preterm infants after their feeding reached 30 mL/kg/day.[193]

Three studies excluded infants with serious congenial abnormalities affecting growth and development, major surgery before randomization, perivenricular or intraventricular hemorrhage, maternal incapacity, liquid ventilation asphyxia resulting in severe and permanent neurologic damage, or uncontrolled systemic infection at the time of enrollment.[207,258,272]

The baseline characteristics of the patients in the Bougle et al. study were nonstatistically significant for the electrophysiological studies (i.e., motor and sensory nerve conduction studies, auditory evoked potentials).[254]

Only three trials measured the blood content of FAs at baseline.[207,272] O'Connor et al. found a nonsignificant difference between groups in the plasma or RBC (lipid fractions) levels of AA and DHA.[207] van Wezel-Meijler et al. observed the same finding.[272] Bougle et al.'s plasma phospolipid composition of EPA was significantly lower in the low LCPUFA supplemented formula than in the DHA/EPA/AA supplemented formula and human milk.[254] However, the RBC content of omega-3 and omega-6 did not differ between groups. The Bougle et al. study was the only one to describe the FA content in human milk i.e., 0.5% (SD 0.1) total FA DHA and ALA (omega-3) plus 0.9% (SD 0.2) total FA AA.[254]

None of the studies reported the presence of concurrent conditions in the study population and/or the use of medications. However, van Wezel-Meijler et al. reported that 13 patients were excluded from the analyses for the following reasons: necrotizing enterocolitis (n=2, 1 each group); chronic lung disease (n=3; n=2 DHA-AA vs n=1 control); grade 4 retinopathy of prematurity (n=1 AA+DHA); cystic periventricular leucomalacia (n=1 control); and, duration of artificial ventilation at baseline.[272] No differences were found between groups.[272] None of the

studies included information regarding maternal concurrent conditions, medications or background diet, that could be relevant for the infants consuming breast milk.

No other pre-study medications or treatments were mentioned in the included studies. The infants in the O'Connor et al. study were formula and/or human milk fed before study entry,[207] whereas, van Wezel-Meijler et al.'s infants received parenteral nutrition using glucose/Vaminolact 6.75%/Intralipid 20% (Kabi-Fresenius, Stockohlm, Sweden) for an average of 12 to 17 days, from 24 hours after birth.[272] This parenteral nutrition contained negligible amounts of LCPUFA. Three to 7 days after birth, enteral feeding was introduced using preterm formula (without LCPUFA). Total enteral nutrition was usually achieved within 2 to 3 weeks after birth.[272]

Intervention/exposure characteristics. The intervention groups in each trial received different types of supplemented infant formula, therefore, each study will be discussed separately.

Bougle et al.'s small sample were randomized to receive a formula with 17.7% total FA of LA (omega-6), AA (0.1%), ALA (omega-3: 1.2%), EPA (0.1%) and DHA (0.6%), for at least 30 days.[254]

O'Connor et al. randomized their participants to receive one of three study formulas with or without the addition of LCPUFA until term CA. The intra-hospital preterm formula was a modified version of Similac Special Care ready-to-feed (Ross Products Division, Columbus, OH, U.S.) with or without AA- and DHA-enriched oils. At term CA, postdischarge nutrient-enriched formula (modified version of NeoSure powder) with and without the same sources of AA+DHA and/or human milk was given to the infants until 12 months CA.[207] The first group received a supplemented formula with fungal and low-EPA fish oil (DHA/EPA ratio: 3.5/1) providing 0.27 g DHA, 0.08 g EPA and 0.43 g AA (per 100 mL) in the Similac Special Care formula and 0.16 g DHA and 0.43 g AA in the NeoSure formula. In the other group, egg-tryglyceride (TG) and low-EPA fish oil provided 0.24 g DHA and 0.41 g AA to the Similac formula, but 0.15 g DHA to NeoSure. The purveyors of the fish, fungal and egg-TG oils were Mochida International (Japan), Suntory Ltd. (Japan) and Eastman Chemicals Co (U.S.), respectively. The duration of the treatment was until 12 months CA.[207]

In van Wezel-Meijler et al., the neonates were randomized to receive preterm liquid formula supplemented with (4.4 g/100mL fat) a 2/1 ratio of DHA (0.015 g/100mL [0.34% fat]) as DHASCO® oil produced by microalgae (Martek Inc., Columbia, U.S.) and AA (0.031 g/100 mL [0.68% fat] as ARASCO® oil produced by fungi (Martek Inc.). The formula was continued from enrollment until a weight of 3000 g was reached. Subsequently, this group continued with a supplemented term formula (3.5 g/100 mL fat) with a reduced absolute amount of DHA (0.012 g/100 mL; 0.34% fat) and AA (0.025 g/100 mL; 0.70 % fat) until 6 months CA.[272]

Fewtrell et al. used a LCPUFA-supplemented preterm formula (n=95) (Prematil, Milupan) with fat blended from vegetable oils (palm coconut, soya, sunflower) and milk fat with derivates of LA and ALA sourced from evening primrose oil (GLA) and egg-lipids (AA [0.31 g/100 mL, DHA [017 g/100 mL], EPA [0.04 g/100mL]). Formula was provided as a ready-to-feed form for a mean of 31 days until neonatal unit care discharge.[273]

Clandinin et al. included two interventional groups. The first group (DAS group) received 17 mg DHA plus 34 mg AA/100 Kcal from single cell oils (SCO) (n=72) as preterm formula (24 Kcal oz), discharge formula (22 Kcal oz) and term formula (20 Kcal oz). The second group (DAF group) received the same formula as the DAS group but with 17 mg DHA/100 Kcal from fish oil and 34 mg AA/100 Kcal from single cell oils (n=90).[193]

Fewtrell et al.'s study 2004 study used a preterm infant formula supplemented with LCPUFA (OsterPrem with LCPUFA) until the infants were discharged from the NICU. Afterwards, a nutrient-enriched postdischarge formula was used (Farley's PremCare with LCPUFA). The fat was a blend of vegetable oils (high oleic sunflower oil, palmolein, palm kernel oil, and canola oil). LCPUFAs were sourced from borage (starflower) oil (GLA [omega-6] 0.9 g/100 mL) and tuna fish oil (high DHA/EPA ratio: DHA 0.5 g/100 mL, EPA 0.1 g/100 mL, AA: 0.04 g/100 mL). Formula was provided in ready-to-feed form during the hospital stay and in powdered form after discharge up to 9 months after CA.[258]

The studies compared the interventional formulas with unsupplemented infant formulas that were identical in appearance and smell,[258,273] contained the same proportion of monosaturated and saturated FAs, and given to the infants during the same period of time as the intervention group. Bougle et al. compared the supplemented formula with a LA (omega-6) and ALA (omega-3) enriched formula.[254]

The studies did not provide information regarding background diet, when introduced, and the purity data the omega-3 supplements. No study report included details as to whether, or how, the presence of methylmercury was tested for, or eliminated from, the omega-3 FA exposure.

Cointervention characteristics. Human milk was the reference standard group, either as a separate arm193,258,273 or as part of the formula groups that did not comply with the intervention.207 Bougle et al permitted the use of supplements, which contained dextrines, proteins and minerals during the study period. The patients received daily supplementation with 1,200 IU of vitamin D and 4.5 mg of vitamin E (Uvesterol ADEC).254 Infant preterm and term formulas in the O'Connor et al. study contained beta-carotene and natural vitamin E.207 Participants in both of Fewtrell et al.'s studies received an identical proportion of minerals and vitamins (A,D,E,K) in their formulas.258,273

Outcome characteristics. Only one study performed electrophysiological studies at baseline and after treatment.254 This study measured the latencies of auditory evoked potentials (BAEP test), motor and sensory nerve conduction studies on the posterior tibial nerve and the flexor hallucis brevis muscle.254

The Bayley's PDI was assessed in five of six studies.[193,207,258,272,273] O'Connor et al.'s average percent of agreement on scoring between site testers and central testers was 93% (range: 73%-100%).[207]

The first Fewtrell et al. study utilized the Knobloch, Passamanick and Sherrard's Developmental Screening Inventory (five subscales: adaptative, gross motor, fine motor, language and personal–social) to assess neurodevelopment at 9 months, as well as neurologic impairement at 9 and 18 months of followup (diagnosed by examining pediatrician).[273]

Study quality and applicability. Five RCTs received a mean Jadad total quality score of 4.2, indicating a good internal validity (Summary Matrix 6). One abstract was not assessed.311

Three trials received a score of 5,258,272,273 Bougle et al. and O'Connor et al. each received a score of 3.207,254 Bougle et al. failed to report the method of randomization and double-blinding,319 while O'Connor et al. was unblinded.310

Summary Matrix 14: Study quality and applicability of evidence for the effect of LCPUFA supplementation on the neurological development in preterm infants

		Study Quality								
		A			B			C		
Applicability	I	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
					O'Connor[U]	2001	470			
	II	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Fewtrell[A]	2002	283						
		Fewtrell[A]	2004	238						
	III	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		van Wezel-Meijler[A]	2002	55	Bougle[U]	1999	40			
n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment										

Qualitative synthesis of individual study results

The latencies of auditory evoked potentials (i.e., Wave I, Wave III, Wave V and I-V interpeak latency) difference between day 0 and day 30 were not significant in the study of Bougle et al.[254] The change in the motor nerve conduction test (m/s) was significantly higher in the group receiving the DHA/EPA/AA-supplemented formula and in the human milk groups, from day 0 to day 30. However, the change in the sensory test (m/s) was nonsignificant during the same period.[254]

Five studies evaluated Bayley's PDI after the administration of supplemented formula, unsupplemented formula, and/or human milk only (reference standard).[193,207,258,272,273] In O'Connor et al., a statistically significant feeding by birth weight stratum interaction was observed for Bayley PDI ($p=0.005$) among infants who consumed >80% of their feeding as study formula and/or human milk.[207]

van Wezel-Meijler et al. observed a statistically significant higher PDI score for the unsupplemented group compared with supplemented formula group, at 3, 6, 12 and 24 months.[272] The first Fewtrell et al. study did not find a statistical difference between formula groups at 18 months. Although the human milk group was not randomized, since it was used as reference standard, the PDI was significantly higher in the breastfed group compared with both formula groups.[273]

Clandinin et al., using ANOVA analysis, found that the control group had a significantly lower PDI score than the formula groups (DAS, DAF) and the human milk group (reference standard).[193] The second Fewtrell et al. study showed that there was a nonstatistical diference in Bayley's PDI scores between formula groups at 18 months.[258]

Fewtrell et al. found that The Knobloch, Passamanick and Sherrard's Developmental Screening Inventory scores (quotient) at 9 months did not differ significantly between the formula groups, whereas, the breastfed group had a significantly higher quotient compared with

the formula groups.[273] This study also failed to find a difference in neurological impairment between formula groups, at 9 and 18 months of followup.[273]

Bougle et al.'s cohort of healthy preterm infants had seven dropouts during the study. The main reasons were NEC (n=1) in the human milk group, hydrocephalus in the control formula group (n=5), and transfer to their referring hospital (n=3 human milk group, n=1 control, n=1 supplemented formula group).[254]

O'Connor et al.'s had 94 withdrawals (80%) at 12 months CA.[207] There was no statistical difference in the number of withdrawals between groups. The main reason for withdrawals was symptoms related to feeding intolerance. During the study 6 infants in the control group, 3 in the AA+DHA (fish/fungal) group, 6 in the AA+ DHA (egg-TG/fish) group, and none in the human milk groups, died. None of the infant deaths were related to study feedings.[207]

There were 13 dropouts in the van Wezel-Meijler et al. study.[272] Reasons for withdrawal were: necrotizing enterocolitis; chronic lung disease; grade 4 retinopathy of prematurity; cystic periventricular leucomalacia; change from formula feeding to mother's expressed milk; and, home-to-hospital distance. There were no losses to followup.[272]

In the first Fewtrell et al. study, six patients randomized to the control formula withdrew from the trial before 3 weeks for the following reasons: early discharge (<3 weeks of age; n=3); necrotizing enterocolitis (n=1); intolerance of feeds (n=1); and, breastfed (n=1).[273] Fourteen infants withdrew in the supplemented formula group. Reasons for withdrawal were: early discharge (n=2); necrotizing enterocolitis (n=5); maternal concern (n=2); and, death(n=2).[273] There were 14 lost to follow up at 9 months in the control group, whereas, only one infant withdrew in the supplemented formula group and three in the human milk group. There were two deaths in the supplemented formula group and three in the human milk group.[273] Clandinin et al. failed to report the dropouts.[193] Fewtrell et al.'s reasons for dropouts were: in the control group—abdominal distention (n=1); death due to bronchopulmonary dysplasia at 25 days of age (n=1); and lost to follow up at 18 months (n=21).[258] In the supplemented formula group, the reasons for dropouts were: necrotizing enterocolitis (n=1); and, lost to follow up at 18 months (n=15).[258]

Quantitative synthesis

The inclusion criteria for meta-analysis in this population were: 1. Formula with same content of omega-3 FA supplements (e.g., DHA+ AA or DHA alone) compared with a control formula without omega-3 FA; 2. same outcome measure; 3. same follow-up period or timepoint of outcome measure; 4. at least two trials. Only five studies measured the Bayley's Developmental Index (PDI). This outcome was chosen to evaluate the possibility of meta-analysis. However, outcome results were only available for more than one study at two follow-up times: CA 12 months and 18 months. At 12 months CA, outcomes were available for two studies.[207,272] In Wezel-Meijler et al.,[272] the experimental group received supplemented formula from the first enteral feeding time until 6 months CA. In O'Connor et al.,[207] however, supplemented formula was used until 12 months CA. We would have combined data at 6 months follow-up, however, this data was not available in O'Connor et al.[207] Thus, meta-analysis was not possible for this outcome.

Impact of covariates and confounders

O' Connor et al.'s Bayley's PDI score in <1,250 g birth weight infants who strictly followed the feeding protocol was greater in infants fed AA+DHA (egg-TG/fish) than control infants, even after adjusting for a number of covariates including the HOME inventory, maternal WAIS-R, and human milk intake.[207] The score did not differ statistically from either the control or AA+DHA (fish/fungal) groups. In an ITT and subgroup population analysis, the percentage of participants who had a significantly delayed motor performance did not differ statistically by study formula group.[207]

In van Wezel-Meijler et al., after adjusting for birth weight and number of SGA infants, there was no difference in PDI between the groups.[272]

To explore the possible influence of maturity on the response to LCPUFA supplementation, the first Fewtrell et al. study stratified the cohort by GA (<30 weeks). Infants who had a GA <30 weeks and received LCPUFA supplemented formula, had a Bayley PDI of 5.8 points higher than the control group, although the difference was nonsignificant.[273] There were no differences in Bayleys PDI between supplemented and control groups with a GA >30 weeks.[273] In this study, there was no significant interaction between formula and duration or volume of formula consumed on later outcome. At 18 months of age, breastfed infants had a significantly higher PDI score than formula groups. This result persisted after adjusting for effect modifiers (social class, level of maternal education, birth order and marital status).[273]

The second Fewtrell et al. study did not find a significant difference between groups when the PDI scores were adjusted by gender, GA and birth weight.[258]

The remaining studies did not report on the control for effect modifiers.

The power calculation was reported in three trials,[310,321,322] while the intention-to-treat analysis approach was reported in both Fewtrell et al.'s trials.[321,322]

Infant Formula Intake—Term Infants

Eight unique parallel design RCTs met eligibility criteria. These trials were published between 1995 and 2003. Seven trials were described in the Growth Pattern Outcomes section (see key question: Growth Patterns-Term Infant Formula Intake).[124,132,151,325,327,329] (Summary Tables 29-30)

Overview of relevant studies

All of the included studies evaluated the influence of supplemental omega-3 FA intake on neurological function of term infants. All but two studies—Birch et al.[182] and Jensen et al.,[203]—included a non-randomized group of breastfed infants that served as a reference standard. Agostoni et al. randomized Italian healthy term infants to receive LCPUFA-(AA+DHA+EPA) supplemented formula or a control formula. The main outcomes were the Brunet-Lézine test (Italian edition) of the graded psychomotor developmental test at 4 months, and the FA composition of venous blood (plasma and RBC PL composition).[176] (Summary Table 29)

Summary Table 29: Omega-3 fatty acids and its influence on neurological development in term infants

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)				
Agostoni, 1995, Italy: 4 mo Parallel RCT[176]	DHA+ EPA+ AA formula (n=27)	Control formula (n=29)/ HM (n=30)	S better score in DHA+EPA in Brunet-Lezine test (DQ) at 4^{++} NS at 24 mo	RBC DHA at 4 mo S + correlation with DQ at 4 mo NS at 24 mo	Jadad total: 4 [Grade: A]; Schulz: Unclear	II
Auestad, 1997, US: 12 mo parallel RCT[104]	Formula DHA+AA (n=46)/ HM (n=63)	Formula DHA (n=43)/ control formula (n=45)	S better in control gp vs. DHA+AA in PDI at 12 mo NS among 3 gps	n/a	Jadad total: 3 [Grade: B]; Schulz: Unclear	I
Lucas, 1999, UK: 6 mo parallel RCT[265]	Formula LCPUFA (n=154)	control formula (n=155)/ HM (n=138)	NS in PDI at 18 mo; NS in KPS at 9 mo (ITT)	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	II
Birch, 1998, US: 17 wk parallel RCT[182]	Formula DHA+AA (n=27)	Formula DHA (n=26)/ NR pb (n=26)	NS in PDI at 18 mo; NS in BRS at 18 mo	NS correlation of PDI & BRS at 18 mo and plasma & RBC LA, ALA, AA, EPA, or DHA at 4 mo & 12 mo	Jadad total: 5 [Grade: A]; Schulz: Unclear	I

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = linolenic acid; LA = alpha linoleic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; HM = human milk group; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; PDI = psychomotor developmental index, Bayley scale; KPS = Knobloch, Passmark, and Sherrard's test; BRS = behavioral rating scales; RBC = red blood cells; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); DQ = developmental quotien; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001

Summary Table 30: Omega-3 fatty acids and its influence on neurological development in term infants

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)				
Makrides, 1999, Australia: 12 mo parallel RCT[205]	Formula DHA+AA (n=28)/ NR HM (n=63)	Formula DHA (n=27)/ NR pb (n=28)	NS in PDI at 12 & 24 mo	S correlation between PDI at 12 mo & plasma AA levels at 12 mo	Jadad total: 5 [Grade: A]; Schulz: Adequate	III
Auestad, 2001a, US: 12 mo parallel RCT[227]	DHA+ AA (egg-TG) formula (n=80)	DHA+ AA (fish/fungal) formula (n=82)/ control formula (n=77)	NS in PDI at 6 & 12 mo	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	I
Auestad, 2001b, US: 1 y, parallel RCT[227]	DHA + AA formula/ HM (n=83)	Control formula/ HM (n=82)	NS in PDI at 6 & 12 mo	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	I
Jensen, 1997, US: 120 d parallel RCT[203]	**Formula 1** LA/ALA 44/1 (n=20)/ **F 3** LA/ALA 9.7/1 (n=20)	**Formula 2** LA/ALA 18.2/1 (n=20)/ **F 4** LA/ALA 4.7/1 (n=20)	NS in PDI at 12 mo S ↓ score (Gross motor DQ) in F 1 & F 3 vs. F2 & 4^{+}	S correlation between plasma DHA & PDI NS correlation between RBC DHA & PDI	Jadad total: 2 [Grade: C]; Schulz: Unclear	II

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; LA = linolenic acid; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; HM = human milk group; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = significant statistical difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; PDI = psychomotor developmental index, Bayley scale; CLOG = cod liver oil group; COG = corn oil group; RBC = red blood cells; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase(d)/higher; ↓ = decrease(d)/reduction/lower; DQ = developmental quotien

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. All studies were parallel RCTs with at least two arms. Countries where the studies were conducted included the United States,104,182,203,227 Italy,176 Australia,205 and the U.K.265

Agostoni et al. did not report their funding source.[176] Auestad et al.'s[104] study was supported by Ross Products Division, Abott Laboratoris, Columbus, OH and the U.S. Maternal and Child Health Bereau, Rockville, MD. Lucas et al.'s study was funded by Nestec Ltd (Switzerland).[265] The study of Birch et al. was supported by an NIH grant and Mead Johnson Nutritional Center (Evansville, IN).[183] Makrides et al.'s study was sponsored by Nestec Ltd (Switzerland), the MS

McLeod Research Trust and the Australian National Health and Medical Research Council.[205] Both of Auestad et al.'s trials were supported by Ross Products Division, Abott Laboratoris, Columbus, OH.[227] The study of Jensen et al. was sponsored by federal funds from the U.S. Department of Agriculture, Agricultural Research Service.[203]

Population characteristics. Maternal and infant characteristics were analyzed separately. Across the seven RCTs, sample sizes ranged from 60176 to 447265 infants. The maternal sample size was provided in only one study.265

The definition of a term infant (at least 37 weeks GA) was described in seven studies.[104,176,182,205,227,265]

Of eight RCTs, the mean GA of randomized infants was reported in six studies and ranged from 39 to 40.3 weeks).[176,203,205,227] The percentage of males of randomized infants was reported in five studies and ranged from 46.4% to 52.5% of infants.[176,182,205,227]

The gender ratio of the infants, among the different diet groups, was evenly distributed in four studies;[176,182,227] however, in the study of Makrides et al., there was a tendency for proportionally more boys to be enrolled in the group that received the highest dose of DHA.[205]

The mean age of the infant's mothers across the eight trials was impossible to determine given that the full sample size was not reported in four of the trials.[104,182,203,205] Excluding the studies failing to report the mean maternal age, the mean age of participants in the other four studies ranged from 27.0 (SD=5.12) to 32.4 (SD=5.7) years.[176,227,265] Auestad et al. did not report the age, gender distribution, or racial/ethnic background of either participating women or their children.[104]

Four studies failed to report the racial/ethnic background of the trial population,[176,203,205,265] In four studies, most of the participants were White, accounting for 68.4% to 90.2% of the study population and the distribution of race/ethnicity between the study groups of randomized infants was closely matched [104,182,227] Different variables were used to demonstrate family sociodemographic status in these studies (i.e., parental education, social score, smoking, marital status, birth order, number of siblings, HOME screening questionnaire score).[104,182,227]

Maternal social status was reported in five studies,[182,205,227,265] as well as information about maternal and/or paternal education and/or maternal marital status. There were no differences in sociodemographic variables among the study groups of randomized infants in all these studies.

Five studies failed to provide the maternal smoking history before and during pregnancy.[104,176,182,203,265] The studies that provided information about maternal smoking history, there was a tendency for less maternal smoking during pregnancy among the breastfed infants compared with the formula-fed groups.[205,227]

The inclusion/exclusion criteria were described in four of eight studies.[104,205,227] Only exclusion criteria were reported in two studies.[104,265] All the studies included only healthy term infants.

Intervention/exposure characteristics. Lucas et al. reported that by the protocol, the volume of formula consumed, calculated as the difference in amounts in the bottle at the start and end of the feed, was recorded.[265]

The duration of formula feeding was reported in all studies and ranged from 3 months[203] to 12 months.[104,205,227] Three studies[104,227] failed to report the name of the infant formulas. Seven trials reported the manufacturer of the omega-3 FA intervention.[176,182,203,205,265] Agostoni et al. used Aptamil with Milupan supplied by Milupa od Friedrichsdorf, Germany.[176] Lucas et al.[265] and Makrides et al.[205] used Nestec formula (Nestec Ltd, Switzerland), and both Birch et al.[182] and Jensen et al.[203] administered Enfamil, by Mead Johnson Nutritional Center (Evansville, IN). Both Auestad et al. trials used fish oil provided by Mochida International Co, Ltd, Tokyo, Japan and fungal oil by Suntory Ltd., Osaka, Japan; egg-derived trigycerides was provided by Eastman Chemical Co, Kingsport, TN.[227]

The duration of the intervention in children in four studies[104,205,227] was 12 months, at least 6 months in Lucas et al.,[265] and 4 months in three studies.[176,182,203]

In many of the studies, information regarding the time of introduction of solid food, caloric composition of formulas, source of omega-3 FA, micronutrient and vitamin content of formulas as well as presence of omega-3 FA stabilizing antioxidants, and attempts to deodorize any odor, were not clearly reported. Only five studies reported about the time of solid food introduction.[104,182,227,265] All the Auestad et al. studies permitted solid foods in all study groups beginning at 4 months of age.[104,227] Birch et al. did not introduce any solid food until 17 weeks of age.[182] Lucas et al. reported that the mean age of first introduction of any solid food did not differ between those fed LCPUFA and control formula (12.5 [SD=0.4] vs 11.8 [SD=0.4] weeks).[265] Information about caloric balance of feeding formulas was reported in five RCTs.[176,182,227,265] Infant formulas provided 670 kcal/L, 2805kJ/L and 670 to 694 kcal/L energy, respectively.

The source of omega-3 FA varied across the trials. Agostoni et al.'s fat blend was derived from palm oil, coconut and palm kernel fats, soybean oil, sunflower oil (parents PUFA), evening primrose oil (GLA) and egg lipids (LCPUFA PL and TGL).[176] The content of AA was 0.44 g/100 g fat; EPA 0.05 g and DHA 0.30 g.[176] However, the control formula also contained LA (omega-6) and GLA (omega-6).[176] Auestad et al.[104] described the source of DHA as fish oil in one of the formulas (DHA group) and the source of DHA and AA as egg-derived phospholipids in another formula (DHA+AA group). Not only the sources but the content of the omega-3 FAs were different in this trial: the DHA group contained 0.2wt% of DHA, while the DHA+AA group contained 0.12wt% DHA and 0.43wt% AA.[104] Lucas et al. reported the source of LCPUFAs as egg-derived phospholipids and triglyceride fractions (0.32wt% DHA, 0.3wt% AA and 0.01wt% EPA).[265]

Birch et al. compared two DHA-supplemented (0.35wt% DHA) and DHA+AA-supplemented formulas (0.36wt% DHA and 0.72wt% AA), containing single-cell oils (DHASCO® and ARASCO®; Market Biosciences, Columbia, MD, USA) with a control, LCPUFA-unsupplemented formula.[183] Makrides et al.'s study formula contained either no LCPUFAs (placebo) or 0.35wt% DHA from tuna oil or 0.34wt% DHA and 0.34% AA from egg phospholipids fraction.[205]

The first Auestad et al. trial[227] randomized infants to receive a control formula or one of two formulas supplemented with DHA and AA: 1) fish oil and fungal oil containing DHA (0.13wt%), AA (0.46wt%) and EPA (0.04wt%) or 2) egg-derived triglyceride, containing DHA (0.14wt%) and AA (0.45wt%). Only this study mentioned that study formulas were

indistinguishable in appearance and odor.[227] The second Auestad et al. trial[227] randomized the breastfed infants to receive a control formula or the AA+DHA (egg-TG) formula for 12 months. The infants were allowed water ad libitum, solid foods after 4 months and alternate formulas for up to 5 days.[227]

Jensen et al. randomly and blindly assigned each infant to receive one of four formulas from birth to 120 days of age.[203] There were no significant differences among formulas in the content of FAs other than ALA, which ranged from 0.4wt% to 3.2wt%. The LA:ALA ratio ranged from 44 to 4.8. No information was provided by the investigators regarding the delivery method of omega-3 FA exposure or attempts to deodorize the oil supplements.

Cointervention characteristics. Only one study[265] reported the content of vitamin and mineral supplements of feeding formulas and oils taken by women. No studies reported the pre-study or on-study medication use by either pregnant or breast feeding mothers or infants.

Outcome characteristics. The most frequently employed outcome assessing infants neurological development was the Bayley Scales of Infant Development, from which were derived a PDI. Two trials[104,205] used Bayley I Scales (1st edition) and five studies employed Bayley II Scales (2nd edition).[182,203,227,265] Lucas et al.[265] administered two tests to assess infant neurological development: as a primary outcome measure they used Bayley Scales, 2nd edition and as a secondary outcome measure–Knobloch, Passamanik and Sherrards Developmental Screening Inventory at 9 months. The latter test comprises of five scales: adaptive, gross motor, fine motor, language, and personal-social. Agostoni et al. used the Italian edition of the graded psychomotor developmental test by O. Brunet and I. Lezine for French children to rate the global neurodevelopment at 4 and 24 months of age. It explores four developmental areas: posture and gross notor function, adaptation and fine motor function, social reactions and language. This test was statistically validated.[176]

As for infants neurological development outcome assessment four studies evaluated these indices at 12 months[203,227,327], two studies at 18 months,[182,265] whereas in two other studies the assessment has been done at 12 and 24 months.[205]

Study quality and applicability. The eight RCTs received a mean Jadad total quality score of 4.25, indicating a good internal validity (Summary Matrix 15). Five trials received a score of 5,[124,205,265,329] Agostoni et al. received a score of 4,[176] Auestad et al. received a score of 3,[104] and Jensen et al. received a score of 2.[203] Jensen et al. failed to report the method of randomization,[325] Auestad et al. 1997 was unblinded,[327] and Agostoni et al. did not report the method of double-blinding.[134]

Summary Matrix 15: Omega-3 fatty acids and its influence on neurological development in term infants.

		Study Quality								
		A			B			C		
Applicability	I	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Birch[U]	1998	79	Auestad[U]	1997	274			
		Auestad[A]	2001	239						
		Auestad[A]	2001	165						
	II	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Agostoni[U]	1995	60				Jensen[U]	1997	80
		Lucas[A]	1999	447						
	III	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Makrides[A]	1999	146						
n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment										

Qualitative synthesis of individual study results

All but one study addressing the issue of child neurological development used Bayley's Developmental Scales (PDI) as a primary outcome measure.[104,182,203,205,227,265] The included studies did not find a statistically significant difference in PDI between the formula groups at 6, 12, 18 and 24 months of age. Makrides et al. (1997) reported a nonsignificantly difference among the 3 formula arms,[327] however when we compared the scores (PDI) between the DHA+AA group versus control formula (see Figure 11 below) the score was favouring significantly the control group. Lucas et al. did not find a significant difference in Bayley PDI at 18 months or in Klobloch, Passamanick and Sherrard's test performance at 9 months between control and LCPUFA groups.[265] Birch et al. also measured the developmental ages on the cognitive, language and motor subscales.[182] The cognitive and motor subscales were significantly poorer in the control group compared with both supplemented formula groups (DHA+AA and DHA).[182] No significant differences were found among diet groups on the language subscale.[182] The Behavioral Rating Scale (BRS) did not differ significantly among diet groups at 18 months of age.[182] Both of the Auestad et al. trials, with and without human milk, failed to find a significant difference in the BRS among diet groups at 6 and 12 months of age.[227]

Jensen et al found a statistically significant difference among the study groups in gross motor developmental quotient (GM DQ) index at 12 months of age.[203] Group 1 (i.e., lowest ALA intake) and Group 3 (LA/ALA ratio 9.7) had significantly lower mean GM DQ than Group 2 (LA/ALA ratio 18.2) and Group 3 (LA/ALA ratio 4.8).[203]

Agostoni et al. found that the Brunet Lezine test DQ was significantly higher in the supplemented group compared with the control group at 4 months of age; the difference was, however, not statistically significant at 24 months.[176]

Four investigators tried to find a correlation between different covariates as well as plasma and/or RBC phospholipid content of omega-3 and omeda-6 LCPUFAs and each neurodevelopmental index.[176,182,203,205] Agostoni et al. found that the RBC DHA content at 4 months was positively correlated with the DQ at 4 months but not at 24 months.[176]

Birch et al. found that PDI and BRS scores at 18 months of age were not significantly correlated with plasma or RBC LA, ALA, AA, EPA, or DHA at 4 months or at 12 months of age.[182] The PDI score was negatively correlated with VEP acuity at 4 months of age, i.e. better

visual acuity was associated with a better PDI score.[182] There was no significant correlation between PDI score at 18 months and FPL acuity at 4 months of age.[182] PDI score at 18 months was not correlated with normalized height, weight, or weight-for-length z scores at 4 months.

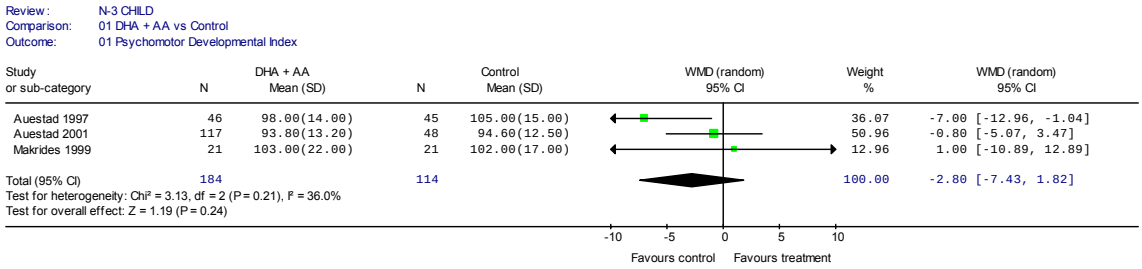
Jensen et al. found a positive correlation between both PDI and GM DQ scores and the plasma, but not the RBC, phospholipid content of DHA at 120 days of age.[203]

In the study performed by Makrides et al., the only FA variable to significantly influence the PDI was plasma AA at 1 year of age.[205] The same study established significant environmental variables that influenced PDI scores at 1 year—i.e., the assessor, maternal education, number of siblings, and the infant's age at testing. HC, number of siblings, and maternal smoking predicted PDI at 2 years of age, and PDI at 1 year was correlated with PDI at 2 years.[205]

Quantitative synthesis

The outcome measure selected to conduct meta-analysis was the Bayley's Developmental Index at 4 and 12 months of age. All the infants that were followed-up at 12 months, were exclusively breastfed until 4 months of age. At 12 months, outcomes were noted in three studies that were using the same comparators—DHA+AA versus unsupplemented formula.[104,205,227]

Figure 11. Bayley's Developmental Index (PDI). Meta-analysis was performed using the random effects weighted mean difference (WMD).



The WMD in the Bayley's PDI score at 12 months was nonstatistically significant (WMD: -2.80, CI 95%: -7.43; 1.82).[104,205,227]

Impact of covariates and confounders

Most of the RCTs did not reveal enough evidence regarding the comparability of the study groups in terms of infant gender, ethnic/racial distribution, birth characteristics, parental socioeconomic background, education or maternal and/or paternal smoking. Only one study failed to report the baseline characteristics of randomized groups so it was impossible to estimate the impact of potential covariates and confounders on the study results.[182] Differences in some characteristics at enrollment were noted between breastfed and formula fed infant groups only.

In the Makrides et al. study, breastfed infants had parents who were less likely to smoke, had attained a higher level of education and had more prestigious social scores compared with formula fed infants.[205] Bayley's PDI scores at 12 and 24 months were adjusted for different covariates. The only variables that significantly influenced (regression model) the PDI scores at

12 months were the assessor, maternal education, number of siblings and the infant's age at testing. At 24 months, HC, number of siblings and maternal smoking predicted the PDI.[205] Auestad et al.[227] reported that maternal age and GA in a breastfed infant group were significantly higher than that in the randomized formula-fed groups. In the same study, infants assigned to breast milk also had a significantly smaller percentage of mothers having no postsecondary education and smaller prevalence of smoking exposures both in utero and in the household.[227] In Lucas et al., the results were unaffected when Bayley's score was adjusted for center or observer.[265]

Jensen et al. used a multiple regression model to adjust the scores for effect modifiers such as birth weight, weight at 120 days of age, chronological age on the assessment day.[203] However, none of these variables seemed to affect the results.

The power calculation was reported in seven trials,[124,132,134,151,325,329] while the intention-to-treat analysis approach was reported in only one study.[132]

Neurological Development Outcomes in Light of Biomarker Data

What is the Evidence That Term or Preterm Human Infants' Neurological Development is Associated With the Omega-3 or Omega-6/Omega-3 Fatty Acid Content of Maternal Biomarkers During Pregnancy?

One cross-sectional study was identified to answer this question. Cheruku et al. was conducted in the United States and founded by the National Institutes of Health, the US Department of Agriculture, the Donaghue Medical Research Foundation, and the University of Conneticut Research Foundation.[274] This study was published in 2002.[274] (Summary Table 31)

Overview of relevant study characteristics and results

Cheruku et al. assessed the association between the content of LCPUFA in maternal blood and the Central Nervous System (CNS) integrity of their newborns.[274]

Healthy pregnant women (n=17) were included after their admission for delivery. The exclusion criteria were: women with a history of chronic hypertension, hyperlipidemia, renal or liver disease, heart disease, thyroid disorders, multiple gestations, pregnancy-induced complications (e.g., hypertension, preterm labor, or premature rupture of membranes), treatment during labor with drugs that affect respiration of newborns, or any infant with lower than 4 hours of crib time in the first and second days postpartum.[274]

The maternal blood samples to measure the plasma FA content were taken at delivery. The CNS integrity was measured using the Motility Monitoring System to record the sleep patterns (i.e., quiet sleep, active sleep, sleep-wake transition, wakefulness, time spent out of the crib) on postpartum day 1 and postpartum day 2.[274] Infant sleep patterns are an expression of central

integrative control. Multiple mechanisms involving both neural and humoral processes in various regions of the brain interact to produce sleep and wakefulness. Changes in the sleep architecture may be associated with neurologic changes during development and that deviant sleep patterns may be associated with neuroogic deficits.[338]

Summary Table 31: Association of neurological development outcomes and biomarkers content in infants (observational study)

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable associations[2,3]	Internal validity	Applicability
Cheruku, 2002, US: Cross-sectional[274]	Healthy pregnant women high DHA (n=10)	Healthy pregnant women low DHA (n=7)	Maternal DHA was (-) associated with AS, AS:QS & sleep-wake transition^{+} (d 2) Maternal DHA (+) associated with wakefulness^{+} (D2) n-6:n-3 ratio in maternal plasma was (+) associated with AS, AS:QS &sleep-wake transition^{+} (d 1) n-6:n-3 ratio in maternal plasma was (-) associated to wakefulness^{+} (d 1)	Quality score: 6 [Grade B]	I

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; N/A = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ↑ = increase; ↓ = decrease/reduction; AS = active sleep; QS = quiet sleep

Regression analysis used to describe the associations of maternal plasma PL FA concentrations with infant sleep and wake states indicated that, among the omega-6 and omega-3 LCPUFAs, only the omega-3 FAs specially DHA, and the n-6:n-3 ratio showed strong correlations on both postpartum days 1 and 2.[274] The following correlations were the most significant among all the statistically significant correlations for this population. On postpartum day 2, maternal DHA was negatively associated with active sleep (AS), AS:QS (quiet sleep) and sleep-wake transition, and positively associated with wakefulness.[274] On postpartum day 2, the ratio of n-6:n-3 LCPUFAs in maternal plasma was positively associated with AS, AS:QS and sleep-wake transition and negatively associated to wakefulness.[274]

On postpartum day 1, the ratio of n-6:n-3 LCPUFAs in maternal plasma was negatively associated with QS and positively associated with arousals in QS.

When the cohort was analyzed by maternal DHA plasma concentration, the high DHA group (>3.0% by wt of total FAs) did not differ significantly from the low DHA group (≤3.0% by wt of total FAs) in terms of maternal age, race, parity, duration of gestation, maternal education, infant birth weight and length, infant HC and Apgar score at 1 and 5 minutes.[274] However, infants from mother with high DHA concentrations had significantly less AS and had a lower AS:QS compared with infants of mothers with low DHA concentrations. Furthermore, infants in the high DHA group had significantly less sleep-wake transition and more wakefulness than did infants in the low DHA group on postpartum day 2.[274]

Study quality and applicability. The quality score was 6 and the applicability level was III.

Summary Matrix 16: Association of neurological development outcomes and biomarkers content in infants

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author Cheruku	Year 2002	n 17	Author	Year	n
	II	Author	Year	n	Author	Year	n	Author	Year	n
	III	Author	Year	n	Author	Year	n	Author	Year	n
n = number of allocated/selected participants										

What is the Evidence That Term or Preterm Human Infants' Neurological Development is Associated With the Omega-3 or Omega-6/Omega-3 Fatty Acid Content of Child Biomarkers?

Five studies were identified to answer this question, including four RCTs that were described in the Growth Pattern Outcomes and Neurological Development Outcomes sections (see key questions: Growth Patterns & Neurological Development-Term Infant Formula Intake),[176,182,203,205] and a prospective single cohort study published in 2001.[271] (Summary Table 32)

Overview of relevant study characteristics and results

Innis et al. selected a cohort of 83 Canadian term infants who were exclusively breastfed, with birth weights in the range of 2,500 g to 4,500 g.[271] The objective of the study was to measure the infant RBC DHA content and its association with the visual, neurological or cognitive development.[271]

Innis et al. was funded by the Medical Research Council (MRC) of Canada and Ross Laboratories, OH.[271]

The infants were enrolled within 2 weeks of age and to be eligible, their mothers were required to intend to breastfeed their infant without providing infant formula or cow's milk for at least 3 months and without introducing solid foods for at least the first 4 months after birth. The infants were excluded if their mothers had substance abuse, metabolic or physiologic problems, communicable diseases, and infants with evidence of metabolic or physical abnormality.[271]

Only one mother was taking FA supplements with LA and DHA. The maternal diet was not reported or controlled. Only five mothers were smokers during the study.[271]

The outcome assessed included the Bayley's PDI at 6 and 12 months and its correlation with the RBC DHA and AA content in infants.

Multiple linear regression analysis was used to determine the impact of the FA variables on the outcomes. The analysis controlled statistically for the duration of breast-feeding, maternal education, family income, gender, maternal smoking, birth order and birth weight, length and HC.

Summary Table 32: Association of neurological development outcomes and biomarkers content in infants (observational study)

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable associations[2,3]	Internal validity	Applicability
Innis, 2001, Canada: Prospective single cohort[271]	Term breastfed infants (n=83)	n/a	NS RBC DHA or AA at 2 mo & Bayley's PDI score (6-12 mo)	Quality score: 8 [Grade A]	II

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; N/A = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; RBC = red blood cells; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ↑ = increase; ↓ = decrease/reduction

No statistically significant relation was found between the infant DHA or AA status (RBC) at 2 months of age and the Bayley's PDI score at 6 and 12 months of age.[271]

There were 31 dropouts at 12 months due to different reasons, like lost to follow up, fed with formula before 3 months of age, or lack of blood samples.

Study quality and applicability. This study had a quality score of 8 and a level of applicability of II.

Summary Matrix 17: Association of neurological development outcomes and biomarkers content in infants

		Study Quality								
		A			B			C		
Applicability	I	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
	II	**Author** Innis	**Year** 2001	**n** 83	**Author**	**Year**	**n**	**Author**	**Year**	**n**
	III	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**

n = number of allocated/selected participants

Visual Function Outcomes

What is the Evidence That Maternal Intake of Omega-3 Fatty Acids During Pregnancy Influences Visual Function in Term or Preterm Human Infants?

Overview of relevant study characteristics and results

One double-blinded RCT[235] and one cross-sectional study[275] evaluated the influence of maternal intake of omega-3 FAs during pregnancy on the visual function. The RCT was conducted in the United Kingdom and was funded by the Scottish Office Health Dept.[235] The cross-sectional study was conducted in Cuba and was funded by Canadian International Development Agency.[275] (Summary Table 33)

Malcolm et al.[235] investigated the photoreceptor function of healthy term infants (mean 279.7 [SD:9.5] days; males 52%) at approximately 1 week of age, whose mothers (ages 17-36 years) received fish oil capsule supplements from a mean of 15.4 wk gestation until delivery (Marinol D40, 100 mg DHA per capsule, R.P. Scherer Ltd, Swindon, UK) compared with infants (279.6 [SD:8.5] days; males 37.9%) whose mothers received sunflower oil capsules from the same time point. Women were excluded if they had had a twin pregnancy, placental abruption, postpartum hemorrhage, allergy to fish products, a thrombophilic tendency, or receiving drugs affecting thrombocyte function. Healthy full-term infants with an Apgar score above 7 and with no visual, medical or developmental disorders were included. The tests used to measure photoreceptor function were intensity-series electroretinogram (ERG) (rod photoreceptor function) and standard maximum combined ERG (mixed rod and cone function). In addition, the b wave amplitudes were fitted to the Naka-Rushton function as another assessment of rod photoreceptor function and the derived log δ was used as a measure of retinal sensitivity.[235]

Summary Table 33: Omega-3 fatty acids intake during pregnancy and its influence on visual function in term infants

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n) Group 5	Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
Malcolm , 2003, Denmark: 15 wks Parallel RCT[235]	DHA (fish oil) capsules (n=50)/ term infants (n=31)	Pb capsules (oleic sunflower oil) (n=50)/ term infants (n=29)	NS in b wave implicit time NS in Naka-Rushton function NS in log δ NS in maximium combined ERG	NS correlation of max combined ERG & cord blood DHA NS (-) correlation of log δ & cord blood AA S (+) correlation of log δ & cord RBC proportion DHA^{+} & total n-3 FA $^{+}$, n-6/n-3^{+} S correlation of log δ & cord RBC quartiles of DHA^{+++}, AA^{+}, total n-3 LCPUFAs $^{+}$	Jadad total: 3 [Grade: B]; Schulz: Unclear	II
Krasevec, 2002, Cuba: cross-sectional[275]	Healthy pregnant women (n=56) Breastfed infants (n=56)	HM + formula infants	Visual acuity scores 99% prediction for 2.5 mo old infants NS Mean values for visual acuity between HM vs. HM + formula infants	NS correlation visual acuity & any individual PUFA concentration, ratio of PUFA concentrations or concentrations of groups of PUFAs in infant tissues	Quality score: 7 [Grade B]	III

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; N/A = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; RBC = red blood cells; PL = phospholipid; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ↑ = increase; ↓ = decrease/reduction; HM = human milk

Malcolm et al. showed that maternal fish oil supplementation during pregnancy, from a mean of 15.4 weeks gestation to delivery, had no significant effect on retinal function (rod photoreceptor function, rod and cone photoreceptor function, or retinal sensitivity) assessed within the first week of life in healthy term infants.[235] There were no differences between the fish oil and placebo groups in the maternal self-selected diets, fish intake, or consumption of DHA-containing dietary supplements at study entry, or in the time period between study entry and delivery (assessed by interview at 15 and 28 weeks gestation, and at delivery).

In addition, the groups did not differ significantly in age, previous obstetric history, socioeconomic status, smoking habits, alcohol intake and exercise patterns. Satisfactory intensity-series ERGs were recorded in 41/60 infants (fish oil, n=22, placebo, n=19), and maximum combined ERGs were recorded in 44/60 infants (fish oil, n=25, placebo, n=19). Regardless of mother's supplementation group, significant correlations were found between retinal sensitivity and cord RBC levels of DHA, AA, omega-6/omega-3 FA and total omega-3 FAs.[235]

Krasevec et al. evaluated the visual acuity in 2-month old term infants (mean age:40.4 [SD:1.5] weeks; males NR) born to Cuban mothers (mean age 26.8 [SD:4.0] years) who had received a high fat fish diet during pregnancy and breast feeding.[275] Included were pregnant women with a history of normal pregnancy, no medical risks affecting FA metabolism (i.e. heart, kidney, hypertensive, gallbladder, or thyroid diseases and gestational or other diabetes), resident of Havana, and a range of age from 17 to 36 years; exclusion criteria were not reported for the mothers. Neither inclusion or exclusion criteria were reported for the infants.[275]

All Cubans received 227 g of a high fat fish every week through the ration system before and during pregnancy, and a higher amount during breast feeding. Infants were exclusively breastfed (55%), fed a combination breastmilk and bottle-feeding (39%), or not fed any breastmilk (5%). Supplemental milks were fed for an average of 2 to 4 weeks before the 2-month study. Binocular visual acuity was assessed at 2 months of age using the Teller Acuity Cards with acceptable reliability.

Krasevec et al.[275] observed that there were no significant correlations between visual acuity and any individual PUFA concentration, ratio of PUFA concentrations or concentrations of groups of PUFAs in the infants' plasma and RBCs, in term infants born to Cuban women who received high fat fish during pregnancy and breast feeding. Fatty acid composition was analyzed in 31/56 infants's plasma and 33/56 infants' RBCs. Infant RBC PUFA contents were compared with values reported in the literature without statistical evaluation. Visual acuity was tested in 54/56 infants. The visual acuity scores for all tested infants were within the 99% prediction limits for 2.5 month old infants. The group mean visual acuity score was within a range of data obtained from full-term, normally developing infants. Mean values for visual acuity were not significantly different between the exclusively breastfed or not exclusively breastfed infants.

Study quality and applicability. Malcom et al.'s Jadad total quality score was 3 (failed to report methods of randomization and double-blinding), indicating a good internal validity.[235] The allocation concealment was unclear. Krasevec et al. quality score was of 7.

Summary Matrix 18: Association of maternal intake of omega-3 fatty acids during pregnancy with full-term infant visual function

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author	Year	n	Author Malcolm[U]	Year 2003	n 100	Author	Year	n
	III	Author	Year	n	Author Krasevec	Year 2002	n 56	Author	Year	n
n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment										

What is the Evidence That the Omega-3 Fatty Acid Content of Maternal Breast Milk, With or Without Known Maternal Intake of Omega-3 Fatty Acids, Influences Visual Function in Term or Preterm Human Infants?

Two RCTs and two observational studies published from 1997 to 2001 met eligibility criteria regarding the influence of maternal breast milk intake in term infants.[138,140,248,276] Krasevec et al.[275] also addressed the issue of maternal intake during breastfeeding and visual function in term infants, and is fully described above (see key question: Maternal Intake/Visual Function).[275] No reports were identified in the preterm population. (Summary Table 34, 35)

Overview of relevant study characteristics and results

Gibson et al. was a double-blind RCT that investigated the maternal intake effect on breastfed infant's neurological and visual function outcomes in Australia.[138] This study included mothers of term infants (>37 weeks of GA) who intended to breast feed for at least 12 weeks (n=52, means age: 30 [SD=4] years). These mothers were randomized to receive one of five doses (0, 0.2, 0.4, 0.9, or 1.3 g DHA/day) of a DHA-rich algal oil (DHASCO, Market Biosciences, MD, US) between day 5 and week 12 postpartum. The oil contained 43% DHA, 1% omega-6 PUFA, 38% saturates and 18% monosaturates. Infants who were exclusively breastfed for 12 weeks were assessed. Infants (n=20) were healthy, appropriate weight for GA, Apgar scores greater than 7 at 5 minutes.[138]

Infant's visual function using VEP (logMAR) was assessed at 12 and 16 weeks of life, and for global development (Bayley's Scales of Infant development) at 1 and 2 years of age. Mothers were from middle class families and completed year 12 education. The five groups were compared in terms of maternal age, maternal BMI, GA, infant's gender, birth weight, birth length, birth HC, Apgar score, siblings, maternal social score, smoking, education, home stimulation, and length of breast feeding, at baseline. There was a predominance of boys in the group that received the highest dose of DHA.[138]

Jensen et al. investigated the effect of DHA supplementation in lactating women on the visual function and growth of their infants.[248]

Mothers were randomaly assigned to receive 200 to 250 mg DHA per day as either algal DHA (n=42), refined high-DHA fish oil (n=42) or placebo (n=42), for 120 days after delivery. Infant characteristics, as well as the maternal characteristics, were not described in this abstract.[248]

Summary Table 34: Omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences visual function in term or preterm human infants

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n) Group 5	Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
Gibson, 1997, Australia: 12 wk parallel RCT[138]	1.3g/d DHA (n=8)/ 0.2g/d DHA (n=10)	0.9g/d DHA (n=10)/ 0.4g/d DHA (n=12)/ pb (n=12)	NS VEP acuity between dietary groups at 12 & 16 wks	No correlation VEP & DHA HM, infant plasma or RBC LCPUFA	Jadad total: 3 [Grade: B]; Schulz: Unclear	II
Jensen, 1999, US: 12 mo parallel RCT [248]	Algal DHA (n=42)	Fish oil DHA (n=42)/ Placebo (n=42)	NS VEP latency, sweep VEP acuity or Teller Card Acuity at 120 or 240 d	NS correlation visual function & infant plasma PL DHA at 120 d	Not assessed	X

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; n = sample size; pts = study participants; NR = not reported; ; S = statistically significant difference; NS = nonsignificant statistical difference; N/A = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; RBC = red blood cells; PL = phospholipid; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ↑ = increase; ↓ = decrease/reduction; BF = breast feeding; VEP = visual evoked potentials; HM = human milk

Jorgensen et al. investigated, in a cross-sectional study, whether the variation in milk DHA content between Danish mothers is large enough to cause differences in visual acuity in their healthy term 4-month-old infants, and to evaluate the influence of frequency of fish intake on the DHA level of milk.[140] The study included term infants (GA 37-42 weeks) with normal birth weight for GA; uncomplicated pregnancy, delivery, and neonatal period; Apgar score > 8 after 5 minutes; fully breastfed at the time of the examination (i.e., no energy drinks and < 100 mL of formula per day). Infants were excluded if they were SGA (< 10th PC of birth weight), had strabismus, or operation of pyloric stenosis. Seventy infants were enrolled, of which 39 completed the study (mean age: 17.4 [SD=0.7] months; 51% males).[140] The study was conducted in Denmark and was supported by the Danish Research and Development Programme for Food Technology (FOTEK), and BASF Health and Nutrition A/S.[140]

The study by Williams et al. was a population-based cohort study that compared the stereoacuity at age 3.5 years in healthy term children who were breastfed with similar children who had not been breastfed after adjustement for socioeconomic status and maternal diet.[276] The study included a random selected subset of children born in the last 6 months of the Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC) enrollment period.

The ALSPAC was a prospective population birth cohorth study.[339] Infants were excluded if they had strabismus, reduced vision, high refractive error, preterm infants (GA <37 weeks).[276] Williams et al. enrolled 641 children aged a mean of 43.2 (SD=0.6) months (i.e., 3.5 years), of which 435 completed the study period (52.1% males).[276] The study was based in the United Kingdom, and was funded by the Medical Reseach Council, the Wellcome Trust, Ministry of

Agriculture, Foods and Fisheries, the Departments of Health and Enviroment, the South West Regional Health Authority, the National Eye Research Centre, Cow and Gate, and Milupa.[276]

Jorgensen et al.'s infants were exclusively breastfed until 14 weeks of age. The median DHA content of the milk was 0.31 wt% of total FAs (range: 0.12-1.20 wt%), AA was 0.30 (SD=0.07) wt% and the content of EPA was 0.39 (SD=0.07) wt%. The study described the details regarding the maternal age, weight gain during pregnancy, Apgar score at 5 minutes, gender, GA, birth weight, length at birth and HC, as well as growth parameters at the time of the examination.[140] Jorgensen et al.' mothers did not take any fish oil supplements regularly, however one of the ninemothers that ate fish the day before the milk sample was taken, ate lean fish while the remaining ate fatty fish.[140] Outcomes measures were visual acuity, using VEP acuity (expressed in LogMar) in the 4-month infants, and the correlation with the LCPUFA content of maternal breast milk and/or maternal fish intake.[140]

Williams et al. used different questionnaires to collect information related to the infant's feeding practices from 4 weeks to 6 months of age and at 36 months (3.5 years), as well as other information like socioeconomic status, smoking status, and housing.[276] During the study period, no formula milks supplemented with DHA were commercially available in the United Kingdom.[276] This study did not report the DHA/AA/EPA FA content in human milk. Williams et al. measured the stereoacuity (peripheral or poor; macular or moderate; foveal or adult) in the 3.5 year-old children. They also measured the possible correlation with the percentage of DHA content of the maternal RBC PL during pregnancy, as well as with the mother's intake of fish oil.[276]

Both Jorgensen et al.'s and Williams et al.'s studies collected the maternal diet intake using a food-frequency questionnaire, including questions regarding fish intake.[140,276]

Summary Table 35: Omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences visual function in term or preterm human infants

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n) Group 5	Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
Jorgensen, 2001, Denmark: Cross-sectional[140]	Term infants HM (BF) (n=39)	n/a	S association between visual acuity (VEP) at 4 mo & mother's milk DHA^{+}	NS association between AA, EPA, LA & ALA (n-3) with visual acuity	Quality score: 9 [Grade A]	III
Williams, 2001, UK: Prospective cohort[276]	Term infants HM (BF) (n=334)	Term infants never breastfed (n=101)	BF was S correlated to foveal (adult) stereacuity Maternal oily fish intake during pregnancy was S correlated with foveal stereoacuity	S correlation between child's stereoacuity at 3.5 y & antenatal mother's RBC DHA content	Quality score: 9 [Grade A]	III

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; N/A = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; RBC = red blood cells; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ↑ = increase; ↓ = decrease/reduction; BF = breast feeding; VEP = visual evoked potentials; HM = human milk

Gibson et al.'s VEP acuity did not differ significantly between the dietary DHA groups at either 12 or 16 weeks of age, although numbers were limited in each treatment group. VEP acuity (n=19) significantly improved with age (0.83 [SD=0.13 logMAR at 12 weeks vs. 0.73 [SD=0.09] log MAR at 16 weeks, p<0.01). There was no association between VEP acuity and the level of DHA in the breast milk, infant plasma or RBC as well as with any socio-demographic variables.[138]

Jensen et al. failed to find a statistical difference among groups in VEP latency, sweep VEP acuity or Teller Card acuity at 120 or 240 days of age in term infants. However, transient VEP amplitude was lower in infants of mothers who received the algal DHA supplement than infants in the other two groups at 120 days but not at 240 days of age. There were no significant correlations betweeb the visual function and the milk DHA or infant plasma PL DHA content at 120 days of age.[248]

Jorgensen et al. observed a significant association between visual acuity of the infant at 4 months of age and the mother's milk DHA.[140], controlling for intake of fatty fish the day before sampling. The visual acuity of infants of mothers who ate fish the day before sampling did not differ from the rest of the group. Neither did AA, EPA, LA and ALA (omega-3) correlate with

visual acuity, nor did any of the antropometric data (i.e., GA or age at examination). No association was found between educational level of the mother and visual acuity or educational level and milk DHA.[140] In a general linear model, including frequency of consumption of lean and fatty fish, and fatty fish intake the day before sampling, all three variables were associated positively with milk DHA.[140]

Williams et al., in an univariate analysis, found that breast feeding, greater maternal age, and consumption of oily fish by the mother antenatally or by the child up to the age of 3.5 years were all associated with an increased likelihood of the child having foveal (adult) stereacuity.[276] As these variables were interrelated, a multiple logistic regression analysis was used to determine which factors might be independently associated with the child's stereoacuity. The variable most associated with an increased likelihood of foveal as opposed to worse-than-foveal stereoacuity was breast feeding.[276] This result was consistent even when it was stratified by age (< or >4 months), compared with a diet without breast milk. A second variable was the mother's intake of oily fish. The mothers who ate oily fish at least once every 2 weeks during pregnancy were more likely to have children who achieved foveal stereoacuity than were the mothers who never ate oily fish (adjusted OR: 1.57; 95%CI: 1.00-2.45). There was no statistical evidence of interaction between the effects on stereoacuity and whether or not the mother ate oily fish, or for breast feeding compared with formula feeding. When only the children whose mothers never breastfed (n=101) were selected, foveal stereoacuity in the children (n=20) was still more likely if the mothers ate oily fish during pregnancy than if they did not, however, the difference was not significant. The correlation between maternal age and children eating oily fish did not reach statistical significance using the multiple regression model.[276] There was a correlation between the child's stereoacuity and the antenatal mother's RBC DHA content.

None of the RCTs reported the power calculation or intention-to-treat approach.[336,340]

Study quality and applicability. Gibson et al. had a total quality score of 3 (did not report the randomization and double-blind method), indicating a good internal validity.[336] However, Jensen et al. could not be assessed given it was an abstract.[340] The allocation concealment was unclear in both. The observational studies had a mean quality score of 9.

Summary Matrix 19: Omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences visual function in term or preterm human infants

		Study Quality								
		A			B			C		
Applicability	I	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
	II	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
					Gibson[U]	1997	52			
	III	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Jorgensen	2001	39						
		Williams	2001	435						

n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment

What is the Evidence That the Omega-3 Fatty Acid Content of Infant Formula Influences Visual Function in Term or Preterm Human Infants?

What is the Evidence That the Omega-3 Fatty Acid Content of Maternal Breast Milk, With or Without Known Maternal Intake of Omega-3 Fatty Acids, and Together with the Omega-3 Fatty Acid Content of Infant Formula, Influences Visual Function in Term or Preterm Human Infants?

Infant Formula Intake - Preterm Infants

Nine unique studies met the eligibility criteria in investigating the effect of omega-3 FAs on visual function in preterm infants. All these studies were parallel design RCTs published between 1992 and 2003. All the studies were summarized in the Growth Pattern Outcomes and Neurological Development Outcomes sections (see key questions Growth Patterns & Neurological Development-Preterm Infant Formula Intake). (Summary Tables 36, 37)

Overview of relevant studies

All the included studies evaluated the influence of intake of infant formula, supplemented with omega-3 FAs on visual function (i.e., visual acuity, retinal development, visual behavior and attention) in preterm infants. The effect on visual function in the groups receiving omega-3 FA-supplemented formulas, were compared with the effect in the groups (control) receiving standard infant formulas (without omega-3 FA supplementation) and/or human milk. In five of the nine studies, visual function measured in infants receiving formulas with or without omega-3 FA supplementation was compared with visual function in infants receiving breast milk.198,207,212,251,254 In all these studies, the breastfed arms were non-randomized and served as reference groups.

Summary Table 36: Omega-3 fatty acids intake associated with the visual function in preterm infants

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Notable clinical-biomarker[2,3] correlations	Internal validity	Applicability
Birch, 1992, US: 6 mo parallel RCT[212]	Soy/marine oil: EPA+ DHA (n=26)/ HM (n=8)	Soy oil: ALA (n=22)/ Corn oil (n=18)	S↓ in VEP for all grps at 57 wks S ↓ VEP in DHA+EPA vs. grps 2-3 at 36-57 wks^{+} NS b-Rod ERG at 36-57 wks	S correlation between: RBC-DHA/DPA & VEP^{+++} RBC-DHA/DPA & FPL^{+} at 57 wks	Jadad total: 2 [Grade: C]; Schulz: Unclear	II
Carlson, 1992, US: 12 mo parallel RCT[185]	Marine oil: DHA + EPA (n=33)	Control: LA (n=34)	S ↑ resolution acuity in DHA +EPA vs. control at 2 & 4 mo $^{+++}$	S correlation (+) RBC DHA at 2 mo with visual acuity at 2,4 mo	Jadad total: 4 [Grade: A]; Schulz: Adequate	II
Koletzko, 1995, Germany: 21 d parallel RCT[251]	LCPUFA-enriched: DHA + EPA + ALA (n=9)	Control: (n=10)/ HM (n=8)	NS difference in visual acuity across at 21 d	n/a	Jadad total: 2 [Grade: C]; Schulz: Unclear	III
Carlson, 1996, US: 4 mo parallel RCT[191]	Marine oil: DHA +EPA (n=26)	Control ALA (n=23)	S↑ higher acuity in DHA+EPA vs. control at 2 mo^{+} NS at 4-12 mo	n/a	Jadad total: 3 [Grade: B]; Schulz: Unclear	II
Faldella, 1996, Italy: 5 mo parallel RCT[198]	LCPUFA-enriched: DHA +EPA+ ALA (n=21)	Control EPA + ALA (n=25)/ HM (n=12)	S shorter wave (N4 & P4) latencies VEP in DHA+EPA vs. control at 52 wks PCA^{++} NS in BAEP & ERG (a & b) latencies) across grps1-3	At 52 wks PCA, inverse correlation between: RBC-DHA & N4 wave latency $^{+}$ & RBC-DHA & P4 wave latency^{++}	Jadad total: 1 [Grade: C]; Schulz: Unclear	III
Bougle, 1999, France: 30 d parallel RCT[254]	LCPUFA-enriched: DHA + EPA + ALA (n=14)	Control (n=11)/ HM (n=15)	NS in VEP (N1 wave latency) at 30 d	n/a	Jadad total: 3 [Grade: B]; Schulz: Unclear	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; RBC = red blood cells; PL = phospholipid; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; HM = human milk; GA = gestational age; PCA = postconception age; CA = corrected age; ERG = electroretinogram; BAEP = brainstem acoustic evoked potential

Summary Table 37: Omega-3 fatty acids intake associated with the visual function in preterm infants

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Internal validity	Applicability
O'Connor, 2001, US, UK, Chile: 14 mo parallel RCT[207]	Fish/fungal oil: DHA 0.27% EPA 0.08% ALA 2.60% (n=140)/ HM (n=43)	Egg-TG/fish oil: DHA 0.24% ALA 2.50% (n=143)/ Control ALA 2.4% (n=144)	(ITT) NS in VEP/FPL acuity at 4 mo CA S ↑VEP acuity in grps1-2 vs. grp3 at 6 mo CA ++ NS VEP acuity across both DHA+AA grps	Jadad total: 3 [Grade: B]; Schulz: Unclear	I
van Wezel-Meijler, 2002, Netherlands: 8 mo parallel RCT[272]	LCPUFA-enriched: DHA 0.34% AA 0.70% (n=22)	Control (n=20)	NS in VEP (P200 & N300) wave latencies at 3 & 12 mo CA NS mean visual acuity at 3,6,12 mo CA	Jadad total: 5 [Grade: A]; Schulz: Adequate	III
Innis, 2002, US, Canada: 28 d parallel RCT[201]	LCPUFA-enriched: DHA 0.33% AA 0.60% (n=66)/ HM (n=90)	LCPUFA-enriched: DHA 0.34% (n=66)/ Control (n=62)	NS in FPL visual acuity at 48 & 57 wks PCA S↑ visual acuity in HM than grps1-3 at 57 wks PCA +	Jadad total: 3 [Grade: B]; Schulz: Unclear	I

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; +p<.05 or significant with 95% confidence interval; ++p<.01; +++p<.001; ++++p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; GA = gestational age; PCA = postconception age; CA = corrected age; ERG = electroretinogram; VEP = visual evoked potential; FPL = forced-choice preferential looking using Teller's card test

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. All nine studies had at least two randomized groups. Three studies involved three randomized groups.[201,207,212] Of the nine trials, five used non-randomized groups of infants receiving human milk (reference standard).[198,207,212,251,254]

The trials had been conducted in the following countries: the United States,[185,191,212]the Netherlands,[272] France,[254] Italy,[198] and Germany.[251] Two trials[201,207]were conducted multinationally in different centers in the United States, United Kingdom, and Chile,[207] and in Canada and United States.[207] The Birch et al. study was funded by National Eye Institute, National Institute of Child Health and Development, United Cerebral Palsy Research Foundation, and Pediatric Subunit United States Public Health Service grants.[212] The Carlson et al. study was supported by National Eye Institute and Ross Laboratories, Columbus, OH.[185] Koletzko et al. was funded by Deutsche Forschungsgemeinschaft and Milupa AG, Germany.[251] Carlson et al. was supported by National Eye Institute, Ross Products Division, National Institute of Child Health and Development, and Abbott Laboratories.[191] O'Connor et al. was supported by Ross Products Division and Abbott Labs.[207] van Wezel-Meijler et al. was funded by Numico

Research.[272] Innis et al. was supported by Mead Johnson Nutritionals.[201] Faldella et al.and Bougle et al. did not report their funding source.[198,254]

Population characteristics. The total number of enrolled infants, including reference non-randomized breastfed infants, across the nine trials was 1,171 with a range from 27251to 470207infants.

All nine trials reported the infants' study arm-specific means of GA. The range of study arm-specific mean GA across all trials was 27.0[191] to 33.9 weeks.[254] In all nine trials, the GA was relatively evenly distributed amongst the study arms. Four studies did not report the percentage of males (or females) across study groups (randomized as well as reference/breastfed).[198,212,251,254] The total percentage of males across the remaining five studies was from 35.5%[272] to 53.4%.[207] In four studies, the percentage of male infants across the randomized groups was similar. One study failed to report the study arm-specific distribution of sex.[191]

Racial/ethnical composition was reported only in three studies,[185,191,207] of which two reported arm-specific percentages of White/Black[185]and White/Black/Hispanic/Other infants.[207] The race distribution across the randomized groups in these studies was more or less balanced. The remaining one trial reported the race percentage (%White/Black) of the total study sample.[191] In two studies by Carlson et al., the majority of participants belonged to the Black race.[185,191] All studies reported the birth weight of the infants.

Six studies reported ranges of birth weight for the entire sample, as well as the arm-specific means of birth weight.[185,201,207,212,251,272] Two studies reported only arm-specific means of birth weight.[198,254] Birth weight, across the majority of the studies ranged from 750 to 1,850 grams.[185,201,207,212,251,272] In six studies, birth weight was similarly and evenly distributed across the randomized study arms.[185,201,207,212,251,272]

Of the nine studies, six described both the inclusion and exclusion criteria with enough detail.[185,201,207,212,251,272] Two trials reported only inclusion or exclusion criteria.[191,254] One trial reported neither inclusion nor exclusion criteria.[198]

The infants in most of these studies were healthy preterm infants (< 37 weeks GA), free of respiratory or neurological disease, able to receive enteral feeding, had no severe intrauterine growth retardation, and did not require long-term mechanical ventilation or gastrointestinal surgery after birth. The studies excluded infants with risk factors for visual development, congenital abnormalities, retinopathy (> stage 2), intraventricular or periventricular hemorrhage (> grade 2), metabolic abnormalities, or history of maternal drug abuse. The study sample of one trial consisted of 49% infants suffering from bronchopulmonary dysplasia.[191]

Only three studies reported parental education.[185,191,207] O'Connor et al. presented only maternal education (in years and earned degrees).[207] In this study, randomized formula groups had similar mean duration of maternal education. However, the breastfed arm had a higher mean maternal education compared with the formula groups (breastfed group:15.1 years vs. formula groups:12.9, 13.1, and 12.8 years). In two other trials, years of parental (mother's and father's) education were balanced across the randomized study arms,[185,191] and the number of years of education in these trials ranged from 11.4 to 12.2. Only one study reported percentage of maternal smoking during pregnancy.[207] The formula (randomized) groups had strikingly high

rates of maternal smoking during pregnancy compared with the breastfed group (formula groups: 28%, 25.4%, 29.3% vs. breastfed group: 4.7%).

Intervention/exposure characteristics. In all nine trials, the study intervention was the assignment of dietary standard infant formulas (preterm/term) with or without the supplemental omega-3 and/or omega-6 LCPUFAs. In six trials, breast milk was the study exposure aside from the randomly assigned intervention (infant formula: with or without the supplementation of omega-3 and/or omega-6 LCPUFA).[198,201,207,212,251,254]

Some of these studies defined breastfed infants as those whose dietary intake of human milk accounted for 75% to 85% of their total dietary intake.[198,201,207,212] The amount/content (i.e., mean g/100 g, % of total FAs), type (i.e., ALA, LA, DHA, AA, EPA), and source (i.e., egg-TG, corn-, soy-, marine-, fish-, and/or fungal-oil) of omega-3 and/or omega-6 LCPUFA supplementation differed slightly across the studies. The formula content of DHA supplementation in the experimental arms across the trials ranged from 0.14%[201] to 0.60%.[254] For the majority of trials, the content of DHA supplementation was confined between 0.20% and 0.35% inclusively.[185,191,198,207,212,251]

Of the nine studies, two did not report the formula content/amount (%) of EPA supplementation in the experimental arms.[201,272] Across the remaining seven trials, the formula content/amount (%) of EPA supplementation in the experimental arms, ranged from 0.03%[251]to 0.65%.[212] Mostly, the EPA formula content was confined between 0.03% to 0.1%.[191,198,207,251,254]

The formula content/amount (%) of ALA supplementation in the experimental arms was reported in seven trials,[185,191,198,207,212,251,254]and ranged from 0.20%[251] to 3.10%.[185] Amongst the studies, the most common source of omega-3 and/or omega-6 LCPUFA supplementation was marine oil.[185,191,341]

In three trials, the sources used for omega-3 and/or omega-6 LCPUFA supplementation were oils derived from the alga and fungus.[201,207,272] One study used egg-derived triglyceride (egg-TG).[207] Two trials did not report the source of omega-3 and/or omega-6 LCPUFA supplementation.[198,254] Birch et al. used corn and soy oils as sources of FA supplementation.[212]

In almost all studies, the infants were enrolled and assigned to the interventions within ten days after their birth. Only one study reported the rate of formula intake (at least 0.72 L formula/day through 79 weeks PCA.[185] The duration of preterm formula intake varied between the studies, ranging from 21 days[251]to 12 months.[207] Upon reaching the weight of 1,800 g, the infants were switched from preterm to term formulas with or without LCPUFA supplements.[185,207] In one study, standard and supplemented formulas differed from their commercial versions in that the former contained nucleotides, β-carotene, lactose, and α-tocopherol.[207]

Two studies reported that the assigned study formulas had nutritionally similar content (except for fat composition), and the only difference between them was the composition of omega-3/omega-6 FAs.[191,212] In two trials, the manufacturer of the study formula (Enfamil) was Mead-Johnson Nutritional Group, Evansville, Indiana.[201,212] In other trials, the manufacturer of the study formulas included Milupa AG, Friedrichsdorf, Germany (Preaptamil with Milupan),[198,251] Ross Products Division, Columbus, OH (Similac Special Care),[191] and Nutricia,

Zoetermeer, The Netherlands (product name not reported).[272] None of the studies reported well-documented data on compliance.

The study protocol in the trial by O'Connor et al. did not limit the amount or duration of human milk feeding. Whenever the study infants were being weaned from human milk, the protocol required that the infant be fed the assigned study formula unless there was a medical indication to do otherwise.[207]

Cointervention characteristics. None of the studies described how the dietary formula intake and background diet was monitored. Three studies reported vitamin intake.[212,251,254] For example, one trial reported that the study groups were given a daily supplementation of 1,200 IU of vitamin D and 4.5 mg of vitamin E.[254] Another trial reported that all infants in the study were given daily multivitamin drops (A, C, and D) and vitamin E (25 IU per day) for 14 days, after feeding was well tolerated.[212] In the trial by Koletzko et al., the infants received oral vitamin supplement providing 0.8 mg α-tocopherolacetate/kg body weight.[251]

Outcome characteristics. Visual acuity parameters (FPL and/or VEP) were evaluated in all the trials. In these studies, FPL was measured by Teller Acuity Card Procedure.[185,191,201,207,212,251,272] The FPL values were expressed as means of Log10 (cycles/cm) [SD] and were derived from threshold of the finest grating size identified (cycles/cm), based on the infant's behavior, and the distance between the infant and visual stimulus.

The SD was expressed in octaves (SD of log acuity score/0.301). The VEP values were measured in five studies.[198,207,212,254,272] For the VEP responses, peak-to-peak amplitude (in mvolts) and latency (in msec) for each check size were determined. The VEP acuity (in logMAR) was obtained from linear functions relating amplitude to the logarithm of each check size. Lower LogMAR values indicate better visual acuity.

Two trials reported ERGs.[198,212] The ERGs were reported as latencies and rod/cone ERG amplitudes (Naka-Rushton parameters: log threshold, LogVmax, and log k).

Study quality and applicability. The nine RCTs received a mean Jadad total quality score of 2.9, approaching a good internal validity (Summary Matrix 20). van Wezel-Meijler et al. received a score of 5,[272] Carlson et al. received a score of 4,[185] four reports received a score of 3,[191,201,207,254] two reports received a score of 2,[212,251] and Faldella et al. received a score of 1.[198] Six trials failed to report the method of randomization,[123,305,312,316,317,319] three were unblinded,[310,316,317] five trials did not decribe the method of double-blinding,[123,150,305,312,319] and two did not report the reasons for dropouts.[305,317]

Summary Matrix 20: Omega-3 fatty acid intake associated with the visual function in preterm infants

		Study Quality								
		A			B			C		
Applicability	I	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
					O'Connor[U]	2001	470			
					Innis[U]	2002	194			
	II	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Carlon[A]	1992	79	Carlson[U]	1996	36	Uauy[U]	1992	81
	III	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		van Mezel-Meijler[A]	2002	55	Bougle[U]	1999	40	Koletzko[U]	1994	27
								Faldella[U]	1996	66
n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment										

Qualitative synthesis of individual study results

The study results regarding the effects of LCPUFA supplementation on visual acuity in healthy preterm infants are not consistent. Five studies observed that preterm infant formula supplemented with LCPUFA is associated with better FLP and/or VEP acuity.[185,191,198,207,212] In contrast, the remaining studies did not observe any relationships between LCPUFA supplementation and the development of visual acuity (FLP and/or VEP).[201,251,254,272] In two studies, ERG responses were not statistically significantly different across the formula groups at 52 to 57 weeks postconceptional age (PCA).[198,212]

According to study results obtained by Birch et al.,[212] the soy/marine oil-supplemented formula (DHA+EPA: 1.0 g/100 g) group had a better VEP acuity than the corn oil-based formula (LA: 24.2, ALA: 0.5 g/100g) group at 36 weeks PCA. At 57 weeks PCA, the soy/marine oil-supplemented formula group had a statistically significantly better VEP acuity than the corn oil- and soy-based formula groups. The soy/marine oil-supplemented formula group had a better FPL acuity (of borderline statistical significance) than the corn oil-supplemented formula group at 57 weeks PCA. Statistically significant differences were observed for rod threshold at 36 weeks PCA between the study arms (higher in corn-oil supplemented formula group vs. human milk, soy- or soy/marine-oil supplemented formula groups). The rod ERG responses between the groups were not statistically significantly different at 57 weeks PCA. There was no statistically significant differences for cone ERG parameters between the groups at 36 and 57 weeks postconception.[212]

Carlson et al.[185] found a statistically significant effect of marine-oil supplementation on visual acuity at the age of 2 and 4 months. Specifically, infants fed with marine oil-supplemented formula (DHA: 0.2 and EPA: 0.3 g/100 g) had a better visual acuity than those fed with standard formula (ALA: 3 g/100 g) group.

The study by Koletzko et al. did not demonstrate any statistically significant effect of LCPUFA supplementation on visual acuity.[251]

The results obtained by Carlson et al.[191] demonstrated that healthy infants (i.e., those without bronchopulmonary dysplasia) who were fed LCPUFA supplemented formula had a better visual acuity at 2 months of age than those infants who were fed formula with no supplementation (2.90 vs. 2.15 cycles/degree, $p< 0.05$). In contrast, there was no difference in visual acuity between the formula groups at 4 months. The study authors detected an interaction between bronchopulmonary dysplasia and diet at 0 and 2 months ($p<0.03$ and $p<0.005$, respectively). Namely, in infants without bronchopulmonary dysplasia the LCPUFA supplementation was related to an improved visual acuity. In contrast, this supplementation in infants with bronchopulmonary dysplasia was related to poorer visual acuity.

Faldella et al. found that the mean latencies of flash VEP (N4 and P4 waves) at 52 weeks PCA were significantly shorter in infants from LCPUFA supplemented formula (DHA: 0.23% and EPA: 0.08%) and breast milk groups compared with infants from standard/control formula groups.[198] No significant differences were observed across the study groups (control formula vs. LCPUFA-supplemented formula vs. human milk) in ERG and BAEP parameters (latencies a and b) measured at 52 weeks postconception.[198]

Bougle et al. did not find any difference amongst the breastfed and the formula (with and without LCPUFA supplement) fed groups in VEP responses (latency of wave N1) after 30 days of diet.[254]

Results in the trial by O'Connor et al. suggested that study diet did not have any significant effect on FPL and VEP acuity at 4 months CA.[207] However, at 6 months CA, infants randomized to either fish/fungal oil-supplemented or egg-TG/fish oil-supplemented formula had higher mean VEP acuity than infants in the control formula group. Infants in the fish/fungal oil- and egg-TG/fish oil-supplemented formula groups had similar VEP acuity at 4 and 6 months CA. There was no difference with respect to FPL acuity between the study groups at 6 months CA.[207]

van Wezel-Meijler et al. did not reveal any statistically significant differences in flash VEP latencies (P200 and N300) and FLP acuity responses at any stage of follow up (at 3 and 12 months of age) between the supplemented and non-supplemented formula groups.[272] The authors reported that VEP responses could not be obtained from three infants at 3 months of age for technical reasons, and because of lack of parents' permission.

According to Innis et al., three randomized groups of infants had similar mean FLP values of visual acuity at 48 and 57 weeks PCA (differences were not statistically significant).[201] At 57 weeks PCA, breastfed term infants had a significantly higher visual acuity than preterm infants randomized to receive either control (without LCPUFA supplement) or LCPUFA supplemented formulas.[201]

In Birch et al, the LCPUFA content of RBC-DHA/DPA ratio correlated with both FPL and VEP at 57 weeks PCA.[212] Based on ANOVA, there was a statistically significant correlation between RBC-PE-DHA at 2 months and visual acuity at 2 and 4 months in Carlson et al.[185]

Faldella et al. found a negative correlation between the RBC DHA and the N4 and P4 wave latency of the VEP at 52 wks PCA.[198]

The study by Birch et al.[212] reported the number (n=2) and reasons (medical complications) of dropouts/withdrawals. Carlson et al.[185] reported that of the 79 infants, there were ten non-completers (reasons for not completing the study not given) who eventually were replaced. At the end of the study, the authors also excluded four infants who had received enteral nutrition. Carlson et al.[191] reported that of the 94 enrolled infants, 35 were lost at 2-month follow up. Of those, 19 infants were lost for their intolerance to enteral feeding leading to sepsis and necrotizing enterocolitis, and an additional 14 infants dropped out of the study because their parents moved or refused any further participation in the study.

The study authors stated that the reasons for non-participation were not related to the type of study diet. Although Faldella et al. reported that eight infants could not complete the follow up, they failed to provide information on the reasons for the loss/withdrawal of infants.[198] In their study, Bougle et al. presented the data and reasons for dropouts: necrotizing enterocolitis (n=1), hydrocephalus (n=1), and transfer to referring hospital (n=5).[254]

According to O'Connor et al., the percentage of study completers at 12 months of observation was about 80%.[207] van Wezel-Meijler et al. reported that of the 55 enrolled infants, 13 were excluded due to different reasons such as necrotizing enterocoloitis (n=2), chronic lung disease (n=3), grade 4 retinopathy of prematurity (n=1), cystic periventricular leucomalacia (1), practical reasons (n=4), and change from formula feeding to mother's expressed milk (n=2).[272]

According to Innis et al., 21 infants did not complete the pre-term diet protocol due to necrotizing enterocolitis or other gastrointestinal disease, complications unrelated to the study, formula intolerance, receiving oxygen at discharge, and protocol violation.[201]

Quantitative synthesis

Visual acuity was measured both through behavioral and electrophysiologic tests. For the behaviorally-based tests, we extracted data from the studies using the Teller Acuity Card Procedure (ACP) or the Forced Choice Preferential Looking Procedure (FPL). For all of the behaviorally-based tests, stimuli were high-contrast square-ware grating of two discrete luminance presented in equal duty cycles. Grating acuity can be expressed in units of cycles per degree (cy/degree) of visual angle. Higher values of cy/degree indicate better visual acuity. For electrophysiologic tests, we extracted data from studies using steady-state or transient VEP tests. Visual acuity was expressed as the minimal angle of resolution (MAR). Opposite to cy/degree, the lower the MAR value, the better the visual acuity. In this systematic review, visual acuity was measured at age 0, 2, 3, 4, 6, 7, 8, 9, 12, 24, and 39 months. In visual acuity research, measures of dispersion, such as the standard error of the mean are commonly represented in units of octaves. A one octaves change represents a doubling or a halving of the stimulus spatial frequency (or a thinning of the width of the individual stimulus lines by one half). In the studies included in this systematic review, visual acuity data were reported in cy/degree, MAR, log cy/degree, and log MAR. Most of the standard deviations of the visual acuity are in these units as well, although some are in octaves. For standardization, all the data has been converted into octaves.

Almost all of the studies included in this review only reported the mean of the visual acuity for each dietary group, not the difference of visual acuity between the groups. Thus, the visual acuity difference between groups consuming a source of DHA and groups not consuming a source of DHA needed to be calculated from individual values. Due to the different meanings of the magnitude of cy/degree and MAR, when the unit of the visual acuity is cy/degree, the visual acuity difference was calculated by subtracting the mean of visual acuity of the no-DHA intake group from the mean of visual acuity of the DHA intake group; however, if the unit was MAR, the reverse was done. Finally, these results (mean visual acuity difference with the standard error) were recorded into the database as the outcome data for each trial. Some studies did not report the actual data, but a graph. In these cases, data were extracted from the graph. The visual acuity development is very sensitive to the age of the infants. One of the complexities of this systematic review was that each included study started at different ages, for example, some visual acuity data were tested at the very beginning of the study. Since this data is actually baseline information, it was excluded from the final analyses since the data would confound observed treatment effects. For example, in the Hoffman 2003 study, there were two measurements, one obtained from 4 to 6 months, and the other at 12 months. However, the treatment was introduced from 4 to 6 months. This means that visual acuity data obtained at 4 to 6 months was actually baseline information. If we combine this data with data obtained from the other studies at the same age, the treatment effectiveness would be confounded by this baseline information.

Since the durations of the supplementation differed across trials, some studies tested visual acuity after the supplement was stopped. In order to separate "during", and "post"supplement effectiveness, the data were split into two sets according to the duration of the supplementation reported in each trial. The effectiveness of the supplement was evaluated by using the database for "during" supplement.

Statistical analysis

It was not reasonable to combine the results of visual acuity difference obtained from fullterm infants and preterm infants, or from different test ages, or from different visual acuity tests (behaviorally-based or electrophysiological-based), or from different study components (randomized and non-randomized components). One meta-analysis is required for each subgroup in which all the factors are the same across the studies. Therefore, the number of different combinations of these factors determined the number of meta-analyses needed for this systematic review.

Although all the included studies have a common interest, i.e., the effectiveness of omega-3 on child visual acuity, most of the studies (73%) included more than two dietary groups.

The fixed effect model was used to obtain combined estimates of visual acuity differences and their standard errors within each category in some meta-analyses. The weights of each study were taken by using the reciprocal of the variance of the visual acuity difference of each study. When heterogeneity was present between studies, a Dersimonian and Laird random-effect method was used instead to get the pooled estimates of the visual acuity difference across the studies. However, it is notable that in some meta-analyses that only included a small number of studies, the test for heterogeneity should be interpreted carefully.

The median of the number of dietary groups in a single study was 3.5. The dietary groups could be classified into 4 major groups—no-DHA intake, DHA intake, DHA+AA intake and human milk (HM) groups. Thus, the comparisons conducted were: DHA vs no-DHA intake and DHA+AA vs no-DHA. These were two randomized comparisons, whereas, a non-randomized comparison of HM vs no-DHA intake was also conducted as a reference.

DHA vs no-DHA

Meta-analysis for behaviorally-based test at 0, 2, 4, 6, and 9 months (Table 4, Figure 12). There was no statistically significant difference in visual acuity between DHA intake and placebo groups for preterm infants based on the behavioral test at age 0, 2, 4, 6, and 9 months.

Table 4. Meta-analysis of visual acuity difference (DHA vs. no-DHA) for preterm infants based on behavioral test

Age	Studies	Heterotest	Point estimate	95% C.I.	P-value
0	[1]Carlson 1992	-	0.20	(-0.11, 0.51)	0.21
2	[1]Carlson 1992, [2]Innis 2002	0.07	0.27	(-0.18, 0.71)	0.24
4	[1]Carlson 1992, [2]Innis 2002	0.02	0.15	(-0.23, 0.52)	0.44
6	[1]Carlson 1992	-	0.19	(-0.03, 0.41)	0.08
9	[1]Carlson 1992	-	0.20	(-0.02, 0.42)	0.07

Age: in months; **Studies:** superscripts correspond to the report number in the graphs; **Heterotest:** P-value for the heterogeneity test; **Point estimate:** (in octaves); positive value means the point estimate favors DHA intake over no-DHA intake; **P-value:** P-value for the effectiveness–only one study in this meta-analysis.

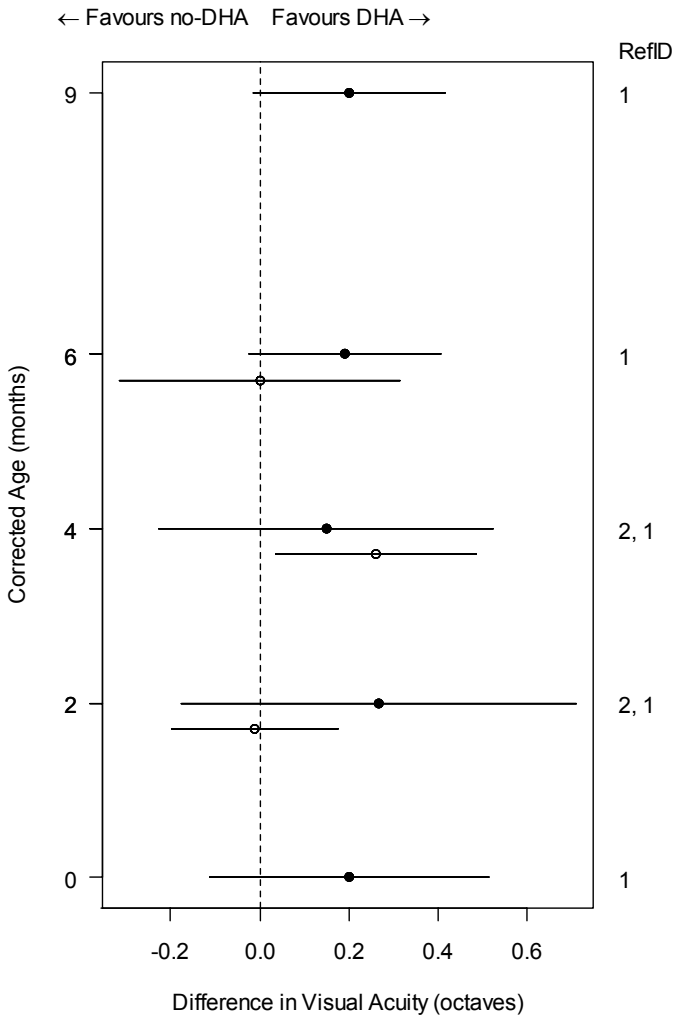


Figure 12. Meta-analysis of visual acuity difference (DHA vs. no-DHA) in preterm infants based on the behavioral test. Shaded circles represent the pooled estimates of visual acuity difference from the meta-analysis of randomized comparisons (formula fed DHA vs formula fed no-DHA) tested at certain ages. The open symbols represent the pooled estimates of visual acuity difference from the meta-analysis of nonrandomized comparisons (human milk vs formula without DHA).

DHA+AA vs no-DHA

Meta-analysis for behaviorally-based test at 0, 2, 3, 4 and 6months (Table 5, Figure 13). There is no statistically significant difference on visual acuity between DHA+AA intake and placebo groups for preterm infants based on the behavioral test at age 0, 2, 4, 6, and 9 months.

Table 5. Meta-analysis of visual acuity difference (DHA+AA vs. no-DHA) for preterm infants based on behavioral test

Age	Studies	Heterotest	Point estimate	95% CI	P-value
0	[6]Carlson 1996	-	0.24	(-0.37, 0.85)	0.44
2	[2]Innis 2002, [4]O'Connor 2001, [6]Carlson 1996	0.12	0.12	(-0.08, 0.33)	0.24
3	[3]Wezel-Meijl 2002	-	0.30	(-0.03, 0.63)	0.08
4	[2]Innis 2002, [4]O'Connor, [5]Birch 1992	<0.01	0.10	(-0.18, 0.38)	0.50
6	[3]Wezel-Meijl 2002, [4]O'Connor	0.20	0.06	(-0.11, 0.23)	0.46

Age: in months; **Studies:** superscripts correspond to the report number in the graphs; **Heterotest:** P-value for the heterogeneity test; **Point estimate:** (in octaves); positive value means the point estimate favors DHA intake over no-DHA intake; **P-value:** P-value for the effectiveness–only one study in this meta-analysis.

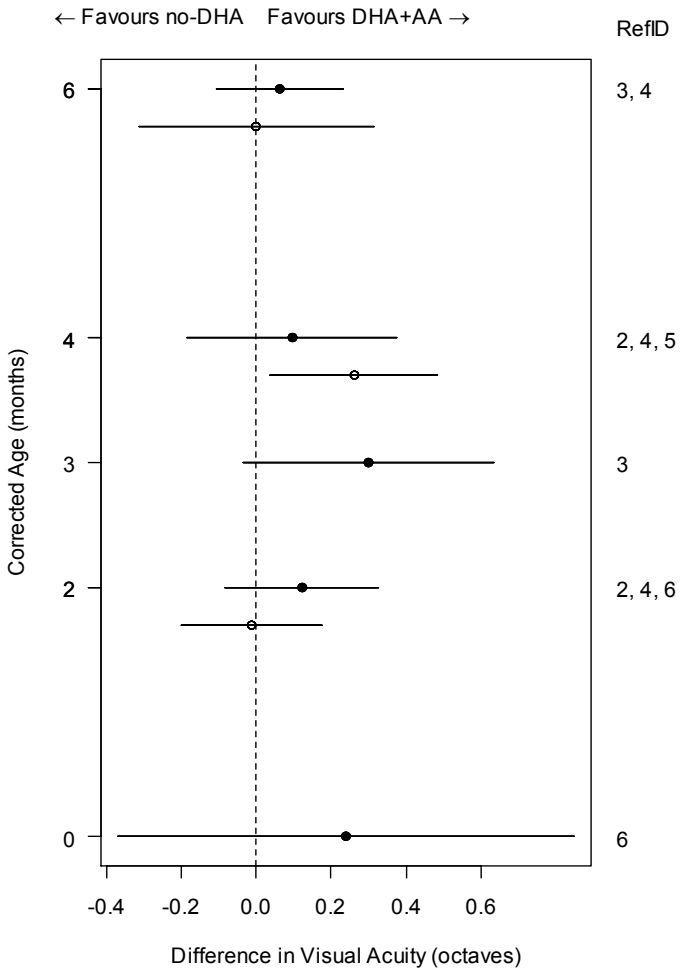


Figure 13. Difference in visual acuity (DHA+AA vs no-DHA)in preterm infants based on the behavioral based test. Shaded circles represent the pooled estimates of visual acuity difference from the meta-analysis of randomized comparisons (formula fed DHA vs. formula fed no-DHA) tested at certain ages. The open symbols represent the pooled estimates of visual acuity difference from the meta-analysis of nonrandomized comparisons (human milk vs. formula without DHA).

Meta-analysis for Electrophysiologically based test at 0, 4 and 6 months (Table 6, Figure 14). Except for the results at 4 month, the results show that at 0 and 6 month, DHA+AA intake group show better visual acuity than the placebo group. Notably, there is only 1 study at each month support the results.

Table 6. Meta-analysis of visual acuity difference (DHA+AA vs. no-DHA) for preterm infants based on electrophysiological test

Age	Studies	Heterotest	Point estimate	95% CI	P-value
0	[5]Birch 1992	-	0.3	(0.16, 0.44)	<0.01
4	[4]O'Connor, [5]Birch 1992	<0.01	0.44	(-0.41, 1.28)	0.31
6	[4]O'Connor	-	0.51	(0.22, 0.8,)	<0.01
Age: in months; **Studies:** superscripts correspond to the report number in the graphs; **Heterotest:** P-value for the heterogeneity test; **Point estimate:** (in octaves); positive value means the point estimate favors DHA intake over no-DHA intake; **P-value:** P-value for the effectiveness–only one study in this meta-analysis.					

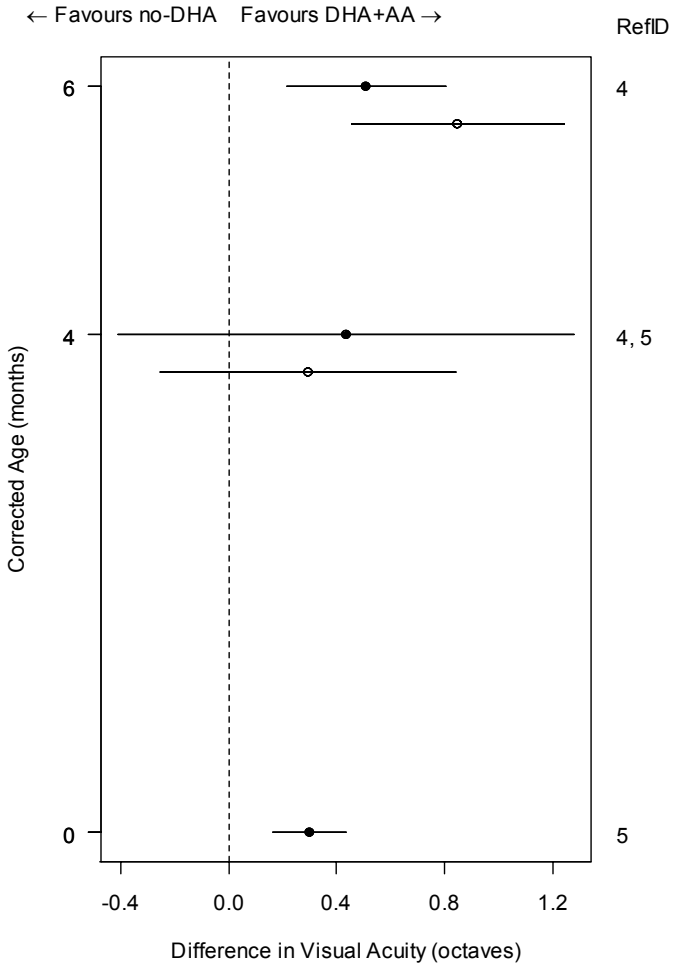


Figure 14. Difference in visual acuity (DHA+AA vs. no-DHA) in fullterm infants based on Electrophysiological test. Shaded circles represent the pooled estimates of visual acuity difference from the meta-analysis of randomized comparisons (formula fed DHA vs. formula fed no-DHA) tested at certain ages. The open symbols represent the pooled estimates of visual acuity difference from the meta-analysis of nonrandomized comparisons (Human milk vs. formula without DHA).

Human Milk vs no-DHA

Meta-analysis for Behaviorally based and Electrophysiologically based test at 0, 2, 4 and 6 months (Table 7).

Table 7. Meta-analysis of visual acuity difference (HM vs no-DHA) in preterm infants based on the behavioral and electrophysiological tests

Test	Age	Heterotest	Point estimate	95% CI	P-value
B	2	0.71	-0.01	(-0.2, 0.17)	0.9
B	4	0.02	0.26	(0.04, 0.49)	0.02
B	6	-	0	(-0.31, 0.31)	1
E	0	-	0.6	(0.46, 0.74)	<0.01
E	4	0.03	0.3	(-0.25, 0.84)	0.29
E	6	-	0.85	(0.46, 1.24)	<0.01

B: Behavioral test. **E:** Electrophysiological test; **Age:** in months; **Heterotest:** P-value for the heterogeneity test; **Point estimate:** of meta-analysis of certain age (in octaves); The positive value means the point estimate favors DHA intake over no-DHA intake; **P-value:** P-value for the effectiveness; only one study in this meta-analysis

Impact of covariates and confounders

Carlson et al. controlled the results for potential independent variables related to visual function such as birth weight, gestational age, oxygen supplementation, enrollment weight, RBC DHA at 2 months, and sex. Oxygen supplementation was negatively related to visual acuity at term in the whole population, whereas, birth weight and gestational age were derectly associated with visual acuity.[185] In a second trial, Carlson et al. used a regression analysis and correlations among neonatal and perinatal characteristics and 4 months grating acuity.[191] Variables were mechanical ventilation, birth weight, age and RBC DHA in infants, among others. Total hours of mechanical ventilation, volume of packed RBCs and days required to reach enteral intake of 418 kj/kg/d were significantly negatively correlated with visual acuity at 4 months of CA. Birth weight was positively correlated with a higher visual acuity at 4 months.[191]

The power calculation was reported in four trials,[123,310,312,316] while the intention-to-treat analysis approach was reported in only one study.[310]

Infant Formula Intake - Term Infants

Thirteen unique parallel design RCTs met eligibility criteria. These studies were published between 1995 and 2003. All but one trial[126] were summarized in the Growth Pattern Outcomes section (see key question: Growth Patterns-Term Infant Formula Intake). (Summary Tables 38-40)

Overview of relevant studies

Carlson et al evaluated the effect of feeding DHA + AA (0.1 wt% and 0.43 wt%, respectively) supplemented formula compared with unsupplemented formula from birth to 12 months of age on visual acuity using the Teller Acuity Card protocol at 2, 4, 6, 9 and 12 months of age.[277](Summary Table 38)

Summary Table 38: Omega-3 fatty acids intake associated with visual function in term infants

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Notable clinical-biomarker correlations	Internal validity	Applicability
Makrides, 1995 Australia: 30 wk parallel RCT[262]	DHA+GLA (n=13)	control (n=19)	S improved VA of DHA+GLA infants at 16 wk^{+++} & 30 wk^{++} S ↑ % of DHA+GLA infants were able to evoke cortical responses to the smallest checkerboard pattern^{+++}	S correlation RBC DHA & VEP acuity at 16 wk^{+++} & 30 wk^{++} of age	Jadad total: 2 [Grade: C]; Schulz: Unclear	II
Carlson, 1996, US: 12 mo parallel RCT[277]	DHA+AA (n=19)	Control (n=20)	S better VA with DHA+AA at 2 mo of age $^{++}$	n/a	Jadad total: 3 [Grade: B]; Schulz: unclear	II
Jorgensen, 1998, Denmark: 4 mo parallel RCT[264]	DHA+EPA (n=12)	DHA+EPA+ GLA (n=14)/ control (n=11)	NS effect of DHA on VA	NS VA at 4 mo & RBC DHA, EPA, or AA S negative correlation VA & RBC CPG LA^{+}	Jadad total: 2 [Grade: C]; Schulz: Unclear	III
Auestad, 1997, US: 12 mo parallel RCT[104]	DHA 0.01% fa (n=43)	DHA+AA (n=46)/ control (n=45)	NS acuity thresholds at 2,4,6,9 or 12 mo of age using either VA method NS FPL at 2,4,6,9, 12 or 39 mo of age	n/a	Jadad total: 3 [Grade: B]; Schulz: Unclear	I
Innis,1997, US, Canada: 90 d parallel RCT[263]	LA/ALA 9.5/1 (n=59)	LA/ALA 7.3/1 (n=57)	NS FPL at 90 d of age	NS VA & plasma & RBC CPG DHA	Jadad total: 2 [Grade: C] Schulz: Unclear	I

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; RBC = red blood cells; PL = phospholipid; CPG = choline phosphoglycerides; EPG = ethanolamine phosphoglycerides; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; VA = visual acuity; PL = phospholipids; d = day(s); GLA = γ-linolenic acid; FPL = forced-choice preferential looking using Teller's card test

Summary Table 39: Omega-3 fatty acids intake associated with visual function in term infants

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Notable clinical-biomarker correlations	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)				
Jensen,1997, US: 120 d parallel RCT[203]	F1 (LA/ALA 44) (n=20)/ F4 (LA/ALA 4.8) (n=20)	F2 (LA/ALA 18.2) (n=20)/ F3 (LA/ALA 9.7) (n=20)	NS latency VEP among gps at 120 & 240 d NS amplitude VEP among gps at 120 & 240 d	NS plasma & RBC PL DHA & amplitude at 120 & 240 d	Jadad total: 2 [Grade: C]; Schulz: Unclear	II
Birch, 1998, US: 4 mo parallel RCT[182]	DHA (n=20)	DHA + AA (n=19)/ control (n=21)	S poorer sweep VEP acuity in control than DHA^{+++} or $DHA^{+}AA^{+++}$ at 6 wk; DHA^{++} or $DHA^{+}AA^{+}$ at 17 wks; DHA^{++} or $DHA^{+}AA^{++}$ at 52 wks NS diet on FPL acuity S better ERG & DHA or $DHA^{+}AA$ at $6\ wk^{+}$	S RBC DHA 17 wks & better sweep VEP acuity at $6\ wk^{+++}$, 17 wk^{++} & 52 wk^{+++} S 6 wk sweep VEP acuity & 17 wk RBC n-3/n-6 $LCPUFA^{+++}$ S 17 wk RBC n-3/n-6 LCPUFA & sweep VEP at $6\ wk^{+++}$, 17 wk+, 52 wk^{+++} S log k & RBC CPG DHA at $6\ wk^{+}$	Jadad total: 5 [Grade: A]; Schulz: Adequate	I
Auestad, 2001a, US: 4 mo parallel RCT[227]	DHA+ AA (egg-TG) formula (n=80)	DHA+ AA (fish/fungal) formula (n=82)/ control formula (n=77)	NS FPL at 2,4,6 & 12 mo of age	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	I
Auestad, 2001b, US: 4 mo parallel RCT[227]	DHA + AA formula/ HM (n=83)	Control formula/ HM (n=82)	NS VA between groups	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	I

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; RBC = red blood cells; PL = phospholipid; CPG = choline phosphoglycerides; EPG = ethanolamine phosphoglycerides; $^{+}p<.05$ or significant with 95% confidence interval; $^{++}p<.01$; $^{+++}p<.001$; $^{++++}p<.0001$; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; VA = visual acuity; PL = phospholipids; d = day(s); FPL = forced-choice preferential looking using Teller's card test; HM = human milk; VEP = visual evoked potential; F = formula; ERG = electroretinogram

Summary Table 40: Omega-3 fatty acids intake associated with visual function in term infants

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Study groups[1] Group 2 (n)/ Group 3 (n)	Notable clinical effects	Notable clinical-biomarker correlations	Internal validity	Applicability
Makrides, 1999, Australia: 1 y Parallel RCT[205]	DHA (n=22)	DHA+AA (n=19)/ control (n=19)	NS VEP acuity at 16 or 34 wk	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	III
Makrides, 2000, Australia: 34 wk Parallel RCT[266]	LA/ALA 10/1 (n=30)	LA/ALA 5/1 (n=28)	NS VEP acuity at 16 & 34 wk	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	II
Birch, 2002 US: 46 wk Parallel RCT[269]	DHA+AA (n=30)	Control (n=28)	NS DHA^{+}AA on sweep VEP at 6 wk S DHA^{+}AA & better sweep VEP 17^{++}, 26^{+++} & 52 wk $^{+++}$ S DHA+AA & better FPL at 17^{++}	S better sweep VEP & plasma AA at 17^{++}, 52^{+++} wk & plasma DHA at 17^{+++}, 52^{+++} wk S better sweep VEP & RBC AA at 52 wk^{++} & RBC DHA at 17^{++} & 52^{+++} wk NS sweep VEP & plasma or RBC LA or ALA at 17 or 52 wk S better FPL & plasma DHA at 17 wk^{++} or RBC LA at 17 wk^{+++} NS FPL & plasma or RBC ALA, AA, plasma LA, or RBC DHA	Jadad total: 5 [Grade: A]; Schulz: Adequate	II
Hoffman, 2003 US: 7 mo Parallel RCT[270]	DHA+AA (n=30)	Control (n=31)	S better sweep VEP & DHA+AA at 12 mo^{+++} NS DHA+AA & FPL at 4,6,9, & 12 mo	S better sweep VEP at 12 mo & RBC DHA^{+++}, Σ n-3 LCPUFAs^{+++}, n-3/n-6 LCPUFAs^{++}, DHA/DPA^{++}, n-6 unsaturation index^{++} S poorer sweep VEP at 12 mo & RBC LA^{++}, AA^{++} NS FPL & RBC n-3 or n-6 FA	Jadad total: 3 [Grade:B]; Schulz: Adequate	II

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; RBC = red blood cells; PL = phospholipid; CPG = choline phosphoglycerides; EPG = ethanolamine phosphoglycerides; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; VA = visual acuity; PL = phospholipids; d = day(s); FPL = forced-choice preferential looking using Teller's card test; VEP = visual evoked potentials

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. All studies were parallel RCTs with at least two groups. Countries where the studies were conducted included Australia,[205,262,266] United States,[104,182,203,227,263,269,277,342] Denmark,[264] and Canada.[263] Makrides et al.'s study was funded by grants-in-aid from Channel 7 Children's Medical Research Foundation, Nestle Australia, Scotia Pharmaceuticals UK and Flinders Medical Research Foundation.[262] Jorgensen et al's study was funded by Food Technology Research and Development Program (FOTEK), DanoChemo AS, BASF Health & Nutrition (Denmark), Swedish Medical Research Council, and Semper AB supplied infant formula.[264] Carlson et al.'s study was funded by the National Institute of Child Health and Human Development Grant and infant formula supplied by Ross Products Division, Abbott Lab.[277] Auestad et al.'s 1997 study and the secondary report by Austed et al. were funded by Ross Products Division, Abbott Laboratories and a US Maternal & Child Health Bureau grant.[104] Innis et al.'s study was funded by the Mead Johnson Research Center.[263] Jensen et al.'s study was funded by Federal funds from the US dept of Agriculture, Agriculture Research Services, Mead-Johnson Nutritional Group, the Foundation Fighting Blindness, Research to Prevent Blindness Inc., and the Retina Research Foundation.[203] Formulas for this study was provided by Mead-Johnson Nutrition Group and weaning foods were provided by Gerber Products Co.[203] Birch et al. was funded by an NIH grant and Mead Johnson Nutritional Research.[182] Both of Auestad et al.'s 2001 studies were funded by Ross Products Division, Abbott Laboratories.[227] Makrides et al.'s study was funded by Nestec Ltd, the MS McLeod Research Trust, and the Australian National Health & Medical Research Council.[205] Makrides et al.'s study was funded by Wyeth Nutritionals International, the Australian National Health and Medical Research Council, and the MS McLeod Research Trust.[266] Birch et al.'s study was funded by an NIH grant and Mead Johnson Nutritional Group.[269] Hoffman et al.'s study was funded by NIH.[270]

Population characteristics. The range of sample sizes were from 33 to 274 infants across the included studies.

The inclusion criteria were described by all of the included term infant studies. The definition of a term infant (range: 37-43 weeks GA) was described in ten studies.[104,182,205,227,262-264,269,277]

All but one study described the exclusion criteria.[203] Enough detail was provided for the selection of healthy infants in nine studies.[104,182,205,227,264,266,269,270]

Opthalmologic examination criteria for the exclusion of infants from visual acuity assessments after enrolment were described in four studies.[104,262,266,277] These infants participated in all the other assessments of the studies.

Exclusion of infants at risk for lipid metabolic abnormalities based on maternal risk factors was described in six studies.[104,182,262,266,269,270]

In the RCTs, the mean GA of randomized infants (range:39.0 - 40.3 weeks) was reported in nine studies.[104,203,205,227,262,264,266,277] The GA was not reported in four studies.[182,263,269,270] The percentage of males of randomized infants was reported in ten studies and ranged from 37.5% to 69.2%.[182,203,205,227,262,264,266,270,277] This information was not reported in three studies.[263,269,270] Ten of the RCTs reported the race and/or ethnicity data.[104,182,203,205,227,266,269,270,277] Randomized

infants were matched for GA at birth in nine studies,[104,203,205,227,262,264,266,277] and not reported in four studies.[182,263,269,270] The proportion of male to female randomized study infants was evenly distributed in four trials,[182,227,277] not reported in two studies,[263,266] and reported for all study infants but not for each study group in one study.[270]

There was a disproportionately higher percentage of males in the control formula group in two studies[104,269] and in one of the supplemented formula groups in three studies.[205,262,264] There was a disproportionately lower percentage of males in one of the supplemented formula groups in one study.[203] The race and/or ethnicity of the randomized infants was reported in ten studies,[104,182,203,205,227,266,269,270,277] The majority of randomized infants were White in eight studies,[104,182,205,227,266,269,270] and Black/Hispanic in two studies.[203,277] The distribution of race/ethnicity among the study groups of randomized infants for these studies was closely matched in three studies,[205,266,269] somewhat matched four in studies,[104,182,227] discrepant in two studies,[203,277] and not reported in one study.[270]

Parental sociodemographic factors were reported in nine studies.[182,205,227,262,266,269,270,277] Different variables were used to demonstrate family sociodemographic status in the various studies (parental education, social score, income, adults in household, children in household, smoking, marital status, birth order, HOME screening questionnaire score). There were no differences in sociodemographic variables among the study groups of randomized infants in five trials,[227,262,269,277] significantly different parental post-secondary education ($p < 0.005$) in one study[270] and reported but not analysed in three trials.[205,266,343] Two of these studies took the sociodemographic factors into account in comparing VEP acuity between randomized formula groups with analysis by covariance [205,266] and multiple linear regression.[266]

Intervention/exposure characteristics. Randomized infants in all studies were fed ad libitum with a standard cow's milk based infant formula with or without the addition of omega-3 and/or omega-6 LCPUFA,[104,182,205,227,262,264,269,270,277] ALA and/or LA,[203,263,266] and/or GLA.[262,264]

The sources of DHA in the studies included fish oil,[104,205,227,248,262,264] or single cell oils (DHASCO®).[182,269,270] The sources of DHA and AA included egg PL,[104,205,227,277] and sources of AA in the studies included single cell oils (ARASCO®)[182,269,270] and fungal lipid.[227] Sources of ALA included canola oil,[203,266] and sources of GLA included evening oil,[262] and borage oil.[264]

The source of ALA and AA were not reported in one study.[263] Only one study monitored the volume of formula intake of the groups of study patients and found no difference.[203] Study infants were placed on the study formulas within the first week of life in the majority of the studies.[104,182,203,205,227,262,263,266,277] The second Auestad et al. study randomized the infants at 11 days of life, but had them begin formula feeding after 3 months of being exclusively breastfed.[227] Study formulas were started within the first month of life in Jorgensen et al's study,[264] from the beginning of week 7 in Birch et al's study,[269] and after weaning from breast feeding at 4 to 6 months of age in Hoffman et al's study.[344]

The introduction of solid foods, usually starting with cereals, will not contribute to the omega-3 and omega-6 FA intake, and thus would have very little impact on the study diets. However, if a significant proportion of the diet is from supplementary foods and beverages other than the study formula, this may contribute to decreased study formula intake. Dietary intake information was not well documented in all the studies. Five studies in which study formula was

initiated within the first week of life, supplementary foods and beverages were discouraged for varying durations during the intervention phase.[104,182,203,262,266]

Innis et al.[263] specified that an infant would be withdrawn if more than 10% of dietary energy came from sources other than assigned formula for 5 days or more. Jorgensen et al.'s study documented that no supplementary food was consumed.[264] Infants were started on the study formulas from the beginning of week 7 of life in Birch et al's study, and it was documented that none of the infants consumed solid food before 17 weeks and most had no solid food other than cereal until 26 weeks of age.[269] In Hoffman et al's study, study formula started at 4 to 6 months of age and the diet was not controlled.[270] Criteria for inclusion/exclusion of supplementary foods or beverages was not stated in two studies.[205,277]

Cointervention characteristics. Only two studies reported the use of tocopherol (vitamin E) in their formulas.[182,264] Auestad et al. also allowed the use of breast millk as a cointervention.[227]

Outcome characteristics. Assessment of visual acuity was evaluated using the following methods: FPL with Teller Acuity Cards[104,182,227,263,277] or infant random dot stereocards,[269,270] VEP,[203,205,262,266] and sweep VEP.[104,182,264,269,270] Some studies employed more than one method for the assessment of visual acuity.[182,269,270] Birch et al. also employed electroretinography (ERG) to assess maturity of retinal function.[182] FPL using Teller Acuity Cards is reported as 1) threshold—the finest gating at which the tester can locate the grating based on the infant's behaviour, and 2) Log10 acuity score—represents the log transformed acuity based on the conversion of the finest grating recognized (cycles/cm) and the distance of the subject from the visual stimulus. The SD of Log10 acuity score is expressed in octaves (SD log acuity scores/0.301). A difference between groups of 1.0 octave means that the smallest stripe detected by one group is twice as large as the smallest stripe detected by the other. Only tests with confidence ratings of 3 to 5 (1-5, low to high) and good inter-rater reliability were included in the analyses.

Random dot stereoacuity by FPL using the infant random dot stereocards is reported as log10 s—log of the minimum detectable binocular disparity. VEP responses to a pattern-reversal stimulus at 2 hertz are reported as 1) latency—time between stimulus and maximal electrical response of the occipital cortex (msec), 2) amplitude—maximal height of the electrical response of the occipital cortex (mvolts), amd 3) logMAR (log10 minimal angle of resolution)—peak to peak amplitude of the VEP response is plotted against log of the angle subtended by each check size and the linear portion of the plot is extrapolated to 0 to give the theoretical value that would just elicit a response (valid only if there were at least three points and r2 was > 0.8 and $p < 0.05$). Sweep VEP responses to sine-wave gratings are reported as logMAR—the log10 transformed data of the extrapolation of the VEP amplitude versus spatial frequency function to zero amplitude. Some studies specified that only the trials that met a 3:1 signal-to-noise and phase coherence criteria were used in the estimates of logMAR. Lower values of logMAR represent better visual acuity. ERG responses are reported as 1) maximum response amplitude (Vmax), rod thresholds (light required to generate a 2 mV response), and 2) semisaturation constant (log k). Maturation = higher Vmax, lower rod threshold & log k.

Study quality and applicability. The 13 RCTs received a mean Jadad total quality score of 3.61, with a good internal validity (Summary Matrix 21). Six trials received a score of

5,[182,205,266,269,329] three studies received a score of 3,[104,270,277] and four reports received a score of 2.[203,262-264]

Summary Matrix 21: Omega-3 fatty acids intake associated with the visual function in term infants

		Study Quality								
		A			B			C		
Applicability	I	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Birch[U]	1998	79	Auestad[U]	1997	274	Innis[U]	1997	238
		Auestad[A]	2001	239						
		Auestad[A]	2001	165						
	II	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Makrides[A]	2000	176	Carlson[U]	1996	94	Makrides[U]	1995	89
		Birch[A]	2002	65	Hoffman[A]	2003	68	Jensen[U]	1997	80
	III	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Makrides[A]	1999	146				Jorgensen[U]	1998	39
n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment										

Qualitative synthesis of individual study results

The 13 relevant unique trials that were reviewed reported their results on the effect of LCPUFA on visual function in term infants. These trials employed different methods for measuring the development of visual acuity. Therefore, the qualitative synthesis of the results will be presented in a stratified manner with respect to the types of visual acuity (i.e., VEP, Teller's visual acuity, random dot stereo-acuity, FPL).

Of the 13 unique trials, five[104,182,264,269,270] reported their results on the effect of LCPUFA supplementation on the development of sweep VEP measured in cycles/degrees or log MAR. Of the five trials, similar mean sweep VEP values between the randomized groups of infants were found in four trials[104,182,264,269] at 1.5,[269] 2,[104] 4,[104,264] 6,[104] 6.5,[182] and 9[104] months of age (differences were statistically non-significant).

In contrast, the findings of the same two trials[182,269] and another trial[270] suggested that infants in the LCPUFA-supplemented formula groups had a lower sweep VEP log MAR values, meaning a significantly better visual acuity than those in the control formula groups at 1.5,[182] 4,[182,269] 6.5,[269] 12,[270] and 13[182,269] months of age.

Five trials,[182,203,205,262,266] reported the effect of LCPUFA supplementation on the VEP), measured in log MAR. Of these, three[203,205,266] did not find any between-arm differences in VEP values at 4 and 8 months of age, i.e., the mean VEP values of the infants in the LCPUFA supplemented and control groups were not statistically significantly different. However, findings from the remaining two trials indicated that infants at 4[182,262] and 13[182] months of age, who had been fed with breast milk and LCPUFA supplemented formula, had lower mean log MAR values, i.e., better visual acuity, compared with those fed with control formula without the LCPUFA supplementation. In both studies,[182,262] the breastfed and LCPUFA groups of infants had very similar VEP acuities.

Teller's visual acuity, as an outcome measured in cycles/degrees, was explored in five trials.[104,227,263,277] In all but one,[277] the observed values of Teller's visual acuity in the LCPUFA-supplemented and control groups of infants did not differ statistically between groups at 2[104,227]

3,[263] 4,[104] 6,[104] 10,[104] and 12[227] months of age. In the trial by Carlson et al.,[277] breastfed infants and those randomized to receive the LCPUFA supplemented formula, had on average a significantly higher visual acuity score (i.e., better Teller's visual acuity) than those randomized to receive the control formula at 2 months of age. In the same trial,[277] the observed effect of LCPUFA supplementation at 2 months of age was transient and was no longer present at 4, 6, 9, and 12 months of age.

Two trials[269,270] investigated the effect of supplementary LCPUFA on the random dot stereoacuity in term infants, measured in log seconds. The results of both trials indicated that stereoacuity did not differ statistically significantly across the randomized groups of infants at 8, 9, 12, and 13 months of age. Note that Hoffman et al.[270] found a trend for better stereoacuity in the infants receiving LCPUFA-enriched formula at 9 and 12 months of age. However, none of the observed differences was statistically significant at 9 and 12 months. Results of these two trials were less consistent for the effect of LCPUFA in the infants at 4 months of age. Specifically, Birch et al.[269] found that the infants randomized to receive LCPUFA-enriched formula had a better stereoacuity at 4 months of age than those randomized to receive the control formula (numerical data was not given). Whereas in the other trial, Hoffman et al.[270] suggested that the measures of stereoacuity did not differ across the randomized groups of infants receiving either LCPUFA-enriched or control formula at 4 months of age.

Of the 13 trials, only one trial[182] assessed the effect of LCPUFA supplementation on FPL acuity. In this trial, the mean FPL acuity, measured in log MAR at 1.5, 4, 6.5, and 13 months of age, did not differ across the groups of infants fed breast milk, LCPUFA-supplemented formula or control formula.

The effect of LCPUFA (DHA and AA) supplementation on the maturity of retinal function as measured by Naka-Rushton parameters (log k, log Vmax, and rod threshold) in term infants was only investigated in one trial.[182] The evaluation of the retinal function maturity was based ERG responses determined by electroretinography.

The results of this trial indicated that at 1.5 months of age, the log k (semisaturation constant) was statistically significantly lower (i.e., more mature ERG response) in the infants receiving DHA + AA supplemented formula, compared with those receiving DHA supplemented formula or control formula. This effect was no longer present at 4 months of age. Other two Naka-Rushton parameters, log Vmax and rod threshold, were not statistically significantly different across the diet groups at either 1.5 or 4 months of age.[182]

Of the 13 trials, six[104,205,227,266,277] did not report any information regarding the associations (i.e., correlation, linear regression) between maternal/infant blood biomarkers (i.e., plasma-, RBC-LCPUFA content) and the measures of visual acuity (i.e., VEP, FLP, Teller's acuity) or ERG responses in infants. Seven trials[182,203,262-264,269,270] reported some information concerning the above-mentioned associations.

Of the seven trials, four[182,264,269,270] reported associations between milk or blood biomarkers (plasma/RBC-DHA and/or –AA content) and the sweep VEP acuity measures. Of these trials, three[182,269,270] found statistically significant negative linear regression coefficients indicating that higher RBC-DHA content was associated with a better sweep VEP acuity in infants at 1.5, 4,[182,269] 6.5,[182] 12,[270] and 13[182,269] months of age. Results of the remaining study[264] suggested that milk- or RBC-DHA content was not associated with the measured sweep VEP acuity at 4

months of age. The results of both trials[182,264] that looked at the RBC-EPA and RBC-AA content in relation to the measure of sweep VEP acuity, indicated that neither RBC-AA nor RBC-EPA content was associated with the sweep VEP acuity during the first year of the infants' life. One study,[269] that investigated the relationship between infant's plasma-DHA and -AA content, found that higher plasma contents of both DHA and AA were associated with better sweep VEP acuity at 4 and 13 months of age.

The relationship between human milk or the infants' blood biomarkers (plasma/RBC-DHA and/or -AA content) and the measures of infant VEP acuity were reported in two trials.[203,262] Both trials suggested that RBC-DHA correlated negatively with the amplitude of VEP acuity (in log MAR), measured at 4[203,262] and 7.5[262] months of age (i.e., infants at 4 and 7.5 months of age who on average had a higher RBC-DHA content, tended to have a lower log MAR or better VEP acuity). The squared correlation coefficients for the association between RBC-DHA and the amplitude of VEP acuity, measured at 4 and 7.5 months of age were 0.23 ($p < 0.001$) and 0.12 ($p < 0.005$), respectively.[262] The former trial[203] also showed that there was no correlation between either plasma- or RBC-DHA content at 4 months of age, and the latency measure of VEP acuity obtained at either 4 or 8 months of age. The same trial,[203] however, found a statistically significant negative correlation between plasma-DHA content and the amplitude of VEP acuity both measured at 4 months of age.

Of the reviewed trials, only one[263] reported the association(s) for human milk and/or the infants' blood lipid composition (plasma-DHA and/or RBC-DHA content) in relation to the measure of Teller's visual acuity. The plasma-DHA content did not correlate with the Teller's acuity, measured at 3[263]months of age. Similarly, the associations relating the infants' RBC-DHA[263] content to Teller's visual acuity did not reach the traditional level of statistical significance.

Only two trials reported the associations between the infants' RBC-DHA content and their stereoacuity (in log seconds) measured at 4[269] and 12[270] months of age. Both trials found that there was no association/correlation between the two factors. For example, in one trial,[269] the reported linear regression coefficient estimate was $\beta = -0.31$ ($p > 0.05$). In the same trial, the infants' plasma-DHA content was negatively correlated with their stereoacuity at 4 months of age, meaning that, on average, infants with higher plasma-DHA content tended to have a better stereoacuity.

The relationship (correlation) between blood lipid content (plasma- and RBC-DHA) and ERG parameters (measured by Naka-Rushton indicators) in infants was reported in one trial.[182] None of the Naka-Rushton parameters except for log k (in scotopic troland seconds) was statistically significantly correlated with plasma- or RBC-DHA content at either 1.5 or 4 months of age. There was a statistically significant negative correlation between the RBC-DHA content and log k in the infants at 1.5 months of age.

All the trials reported some information on the losses to follow up/non-completers/withdrawn. The trial that failed to report this information was presented in a form of an abstract. The most common reported reasons for the non-completion/withdrawal of study protocol were: intolerance to lactose or cow's milk/dietary hypersensitivity, poor compliance to the study regimen, early cessation of breast feeding, illness (cataract, meningitis, pyloric stenosis, allergic asthma, and phenylketonuria), declined to participate in the trial, and relocation.

The number of non-completers/drop-outs varied across the trials ranging from two[264] to 116.[104] Across majority of the trials,[182,203,205,262-264,266,269,270,277] the number of non-completers ranged from two[264] to 47[263], with a mean of 20 per trial.

Carlson et al.[277] reported one infant was withdrawn for an abnormal ophthalmologic examination. It should be noted that in all studies, that not all infants who completed the study feeding protocol were successfully assessed for visual acuity. It was not always reported as to which study group the unsuccessful visual acuity assessments were in.

In the Makrides et al. study, there were 66 of 79 of all infants who completed the feeding study who had successful VEP assessments at 16 weeks, and 60 who has successful feeding assessments at 30 weeks;[262] the sample size calculation was not reported. In the Jorgensen et al. study,[264] there were 26 of 37 formula study infants with successful sweep VEP (DHAGF:18, STF:8). Auestad et al.[104] withdrew two from the control group, nine from the DHA + AA group, and four from the DHA group, due to abnormal ophthalmologic examination; one from the DHA group was excluded from the acuity card procedure. Some studies reported the exclusion of values from visual acuity assessments due to low tester confidence.[104]

In Makrides et al.,[205] one infant in the formula group was withdrawn due to cataracts. This resulted in smaller sample sizes that were determined a priori. Hoffman et al.[270] reported that 16 were lost to follow up or had unsuccessful stereoacuity testing, however, information regarding which group these participants belonged to was not specified, so it is unknown if the sample sizes were too small based on a priori sample size calculations.[182]

Quantitative synthesis

Quantitative analysis of visual acuity was as described previously for pre-term infants (see above).

DHA vs. no-DHA.

Meta-analysis for behaviorally based test (Teller's Card test) at age 2, 4, 6, 9 and 12 months (Table 8, Figure 15). There is no statistically significant difference on visual acuity between DHA intake and placebo groups for term infants based on the behavioral test at age 2, 4, 6, 9 and 12 months.

Table 8. Meta-analysis of visual acuity difference (DHA vs. no-DHA) for term infants based on behavioral test

Age	Studies	Heterotest	Point estimate	95% CI	P-value
2	[1]Auestad 1997, [2]Birch 1998	0.72	0.20	(-0.1, 0.51)	0.19
4	[1]Auestad 1997, [2]Birch 1998	0.38	-0.14	(-0.37, 0.10)	0.25
6	[1]Auestad 1997, [2]Birch 1998	0.96	-0.07	(-0.27, 0.12)	0.45
9	[1]Auestad 1997	-	-0.18	(-0.42, 0.06)	0.13
12	[1]Auestad 1997, [2]Birch 1998	0.97	-0.19	(-0.38, 0)	0.05

Age: in months; **Studies:** superscripts correspond to the report number in the graphs; **Heterotest:** P-value for the heterogeneity test; **Point estimate:** (in octaves); positive value means the point estimate favors DHA intake over no-DHA intake; **P-value:** P-value for the effectiveness–only one study in this meta-analysis.

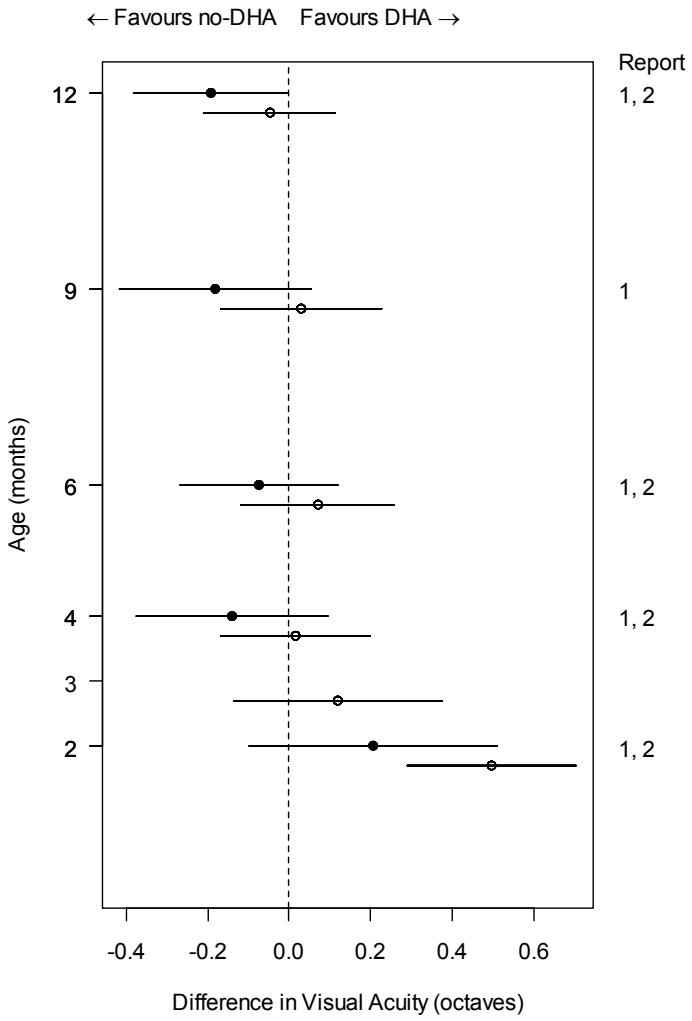


Figure 15. Meta-analysis of visual acuity difference (DHA vs no-DHA) in term infants based on behavior test Shaded circles represent the pooled estimates of visual acuity difference from the meta-analysis of randomized comparisons (formula fed DHA vs. formula fed no-DHA) tested at certain ages. The open symbols represent the pooled estimates of visual acuity difference from the meta-analysis of nonrandomized comparisons (Human milk vs. formula without DHA).

Meta-analysis for electrophysiologically-based test (VEP) at 2, 4, 6, 9 and 12 months (Table 9, Figure 16). There is no statistically significant difference on visual acuity between DHA intake and placebo groups for term infants based on the Electrophysiologically based test at age 2, 4, 6, 8, 9 and 12 months.

Table 9. Meta-analysis of visual acuity difference (DHA vs. no-DHA) for term infants based on electrophysiological test

Age	Studies	Heterotest	Point estimate	95% CI	P-value
2	[1]Auestad 1997, [2]Birch 1998	0.01	0.25	(-0.36, 0.86)	0.42
4	[1]Auestad 1997, [2]Birch 1998, [3]Makrides 1999	0.04	-0.01	(-0.25, 0.24)	0.96
6	1Auestad 1997, 2Birch 1998	0.07	-0.02	(-0.36, 0.32)	0.91
8	[3]Makrides 1999	-	-0.27	(-0.64, 0.1)	0.16
9	[1]Auestad 1997	-	-0.11	(-0.33, 0.11)	0.32
12	[1]Auestad 1997, [2]Birch 1998	<0.01	0.12	(-0.45, 0.69)	0.68

Age: in months; **Studies:** superscripts correspond to the report number in the graphs; **Heterotest:** P-value for the heterogeneity test; **Point estimate:** (in octaves); positive value means the point estimate favors DHA intake over no-DHA intake; **P-value:** P-value for the effectiveness–only one study in this meta-analysis.

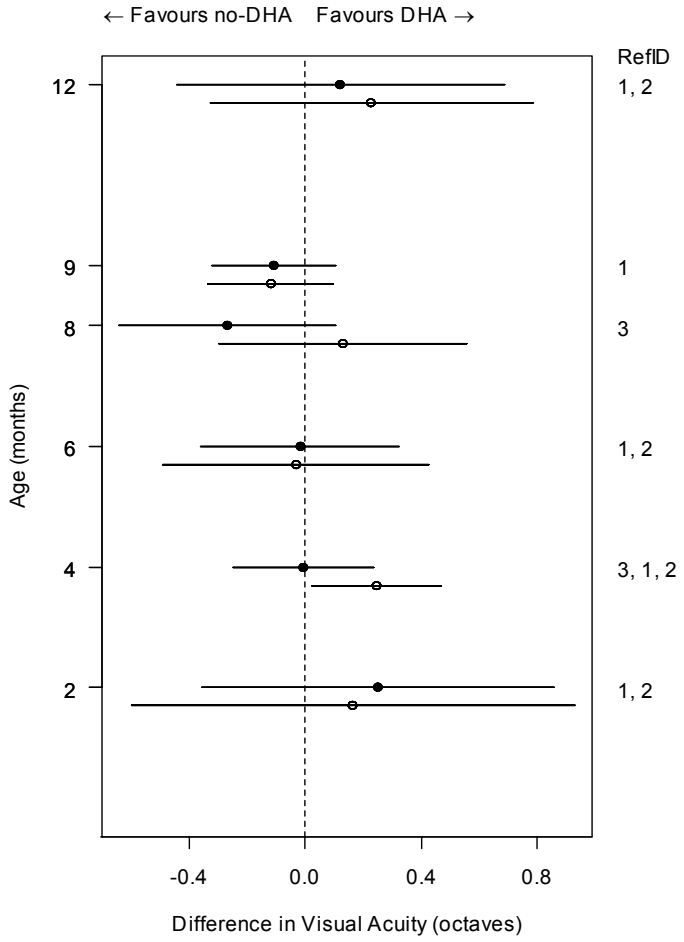


Figure 16. Meta-analysis of visual acuity difference (DHA vs. no-DHA) in term infants based on electrophysiological test. Shaded circles represent the pooled estimates of visual acuity difference from the meta-analysis of randomized comparisons (formula fed DHA vs. formula fed no-DHA) tested at certain ages. The open symbols represent the pooled estimates of visual acuity difference from the meta-analysis of nonrandomized comparisons (Human milk vs. formula without DHA).

DHA+AA vs no-DHA

Meta-analysis for Behaviorally based test (Teller's Card test) at 2, 4, 6, 9 and 12 month (Table 10, Figure 17). Except for results at 2 months, there is no statistically significant difference in visual acuity between DHA + AA intake and placebo groups for term infants based on the behavioral test at age 4, 6, and 9 and 12 months.

Table 10. Meta-analysis of visual acuity difference (DHA+AA vs. no-DHA) for term infants based on behavioral test

Age	Studies	Heterotest	Point estimate	95% C.I.	P-value
2	[1]Auestad 1997, [4]Carlson 1996, [2]Birch 1998	0.32	0.37	(0.15, 0.6)	<0.01
4	[1]Auestad 1997, [4]Carlson 1996, [2]Birch 1998	0.55	-0.14	(-0.33, 0.05)	0.16
6	[1]Auestad 1997, [2]Birch 1998	0.29	0.07	(-0.16, 0.3)	0.57
9	[1]Auestad 1997	-	-0.04	(-0.31, 0.23)	0.78
12	[1]Auestad 1997, [2]Birch 1998	0.63	-0.04	(-0.26, 0.17)	0.7

Age: in months; **Studies:** superscripts correspond to the report number in the graphs; **Heterotest:** P-value for the heterogeneity test; **Point estimate:** (in octaves); positive value means the point estimate favors DHA intake over no-DHA intake; **P-value:** P-value for the effectiveness–only one study in this meta-analysis.

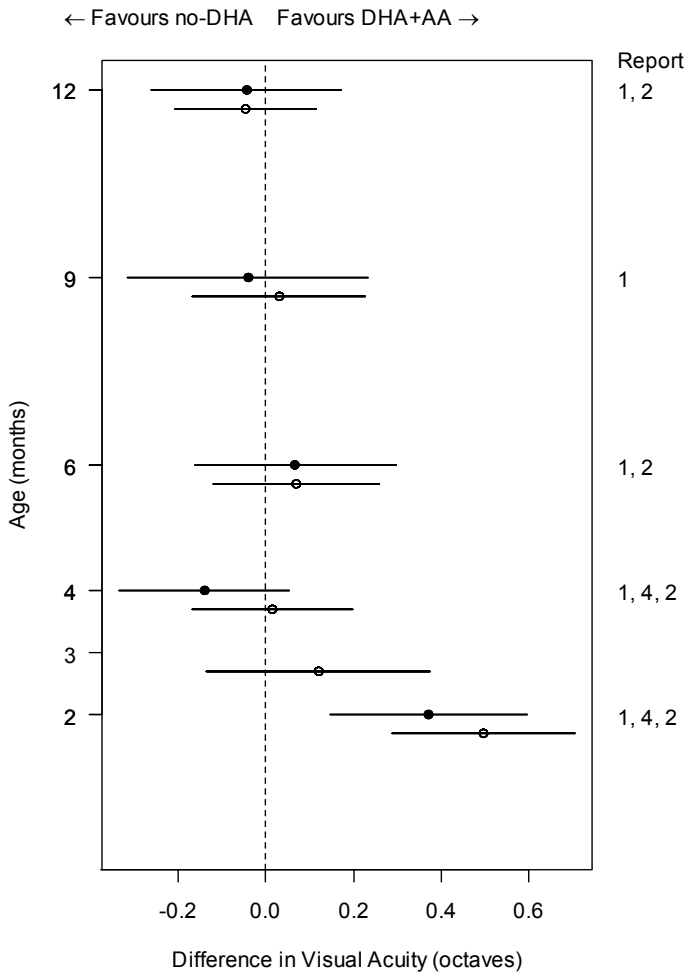


Figure 17. Meta-analysis of in visual acuity difference (DHA+AA vs no-DHA) in term infants based on behavior test. Shaded circles represent the pooled estimates of visual acuity difference from the meta-analysis of randomized comparisons (formula fed DHA+AA vs. formula fed no-DHA) tested at certain ages. The open symbols represent the pooled estimates of visual acuity difference from the meta-analysis of nonrandomized comparisons (human milk vs. formula without DHA).

Meta-analysis for Electrophysiologically based test (VEP) at 2, 4, 6, 7, 9 and 12 months (Table 11, Figure 18).Except results at 12 month, there is no statistically significant difference on visual acuity between DHA +AA intake and placebo groups for term infants based on the Electrophysiologically based test at age 2, 4, 6, 8, and 9 months.

Table 11. Meta-analysis of visual acuity difference (DHA+AA vs. no-DHA) for term infants based on electrophysiological test

Age	Studies	Heterotest	Point estimate	95% CI	P-value
2	[1]Auestad 1997, [2]Birch 1998	<0.01	0.29	(-0.32, 0.91)	0.35
4	[5]Birch 2002, [3]Makrides 1999, [1]Auestad 1997, [7]Makrides 1995, [8]Jorgensen 1997, [2]Birch 1997	<0.01	0.17	(-0.01, 0.36)	0.07
6	[5]Birch 2002, [1]Auestad 1997, [2]Birch 1998	<0.01	0.16	(-0.13, 0.45)	0.28
8	[3]Makrides 1999	-	0	(-0.35, 0.35)	1
9	[1]Auestad 1997	-	-0.12	(-0.32, 0.08)	0.23
12	[5]Birch 2002, [1]Auestad 1997, [2]Birch 1998, [6]Hoffman 2003	<0.01	0.32	(0.09, 0.56)	0.01

Age: in months; **Studies:** superscripts correspond to the report number in the graphs; **Heterotest:** P-value for the heterogeneity test; **Point estimate:** (in octaves); positive value means the point estimate favors DHA intake over no-DHA intake; **P-value:** P-value for the effectiveness–only one study in this meta-analysis.

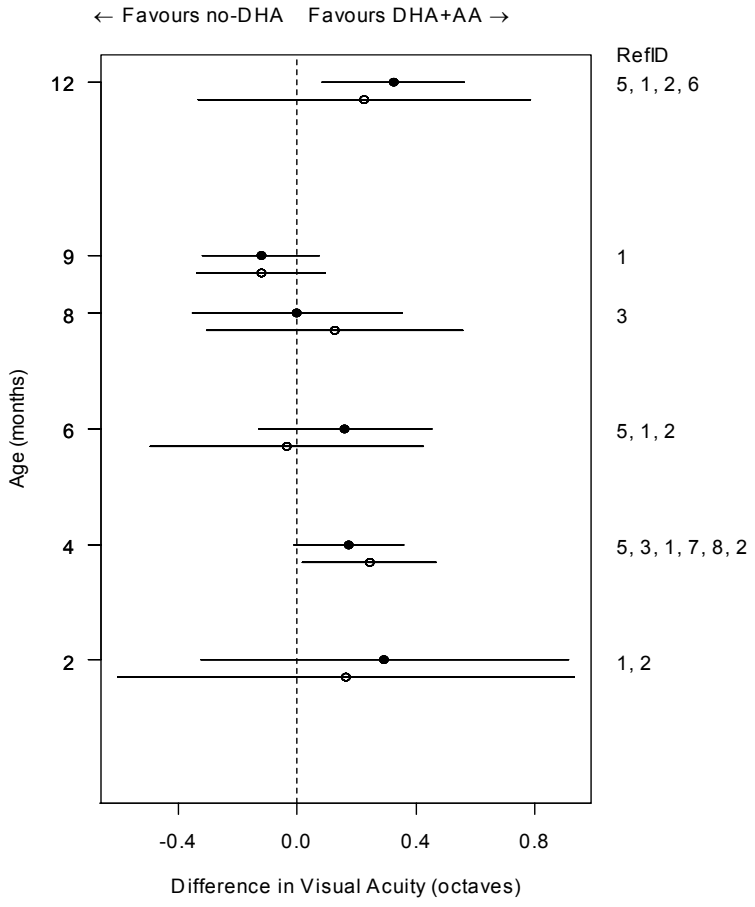


Figure 18. Difference in visual acuity (DHA+AA vs. no-DHA) in term infants based on electrophysiological test
Shaded circles represent the pooled estimates of visual acuity difference from the meta-analysis of randomized comparisons (formula fed DHA vs. formula fed no-DHA) tested at certain ages. The open symbols represent the pooled estimates of visual acuity difference from the meta-analysis of nonrandomized comparisons (human milk vs. formula without DHA).

Human milk vs no-DHA

Here are listed the combined results of visual acuity difference between HM and no-DHA groups based on behavioral- and electrophysiological-based tests at different ages as references.

Table 12. Meta-analysis of visual acuity difference (HM vs. no-DHA) in term infant based on behavioral and electrophysiological test

Test	Age	Heterotest	Point estimate	95% CI	P-value
B	2	0.38	0.5	(0.29, 0.7)	0
B	3	-	0.12	(-0.13, 0.37)	0.36
B	4	0.94	0.02	(-0.17, 0.2)	0.87
B	6	0.48	0.07	(-0.12, 0.26)	0.47
B	9	-	0.03	(-0.17, 0.23)	0.76
B	12	0.93	-0.05	(-0.21, 0.11)	0.57
E	2	<0.01	0.17	(-0.6, 0.93)	0.67
E	4	0<0.01	0.24	(0.02, 0.47)	0.03
E	6	0.01	-0.03	(-0.49, 0.43)	0.89
E	8	0.06	0.13	(-0.3, 0.56)	0.56
E	9	-	-0.12	(-0.34, 0.1)	0.28
E	12	<0.01	0.23	(-0.33, 0.79)	0.42

B: Behavioral test. **E:** Electrophysiological test; **Age:** in months; **Heterotest:** P-value for the heterogeneity test; **Point estimate:** of meta-analysis of certain age (in octaves); The positive value means the point estimate favors DHA intake over no-DHA intake; **Standard error:** of pooled estimates from meta-analysis (in octaves); **P-value:** P-value for the effectiveness; only one study in this meta-analysis

Impact of covariates and confounders

Of the 13 reviewed trials, 12 reported some information on statistical techniques/methods used to estimate the effect of LCPUFA formula supplementation on visual acuity development in term infants. In 11 trials, the effect of interest was estimated using analysis of variance with repeated measures (ANOVA) alone,[262,263] ANOVA and analysis of co-variance (ANCOVA),[104,203,227] ANCOVA and multiple linear regression (MLR),[205,266]or ANOVA and MLR.[182,264,269,270] In one trial,[277] ANOVA together with generalized linear model was used. Five trials reported that the analyses (ANOVA/ANCOVA/MLR) were adjusted for age only.[182,262,263,269,277] The analyses in other trials were reported to be adjusted for some additional covariates such as birth weight,[203,205,264,266] length at birth,[203,205,264] HC at birth,[266] sex,[203,205,270] ethnicity,[203] maternal smoking,[205,266] blood lipid (DHA) composition,[203,266,270] duration of breast feeding,[264] GA,[205,264] maternal education,[205,266] birth order,[205] and social score.[205,266] Three trials[104,227] reported that the adjustment in the analysis was done for the study site.

Several trials reported that the randomized formula study groups at baseline were not well-balanced (statistically significant differences) for the following factors: sex,[205] parental education,[270] ethnicity,[182] maternal smoking,[104,266] and birth weight.[263,264] For example, in one trial,[104] the percentage of infants whose mothers had been smokers were 26, 17, and 11 in the DHA + AA, DHA, and control formula groups, respectively (chi-square test based $p < 0.05$). The authors of this trial reported that the association between the diet and visual acuity was only adjusted for the study site. In the other trial,[266] a baseline distribution of the randomized infants whose mothers were smokers across the two ALA-enriched and LA-enriched formula groups was 51% and 39%, respectively. Furthermore, there was a higher proportion of smoker non-completers in the ALA-enriched than LA-enriched formula group (8% vs. 2%). These factors

had been controlled for, as reported, in three trials.[205,264,266] It is not clear whether the trials reporting to have controlled only for age,[182,262,263,269,277] or site,[104,227] adjusted for additional factors such as maternal smoking, the infants' ethnicity, sex, and size, or other potentially important covariates.

Across the trials, mothers whose infants had been breastfed, tended to be more educated,[104,205,262,277] to have a higher social class,[205] to be non-smokers,[104,205,266] and of White race,[104,182,277] than those in the formula arms.[104,277]

Most trials reported that infant sex,[182,264,269,270] maternal education,[104,182,227,266,269] maternal social score,[262,266,270] maternal age,[182,227,264,269,270] infant length and HC at birth,[104,227,248,262-264,270] GA,[104,262,264,277] and duration of breast feeding[264] amongst the randomized formula groups were evenly distributed (statistically non-significant differences).

Four trials reported those covariates that influenced the outcome (visual function). These covariates were as follows: sex,[205] birth weight,[205,264] duration of breast feeding,[264] maternal smoking,[205,266] anthropometrical measures at birth,[266] partner's social score,[266] and RBC-DHA content.[270] In these trials, female gender,[205] lower rates of maternal/partner smoking,[205,266] higher birth weight,[205,264] greater HC,[264,266] and longer duration of breast feeding[264] were independently related with better visual acuity ($p < 0.05$).

In most trials, the analyzed main effect of age was statistically significant. It correlated with better visual functioning. The statistically significant interaction between age and diet was detected in two trials,[269,270] meaning that the effect of diet was not uniform with respect to the infants' age.

The power calculation was reported in ten trials,[120,124,126,151,325,329,331,334,335] while the intention-to-treat analysis approach was reported in only one study.[120]

Visual Function Outcomes in Light of Biomarker Data

What is the Evidence That Term or Preterm Human Infants' Visual Function is Associated With the Omega-3 or Omega-6/Omega-3 Fatty Acid Content of Maternal Biomarkers During Pregnancy?

One cross-sectional study was identified to respond this question. Krasevec et al.'s[275] study was described in the Visual Function Outcomes questions (see key question: Maternal Intake/Visual Function).

What is the Evidence That Term or Preterm Human Infants' Visual Function is Associated With the Omega-3 or Omega-6/Omega-3 Fatty Acid Content of Child Biomarkers?

There were a total of 21 studies that addressed this question. Eight cross-sectional studies, included in seven reports, published between 1993 and 2002, met the eligibility criteria. Since these were observational studies, and in order to respond to this particular question, the cross-

sectional data was abstracted from three prospective cohort studies.[271,281,282] Krasevec et al.[275] was described above (see key question: Maternal Intake/Visual Function.). There were also nine RCT's described in the term population[138,182,203,248,262-264,269,270] and three RCTs in the preterm population (see above).[185,198,212]. (Summary Tables 38-40 and 41-43)

Overview of relevant studies

The studies that included preterm infants will be described separately from the term infant studies. Birch et al. assessed the association between LCPUFA RBC content of omega-3 FAs and the visual function development after dietary supply of LCPUFA (breast milk) in American infants. This report included two different study populations, one of healthy preterm infants and another of full-term infants. The outcomes were measured at 4 months CA and, for the term infants, also at 36 months CA.[278] (Summary Table 41)

Makrides et al. studied the FA profiles of Australian term infants (5 months of age) fed breast milk and infant formula, and its association with VEP acuity.[280](Summary Table 41)

Summary Table 41: Association between omega-3 or omega-6/omega-3 fatty acid content of biomarkers and visual function development in term and preterm infants

Author, Year, Location: Design	Study groups[1]		Notable associations[2,3]	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)			
Birch, 1993a, US: Cross-sectional[278]	Preterm infants breastfed (n=15)	Preterm infants corn oil formula (n=15)	LogMAR acuity was S correlated with the ratio [DHA n-3/DPA n-6] in total RBC lipids^{+++} FPL acuity LogMAR was S correlated with the ratio DHA n-3/DPA n-6^{++} RBC ratio was S ↑ in HM than in formula fed^{++++}	Quality score: 4 [Grade B]	III
Birch, 1993b, US: Cross-sectional [278]	Term infants 4 mo CA breastfed (n=NR) / Term infants 36 mo CA corn oil formula (n=NR)	Term infants 4 mo CA corn oil formula (n=NR) / Term infants 36 mo CA breastfed (n=NR)	Mean VEP & FPL acuities better in HM than in formula (4 mo)$^{+}$ Mean RBC DHA/DPA in total RBC lipids was S ↑$^{++++}$ HM than in formula group & stereo acuity was S correlated with the end-product ratio^{+} Letter matching (36 mo) was S correlated with ratio, RBC DHA/DPA (4 mo)$^{+}$	Quality score: 4 [Grade B]	III
Makrides, 1993, Australia: Cross-sectional [280]	Term infants breastfed (n=8)	Term infants formula fed (n=8)	HM group S ↓ logMAR (i.e., better VEP acuity) than formula-fed (5 mo) S correlation between logMAR (VEP acuity) & % DHA^{+} & LA^{+} in RBC PL	Quality score: 4 [Grade B]	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; grp = group; wk = week(s); mo = month; wt = weight; RBC = red blood cells; PL = phospholipid; CPG = choline phosphoglycerides; EPG = ethanolamine phosphoglycerides; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; LBM = low breast milk; HBM = high breast milk; FPL = forced-choice preferential looking; HM = human milk; CA = corrected age; ERG = electroretinogram; VEP = visual evoked potential

Innis et al. studied the development of preferential looking acuity in exlusively breastfed or formula-fed Canadian term infants. The goal was to measure the possible association between this outcome and the omega-3 and/or omega-6 FA content of RBC and plasma of the infants at 14 days and 3 months of age.[281](Summary Table 42)

Leaf et al. evaluated the correlation between the FA composition of RBC and plasma in Australian preterm infants of 40 weeks of PCA and the development of visual function, using ERG and the Teller Acuity Card Procedure. The exposure was low breast-milk diet or high breast-milk diet, besides the use of total parenteral nutrition (TPN) until reaching 2,000 g of weight.[279](Summary Table 42)

The aim of Jorgensen et al.'s study was to establish an association between the FA composition of RBC and plasma of term infants and their visual acuity, using the Teller Acuity Card Procedure at 4 months of life.[282] A small sample of Danish term infants were receiving either breast milk or formula without LCPUFA supplementation.[282](Summary Table 42)

Summary Table 42: Association between omega-3 or omega-6/omega-3 fatty acid content of biomarkers and visual function development in term and preterm infants

Author, Year, Location: Length & Design	Study groups[1]		Notable associations[2,3]	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)			
Innis, 1994, Canada: Cross-sectional [281]	Term infants breastfed (n=17)	Term infants formula fed (n=18)	NS between groups in visual acuity test (14 d & 3 mo) Visual acuity NS to diet or plasma PL, RBC PC or PE concentrations of DHA on entire group of infants or within the breastfed or formula-fed group of infants	Quality score: 5 [Grade B]	III
Leaf, 1996, Australia: Cross-sectional [279]	Preterm infants HBM (n=9)	Preterm infants LBM (n=9)	S (+) correlation between scotopic b wave implicit time & % DHA in plasma^{+++} & RBC PL^{+}, total n-3 LCPUFA in plasma^{++} & RBC PL^{++} (+) correlation between RBC AA^{+} & total n-6 LCPUFA^{+} & scotopic a-b amplitude NS relationships were seen between photopic ERGs & plasma or RBC LCPUFAs	Quality score: 6 [Grade B]	III

Jorgensen, 1996, Denmark: Cross-sectional [282]	Term infants breastfed (n=17)	Term infants formula fed (n=16)	Visual acuity ↑ overtime in both feeding groups[+++], S ↑ increase in HM group[+++] NS correlation between RBC DHA & visual between groups (4 mo) NS correlation between AA levels & visual acuity	Quality score: 5 [Grade B]	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; RBC = red blood cells; PL = phospholipid; CPG = choline phosphoglycerides; EPG = ethanolamine phosphoglycerides; [+]p<.05 or significant with 95% confidence interval; [++]p<.01; [+++]p<.001; [++++]p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction

Innis et al. included a sample of Canadian term infants exclusively breastfed for at least 3 months since birth. The aim of the study was to determine the association between the RBC DHA content at 2 months of age and visual and neural development. The visual acuity was measured using the Teller Acuity Card Procedure at 2, 4, 6 and 12 months.[271](Summary Table 43)

Krasevec et al. measured the LCPUFA content in maternal and infant's blood at 2 months of age and its correlation with the visual acuity using the Teller Acuity Card Procedure. The Cuban term infants were either breastfed or formula fed.[275](Summary Table 43)

Summary Table 43: Association between omega-3 or omega-6/omega-3 fatty acid content of biomarkers and visual function development in term and preterm infants

Author, Year, Location: Length & Design	Study groups[1]		Notable associations[2,3]	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)			
Innis, 2001, Canada: Cross-sectional [271]	Term infants breastfed (n=83)	n/a	RBC PE DHA (2 mo) was S (+) correlated to visual acuity at 2 mo[++] & 12 mo[+] NS at 4 & 6 mo Infants with RBC PE DHA <8.53g/100g had S ↓ visual acuity at 2 & 12 mo than infants with > 10.78g/100g FA[+]	Quality score: 8 [Grade A]	III

Krasevec, 2002, Cuba: Cross-sectional [275]	Mother/term infants breastfed (n=31)	Mother/term infants formula fed (n=23)	NS correlations between visual acuity & EFA concentration, ratio of EFA, or group of PUFA in infant tissues NS correlation for full sample & each feeding group (i.e., exclusively breast milk vs. not exclusively breastfed) NS correlation between PUFA profiles of maternal tissues for exclusively breastfed infants & visual acuity	Quality score: 7 [Grade B]	III

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; RBC = red blood cells; PL = phospholipid; CPG = choline phosphoglycerides; EPG = ethanolamine phosphoglycerides; $^{+}p<.05$ or significant with 95% confidence interval; $^{++}p<.01$; $^{+++}p<.001$; $^{++++}p<.0001$; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. Eight unique cross-sectional studies were deemed relevant for the review.271,275,278-282 One report included two unique studies, with preterm and full-term infants. Birch et al. and Leaf et al. included preterm infants,278,279 whereas the remaining six studies selected full-term infants.271,275,278,280-282 Two studies were conducted in the US,278 two in Australia,279,280 two in Canada,271,281 one in Denmark,282 and one in Cuba.275

Leaf et al.[279] did not provide the funding source. Both Birch et al. studies were supported by the National institutes of Health, Delta Gamma Foundation of Dallas, Pediatric Subunit, and the United Cerebral Palsy Foundation.[278] Makrides et al. was funded by Scotia Pharmaceuticals and Nestle Australia,[280] whereas Jorgensen et al. received funds from Food Technology Research and Development Program, DanoChemo A/S, and the Swedish Medical Research Council.[282] The first Innis et al. study was supported by the British Columbia Children's Hospital Investigatorship,[281] and the second[271] by the Medical Research Council of Canada and Ross Laboratories. Finally, Krasevec et al.[275] was funded by a CIDA Award for Canadians.

Population characteristics. All the studies included healthy preterm278,279 or term infants. The elegibility criteria was adequately described in six of eight studies. The preterm infant's studies included 48 patients (sample size range: 18-30), while the term infants' studies selected 296 subjects (sample size range: 16-83).

The preterm infants were included if they were healthy, and born before 32 and 33 weeks of GA; Birch et al. also included infants with birth weights of 1,000 g to 1,500 g and an appropriate weight for GA.[278,279] Birch et al. included two samples of healthy full-term infants, one was composed of 30 infants born at 39 to 41 weeks of GA and tested at 4 months of PCA, and the other was composed of 43 term infants tested at 36 months of age.[278] Makrides et al.' infants were selected at approximately 5 months of age, with an appropriate weight for GA at birth

recorded from immunizations and/or postnatal clinics.[280] The first sample of infants from Innis et al.'s study had an appropriate weight for GA and their mothers had to choose to breast feed or formula feed for at least 3 months.[281] Yet, the second sample of term infants from Innis et al.'s study[271] had a birth weight 2,500 g to 4,500 g and were enrolled within 2 weeks of birth. Their mothers were required to intend to breast feed them without providing infant formula or cow's milk for at least 3 months, and without introducing solid foods for at least the first 4 months after birth. Jorgensen et al.s elected healthy children with birth weights between 2,700g and 4,500g and an Apgar score > 7 after 5 min. Finally, Krasevec et al.[275] was the only study that included healthy Cuban women who experienced a normal pregnancy and their infants at 2 months postpartum.

The preterm infants were excluded if they experienced a major congenital anomaly, severe intra/peri ventricular hemorrhage, 5-min Apgar score below 5,[279] were unable to tolerate enteral feeds by day 10 of life, with respirator treatment for more than 7 days, congenital infection or malformation, retinopathy of prematurity, or grade 3 or 4 intraventricular hemorrhage.[278] Term infants were excluded if they had a known eye disorder, family history of eye disorder, a neurological disorder, or neonatal morbidity.[278,280] Innis et al.[271] also excluded mothers with substance abuse, communicable diseases, metabolic or physiologic problems, infections likely to influence fetal growth, or multiple births and infants with evidence of metabolic or physical abnormality. Three studies did not provide the exclusion criteria.[275,281,282]

None of the studies reported the use of medication and/or supplements before study entry. For both studies that included a preterm population, the weight and length at the time of the evaluation,[278,279] as well as the GA at birth,[279] were comparable between feeding groups.

The studies that evaluated term infants had a between-group nonstatistically difference in terms of birth weight,[271,281,282] GA,[281,282] current weight, length,[278] age, parity, social status,[280] pregnancy weight gain, maternal age, percentage of cesarians and percentage of males.[282]

Only Leaf et al.[279] reported the comorbid condition of their preterm infants during the study period. The conditions were: subependymal intraventricular hemorrhage (n=4), mild ventricular dilatation (n=1), stage 1 retinopathy of prematurity (n=1) and stage 2 (n=1).

Intervention/exposure characteristics. The exposure desciption will be made separately for preterm and term infants. The preterm infants were fed according to nursery protocol and their parent's wishes in Leaf et al.'s study.[279] TPN was commenced in those with birth weight < 1,500 g (Vitamin-N, Pharmacia Ltd.) along with Intralipid 20% (Pharmacia Ltd.), which provided a source of lipids (receiving in 15 mL/kg/day approx. 6.4 mg/kg/day of AA and 5.8 mg/kg/day of DHA from egg-phospholipid). Enteral feed were started as soon as possible by intermittent gavage. Breast milk was given if available. If not, infants were commenced on Premature Enfalac formula until 2,000 g, and then standard on Enfalac, which contains vegetable oils as a source of lipids. No LCPUFAs were found in the formula milks. For breast milk, 150 mL/kg/day provided 32 mg/kg/day of AA and 17 mg/kg/day of DHA. The low breast-milk (LBM) and high breast-milk (HBM) groups did not differ in the amount of TPN and intralipid intake (less AA and DHA than breast milk). In Birch et al.'s study, preterm infants were fed with breast milk or a corn oil-based formula.[278]

The term infant groups in Birch et al.'s study were either breastfed or fed corn oil-based formula. The diets were regulated until 12 months of age to maintain cholesterol and FA profiles

consistent with the two dietary regimes. The breastfed group was provided with a monosaturated FA formula, with high oleic acid supplement and by feeding egg yolk as a solid food (LC PUFA). For the formula-fed group, a high LA supplement was provided and solid foods were selected to maintain a low cholesterol and omega-3 LCPUFA supply.[278]

Makrides et al.'s formula-fed infants (n=8) received one of three infant formulas, each of which had a similar FA composition. LA ranged from 12% to 15% and ALA ranged from 1% to 1.6% of total FAs. The LA:ALA ratios were similar and ranged from 9.4 to 11.3. Both groups were receiving solid foods, like rice cereal and stewed fruit. None of the infants were receiving detectable quantities of DHA or AA from solids.[280] The first term formula group in Innis et al.'s study (no LCPUFA) (n=18) were fed with ready-to-feed Enfalac (by Mead Johnson Nutritionals) from 14 (SD=2) days of age. Enfalac is a whey protein based term formula with 17.9% LA (omega-6) and 2.1% ALA (omega-3). The breast milk was composed of 13.4 % LA, 1.5 % ALA, 0.1% EPA and 0.2 % DHA. This group was also provided with a daily supplement of vitamin D, A and C, while the formula-fed group did not receive supplements (vitamins or minerals). The duration of the intervention was 3 months.[281]

In the second Innis et al. study, the infants were exclusively breastfed for 3 months, and the majority were exclusively breastfed for more than 3 months.[271] The mother's breast milk had 0.26 g DHA, 0.4 g AA, 12.5 g LA (per 100 g of milk FAs).[271]

Jorgensen et al.'s breast feeding infants (n=17) received between 0.44% to 0.56%wt of AA, 0.13% to 0.23%wt of EPA and 0.43% to0.53%wt of DHA, while the formula-fed infants (n=16) received 14.4%wt LA and 1.7% wt of ALA (omega-3). The omega6/omega-3 ratio was comparable between groups. Small amounts of supplementary food (vegetable mashes and cereal-based gruel, one meal per day) were introduced to one breastfed and nine formula-fed infants from the age of 3 months.[282]

Krasevec et al.[275] included data regarding the infant's feeding practices collected at 2 months of age. They were exclusively breastfed (n=31), fed with a combination of breast milk and bottle-feeding (n=22), or not fed any breast milk (n=3). The most common supplemental milk fed to these infants was a cow milk formulation made with skim milk powder and vegetable oil, as well as evaporated milk and yogurt. Supplemental milks had been fed for an average of 2 to 4 weeks before the 2-month study visit. Their mothers were receiving 454 g/week of a high fat fish (*Trachurus mediterraneous*) while breast feeding (source of LCPUFA).[275]

Outcome characteristics. All but one of the studies280 evaluated the visual function with the same test. The FPL was measured with the Teller Acuity Card Procedure. The retinal maturity was measured with an ERG in one study.279 Visual acuity was also assessed using VEP acuity in three studies.278,280 Finally, both Birch et al.'s term and preterm studies evaluated the visual function using the operant FPL acuity, stereo acuity, recognition acuity, color vision, letter matching, picture naming, orthopic exam at 36 months of age. All acuities were expressed in a common unit of measurement, which is independent of the technique, log MAR (log minutes of arc resolution).278

All of the studies drew blood samples from the infants, and in one case from the mothers.[275] The description of the lipid extraction was adequate. The correlation between the RBC or plasma FA composition and the visual function outcomes was calculated.

Study quality and applicability. Although the studies employed different reseach designs, the mean quality score was 5.3 and the applicability assigned was level III.

Summary Matrix 22: Association between omega-3 or omega-6/omega-3 fatty acid content of biomarkers and visual function development in term and preterm infants

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author	Year	n	Author	Year	n	Author	Year	n
	III	Author	Year	n	Author	Year	n	Author	Year	n
		Innis	2001	83	Birch	1993	30			
					Birch	1993	73			
					Makrides	1993	16			
					Innis	1994	35			
					Leaf	1996	18			
					Jorgensen	1996	33			
					Krasevec	2002	56			
n = number of allocated/selected participants										

Qualitative synthesis of individual study results

The results will be analyzed separately for preterm and term infants.

Both preterm studies reported different clinical outcomes related to visual function, thus they will be depicted independently. Birch et al. found that the breastfed infants had a significantly better VEP acuity than the formula-fed infants at 4 months of age. LogMAR acuity was significantly correlated with the end-product ratio [DHA n-3/DPA n-6] in total RBC lipids. For FPL acuity, the results were the same for both the breastfed and formula-fed groups. For the mean RBC end-product ratio, DHA/DPA was significantly higher in the human milk-fed infants compared with the formula-fed infants, and the LogMAR was significantly correlated with this ratio.[278]

Leaf et al. analyzed the ERG (retinal function measure) at 40 weeks PCA in relation to dietary intake and to plasma and RBC LCPUFA content, and also to infant variables such as GA and age at recording, as possible effect modifiers.[279] The infant group was separated by the predominance of breast milk intake (nine infants had a high breast milk intake [mean 74% of total diet] and nine had a low breast milk intake [mean 17.5% of total diet]) in order to make comparisons. Scotopic and photopic ERG results were analyzed in relation to plasma and RBC FAs: AA and DHA, total omega-6 LCPUFA and total omega-3 LCPUFA as continuous variables. There was a positive correlation between scotopic b wave implicit time and percentage composition of DHA in both plasma and RBC PL. A similar relationship was seen with total n-3 LCPUFA in both plasma and RBC PL. There was a positive correlation between both RBC AA and total n-6 LCPUFA and scotopic a-b amplitude. No significant relationships were seen between photopic ERGs and either plasma or RBC LCPUFAs. The correlation between the Teller Card visual acuity test and blood biomarkers was not measured.

For the term population, since the exposure characteristics as well as the population characteristics were so different across the studies, the outcome measures will be described separately for each study.

Birch et al.'s mean VEP and FPL acuities were better in human milk-fed infants than in the formula-fed infants at 4 months of age. No correlation with RBC FA content was measured.[278] However, the 36-month evaluation of the full-term infant group showed that there was no statistical differences between groups (human milk vs. formula fed) in terms of mean OPL grating acuities, near recognition acuity and distance recognition. Human milk-fed infants had significantly better OPL stereoacuity than the formula-fed group at 36 months. The mean RBC end-product ratio, DHA/DPA in total RBC lipids, was significantly higher in the human milk group compared with the formula group, and stereo acuity was significantly correlated with the end-product ratio. The human milk group was significantly better in letter matching than the formula group. Performance on this outcome at 36 months was significantly correlated with the end-product ratio, DHA/DPA in total RBC lipids at 4 months. No significant differences were found between the two diet groups in picture naming or color vision.[278]

Makrides et al.'s breastfed infants had a significantly smalled logMAR (i.e., better VEP acuity) than those who had been formula-fed at 5 months of age. There was no correlation between postnatal age and VEP acuity. Infants fed with breast milk had a greater proportion of RBC DHA and less RBC LA relative to those who had received infant formula. There was a significant correlation between logMAR (VEP acuity) and the proportion of DHA and LA ($p < 0.01$) in RBC PL.[280]

The Innis et al.'s first study, the covariates used in the analysis were age (14 days of age vs. 3 months) and diet (human milk vs. formula). There was a nonsignificant difference between groups in visual acuity test at 14 days and at 3 months. Regression analysis indicated that visual acuity was not related to dietary intake or to plasma PL, RBC PC or PE concentrations of DHA, when tested for the entire group of infants, or just within the breastfed or formula-fed group of infants.[281]

In the second Innis et al. study, the RBC PE DHA at 2 months was significantly and positively correlated to visual acuity at 2 and 12 months, but not at 4 and 6 months of age. Infants with an RBC PE DHA concentration < 8.53 g/100 g had significantly lower visual acuity at 2 and 12 months than infants with an RBC PE DHA > 10.78 g/100 g FAs.[271]

Jorgensen et al.infant's visual acuity increased over time in both feeding groups, with a significantly higher increase in the breastfed group. There was no significant correlation between RBC DHA and visual acuity within the two feeding groups at 4 months. However, when the two groups were combined, the correlation became significant. There was no significant correlation between AA levels and visual acuity.[282] Finally, Krasevec et al.'s study did not find significant correlations between visual acuity scores and any individual EFA concentration, ratio of EFA concentrations, or group of EFA in infant tissues. The same results were obtained when the correlation was measured in the entire sample, and when assessing each feeding group (i.e., exclusively breast milk vs. not exclusively breastfed) separately. There were no relations between EFA profiles of maternal tissues for exclusively breastfed infants and visual acuity.[275] The mean visual acuity scores did not differ between feeding groups.

Cognitive Development Outcomes:

What is the Evidence That Maternal Intake of Omega-3 Fatty Acids During Pregnancy Influences Cognitive Development in Term or Preterm Human Infants?

One RCT published in 2001 was identified which answered this question.[141] This study also answered the question regarding cognitive outcomes in breastfed infants whose mothers received the LCPUFA intervention. (Summary Table 44)

Overview of relevant study characteristics and results

Helland et al. assessed the gestational length, birth weight, and neurologic and cognitive outcomes in a sample of healthy pregnant women. They were randomized to receive cod liver oil (1183 mg/10 mL DHA, 803 mg EPA) or corn oil (LA and ALA) from week 18 of pregnancy to 3 months post delivery.[141]

Helland et al. was conducted in Norway and funded by Peter Moller, Avd. Orkla ASA, and "Aktieselskabet Freia Chocoladefrabriks Medicinske Fond."

The participants (n=590 enrolled) were included if they were healthy women with single pregnancies between 19 and 35 years of age, and intended to breastfeed their infant. They should not have taken any supplements of omega-3 FAs earlier during the pregnancy. The exclusion criteria were premature births, birth asphyxia, infections, and anomalies in the infants that required special attention.[141] Helland et al. had a high rate of dropouts, leaving 341 women in the final analysis (57%).[288]

There was no difference between groups in body mass index before pregnancy and at birth, parity, smoking, or maternal and paternal education at baseline.[141] The mean age of mothers receiving cod liver oil was significantly higher than the age of mothers receiving corn oil.

The subjects were randomly assigned to either 10 mL/day of cod liver oil (Peter Moller, Avd Orkla ASA, Oslo, Norway), or identical 10 mL/day of corn oil from 18 week of pregnancy to 3 months after delivery.[141] The cod liver oil contaned 1,183 mg/10 mL DHA, 803 mg/10 mL of EPA. The corn oil contained 4,747 mg/10 mL LA (omega-6) and 92 mg/10 mL ALA (omega-3). The amount of fat-soluble vitamins was identical in both oils. There was no significant difference between groups in the maternal dietary intake of nutrients at baseline.[141] There was no significant difference in maternal plasma PL concentration of DHA before entering the study.

The cognitive outcomes were assessed using the Fagan test of Infant Intelligence at 27 and 39 weeks of age (6-9 months). A subpopulation analysis (n=90) was performed at 4 years of age, assessing the children's intelligence using the Kaufman Assessement Battery for Children (K-ABC).[141]

Summary Table 44: Omega-3 fatty acids and its influence on cognitive development in infants after intake during pregnancy and breast feeding

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Internal validity	Applicability
Helland, 2001, Norway: 34 wks parallel RCT[141]	Cod liver oil (DHA+EPA) (n=301)	Corn oil (LA+ALA) (n=289)	NS novelty preference (Fagan test) at 6 & 9 mo NS correlation between level DHA & novelty preference (6 & 9 mo) Cod liver oil > Mental Processing K-ABC score than corn oil (4 y)$^{+}$	Jadad total: 4 [Grade: A]; Schulz: Unclear	III

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; N/A = not applicable; grp = group; wk = week(s); mo = month; RBC = red blood cells; PL = phospholipid; $^{+}$p<.05 or significant with 95% confidence interval; ++p<.01; +++p<.001; ++++p<.0001; ↑ = increase; ↓ = decrease/reduction

Helland et al. did not observe a significant difference between groups in the novelty preference at 6 or 9 months of age. When the score from 6 and 9 months of age were combined, there still was no difference between the two groups.[141] When infants with high DHA concentration in umbilical plasma PL were compared with infants with low DHA concentration, there were no differences in novelty preference. Neither did they find differences in DHA concentrations between infants with high and low novelty preference.[141]

Children in the cod liver oil group had significantly higher scores than the corn oil group on Mental Processing Composite of the K-ABC test at 4 years of age. There was nonsignificant difference between groups in the rest of the test composites (simultaneous processing scale and nonverbal scale). The Mental Processing Composite correlated significantly with HC at birth, but not with birth weight or gestational length.[141] No correlation was found between LCPUFA content in umbilical plasma PL and intelligence scores. Yet intelligence scores at 4 years correlated with plasma PL concentrations of DPA (omega-3) and DHA at 4 weeks of age. Mental processing skills of the children correlated significantly with maternal intake of EPA and DHA during pregnancy.[141]

There were 153 withdrawals from randomization to the second Fagan test assessment. The reasons were congenital anomalies, infections in the mothers or infants, miscarriages, premature births, and before giving birth (lack of compliance, discomfort taking the oil).[141]

Study quality and applicability. Helland's Jadad total quality score was 4, indicating good internal validity, yet with an unclear allocation concealment.

Summary Matrix 23: Omega-3 fatty acids and its influence on cognitive development in infants after intake during pregnancy and breast feeding

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author	Year	n	Author	Year	n	Author	Year	n
	III	Author Helland[U]	Year 2001	n 590	Author	Year	n	Author	Year	n
n = number of allocated/selected participants										

What is the Evidence That the Omega-3 Fatty Acid Content of Maternal Breast Milk, With or Without Known Maternal Intake of Omega-3 Fatty Acids, Influences Cognitive Development in Term or Preterm Human Infants?

One RCT and a single prospective cohort study published between 1997 and 2001 were identified to answer this question.[138,284] Helland et al. also addressed this question, which was described above (see key question: Maternal Intake/Cognitive Development). (Summary Table 44 and 45)

Overview of relevant study characteristics and results

Gibson et al. was a double-blind RCT that investigated the maternal intake effect on breastfed infant's neurological and visual function outcomes in Australia.[138] This study included mothers of term infants (> 37 weeks of GA) who intended to breast feed for at least 12 weeks (n=52, means age: 30 [SD=4] years). These mothers were randomized to receive one of five doses (0, 0.2, 0.4, 0.9, or 1.3 g DHA/day) of a DHA-rich algal oil (DHASCO, Market Biosciences, MD, US) between day 5 and week 12 postpartum. The oil contained 43% DHA, 1% omega-6 FA, 38% saturates and 18% monosaturates. Infants who were exclusively breastfed for 12 weeks were assessed. Infants (n=20) were healthy, with appropriate weight for GA and Apgar scores greater than 7 at 5 minutes.[138] (Summary Table 45)

Infant's visual function using VEP (logMAR) was assessed at 12 and 16 weeks of life, and for global development (Bayley's Scales of Infant development) at 1 and 2 years of age. Blood was drawn for biomarker analysis in infants at 12 weeks of age. Mothers were from middle class families and completed year 12 education. The five groups were compared in terms of maternal age, maternal BMI, GA, infant's gender, birth weight, birth length, birth HC, Apgar score, siblings, maternal social score, smoking, education, home stimulation, and length of breast feeding, at baseline. There was a predominance of boys in the group that received the highest dose of DHA.[138]

Agostoni et al. evaluated the neurodevelopmental indices at 1 year of age in a single prospective cohort of term infants (n=44; 54.5% males) who were exclusively breastfed for at least 3 months in Italy.[284] (Summary Table 45)

The children received breast milk for at least 3 months, after which weaning foods were introduced in all subjects. They underwent clinical examination at 0, 1, 3, 6, 9 and 12 months.

The mother's milk lipid composition was determined at each time-point. The day before, the control pooled milk was collected from all feedings over 24 hours. There was a progressive reduction of the number of breastfed infants to n=29 (at 6 months), n=17 (n=9 months) and n=10 (at 1 year).

Summary Table 45: Omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences cognitive development in term or preterm human infants

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable clinical effects	Internal validity	Applicability
Gibson, 1997, Australia: 12 wk parallel RCT[138]	1.3g/d DHA (n=8)/ 0.2g/d DHA (n=10)	0.9g/d DHA (n=10)/ 0.4g/d DHA (n=12)/ pb (n=12)	S correlation between MDI & DHA in infants's diet & status (RBC & plasma at 12 wks) at 1 y[+] NS at 2 y S correlation MDI & length of BF at 1 y[+] NS at 2 y	Jadad total: 3 [Grade: B]; Schulz: Unclear	II
Agostoni, 2001, Italy: Single prospective cohort[284]	Term breastfed infants at 1 y-old (n=44)	n/a	S correlation between Bayley's MDI & milk total fat content at 6 mo[++], but NS at 12 mo NS AA, DHA milk content correlation with MDI at 12 mo	Quality score: 8 [Grade A]	III

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; N/A = not applicable; grp = group; wk = week(s); mo = month; RBC = red blood cells; PL = phospholipid; MDI = Mental Developmental Index; [+]p<.05 or significant with 95% confidence interval; ++p<.01; +++p<.001; ++++p<.0001; ↑ = increase; ↓ = decrease/reduction; BF = breast feeding

Gibson et al.'s mean Bayley's Mental Developmental Index (MDI) score did not differ between groups at 1 or 2 years of age.[138] Bayley's MDI score at 1 year of age (n=51) was found to correlate with DHA indices in the infant's diet and status, although no association was found at 2 years (n=49). Length of breast feeding was also significantly correlated with MDI at 1 year, but not at 2 years. Length of breast feeding was collinear with indices of social status, education and home stimulation.[138] All these factors were consistent predictors of Bayleys MDI at both ages. Whether the partner smoked was also related to Bayley's MDI at 1 year, but not at 2 years. In a post hoc analysis, it was observed that at 1 year, home stimulation and RBC DHA were the only significant predictors of Bayley's MDI score. By 2 years of age, the model only included gender plus the social score of the oartner as predictors of Bayley's MDI.[138]

The Bayley's MDI at 1 year old was 93.39 (SD=8.1). After correcting for potential confounders such us parity and mother's characteristics (i.e., age, education, smoking habits), breast-feeding for 6 months or longer was not significantly correlated to the mean MDI result compared with subjects who were breastfed for 3 to 6 months (n=15).[284] Associations between MDI and the milk fat content and composition were measured with a multiple regression

analysis. There was a positive correlation between MDI and the milk total fat content at 6 months, but not at 12 months. There was no correlation between the AA and DHA FA content of breast milk and the MDI result at 12 months.[284]

Study quality and applicability. Gibson et al.'s Jadad total quality score was 3, indicating a sound internal validity. However, the allocation concealment was unclear.138 Agostoni et al. had a quality score of 8.284

Summary Matrix 24: Omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences cognitive development in term or preterm human infants

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author	Year	n	Author Gibson[U]	Year 1997	n 52	Author	Year	n
	III	Author Agostoni	Year 2001	n 44	Author	Year	n	Author	Year	n
n = number of allocated/selected participants										

What is the Evidence That the Omega-3 Fatty Acid Content of Infant Formula Influences Cognitive Development in Term or Preterm Human Infants?

What is the Evidence That the Omega-3 Fatty Acid Content of Maternal Breast Milk, With or Without Known Maternal Intake of Omega-3 Fatty Acids, and Together With the Omega-3 Fatty Acid Content of Infant Formula, Influences Cognitive Development in Term or Preterm Human Infants?

Infant Formula Intake - Preterm infants

Six RCTs met the eligibility criteria. They were published between 1992 and 2004. All the studies were summarized in the Growth Pattern Outcomes and Neurological Development Outcomes sections (see key questions: Growth Patterns & Neurological Development-Preterm Infants). (Summary table 46)

Overview of relevant studies

Summary Table 46: Omega-3 fatty acids and its influence on cognitive development in preterm infants

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)			
Carlson, 1992, US: 6 mo parallel RCT[185]	Supplemented formula (marine oil) (n=31)	control formula (n=34)	DHA-supplemented infants had a S ↓ novelty preference vs. control group	Jadad total: 4 [Grade: A]; Schulz: Adequate	II
O'Connor, 2001, US, UK, Chile: 12 mo parallel RCT[207]	DHA+AA (fish/fungal) (n=140)/ Human milk (reference standard) (n=43)	DHA+AA (egg-TG/fish) (n=143)/ Control formula (n=144)	(ITT) NS Bayley's MDI (12 mo) M novelty preference look (Fagan test) AA+DHA (egg-TG/fish) > control & AA+DHA (fish/fungal) (6 mo)[++]	Jadad total: 3 [Grade: B]; Schulz: Unclear	I
van Wezel-Meijler, 2002, The Netherlands: 6 mo, parallel RCT[272]	AA+DHA preterm formula (n=22)	Control formula (n=20)	NS Bayley's MDI (3, 6, 12 & 24 mo)	Jadad total: 5 [Grade: A]; Schulz: Adequate	III
Fewtrell, 2002, UK: 33 d parallel RCT[273]	AA+DHA+EPA preterm formula (n=95)	Control formula (n=100)/ human milk (n=88)	(ITT) NS Bayley's MDI (18 mo)	Jadad total: 5 [Grade: A]; Schulz: Adequate	II
Clandinin, 2002, Canada: 92 wks parallel RCT[193]	DAS (DHA+AA from SCO) (n=72)/ human milk (n=105)	DAF (DHA from fish oils+AA from SCO) (n=90)/ Control formula (n=83)	Bayley's MDI: DAS & DAF formulas had > scores than control formula[+]. HM had > scores than the other groups (118 wks PMA)[+]	Not assessed	X
Fewtrell, 2004, UK: 9 mo parallel RCT[258]	GLA+ DHA formula (n=122)	Control formula (n=116)	(ITT) NS Bayley's MDI (18 mo). Boys in formula > score vs. control[+]	Jadad total: 5 [Grade: A]; Schulz: Adequate	II

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; grp = group; wk = week(s); mo = month; wt = weight; MDI = Mental Developmental Index; [+]p<.05 or significant with 95% confidence interval; [++]p<.01; [+++]p<.001; [++++]p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; SCO = single-cell oil

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. Six parallel RCTs involving preterm infants were identified to address these questions.193,207,258,272,273 Five of them were published in English scientific journals, while one was published as an abstract.193 Carlson et al. was conducted in the United States.185 Both Fewtrell et al.'s studies were conducted in the UK,258,273 van Wezel-Meijler et al.was located in the Netherlands,225,272 Clandinin et al. was conducted in Canada,193 and O'Connor et al. was sited in the US, UK and Chile.207

Two studies involved three arms comparing the use of supplemented and unsupplemented infant formula with the addition of a fourth reference standard group (i.e., human milk).[193,207] Three RCTs compared only two study groups of formula with or without LCPUFA,[185,258,272] while another study also used a group using human milk as a reference standard.[273] Carlson et al. was supported by the National Eye Institute and Ross Laboratories.[185] van Wezel-Meijler et al. and Fewtrell et al.'s 2002 studies were supported by a private source, Numico Research.[272,273] Clandinin et al. was funded by Mead Johnson & Company (pharmaceutical-nutritional company),[193] while Fewtrell et al.'s 2004 study was supported by H.J. Heinz Company (food company).[258] O'Connor et al. did not report the funding source.[207]

Population characteristics. There were 1,486 preterm infants enrolled across the included studies that were randomized to receive the supplemented or control formulas. The sample sizes ranged from 55 to 470 participants. The mean age of the infants at randomization was nonsignificantly different between study groups across the four RCTs. One study did not report the age of their infants.193 The GA of the preterm infants was below 37 weeks across four studies, except for one study that also included VLBW term infants.193 The between-group difference on the GA was not significant across the studies.

In five studies, the proportion of male participants did not differ significantly between randomized groups, although two studies did not mention this information or the between-group difference.[185,193] The range of percentage of males was from 35% to 56%.

Carlson et al. and O'Connor et al. described the racial composition of their participants.[185,207] Carlson et al. included 86.5% of Black infants,[185] while O'Connor included predominatly White subjects.[207] The rest of the studies failed to provide the race and/or ethnicity of their subjects.

Variables like birth weight, proportion of SGA patients, percentage from multiple pregnancies, Apgar score at birth were nonstatistically different between groups in two studies.[185,207] van Wezel-Meijler et al. matched their population by birth weight and proportion of SGA at baseline.[272] Both Fewtrell et al.'s infants were well matched by birth weight and length, proportion of SGA, proportion from multiple pregnancies, and delivered by C-section, at baseline.[258,273]

Four of six studies analyzed the between-group difference of maternal covariates. Carlson et al.'s sample did not differ in maternal age and parental education.[185] O'Connor et al. matched their study groups by maternal age, education, smoking status during pregnancy and in the home, prenatal care, the HOME inventory score and the maternal intelligence measured with WAIS-R Raw vocabulary score.[207] Statistically siginificant differences in the HOME Inventory Score were observed between the following birth weight groups: 1) Infants with < 1,250 g, the control group had a higher score than infants in the AA + DHA (fish/fungal) group; 2) Infants > 1,250 g,

the control group had a higher score than those in the AA + DHA (egg-TG/fish) group; and 3) Infants with a birth weight higher than 1,250 g in the AA + DHA (fish/fungal) group had a higher score than those in the AA + DHA (egg-TG/fish) group.[207]

The inclusion criteria were described in every included study, however the exclusion criteria were not reported in two studies.[193,273]

The studies included mostly healthy preterm infants with a defined weight range, drawn from neonatal intensive care units (NICU). Carlson et al. included VLBW (between 748 g and 1,390 g) preterm infants.[185] O'Connor et al. selected preterm infants (< 33 weeks of GA) with a birth weight range of 750 g to 1,805 g in NICU that could initiate enteral feeding by 28th day of life, including singleton and twin births, as well as SGA subjects.[207] van Wezel-Meijler et al. included premature infants (< 34 weeks of GA), with birth weight of < 1,750 g, normal neurological examination throughout the neonatal period, normal repeated brain ultrasound or showing minor abnormalities such as isolated subependymal haemorrhage and subventricle, with no ventricular dilation, transient periventricular echodensities, without evolution into cysts or any combination of previous findings.[272] Infants in Fewtrell et al.'s 2002 study had a GA below 37 weeks and a birth weight of < 1,750 g, free of congenital malformations known to affect neurodevelopment, whose mothers decided not to breastfeed at 10 days of age.[273] Preterm infants (GA < 35 weeks) in Fewtrell et al.'s study had a birth weight ≤ 2,000 g, and had received at least one of their enteral feeds as formula milk during their hospital stay.[258] Clandinin et al. included VLBW term and preterm infants after their feeding reached 30 mL/kg/day.[193]

Four studies excluded infants with serious congenial abnormalities affecting growth and development, major surgery before randomization, periventricular or intraventricular hemorrhage, maternal incapacity, liquid ventilation asphyxia resulting in severe and permanent neurologic damage, or uncontrolled systemic infection at the time of enrollment.[185,207,258,272]

Three RCT measured the blood content of FAs at baseline.[185,207,272] O'Connor et al. and van Wezel-Meijler et al. found a nonsignificant difference between groups in the plasma or PE or PC fractions of RBC levels of AA and DHA.[207,272] None of the studies measured the FA content of human milk.

Only two studies reported the presence of concurrent conditions in the study population and/or the use of medications.[185,272] Carlson et al.'s preterm infants had VLBW, and some were in mechanical ventilation and IV nutrition at randomization.[185] van Wezel-Meijler et al.'s study reported that 13 patients were excluded from the analyses for the following reasons: necrotizing enterocolitis (n=2, 1 each group), chronic lung disease (n=3, n=2 DHA-AA vs. n=1 control), grade 4 retinopathy of prematurity (n=1, AA + DHA), cystic periventricular leucomalacia (n=1, control), and the duration of artificial ventilation of their patients at baseline. No differences were found between groups.[272] None of the studies included information related to maternal concurrent conditions or medications, which could be relevant to patients taking human milk.

No other prestudy medications or treatments were mentioned in the included studies.

O'Connor et al.'s infants were formula and/or human milk fed before study entry,[207] whereas van Wezel-Meijler et al.'s study used parenteral nutrition with glucose/Vaminolact 6.75%/Intralipid 20% (Kabi-Fresenius, Stockohlm, Sweden) being administered for an average of 12 to 17 days, starting 24 hours after birth. This parenteral nutrition contained negligible

amounts of LCPUFA. Three to 7 days after birth, enteral feeding was introduced using preterm formula (without LCPUFA). Total enteral nutrition was usually achieved within 2 to 3 weeks after birth.[272]

Intervention/exposure characteristics. The intervention groups in each trial received different types of supplemented infant formula, thus each study will be discussed separately.

Carlson et al.'s patients received either a marine oil-supplemented formula with 0.3 g EPA and 0.2 g DHA as preterm formula until discharge (1,800 g), then term formula until 79 weeks of age.[185] The manufactured was Ross Laboratories.

O'Connor et al.'s study randomized its participants to receive one of three study formulas, with or without the addition of LCPUFA and/or human milk: intrahospital preterm formula (modified version of Similac Special Care [SSC]; ready-to-feed by Ross Products Division, Columbus, OH, US) with AA or DHA enriched oils until term CA; and at term CA, postdischarge nutrient-enriched formula (modified version of NeoSure powder) AA and DHA and/or human milk until 12 months of CA.[207] The first group received a supplemented formula with fungal and low-EPA fish oil (DHA/EPA ratio: 3.5/1) providing 0.27 g DHA, 0.08 g EPA and 0.43 g AA (per 100 mL) in the SSC formula and 0.16 g DHA and 0.43 g AA in the NeoSure formula. In the other group, egg- TG and low-EPA fish oil provided 0.24 g DHA and 0.41 g AA in SSC, but 0.15 g DHA in NeoSure. The purveyors of the fish, fungal and egg-TG oils were Mochida International (Japan), Suntory Ltd. (Japan) and Eastman Chemicals Co (US), respectively. The duration of the treatment was until 12 months of CA.[207]

In van Wezel-Meijler et al., the neonates were randomized to receive preterm liquid formula supplemented with (4.4 g/100mL fat) a 2/1 ratio of DHA (0.015 g/100 mL [0.34% fat]) as DHASCO® oil produced by microalgae (Martek Inc., Columbia, US) and AA (0.031 g/100 mL [0.68% fat] as ARASCO® oil produced by fungi (Martek Inc.). The formula was continued from enrollment until a weight of 3,000 g was reached. Subsequently, this group continued with a supplemented term formula (3.5 g/100 mL fat) with a reduced absolute amount of DHA (0.012 g/100 mL; 0.34% fat) and AA (0.025 g/100 mL; 0.70 % fat) until 6 months of CA.[272]

Fewtrell et al. used a LCPUFA-supplemented preterm formula (n=95) (Prematil, Milupan) fat blended with vegetable oils (palm coconut, soya, sunflower) and milk fat, with derivates of LA, and ALA sourced from evening primrose oil (GLA) and egg-lipids (AA 0.31 g; DHA: 017 g; EPA: 0.04 g [per 100mL]). Formula was provided as ready-to-feed form for a mean of 31 days until neonatal unit care discharge.[273]

Clandinin et al. included two interventional groups. The intervention for the first group (DAS) was 17 mg DHA plus 34 mg AA/100 Kcal from single cell oils (SCO) (n=72) as preterm formula (24 Kcal oz), discharge formula (22 Kcal oz) and term formula (20 Kcal oz). The intervention for the second group (DAF) was the same as for DAS but with 17 mg DHA/100 Kcal from fish oil and 34 mg AA/100 Kcal from SCO (n=90).[193]

Fewtrell et al.'s study used a preterm infant formula supplemented with LCPUFA (OsterPrem with LCPUFA) until the infants were discharged from NICU. Afterwards, a nutrient-enriched postdischarge formula was used (Farley's PremCare with LCPUFA). The fat was a blend of vegetable oils (high oleic sunflower oil, palmolein, palm kernel oil, and canola oil). LCPUFA were sourced from borage (starflower) oil (GLA: n-6 0.9g/100 mL) and tuna fish

oil (high DHA/EPA ratio: DHA 0.5 g/100 mL, EPA: 0.1 g/100 mL, AA: 0.04 g/100 mL). Formula was provided in ready-to-feed form during the hospital stay and in powdered form after discharge up to 9 months after term.[258]

The studies compared interventional formulas with identical appearance and smell,[185,258,273] and unsupplemented infant formulas containing the same proportion of monosaturated and saturated FAs, over the same time period as the intervention group.

The studies did not provide information regarding the background diet, when introduced, and the purity data for the omega-3 supplements. No study report included details as to whether, or how, the presence of methylmercury was tested or eliminated from the omega-3 FA exposure.

Cointervention characteristics. Human milk was the reference standard group, either as a separate arm,193,258,273 or as part of the formula groups that did not comply with the intervention.207 O'Connor et al.'s infant preterm and term formulas contained beta-carotene and natural vitamin E.207 Both Fewtrell et al.'s subjects received and identical proportion of minerals and vitamins (A, D, E, and K) in their formulas.258,273

Outcome characteristics. The instruments used to measure the cognitive development in the preterm infants were the Bayley's Scale of Infant Development (MDI),193,207,258,272,273 the Fagan Test of Infant Intelligence (Infantest), novelty preference (a measure of visual recognition memory) by determining the percentage of total looking time spent looking at a novel versus familiar face stimuli during the test phase, mean duration of individual looks (measure of efficiency of information processing),185 and the vocabulary checklist from the infant version of the MacArthur Communicative Development Inventories (a standardized parent-report instrument.207 O'Connor et al.'s average percent of agreement on scoring between site testers and central testers was 91% (range: 71%-100%) for the Bayley's MDI.207

Study quality and applicability. The six RCTs received a mean Jadad total quality score of 4.4, indicating a good internal validity (Summary Matrix 25). Three trials received a score of 5,258,272,273 Carlson et al. received a score of 4,185 and O'Connor received a score of 3.207 O'Connor et al. was unblinded,310 and Carlson et al. failed to report the method of double-blinding.150

Summary Matrix 25: Omega-3 fatty acids and its influence on cognitive development in preterm infants

		Study Quality								
		A			B			C		
Applicability	I	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
					O'Connor[U]	2002	470			
	II	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Carlson[A]	1992	79						
		FewtrellA	2002	283						
		FewtrellA	2004	238						
	III	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Van Wezel-Meijler[A]	2002	55						

n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment; [I] Inadequate

Qualitative synthesis of individual study results

O'Connor et al. did not find a statistical difference in the Bayley's MDI score between groups at 12 months CA.[207] van Wezel-Meijler et al. and both Fewtrell et al.'s studies failed to observe a statistically different MDI score between groups at any follow up.[258,272,273] Clandinin et al.[193] showed that term infants had higher MDI scores than preterm infants (data not shown). Infants in the DAA and DAF formula groups had significantly higher scores than infants in the control formula group, whereas infants in the human milk group had significantly higher scores than infants from the other groups at 118 weeks of postmenstrual age.

Carlson et al. and O'Connor et al. measured the Fagan Test of Infant Intelligence (Infantest) at 6, 9 and 12 months of CA.[185,207] Carlson et al. observed that, during novelty tests, both diet groups had a significant preference for novelty (i.e., longer looking time viewing the novel stimuli).[185] However, at 12 months DHA-supplemented group had a significantly lower novelty preference compared to control group. Diet influenced the number of discrete looks during the novelty test: the DHA group had more total (novel and familiar) discrete looks compared with the control group, as well as a shorter average look duration.[185] O'Connor et al. found that the mean novelty preference look was significantly greater in AA + DHA (egg-TG/fish) formula group than in the control and AA + DHA (fish/fungal) groups at 6 months.[207] Novelty preference has been interpreted as an early measure of information processing capability and it has validity for performance on standardized intelligent tests in childhood.[345] Shorter visual fixation look duration in infancy has also been shown to be related to superior performance in infancy and childhood. Shorter look duration has been interpreted as evidence of more efficient information processing or enhanced ability to disengage from attended stimuli.[345] However, there was a nonstatistically different result between groups in the Infant version of the MacArthur Communicative Development Inventories (a standardized parent-report instrument) at 9 months CA and 14 months CA.[207]

Although the correlation between the FA content in blood and clinical outcomes was not measured, the level of AA and DHA was significantly higher at hospital discharge (mean time: 41 days) in the supplemented groups compared with the control group in the O'Connor et al. study.[207] With the exception of AA levels in RBC PE at 4 and 12 months CA, infants fed the AA + DHA supplemented formulas had higher levels of AA and DHA in plasma and RBC PL than those infants fed the control formulas. Infants fed AA + DHA (fish/fungal) but not AA + DHA (egg-TG/fish), had higher levels of AA in RBC PE than infants fed the control formulas ($p < 0.02$).

van Wezel-Meijler et al. did not find a statistically difference in AA levels in RBC between groups at 2 to 3 weeks.[272] DHA levels were significantly lower in the control group compared with the group receiving supplemented formula.[272]

O'Connor et al.'s had 94 withdrawals (80%) at 12 months of CA. There were nonstatistically significant differences between groups. The main reason of the withdrawals was symptoms related to feeding intolerance. During the study, the following infant deaths were reported: six infants from the control group, three infants from the AA + DHA (fish/fungal) group, and six infants from the AA + DHA (egg-TG/fish) group; none of the infants from the human milk groups died. No infants deaths were related to study feedings.[207]

There were 13 dropouts in van Wezel-Meijler et al.'s study.[272] The reasons were: necrotizing enterocolitis (NEC), chronic lung disease, grade 4 retinopathy of prematurity, cystic periventricular leucomalacia, change from formula feeding to mother's expressed milk and home to hospital distance. There were no losses to follow up.[272]

In the first Fewtrell et al.'s study,[273] six patients randomized to the control formula withdrew from the trial before 3 weeks for the following reasons: early discharge (< 3 weeks of age) (n=3); NEC (n=1); intolerance of feeds (n=1); and breastfed (n=1). Fourteen infants withdrew from the supplemented formula group for the following reasons: early discharge (n=2); NEC (n=5); maternal concern (n=2); and death (n=2). There were 14 infants lost to follow up at 9 months in the control group, one was lost to follow up in the supplemented formula group, and three were lost to follow up in the human milk groups. There were two deaths in the supplemented formula group and three were lost to follow up in the human milk groups.[273] Clandinin et al. failed to report the dropouts.[193] In Fewtrell et al.'s study, reasons for dropout in the control group included: abdominal distention (n=1), death due to bronchopulmonary dysplasia at 25 days of age (n=1), and lost to follow up(n=21). In the supplemented formula group, reasons for dropout included: NEC (n=1), and lost to follow up at 18 months (n=15).[258]

Quantitative synthesis

Only five studies measured the Bayley's MDI. This outcome was chosen to evaluate the possibility of meta-analysis. Yet, outcome results were only available for more than one study at two follow-up times: CA 12 months and 18 months. At CA 12 months, outcomes were available for two studies.[207,272] In van Wezel-Meijler et al.,[272] the experimental group received supplemented formula from the first enteral feeding time until 6 months CA. In O'Connor et al.,[207] however, supplemented formula was used until 12 months CA. We would have combined data at 6 months follow-up, but it was not available in O'Connor et al.[207] Thus, meta-analysis was not possible for this outcome.

Impact of covariates and confounders

Carlson et al. adjusted (ANOVA) the novelty test results in both groups for diet and study age and failed to find an change in the results; however, at 12 months, the DHA-supplemented group had a significantly lower novelty prederence compared with the control group.[185]

In an intention-to-treat analysis using ANCOVA and taking into consideration covariates like site, gender, birth-weight stratum, feeding per gender, feeding per birth-weight stratum, HOME, maternal WAIS-R raw vocabulary score, GA, human milk intake, birth order, and the first language of the biological mother, O'Connor et al. did not find a statistical difference between groups at 12 months CA in the Bayley's MDI score.[207]

The second Fewtrell et al. study, in a subgroup analysis, observed that the boys in the supplemented formula group had a significantly higher score than those in the control group at 18 months, and there was a significant interaction between diet and sex on the MDI score. These differences were maintained after adjusting for effect modifiers, such as maternal education and social class.[258]

The power calculation and the intention-to-treat analysis approach was reported in three trials.[310,321,322]

Infant Formula Intake - Term Infants

Eight unique studies published between 1997 and 2002 were identified that addressed this set of questions. All the trials were summarized in the Growth Pattern Outcomes section (see key question: Growth Patterns-Term Infant Formula Intake). (Summary Table 47)

Overview of relevant studies

Summary Table 47: Omega-3 fatty acids as supplemental treatment for cognitive development in term infants

Author, Year, Location: Length & Design	Study groups[1]		Notable clinical effects	Notable clinical-biomarker correlations	Internal validity	Applicability
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)				
Auestad, 1997, US: 4 mo parallel RCT[104]	AA+DHA formula (n=46)/ HM (n=63)	DHA formula (n=43)/ Control formula (n=45)	NS Bayley's MDI between grps at 12 mo	n/a	Jadad total: 3 [Grade: B]; Schulz: Unclear	I
Birch, 1998, US: 17 wk parallel RCT[182]	DHA+AA formula (n=27)	DHA formula (n=26)/ Control formula (n=26)	MDI S better in n-3 formulas vs. control at 18 mo	MDI score at 18 mo correlated (+) with plasma & RBC DHA at 4 mo RBC-LA & ALA correlated (-) with MDI at 18 mo	Jadad total: 5 [Grade: A]; Schulz: Unclear	I
Willatts, 1998, UK: 4 mo parallel RCT[223]	DHA + AA formula (n=20)	Control formula (n=20)	NS problem-solving scores, intention score & number of solutions at 3 mo	n/a	Jadad total: 3 [Grade: B]; Schulz: Unclear	II
Lucas, 1999, UK: 6 mo, parallel RCT[265]	LCPUFA formula (n=154)	Control formula (n=155)/ HM (n=138)	NS Bayley's MDI between grps at 18 mo (ITT)	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	II
Makrides, 1999, Australia: 1 y parallel RCT[205]	DHA+ AA formula (n=24)/ HM (n=46)	DHA formula (n=23)/ pb (n=21)	NS Bayley's MDI between groups at 1 or 2 y	NS FA variables correlated MDI scores at 1 or 2 y	Jadad total: 5 [Grade: A]; Schulz: Adequate	III
Auestad, 2001a, US: 1 y, parallel RCT[227]	DHA+ AA (egg-TG) formula (n=80)	DHA+ AA (fish/fungal) formula (n=82)/ control formula (n=77)	NS Bayley's MDI between groups at 6 &12 mo	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	I

Auestad, 2001b, US: 1 y, parallel RCT[227]	DHA + AA formula/ HM (n=83)	Control formula/ HM (n=82)	NS Bayley's MDI between grps at 6 & 12 mo	n/a	Jadad total: 5 [Grade: A]; Schulz: Adequate	I
Jensen, 2002, US: 120 d parallel RCT[203]	↑↑ ALA formula (n=20)/ ↓↓ ALA formula (n=20)	↑ ALA formula (n=20)/ ↓ ALA formula (n=20)	NS Bayley's MDI between grps at 12 mo	NS correlations, CAT/CLAMS DQ & plasma or RBC PL n-3 or n-6 at 120 d CLAMS DQ correlated (+) with RBC PL EPA, not with plasma or RBC PL DHA CAT DQ correlated + with plasma PL LA (n-6) at 120 d	Jadad total: 2 [Grade: C]; Schulz: Unclear	II

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; [2]biomarker source; [3]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acidLength = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; n/a = not applicable; grp = group; wk = week(s); mo = month; wt = weight; RBC = red blood cells; PL = phospholipid; MDI = Mental Developmental Index; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; ITT = intention-to-treat analysis; PP = per-protocol analysis (e.g., completers); ↑ = increase; ↓ = decrease/reduction; HM = human milk; CAT/CLAMS = Clinical Adaptative Test/Clinical Linguistic and Auditory Milestone Scale; DQ = developmental quotien

Qualitative synthesis of relevant studies' key characteristics

Study characteristics. Eight parallel RCTs involving term infants were identified. All of them were published in English scientific journals. Four studies were conducted in the United States,[104,203,227] whereas, two studies were located in the United Kingdom[223,265] and one in Australia.[205]

Four studies involved two arms which compared formulas with or without LCPUFA,[223,227,265] however, Lucas et al. also had a reference standard group (i.e., human milk).[265] Three studies randomized their patients to three study groups, comparing the use of two different LCPUFA supplemented formulas with a standard formula,[104,205,227] yet two of them also included a breastfed group as a reference standard.[104,205] Finally, patients in Jensen et al.'s study received four different formulas with increasing amounts of ALA (omega-3), and decreasing amounts of LA (omega-6) and omega-6/omega-3 ratios.[203]

Jensen et al. was supported by the U.S. Department of Agriculture, Agricultural Research Service, Mead-Johnson Nutritional Group, the Foundation Fighting Blindness, Research to Prevent Blindness, Inc. and the Retina Research Foundation.[203] Auestad et al. (1997 and 2001ab) were funded by Ross Products Division, Abbot Laboratories,[104,227] whereas, Lucas et al.[265]and Makrides et al.[205] were financially supported by Nestec Ltd., Switzerland. Willatts et al. was supported by Milupa Ltd., UK.[223]

Population characteristics. There were 1,470 term infants enrolled across the included studies of infants randomized to receive LCPUFA supplemented formula or control formula.

The sample sizes ranged from 40 to 447 participants. Most studies performed the randomization since birth or from the time when the infant could tolerate enteral feeding (mean time= 7 days of life). The mean age at randomization was only reported in two studies.[104,203] Three studies reported that the mean age of their participants was nonsignificantly different between groups, either at baseline or at the time of the assessment.[104,203,223] Four studies did not provide this information.[205,227,265] Six studies reported that the between-group difference in terms of gender or percentage of males was nonsignificant.[104,205,223,227,265] Jensen et al. did not provide the difference between study arms.[203]

Makrides et al. only selected White participants.[205] Jensen et al.'s racial composition was: Black (62%), Hispanic (28.5%), and White (9.5%) at 120 days, yet statistical differences among groups was not reported.[203] Auestad et al. 1997's subjects were predominantly White among the groups, but this group was significantly larger in the nonrandomized breastfed group compared with the formula groups.[104] Auestad et al. 2001ab's studies included about 80% of European American infants, but the study groups did not differ significantly.[227] Two studies failed to provide the racial and/or ethnical composition of their participants.[223,265]

Birth weight, GA, length and HC at birth, birth order, triceps skinfold thickness at birth, and Apgar score at 5 minutes were measured in most studies. The GA did not differ between groups in the four studies,[104,227,265] however, in Willatts et al.'s study, infants in the LCPUFA formula group had a significantly longer GA than infants in the control group.[223] Seven studies did not find a statistical difference between groups for birth weight.[104,203,205,223,227,265] None of the studies provided information regarding the maternal clinical history and/or medications that could have some influence on the FA composition of the breast milk.

The inclusion and exclusion criteria were reported in five studies.[104,205,227,265] The exclusion criteria were not reported in two studies.[203,223]

The studies included healthy term infants (at least 37 weeks of GA) with appropriate weight for the GA (2,500 g - 4,000 g). Two studies also included babies whose Apgar score was > 7 (at 5 minutes).[227] To receive formula, their mothers had to decide to not breastfeed and viceversa. The patients were excluded if they had congenital abnormalities,[104,205,227,265] Apgar score < 7[104], significant illness,[104,227] IV lipid infusion, blood transfusion,[104] and maternal medical history known to have proven adverse events on the fetus.[227]

The maternal socioeconomic status was not reported in one trial.[203] Seven studies did not observe a statistically different status between group, in terms of maternal education, marital status, housing, and family size. Only Makrides et al.'s breastfed infants (reference standard group) had parents who were less likely to smoke, had attained a higher level of education, and had more prestigious social scores compared with formula-fed infants.[205]

None of the studies reported the use of medications and/or treatments as well as concurrent conditions, at baseline, in the eligible infants or their mothers. The smoking status during pregnancy and at birth (in household) was significantly higher in mothers in the AA + DHA formula group compared with the other groups in Auestad et al. 1997's study.[104] In Makrides et al.'s study, the proportion of smokers in the DHA formula group was higher than in the other groups.[205] Both of Auestad et al. 2001's studies[227] did not reveal a significant difference between groups for maternal smoking status.[227]

The prestudy diet characteristics in the mothers were not reported. Two studies mentioned that their infants received standard formula since birth until enrollment, yet not description was made.[227,265] One study mentioned that their infants were breastfed since birth and during the whole study.[227]

None of the studies measured the biomarkers status in either plasma or RBC PL at baseline, in infants or their mothers.

Intervention/exposure characteristics. The intervention with formula was heterogeneous across the included studies, thus the description will be done separately for each trial.

Auestad et al. 1997 randomized their patients to receive two different liquid ready-to-feed formulas supplemented with LCPUFA. One of them contained AA (0.43 wt% total FAs) and DHA (0.12 wt%) from egg yolk PL (AA + DHA formula). T he second (DHA formula) provided DHA (0.2 wt%) from high DHA, low EPA fish (tuna) oil with a ration of DHA/EPA of ~4:1. The formulas contained the same amount of protein, carbohydrate, fat and energy (670-694 kcal) per liter. The oil blend consisted of high oleic safflower, coconut, and soy oils with or without PL or TG sources of LPUFA. The control formula contained the same amount of nutrients, but without the addition of DHA, EPA or AA. These formulas were provided as the sole source of nutrition for a minimum of 4 months.[104] In the reference standard group, the human milk contained similar amounts of AA and DHA than the supplemented formulas.

The infants were exclusively breastfed for at least 3 months, after which supplementation with commercial formula SW1 was permitted.[104]

Birch et al. compared the use of three different infant formulas: Enfamil with iron; Enfamil with iron supplemented with 0.35% DHA (of total FA); or Enfamil with iron supplemented with 0.36% DHA and 0.72% AA.[182] All formulas provided LA and ALA. The source of the PUFA was single cell oils (DHASCO® and ARASCO®, Martek Biosciences, Columbia, US). All formulas were provided in ready-to-feed cans. The duration of intervention was from a mean of 2.1 days of life until 17 weeks (4 months).[182]

Willatts et al. compared the use of LCPUFA supplemented formula with an unsupplemented formula. The standard formula was the Aptamil brand without DHA and AA. The supplemented formula was ready-to-feed Aptamil/Milupan manufactured by Milupa Ltd., Trowbridge, UK). The fat blend was derived from milk fat, vegetable oils, and egg lipids. While the omega-3 content was 0.15 g to 0.25 g/100 mL of DHA and 0.60 g to 0.65 g/100 mL of ALA, the omega-6 content was 11.5 g to 12.8 g/100 mL of LA and 0.30 g to 0.40g of AA. The intervention length was until 4 months of age. The total amount of formula intake during the trial did not differ between groups.[223]

Lucas et al. compared the use of a supplemented formula (Nestec Ltd, Vevey, Switzerland) that contained 0.30% AA and 0.32% DHA from purified egg PL and TG fractions (Lipid Teknic, Norway), with an identical unsupplemented formula.[265] The duration of the intervention was until the age of 6 months.[265] The reference standard group (n=138) received only breast milk for at least 6 weeks.[265]

In the Makrides et al. study, the LCPUFA supplemented formula (provided by Nestec Ltd., Konolfingen, Switzerland) contained 0.35% DHA as total FAs from tuna oil in one formula, and 0.34% DHA and 0.34% AA from an egg PL fraction in the second formula.[205] The control

formula did not contain LCPUFA, yet the protein, fat and carbohydrate composition of all the formulas was identical, as well as the packaging.[205] The reference standard group's breast milk contained (n=33) 0.9 % EPA, 0.20 % DHA and 0.39% AA.[205]

Auestad et al. 2001's reported trials had different intervention characteristics. The Auestad et al. 2001a compared the use of three formulas, two of them were supplemented with DHA+AA, one derived from fish oil and fungal oil, and the other derived from egg- TG.[227] All were liquid ready-to-use formulas with similar amount of protein, carbohydrate, fat and calories. The fat blend consisted of high-oleic safflower, coconut, and soy oils. They were indistinguishable in appearance and odor. All contained ALA and LA. The DHA+AA (fish/fungal) formula contained (per 100 mL) 0.46g AA, <0.04g EPA and 0.13g DHA, while the DHA+AA (egg-TG) contained (per 100 mL) 0.45g AA and 0.14g DHA. The duration of the intervention was from less than 9 days of life to 12 months of age. The formulas were exclusively administered during the first 4 months, then as sole milk beverage up to 12 months.[227] In Auestad et al. 2001b,[227] breast feeding was supplemented with a DHA+AA (human milk/egg-TG) formula, containing the same amount of DHA and AA described above in one group and a control formula and human milk as the comparator (human milk/control). The breast milk contained (per 100 mL) 0.51g AA, 0.05g EPA and 0.12g DHA. The duration of the breast feeding was exclusively until 3 months, after which only the formula was administered as the milk source.[227]

Jensen et al. compared the use of four formulas with different content of LA (omega-6) and ALA (omega-3). The content of ALA and LA in each formula (from lowest to highest content of omega-3) was 0.4%, 0.95%, 1.7% and 3.2% of total FAs, for ALA, and 17.6%, 17.3%, 16.5% and 15.6% of total FAs, for LA, respectively. The PUFA's were abstracted from canola, safflower, high oleic sunflower and coconut oil. The amount of protein, total fat, energy, carbohydrate, vitamin and minerals were similar to those of Enfamil brand. The formulas were manufactured by Mead Johnson Nutritionals (Evansville, Ind.). The duration of the intervention was from day 1 of life to 120 days of life.[203]

Cointervention characteristics. The background diet during the study period was not reported in two studies.[205,223] In Jensen et al.'s study, infants were exclusively formula-fed for 120 days, after which the diet intake was neither controlled nor monitored.[203] In Auestad et al.1997's study, supplementation with solid foods was permitted for all infants since 4 months of age.[104] The mean age of the first introduction of any solid food did not differ between groups in Lucas et al's study.265 In both of Auestad et al. 2001's studies, infants were allowed to drink water and solid foods after 4 months of age.[227]

Regarding the cointervention characteristics, three studies failed to provide this information.[205,223,227] Jensen et al. did not allow any medication during the study.[203] Auestad et al. 1997 only stated that there was a nonstatistically significant difference between groups in terms of cointerventions, yet did not provide details.[104] Lucas et al.'s LCPUFA group was prescribed more antibiotics (OR 1.3) and had more visits from a medical practitioner (OR 1.8) during the study period, but the differences were not significant compared with the control group.[265] Finally, since the infants in Auestad et al. 2001b[227] received breast milk besides the interventional formula, the former would be considered the cointervention.[227]

Outcome characteristics. Seven studies used the Bayley's MDI scale.[104,182,203,205,227,265] Three studies utilized the MacArthur Communicative Development Inventories (a standardized parent-report instrument that evaluates the early word production, language comprehension, and gesture communication).[104,227]

Jensen et al. also used the DQ for language development (CLAMS DQ), visual problem solving ability (CAT DQ) and overall cognition (mean of CLAMS and CAT DQ).[203] To assess the cognitive and language development at 39 months, the Stanford-Binet Intelligence Scale Form L-M, the Peabody Picture Vocabulary Test Revised (PPVT-R), and the Beery Visual-Motor Index test were used after standardization procedures in the Auestad et al. 1997.[104] Willatts et al.[223] used a problem-solving assessment in two steps, at 3 and 9 months of age. Lucas et al. also utilized the Knobloch, Passamanik, and Sherrards Developmental Screening Inventory at 9 months (as DQ).[265] Both of Auestad et al.2001's studies also assessed the cognitive development with the Fagan test of Infant Intelligence (Infantest) at 6 and 9 months.[227]

Study quality and applicability. The eight RCTs received a mean Jadad total quality score of 4.1, indicating a good internal validity (Summary Matrix 26). Five trials received a score of 5,[124,205,227,265] Auestad et al. 1997 and Willatts et al. received a score of 3,[104,223] and one report received a score of 2.[203] Jensen et al. failed to report the method of randomization,[325] and three trials were unblinded.[325,327,333]

Summary Matrix 26: Omega-3 fatty acids as supplemental treatment for cognitive development in term infants

		Study Quality								
		A			B			C		
Applicability	I	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Birch[U]	1998	79	Auestad[U]	1997	274			
		Auestad[A]	2001a	239						
		Auestad[A]	2001b	165						
	II	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Lucas[A]	1999	447	Willatts[U]	1998	40	Jensen[U]	1997	80
	III	**Author**	**Year**	**n**	**Author**	**Year**	**n**	**Author**	**Year**	**n**
		Makrides[A]	1999	146						

n = number of allocated/selected participants; RCT = [A]Adequate vs [U]Unclear allocation concealment

Qualitative synthesis of individual study results

The Bayley's MDI scale was assessed in seven of eight studies. None of these studies but one observed a between-group significant difference at any follow up point.[104,203,205,227,265] Birch et al. found that the group supplemented with omega-3 FA for 4 months had a significantly higher score compared with the control group at 18 months of age.[182] Jensen et al. recognized that the groups were too small to detect an among-group difference in the neurodevelopmental indices.[203] The purpose of this study was to determine the correlation between the plasma and/or the RBC PL content of any omega-3 FAs, total omega-3 and/omega-6 FAs at 120 days, and neurodevelopmental indices at 1 year of age.[203]

In Makrides et al.'s study, there was no difference when the scores were compared between the human milk with the formula groups at 1 year. There was a significant decrease in MDI scores of formula-fed infants between 1 and 2 years of age that was independent of the diet.[205]

In relation to the Knobloch, Passamanik, and Sherrards Developmental Screening Inventory, Lucas et al. did not reveal a significant difference between study groups at 9 months, including the comparison with the reference standard group.[265]

Only Auestad et al. 2001 evaluated the Fagan Test of Infant Intelligence (Infantest) in both study populations, failing to detect a significant difference between groups at 6 and 9 months.[227] The Infant version of the MacArthur Communicative Development Inventories (a standardized parent-report instrument) was measured in three studies.[104,227] At 14 months, two statistically significant differences were found in the components of this test in Auestad et al. 1997.[104] Vocabulary comprehension was significantly lower in the DHA group than in the human milk group.[104] Vocabulary production in the DHA group was marginally lower than that in the control formula group ($p=0.052$). The DHA + AA group did not differ from the human milk group. When the comparison is made only among the three formula groups, there was a significantly lower Vocabulary Production Score in the DHA group compared with the control group.[104] Both of Auestad et al. 2001's studies had a nonsignificantly different result between groups at 9 months. However, at 14 months, infants fed the DHA + AA (fish/fungal) formula had a slightly significantly higher vocabulary expression score than those fed the DHA + AA (egg-TG) formula.[227]

Auestad et al. 1997 did not find a significant difference between groups for the IQ (Stanford-Binet), Receptive Vocabulary (PPVT-R), Expressive Vocabulary and Visual-Motor Index Score.[104]

Regarding the problem-solving scores, Willatts et al. observed a nonsignificant difference between groups in the intention score and number of solutions at 3 months.[223]

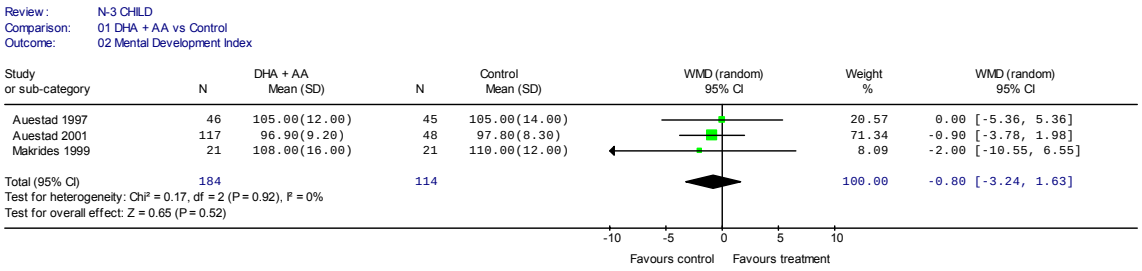
Jensen et al. did not find a correlation between the blood content of omega-3 and/or omega-6 FAs and the Bayley's MDI score at 1 year.[203] There were no statistical correlations, in a multiple regression analysis, between CAT/CLAMS DQ and the plasma or RBC PL content of any omega-3 or omega-6 LCPUFA at 120 days of age. CLAMS DQ (and index of the language development) correlated positively with the RBC PL content of EPA, but not with the plasma or RBC PL content of DHA. The CAT DQ (an index of visual problem solving ability) correlated positively with the plasma PL content of LA (omega-6) at 120 days. Finally, Makrides et al.'s regression analysis found that no FA variables significantly predicted MDI scores at either 1 or 2 years.[205]

Birch et al. found that the MDI score at 18 months was positively correlated with plasma and RBC DHA at 4 months of age. None of the other plasma biomarkers (LA, AA, ALA, EPA) were correlated with the MDI at 18 months, although the RBC-LA and RBC ALA were negatively correlated with the MDI at 18 months of age.[182] None of the biomarkers measured at 12 months of age were correlated with the MDI at 18 months of age.[182]

Quantitative synthesis

The outcome assessed was Bayley's MDI at age 4 and 12 months given that at these ages, the diet was exclusively formula (4 months) or a 12-month followup. At 4 months of age, the outcomes were not available in any of the studies. At age 12 months, outcomes were noted in three studies that were using the same comparators, i.e., DHA+AA versus unsupplemented formula.[104,205,227]

Meta-analysis was performed using the random effects weighted mean difference (WMD).



The WMD for the Bayley's MDI score at 12 months of age in three studies (DHA+AA vs. control) was nonstatistically significant (WMD: -0.80, CI 95%: -3.24; 1.63).[104,205,227]

Impact of covariates and confounders

The effect modifiers that could be influencing the results were controlled in all the studies. Variables like GA, gender, birth weight, length at birth, maternal age, and socioeconomic status were detected in most of the studies. Jensen et al.'s groups were comparable in terms of the study formula's intake.[203] The CAT DQ (an index of visual problem solving ability) correlated positively with weight at 120 days of age.[203]

Auestad et al. 1997 observed that female sex was positively associated with IQ, receptive vocabulary, and visual-motor ability at 39 months.[104] Maternal education was positively associated with IQ and receptive vocabulary, when either all four feeding groups or only the formula groups were included in the regression model. The variable selection model identified which of 22 potentially influential variables contributed significantly to the variance for IQ and expressive language. Approximately one third of the variance for IQ was explained by four factors: sex, years of maternal education, number of siblings, and exposure to cigarette smoke. Positive associations were found for female sex and maternal education, and negative associations were found for the other two variables previously described. Expressive language was positively associated with maternal education, but negatively associated with average hours in childcare per week and hospitalizations since birth, but only when the breastfed group was included in the analysis.[104] At 14 months, there was a significant association between vocabulary production and comprehension. At 39 months, there was a significant association between receptive (PPVT-R) and expressive (MLU) language and between expressive language

(MLU) and IQ. However, no significant associations between vocabulary production at 14 months and expressive language (MLU) at 39 months were found.[104]

In the early peak-fixation infants, none of the covariables was significantly related to number of intentional solutions in Willatts et al.[223] In the late peak-fixation infants, only diet and birth weight were significantly related to the number of intentional solutions. ANCOVA on the intention scores for the effects of diet and peak fixation showed no significant main effects, and diet per peak interaction was not significant.[223] Regarding the problem-solving scores, Willatts et al. observed a nonsignificant difference between groups in the intention score and number of solutions at 3 months.[223] When adjusted by GA, the differences were still nonsignificant. ANCOVA on number of intentional solutions for the effects of diet and peak fixation, covariated with GA and birth weight, showed a significant diet per peak fixation interaction. Simple-effects analysis showed that the number of intentional solutions did not differ significantly between the early-peak fixation infants receiving LCPUFA. In contrast, the number of intentional solutions was significantly reduced in the late peak-fixation infants receiving the standard formula.[223]

In Lucas et al.'s study, the results did not change after adjusting by center or observer (see above).[265] A multiple linear regression with adjustement for possible confounding factors and imbalance at baseline was made between the formula groups and the human milk reference standard group.[265] It did not observe a significant difference between formula groups and breastfed infants, even after adjusting by effect modifiers (sex, center, maternal age, maternal education, maternal marital status, and social class).[265] In relation to the Knobloch, Passamanik, and Sherrards Developmental Screening Inventory, Lucas et al. did not reveal a significant difference between study groups at 9 months, including the comparison with the reference standard group, which was maintained after adjusting by effect modifiers.[265]

Makrides et al. found that the feeding mode was the only nutritional variable to predict MDI with formula feeding resulting in lower MDI scores.[205] Although environmental variables such as parental education, occupational prestige, and Home Screening Questionnaire scores were associated with Bayley's MDI at 1 and 2 years of age, only weight (at 1 year) and birth order, feeding mode, and gender (at 2 years) significantly predicted MDI.[205] At 2 years the MDI scores of breastfed infants were higher than those of the formula-fed group, even after adjusting for the significant covariates of gender and number of siblings (95% CI: 4.4-21.7).[205]

The power calculation was reported in seven trials,[124,132,151,325,329,333] while the intention-to-treat analysis approach was reported in only one study.[132]

Cognitive Development Outcomes in Light of Biomarker Data

What is the Evidence that Term or Preterm Human Infants' Cognitive Development is Associated With the Omega-3 or Omega-6/Omega-3 Fatty Acid Content of Child Biomarkers?

Six studies were identified to answer this question. Two were RCTs and were described above (see key questions: Growth Patterns-Term Infant Formula Intake, and Maternal

Intake/Visual Function).[138,182,203,205] Innis et al. and Ghys et al. were prospective single cohort studies published between 2001 and 2002.[271,285] (Summary Tables 45, 47 and 48)

Overview of relevant study characteristics and results

Innis et al. selected a cohort of 83 Canadian term infants who were exclusively breastfed, with birth weights in the range of 2,500 g to 4,500 g.[271] The objective of the study was to measure the infant RBC DHA content and its association with the visual, neuro or cognitive development.[271]

Ghys et al. evaluated the association between the AA and DHA status at birth and the cognitive development at 4 years of age in a full-term infant cohort.[285]

Innis et al. was funded by the Medical Research Council (MRC) of Canada and Ross Laboratories, OH.[271] Ghys et al. failed to report the funding source.[285]

Innis et al. enrolled infant (n=83) with less than 2 weeks of age and to be eligible, their mothers were required to intend to breastfeed their infant without providing infant formula or cow's milk for at least 3 months, and without introducing solid foods for at least the first 4 months after birth. The infants were excluded if their mothers had substance abuse, metabolic or physiologic problems, communicable diseases, and infants with evidence of metabolic or physical abnormality.[271]

Ghys et al. included full-term newborns (n=246) from healthy Caucasian women born between 1994 and 1995. A total of 128 (mean age 47 [SD=1.3] months, 55% males) infants were assessed for cognitive development outcomes at 4 years of age.[285]

Only one mother was taking FA supplements with LA and DHA in Innis et al. The maternal diet was not reported or controlled. Only five mothers were smokers during the study.[271]

In Ghys et al.'s study, 84% of infants were first born and none had suffered any neurologically damaging disorder or event, and 5% of the families lived on social security.[285]

Innis et al. used the Bayley's MDI at 6 and 12 months to assess the cognitive development and its correlation with the RBC DHA and AA content in infants.[271] Another test used for this outcome, was Novelty preference with the Fagan Test of Infant Intelligence (Infantest) at 6 and 9 month of age.[271] Ghys et al. used the Dutch version of the Kaufman Assessement Battery for Children (K-ABC), and the Groningen Developmental Scale (GOS) for children between 2.5 and 4.5 years of age.[285]

Multiple linear regression analysis was used to determine the impact of the FA variables on the outcomes in both studies.[271,285] In Innis et al.'s study, the analysis controlled statistically for the duration of breast feeding, maternal education, family income, gender, maternal smoking, birth order and birth weight, length and HC.[271]

The covariables used in Ghys et al. were birth weight, breast feeding, maternal intelligence (IQ) and parental educational attainment, which are associated with cognitive development in infants.[285]

Summary Table 48: Association of cognitive development outcomes and biomarkers content in infants (observational study)

Author, Year, Location: Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Notable associations[2,3]	Internal validity	Applicability
Innis, 2001, Canada: Prospective single cohort[271]	Term breastfed infants (n=83)	n/a	No correlation between RBC DHA & AA status & Bayley's MDI (6,12 mo), novelty preference (6,9 mo)	Quality score: 8 [Grade A]	III
Ghys, 2002, the Netherlands: Prospective single cohort[285]	Term infants (n=128)	n/a	No correlation between plasma or RBC DHA & AA & cognitive development (4 y)	Quality score: 8 [Grade A]	III

[1]biomarkers = EPA, DHA, AA, AA/EPA, AA/DHA, AA/EPA+DHA; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; n = sample size; pts = study participants; NR = not reported; S = statistically significant difference; NS = nonsignificant statistical difference; N/A = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; RBC = red blood cells; MDI = Mental developmental scale; y = year(s)

Innis et al. did not find a statistically significant relation between the infant DHA or AA status (RBC) at 2 months of age and the Bayley's MDI score at 6 and 12 months of age, as well as the Novelty Preference at 6 and 9 months.[271]

In a bivariate analysis, Ghys et al. did not observe a correlation between the DHA and AA concentration in infant's plasma or RBC and the cognitive development at 4 years of age. Small but significant associations occurred with maternal IQ, birth weight, duration of breast feeding, maternal smoking during pregnancy, and paternal educational attainment.

Study quality and applicability. Both studies had a mean total quality score of 8 and a level of applicability of III.

Summary Matrix 27: Association of cognitive development outcomes and biomarkers content in infants

		Study Quality								
		A			B			C		
Applicability	I	Author	Year	n	Author	Year	n	Author	Year	n
	II	Author	Year	n	Author	Year	n	Author	Year	n
	III	Author	Year	n	Author	Year	n	Author	Year	n
		Innis	2001	83						
		Ghys	2002	128						

n = number of allocated/selected participants

Safety Issues

What is the Evidence for the Risk, in Pregnant Women, of Short and Long-Term Adverse Events Related to Their Intake of Omega-3 Fatty Acids?

What is the Evidence for the Risk, in Breast Feeding Women, of Short and Long-Term Adverse Events Related to Their Intake of Omega-3 Fatty Acids?

All nine unique relevant trials,[196,230,232,233] that were reviewed, reported some information on safety and/or adverse events (e.g., complications, intolerance) (See Summary Tables in Appendix E*). In one report, Olsen et al.[230] presented pregnancy-related adverse events/outcomes aggregated across six unique trials, four of which were preventive and two of which were therapeutic.[230] In seven of the nine trials, the experimental intervention consisted of LCPUFA enriched (fish oil) capsules.[230,233] The remaining two trials studied LCPUFA-enriched eggs[232] or margarine.[196] Control intervention in the nine trials consisted of the capsules, eggs, and margarine without the LCPUFA-supplementation, respectively.

In seven trials,[230,233] women in the experimental arms reported belching and unpleasant taste more often than those in the control arms. Two of eight studies reported the occurrence of nausea,[196,230] finding similar between-arm rates of nausea as opposed to another trial,[233] which showed that women in the LCPUFA supplementation arm experienced nausea more frequently than those allocated to the regimen of standard intervention (9.7% vs 2.9%). Note that the daily dose of EPA/DHA intake in this trial[233] was greater than that in other trials.[196,230]

In the trial by Onwude et al.,[233] the proportion of women who had had stomach pain was higher in the experimental arm compared with the control arm (4.8% vs. 0%). The aggregated results of six trials[230] showed the rates of stillbirths, stay at hospital after delivery, vaginal bleeding, macrosomia, anaemia, vomiting, constipation, diarrhoea, and nose bleeding were similar between the experimental and control arms. In their trial, Smuts et al.[232]observed fewer adverse events for the omega-3 supplemented than for the control arm (birth of infant with LBW: 0% vs. 26%, preterm delivery: 5.6% vs. 26%, C-section: 11% vs 32%, gestational diabetes: 0% vs. 16%). De Groot et al.[196] observed similar rates of long-term hospitalization, diabetes mellitus, still birth, and postpartum depression in the two randomized groups. In this trial, six women were withdrawn/lost to follow up for the following reasons: morning sickness (n=2), long-term hospitalization (n=2), diabetes mellitus (n=1), and stillbirth (n=1). Of the nine trials, only Smuts et al.'s[232] explicitly reported their opinion on the underlying reasons (breech, preterm delivery, maternal gestational diabetes and chorioamnionitis) for the observed adverse events (admission to intensive care unit).

* Appendices and Evidence tables cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/o3mchtp.htm

What is the Evidence for the Risk, in Term or Preterm Human Infants, of Short and Long-Term Adverse Events Related to Maternal Intake of Omega-3 Fatty Acids During Pregnancy?

What is the Evidence for the Risk, in Term or Preterm Human Infants, of Short and Long-Term Adverse Events Related to Their Intake of Omega-3 Fatty Acids After Birth (e.g., Maternal Breast Milk, Infant Formula Supplemented With Omega-3 Fatty Acids)?

What is the Evidence That These Adverse Events, or any Contraindications, are Associated With the Intake of Specific Sources (e.g., Marine, Plant), Types (e.g., EPA, DHA, ALA) or Doses of Omega-3 Fatty Acids, Including in Specific Populations Such as Diabetics?

Preterm infants

All the eleven relevant trials[193,201,207,212,218,251,257,258,273,286,287] that were reviewed, reported some information on safety/adverse events (e.g., complications, intolerance) (See Summary Tables in Appendix E[*]). The trials reported explicitly that the study infants had experienced similar arm-specific rates of the following adverse events (ascribed or not ascribed to the study participation): neonatal morbidity,[193,212] bleeding time,[212] gastric residuals,[251,257,286] spitting/abdominal distention,[251,258,273] respiratory effects (pharyngitis, rhinitis, bronchiolitis, pneumonia, and increased cough),[218,273] cardiovascular (bradycardia, cardiovascular event), gastrointestinal (increased abdomen, vomiting, diarrhoea, infection), haemic (anemia, hypoxia), lymphatic, urogenital, flatulence, otitis media, apnea,billirubinemia,[218] eczema,[258,273] death,[201,207,218,258,273] chronic lung disease,[207] systemic infection,[207,258,273] hospital readmission,[207,273] feeding intolerance,[207,258,273] retinopathy of prematurity,[201,273]intra-ventricular haemorrhage (IVH),[201,273] pulmonary haemorrhage,[273] necrotizing enterocolitis (NEC),[201,258,273] sepsis,[201,273] vomiting,[257] bradycardia,[286] and stool frequency.[257,258]

The difference in the frequency of adverse events between the study arms was found only in three trials.[218,258,273] Specifically, in one trial[218] at 48 weeks of post-conception age (after 17 weeks of feeding), infants in the omega-3 supplemented arm had a higher rate of diarrhoea (vs. human milk arm) and flatulence (vs. control formula and human milk arms), but lower rates of milk intolerance and anaemia (vs. control formula).

Note that in the same trial,[218] but at 92 weeks post-conception age (after 60 weeks of feeding), the omega-3 FA-supplemented and control dietary arms had similar rates of flatulence, anaemia, and diarrhoea. In another trial, infants in the omega-3 FA-supplemented arm were found to have a lower mean number of stools per day, compared with those in the control arm (1.96 vs. 2.12).[273] In the trial by Fewtrell et al.,[258] infants in the supplemented arm required the use of ventilation and umbilical catheters for a longer period of time than those in the control

[*] Appendices and Evidence tables cited in this report are provided electronically at http://www.ahrq.gov/clinic/tp/o3mchtp.htm

formula arm (median days ventilated: 4 [3-8] vs. 2 [2-5] and median days with umbilical catheters: 4 [3-6] vs. 3 [2-5]).

Information on the consequences (i.e., withdrawals, death) of adverse effects/intolerance was reported in six trials.[201,207,218,258,273,287] McClead et al.,[287] reported that two infants who had developed tachycardia and tachypnea subsequently recovered. In the trial conducted by Vanderhoof et al.,[218] the majority of those who were withdrawn, had cow's milk intolerance (n=8), vomiting (n=5), diarrhoea (n=3), ileus (n=3), enlarged abdomen (n=2), and NEC (n=2). Other more rare events leading to the infant's withdrawal were oesophageal reflux (n=1), constipation (n=1), rash (n=1), and cerebral necrosis (n=1). In another trial,[207] the formula feeding intolerance resulted in 51 (12% of the total number of randomized infants) withdrawals. Innis et al.[201] reported that amongst the 21 infants who were withdrawn from the feeding protocol, three infants had had NEC (n=2) and formula feeding intolerance (n=1). In the trial by Fewtrell et al.,[273] ten infants were withdrawn for the following reasons: death (n=3; had chronic pulmonary disease requiring ventilation), NEC (n=6), and formula feeding intolerance (n=1). In another trial,[258] three infants, each of whom had developed bronchopulmonary dysplasia resulting in death, NEC, and abdominal distension, were withdrawn from the feeding protocol.

Most commonly reported reasons for death were SIDS (n=4; totalled across studies),[201,218] NEC (n=2; totalled across studies),[218,273] and pulmonary disease requiring ventilation (n=4; totalled across studies).[258,273] Only six trials reported explicitly that the adverse events and/or death occurring in these trials could not have been ascribed to the feeding diets.[207,212,218,251,273,287]

Term infants

Of the twelve unique relevant trials that were reviewed, eleven reported some information on safety and/or adverse events (e.g., complications, intolerance).[104,182,203,205,227,261,265,266,268,287] (See Summary Tables in Appendix E) The authors of one trial[263] failed to report any relevant data on the above-mentioned outcome of interest. Given the information provided by the study authors, in general, the experimental regimens had been well tolerated and the trial authors observed either no or very few serious adverse events occurring to the infants. In addition, even if certain adverse events were observed, none of the between-group differences with respect to their occurrence reached the traditional level of statistical significance, regardless of the timing of observation.

For example, six trials[104,205,227,265,266,268] reported that the infants had experienced similar arm-specific rates of the following adverse events (ascribed or not ascribed to the experimental diet): cataracts,[104] viral meningitis,[104] pyloric stenosis,[104,265] phenylketonuria,[104], ≥1 hospitalization,[104,268] prescribed antibiotics,[104,265] otitis media,[104] respiratory infections,[265,268] gastroenteritis,[265,268] eczema,[265] asthma,[265] visit to medical practitioner,[265,268] vomiting,[205,227,265,266] constipation,[205,265,266] diarrhoea,[205,266] stool consistency,[227,268] and allergy.[268]

The inability to find the between-group statistically significant differences in the proportions of infants with adverse events, could have been partially due to the small numbers of these events across these trials and/or insufficient sample size. For example, in one study,[104] the between-arm differences in the number of used prescriptions for antibiotics could not reach the statistically

significant result (formula with [DHA]: 57% vs. formula with [DHA+AA]: 46% vs. breastfed group: 66%). Another study,[265] found that the formula with [DHA+EPA] supplementation group was prescribed more antibiotics (OR = 1.3, 95% CI: 0.8, 2.2) and had more visits to medical practitioner (OR = 1.8, 95% CI: 0.8, 4.2) than the control formula group, but neither of these differences was statistically significant. Of the remaining four trials,[182,203,261,287] three trials[182,261,287] reported explicitly that the experimental regimens had been well tolerated (i.e., trial authors observed either no or very few adverse events in the infants). These adverse events were: tachycardia and tachypnea,[287] diaper dermatitis,[261] unspecified illness unrelated to the diet and lactose intolerance,[182] and dietary protein hypersensitivity.[203] In the trial by Jensen et al.,[203] it was not clear if the study infants had or had not experienced any adverse events (the authors did not state this explicitly). Note that these four trials,[182,203,261,287] on average, had a shorter length of intervention (range: 1-17 weeks) and smaller total sample size (range: 20-108 infants) than the six trials[104,205,227,265,266,268](range: 12-48 weeks and 109-447 infants, respectively) that observed the greater number of adverse events (though with similar arm-specific rates of adverse events).

In seven trials,[104,203,205,227,265,266,268] it was explicitly reported that the infants who had had adverse events were withdrawn/non-completers. Three trials,[104,182,266] explicitly stated that some of the observed adverse events (viral meningitis, pyloric stenosis, cataracts, phenylketonuria, sudden infant death syndrome, and unspecified illness) were not related to the experimental formula feeding.

Chapter 4. Discussion

Overview

A total of 117 reports, describing 89 unique studies, investigated questions pertinent to this systematic review of the evidence concerning the effects of omega-3 fatty acids on child and maternal health. The questions regarding the influence of the intake of omega-3 fatty acids on pregnancy outcomes, such as duration of gestation, preeclampsia, eclampsia or gestational hypertension and infants SGA were address separately, since RCTs were identified that answered each of these questions separately.

The questions regarding the child's outcomes, such as growth patterns, neurological development, cognitive development and visual function are divided in a series of questions: one question is related to the maternal intake of omega-3 fatty acids for each outcome; another question is associated with the infant's intake of human milk; two questions have been lumped together regarding the infant's intake of formula, with or without breast milk; a separate question addressed the infant's intake of omega-3 fatty acids from other sources (diet, supplements); and, a final set of questions relate to biomarkers in maternal, fetal or infant's blood, and the association with the clinical outcomes.

For each group of outcomes, we present a synthesis of the key findings with respect to each question. This includes a critical appraisal of the group of trials from which results are drawn. The broader implications of these findings, including potential future research, are highlighted. We begin with the safety issues concerning all the included studies.

Evidence Synthesis and Appraisal

Adverse events, contraindications, and intolerance are often under-reported in human experimental studies. Many studies do not report any data on *adverse events*, and so it is frequently not clear whether or not an adverse event had actually occurred in these studies. Furthermore, even if a study reports an adverse event, the study authors do not always state explicitly if this adverse event was related to the study intervention or some other factor(s). An additional problem that aggravates the assessment of adverse event data, is that some authors do not clarify whether the number of adverse events reflects the total number of event occurrences across all patients (i.e., a single patient may experience more than one adverse event during the study period), or the number of patients who had experienced at least one adverse event. This information should be reported in order to distinguish between the two scenarios.

Overall, omega-3 fatty acids supplementation in pregnant women, breastfeeding mothers and preterm and term infants, was very well tolerated and did not generate any serious adverse events across the included RCTs. The safety data was reported in 21 RCTs.

In pregnant women, the adverse events related to the omega-3 fatty acids intake were mild and transient, with nausea and gastrointestinal discomfort being the most commonly reported.[230,233]

For both the term and preterm population, change in number of stools and flatulence were the most common adverse events related to the omega-3 supplemented formulas. However, most of the serious adverse events were related to the fact that the infants were premature with low birth weights, which increases the occurrence of necrotizing enterocolitis (NEC), bleeding problems, infections and respiratory failure, among others in the case of preterm infants.[104,182,193,201,203,205,207,212,218,227,251,257,258,261,265,266,268,273,286,287] In general, none of the withdrawals were due to the interventional formula.

Fifteen average poor quality (Jadad: 2.8/5) RCTs addressed the question of the *influence of omega-3 fatty acids intake during pregnancy on the duration of gestation*.[31,41,288,290,291,293-295,295,296] Seven trials included otherwise healthy pregnant women,[141,196,209,231,232,234,235] the remaining eight studies included a high-risk population of pregnant women, yet with different types of risk factors (i.e., IUGR, premature delivery, preeclampsia, etc). Ten studies did not find a significant difference between intervention groups in the duration of gestation measured as mean of gestational age at delivery.[141,196,230-235] However, four average poor quality (Jadad score 2/5) studies observed that the omega-3 fatty acid group had a significantly greater duration of gestation after treatment compared with the unsupplemented group.[209,230]

Omega-3 fatty acids did not have a significant effect on the proportion of premature deliveries in ten studies.[31,209,233,234,238] Only Smuts et al. observed a noticeable lower percentage of premature deliveries in mothers taking omega-3 fatty acid supplements, yet this study was underpowered (small sample) to measure the statistical significance of such observation.[232]

Other variables, such as length of the intervention and background diet, were different among the identified trials. Most studies began the treatment during the second trimester of pregnancy,[141,196,230,231,233,235,238] while the remaining trials enrolled their subjects during the third trimester. Fish consumption in the background diet, one of the most important effect modifiers, was used as a covariate in only one trial.[209] After adjusting for this effect modifier, the results did not change, and the fish oil group still had a longer duration of gestation than the olive oil group.[209]

Other covariates used to control the results were the compliance with the intervention,[209] current smoking status,[233,234] as well as maternal BMI and number of prior pregnancies.[234] The only variable that had an impact on the results was the smoking status in Smuts et al's study.[234] The duration of gestation was significantly longer in the high-DHA group in the nonsmokers.[234]

Meta-analysis of the incidence of premature deliveries was performed pooling the data of eight RCTs that compared the use of capsules containing DHA+EPA,[31,41,291] and two trials using high DHA eggs[294,296] with control group. Both meta-analysis failed to find a statistical difference between groups. The limitation of combining the studies using DHA+EPA versus control, is that the population of pregnant women included in seven trials was high risk for premature delivery in different ways (twin pregnancy,[31] antecedent of premature delivery,[31] antecedent of GHT and IUGR,[31,291] and threatening pre-eclampsia[31]). Only one study included healthy Danish women.[41] Subgroup analysis was not possible given the lack of individual data for each of the six RCTs included in Olsen et al. 2000.[31] Another limitation of this approach is the length of intervention. While five trials started in the second trimester of pregnancy,[31,291] three began the intervention during the third trimester (shorter period of time and likely not meaningful to see a significant effect).[31,41]

These findings suggest that there is inconsistent evidence of the use of omega-3 fatty acids supplements during the second or third trimester of pregnancy to reduce the incidence of premature pregnancies in high and low risk populations. Nevertheless, the overall effect does not show a significant difference between study arms.

The *association between the maternal biomarkers during pregnancy and the duration of gestation* was assessed in four studies.[234,239-241] The study by Smuts et al. was an RCT that compared the use of DHA-enriched eggs intake with ordinary eggs in healthy pregnant women.[234] This study did not observe a significant correlation between the maternal RBC content of DHA and the duration of gestation, however, the study found a significantly positive correlation between the infant RBC DHA at birth and this pregnancy outcome.[234]

Three observational trials,[239,240] found a significantly positive association between the maternal plasma content of AA (at 34-35 weeks of GA) and the duration of gestation, whereas, Rump et al.'s cross-sectional study did not find any correlation between maternal biomarker content and duration of gestation.[241] The study by Elias and Innis was a single prospective cohort of pregnant women that reached a term delivery,[240] and the study by Reece et al.[239] was a case-control study that compared the maternal content of RBC omega-3 and omega-6 fatty acid biomarkers at 34 weeks of gestation and at delivery in preterm and term pregnancies. This study found that the preterm deliveries had a significantly higher content of AA (omega-6) and DPA (omega-6), reflecting a relative reduction in the omega-3 fatty acids. The omega-6/omega-3 ratio was higher in preterm deliveries or in 34-week pregnant women, compared with samples taken after term deliveries.[239]

These findings suggest that there is an uncertain association between the maternal biomarkers during pregnancy and the duration of gestation, independently of the maternal intake.

Eight RCTs addressing the question concerning the *influence of maternal intake of omega-3 fatty acids during pregnancy in the incidence of gestational hypertension (GHT), preeclampsia or eclampsia* were identified with a quality score approaching good internal validity (Jadad: 2.9/5).[209,230,233,236,237] Six studies compared the use of fish oil supplements containing DHA and EPA with placebo (generally olive oil). The population characteristics of these studies were very diverse, since one of them included healthy Danish pregnant women,[209] while the others included high-risk pregnant women (i.e., preeclamptic, twin pregnancies, IUGR or preeclampsia in previous pregnancies, etc).[230,233,236,237] The incidence of GHT in these populations, after the use of omega-3 fatty acids or placebo did not differ in six of seven studies.[209,230,233,237,238] The study by D'Almeida et al. was the only poor quality trial conducted in South Africa that observed a reduction of the incidence of GHT in the magnesium oxide group, compared to the omega-3 FA supplemetation and the placebo groupsa (no significance assessed).[236] Regarding the incidence of preeclampsia (triad of hypertension, edema and proteinuria), six studies showed that compared with placebo, supplementation with omega-3 fatty acids did not have a significant effect.[230,233,234,237,238]

Only one study conducted in South Africa observed a statistically significant difference between groups, showing that the fish oil group had a lower incidence of preeclampsia compared with placebo and magnesium oxide.[236]

Meta-analysis was possible for the outcome related to the incidence of gestational hypertension. Two studies were included in the analysis,[230,233] which selected a population of

women at high risk of developing GHT. The overall effect size was nonsignificant between groups.

It appears that there is some evidence to suggest that supplementation with omega-3 fatty acids during the second or third trimester of pregnancy does not reduce the incidence of gestational hypertension, preeclampsia or eclampsia in healthy or high-risk pregnant women. However, the results were not adjusted for the potential covariates or confounders, such as background diet, grade of risk for GHT or preeclampsia in the current pregnancy, smoking status, and age, among others.

No RCTs were identified to investigate the *association between the omega-3 or omega-6/omega-3 ratio content of maternal biomarkers and the incidence of preeclampsia-eclampsia or gestational hypertension*. We identified five observational trials that addressed this question, yet the incidence of preeclampsia could not be assessed given the study designs.[179,229,242-244] Four studies selected preeclamptic women and normal pregnant women as controls.[229,242-244] Al et al. selected women with GHT and healthy pregnant women as controls,[179] and Craig-Smith et al. also included women with GHT and chronic hypertension.[243] Wang et al. and Hofmann et al. found that the maternal plasma content of AA did not differ significantly between preeclamptic and normal pregnant women.[229,242] On the other hand, Craig-Smith et al. observed that the women with chronic hypertension had a significantly higher plasma content of AA compared with women with preeclampsia, GHT or normal pregnant women.[243] Shouk et al. observed that the women with preeclampsia had a significantly higher AA content compared with normal women, although the plasma measurement was different from the other studies (mcg/L).[244] Results regarding total PUFA content, total omega-3 fatty acids, total omega-6 fatty acids, DHA, EPA and other PUFAs did not follow a consistent pattern across the studies. The results are very inconsistent among the studies.

These discrepancies across the studies can be explained given the differences in the study designs, case ascertainment, severity of preeclampsia, appropriate technique of lipid extraction and manipulation, measurements of FA in plasma (% weight of total FA, mcg/L or mol/L) background diet, age, gestational age, and other variables like alcohol intake, tobacco use and supplements that were not assessed.

Regarding the *influence of omega-3 fatty acids supplementation during pregnancy on the incidence of SGA infants*, fourteen average poor quality scores (Jadad: 2.85/5) RCTs with addressed this question. The definition of SGA was diverse across the included studies, using the smaller percentile (PC) as the upper limit (i.e., PC < 3 or PC < 5 or PC < 10 for gestational age). Most of the studies evaluated the mean birth weight, instead of the incidence of SGA infants. In the majority of the studies, mean birth weight was not influenced by the intervention. Despite the fact that the selected populations in the trials were so different (e.g., high risk vs. healthy women), the results seem to be very consistent across the studies. None of the trials adjusted their results for the maternal background diet, which can be an important effect modifier.

Meta-analysis was performed for two different variables. The birth weight (mean value) was combined in two studies that were comparable in terms of type of intervention and population. The overall size of the effect was nonsignificantly different between groups (supplemented vs. unsupplemented).[230,233] The other outcome was the incidence of infants with IUGR in three

studies,[230,233,238] with a nonsignificant overall effect of supplementation during pregnancy. These findings are consistent with the results of the remaining included studies.

Six studies addressed the question regarding the *association between the omega-3 or omega-6/omega-3 ratio content of maternal biomarkers and the incidence of SGA infants*.[196,240,241,245-247] de Groot et al.'s RCT found a significantly positive correlation between the maternal plasma and RBC DHA content and birth weight, however, this relationship was nonsignificant when measured at delivery.[196] Among the observational studies, three investigators compared the maternal biomarker content in women at risk of IUGR with healthy controls.[245-247] Two of them found that the women with IUGR fetuses had a significantly lower content of LA (omega-6) in the plasma.[246,247] The content of DHA, EPA, AA, total omega-3 and omega-6 fatty acids, however, did not show a constant pattern across the studies. Two observational studies did not observe a correlation between maternal plasma biomarkers and birth weight,[241,247] consistent with the result in the RCT.[196] Elias and Innis did not define the birth weight for GA, so their results are difficult to interpret in the context of correlation of maternal PUFA with SGA infants.[346]

These discrepancies in the study results may be due to many variables that play a relevant role in the lipid profile, such as population characteristics (healthy pregnant women, high risk of IUGR, women with IUGR), background diet, lipid extraction and manipulation, lipid fraction (TGL, PL, CE), and timing of drawing the blood samples.

No studies were identified to address the question of the influence of the omega-3 fatty acids from sources other than formula or human milk, and any of the child's clinical outcomes (e.g., growth patterns, neurological and cognitive development, and visual function).

One good quality RCT addressed the question of the *influence of maternal omega-3 fatty acids intake during pregnancy on the growth patterns outcomes*.[141] There was no statistical differences between infants from mothers that were taking the supplementation with omega-3 and omega-6, or omega-6 fatty acids predominantly, on the weight, length and head circumference (HC) from birth to 12 months of age.[141] The infants were also breastfed exclusively during the first three months of life, and their mothers were still taking the interventional oils. Thus, these results also apply to the question of the maternal breast milk content of omega-3 fatty acids and growth patterns.

Helland et al. included a large sample (n=590) of healthy pregnant women from Norway, yet this study only used the completers in the analysis (n=341) given the large number of dropouts.[141] The fact that only 57% of the included women were included in the analysis, makes the results more difficult to interpret. The intake of marine omega-3 fatty acids is relatively high in Norway compared with other countries.[347,348] The pregnant and lactating women have high concentration of DHA in plasma phospholipids and breast milk, and a great majority of Norwegian mothers also breastfeed their infants up to at least 3 months after giving birth, thus providing their infants with preformed DHA.[141]

One good quality RCT evaluating omega-3 supplementation in Norwegian mothers,[141] one poor quality RCT,[248] and two observational studies were identified to answer the question related to the *influence of omega-3 fatty acid content of maternal breast milk on the growth patterns in term infants*.[249,302] No studies were identified to answer this question for the preterm population.

The two RCTs showed no apparent effects of breast milk, with maternal intake of omega-3 (DHA) or omega-6 fatty acids (AA), on the growth patterns at any time point.[141,248] The single prospective cohort of a small sample of Swedish mother/term infant pairs, where the infants were receiving almost exclusively breast milk for 3 months, showed a positive correlation between the maternal mother's breast milk content of AA/DHA and the infant's rate of increase of HC at 1 and 3 months of age.[249] No associations were found between the HC and LA or ALA, or between HC and AA or DHA in breast milk.[249]

On the other hand, a cross-sectional study that included two different cohorts of term infants from Africa (two different cities with different intakes of PUFAs) was identified.[302] Despite the limitations of including a study with this type of research design, the differences in weight-for-age and weight-for-height z-scores and weight gain (g) were significantly lower in infants from Ouagadougou (low omega-3 fatty acids intake) compared with infants from Brazzaville (high omega-3 intake).[302] There are several problems with the interpretation of these results, such as the fact that the included cohorts corresponded to a completely different population (location, maternal education, home characteristics, feeding practices, maternal diet, etc.). Thus, the differences in the growth patterns could be due to all these baseline discrepancies rather than a real statistical difference. The conflicting findings across the studies demonstrate the need for further appropriate research on this association.

Twenty RCTs, with an overall mean quality score of 2.64/5 (i.e., poor quality), addressed the question of the *influence of omega-3 fatty acid supplement of infant formula on the growth patterns in preterm infants*.[185,191,193,198,201,207,212,218,225,250-259,273] Eighteen studies failed to find an effect of the omega-3 and omega-6 fatty acids supplementation in preterm formulas on the growth parameters at several time points.[185,193,198,201,207,212,218,225,250-259] The growth outcomes measured were the mean (SD) weight, length and head circumference, the normalized z-score of weight, length and HC and the weight, length and HC gain.

Two studies found that the omega-3 fatty acids supplemented group had a significantly lower weight at 6, 9 and 18 months of CA.[191,273] Both studies included healthy preterm infants and provided formulas containing DHA+EPA, as well as a control formula for comparison. The duration of the supplementation was different across the 19 trials (range from 3 weeks to 12 months CA). Interestingly enough, two studies by the same author (Fewtrell et al.) showed opposite effects in the growth pattern outcomes.[321,322] The results were different probably due to the different length of intervention (33 days vs. 9 months), dose of DHA and EPA (DHA 0.17 g/100 ml vs. 0.5 g/100 ml) and source of PUFAs (egg-TGL vs. fish oil).

Meta-analysis was performed for two different growth outcomes—weight and length at 4 months of CA. The results of the meta-analysis performed on the mean weight and length measured at 4 months, in the studies that compared the use of formula supplemented with DHA+AA with control formula,[201,207] showed that the overall effect was nonstatistically significant. No other combinations were possible, given the differences in the intervention length, measuring points and type of growth parameter (mean, z-score, mean change). Overall, there is some evidence from 20 RCTs that the omega-3 fatty acids supplementation may not have an impact on the growth parameters. This findings are consistent with the meta-analysis done by Simmer and Patole in 2003.[349]

Eighteen average good quality (Jadad: 3.2/5) RCTs addressed the question of the *influence of omega-3 fatty acid supplement of infant formula on the growth patterns in term infants*.[104,182,203,205,223,227,260-270]

The effects across these studies on the growth outcomes, such as weight, length and head circumference, were nonstatistically different between study arms. Yet, some inconsistent differences were found across five trials at certain timepoints and subgroup of patients.[120,325,328,329,332] The supplementation with omega-3 and/or omega-6 fatty acids has not demonstrated any benefit regarding the growth of term infants across these trials.

The studies were rather diverse in terms of intervention characteristics (type of formula, content of PUFA, duration of intervention, cointerventions), as well as the timing of the outcome measures (e.g., 2, 4, 6, 9, 12 months of age).

Meta-analysis was only possible for two studies that had the same intervention as well as the timing of the outcomes.[104,205] We decided to measure only two time points that corresponded to the background diet as a potential confounder. Consequently, 4 and 12 months of age were the time points selected. Four months of age is when the infants were exclusively fed with the formula, after which they began solid foods that were not controlled in any of the trials. The overall effect of formulas containing DHA+AA or DHA compared with control formula was nonstatistically significant at 4 or 12 months of age for any of the growth parameters (weight, length or HC in mean (SD)). This is consistent with the rest of the included studies and with a meta-analysis prepared by Simmer in 2003.[350]

Only four trials adjusted the results for potential confounders, such as gender, maternal education, parental socioeconomic status and center, failing to find any change in the results.[203,205,263,266]

Regarding the *association between the growth patterns in preterm and term infants and the omega-3 or omega-6/omega-3 fatty acid content of maternal or fetal biomarkers,* no studies were identified to answer these questions.

A total of 12 studies addressed the question of the *association between growth patterns in preterm and term infants and the omega-3 or omega-6/omega-3 fatty acid content of child biomarkers*. Five RCTs included a preterm population of infants,[185,191,201,207,212] five RCTs[143,203,205,262,263] and a prospective single cohort[271] included a term population of infants and Woltil et al., which was consciously described only in the preterm section of this question, selected a group of VLBW preterm and term infants.[225]

All the RCTs that included a preterm population, assessed the correlation between the infant's plasma and RBC content of AA and the growth outcomes, such as weight (mean, gain), length and HC.[185,191,201,207,212] Carlson et al. found a significantly positive correlation between the weight and length z-scores from 2 to 12 months of CA and the plasma and RBC AA.[185] However, Uauy et al. observed a negative correlation between the RBC AA content and the length z-score at 57 weeks (PCA).[212] Two studies found a positive correlation between the RBC AA and the weight and length at 1 month CA[207] and at 2 months CA.[201] These two studies also found a significantly positive correlation between the same biomarker and weight gain.[201,207] Only Carlson et al. detected a positive correlation between the plasma and RBC AA and the HC at 2 and 4 months.[185]

Carlson et al., in another study, found a negative correlation between the weight-for-length z-score and the RBC DHA at 5 months of age.[191] Woltil et al. found a positive correlation between the weight, length and HC gains, and the plasma and RBC DHA content in preterm and term infants.[225]

Five RCTs measured the correlation between the plasma or RBC PUFAs and growth outcomes in term infants.[143,203,205,262,263] Two studies did not find a significant correlation between the omega-3 fatty acids in plasma or RBC and weight.[203,262] However, Jensen et al. observed a significant positive correlation between the weight at 4 months and the plasma AA content at the same time point.[203] Innis et al., on the contrary, did not find a significant correlation between growth patterns and the plasma and RBC AA content in term infants.[263]

Makrides et al. found a significantly negative correlation between plasma DHA at 16 weeks and weight at 12 and 24 months of age.[205] Consistent with the findings in Innis et al.'s cohort of term infants, with a negative correlation of RBC and plasma DHA and infant's weight at 6 months of age, yet not at 12 months.[271] Guesnet et al. also found a negative correlation between the plasma and RBC EPA at birth and the length gain over 6 weeks.[143]

It appears to be a negative correlation between weight and the plasma or RBC content of DHA, and a positive correlation between weight and the content of AA in plasma or RBC. However, not all of the studies found this association. The content of omega-6 fatty acids (AA) as a biomarker may be related to weight gain in infants. The content of DHA seems to be inversely related to weight gain, yet no significant clinical outcomes were detected.

There was one good quality RCT that addressed the question of the *influence of omega-3 fatty acids intake during pregnancy and the neurological development outcomes.*[141] Helland et al. randomized a sample of pregnant women to receive either cod liver oil (DHA + EPA) or corn oil (LA +ALA) until 3 month post-delivery. This study failed to find a significant difference between groups in maturity as evaluated from the EEGs, neither at day 1 of life nor at 3 months of age.[141]

Two studies, one RCT[138] and one single prospective cohort design,[284] addressed the question of the *omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influence on the neurological development in term or preterm human infants*.[138,284] Gibson et al. randomized healthy mothers of term infants who intended to breastfed with increasing doses of DHA-rich algal oil. The infants were exclusively breastfed for 3 months. There was no difference between groups in the Bayley's Developmental Index (PDI score) at 12 and 24 months of age, however, none of the groups were acting as a control group (no omega-3 fatty acids).[138] Another issue with the interpretation of these results is that the infants were only exclusively breastfed for the first 3 months of life, which introduces potential confounding factors, such as the background diet of the infants after this age. Other potential confounders were controlled in a post-hoc analysis, which found that there were no associations with any sociodemographic variables at 1 year. The only association at 2 years of age was between PDI and the level of education of the partner.[138]

Agostoni et al. evaluated the neurodevelopmental indices at 1 year of age in a single prospective cohort of term infants who were exclusively breastfed for at least 3 months in Italy.[284] After correcting for potential confounders such us parity and mother's characteristics (i.e., age, education, smoking habits), breastfeeding for 6 months or longer was not significantly

correlated to the mean PDI result compared with subjects breastfed for 3 to 6 months (n=15).[284] There was no correlation between PDI and the milk fat content at any time point.

The results of these two different design studies showed that maternal breast milk might not have an influence on the neurological outcome, measured with the PDI scale of the Bayley's Index.

Six average good quality (Jadad: 4.2/5) RCTs were identified to assess the *neurological development of preterm infants (< 37 weeks of GA) supplemented with omega-3 fatty acids in infant formula with or without breast milk intake*.[193,207,254,258,272,273] The outcomes assessed were the PDI scale of the Bayley's Developmental Index, the Knobloch, Passamanick and Sherrards' Developmental Screening Inventory (five subscales), the neurological impairment evaluated by a pediatrician, BAEP, and NCV studies.

The results showed that, for the PDI scale, two of five studies did not observe a significant difference between the supplemented and the control formula.[258,273] Two studies found that the supplemented formula groups had a significantly higher score (better) than the control group.[193,207] However, O'Connor et al. only observed this difference in the group of infants that consumed > 80% infant formula and whose weight at birth were <1,250 g.[207] On the other hand, van Wezel-Meijler et al. found a significantly better PDI score in the control group compared with the supplemented group at 3, 6 and 24 months, yet this difference did not reach statistical significance when adjusted for birth weight and number of SGA infants.[272] Only Fewtrell et al. found that there was no difference between groups in the neurological impairment assessment at 9 and 18 months CA, and in the Knobloch, Passamanick and Sherrards' Developmental Screening Inventory score.[258]

For the studies that measured the Bayley's PDI score, we could not combine them for meta-analysis given the lack of information at certain time points (i.e, 4 or 12 months of age). Two studies included patients who were also breastfed,[207,258] which could have introduced bias given the content of PUFAs in human milk. In some cases, the duration of supplementation was different than the time to outcome measure, or endpoint (e.g., intervention lasted 6 months and PDI was measured at 24 months). Infants that tolerated enteral feeding began their solid food at around 4 months of age. This background diet added to the formulas was not controlled in the trials, which can modify the effect of the intervention. Other factors, such as maternal diet, second hand smoking, and socioeconomic status are potential confounders, as well as parental stimulation at home.

Four studies used a non-randomized reference standard group of mothers who decided to breastfeed exclusively.[193,207,254,273]

Overall, there is not consistent evidence to suggest that the omega-3 fatty acids supplementation of infant formula, with or without breast milk, influences the neurological development in preterm infants. These findings also corresponds with the meta-analysis done by Simmer and Patole.[349]

Eight average good quality (Jadad: 4.25/5) RCTs addressed the question regarding the *influence of omega-3 fatty acids supplement in infant formula, with or without human milk, on the neurological development of term infants*.[104,176,182,203,205,227,227,265] The main outcome measured was the Bayley's Developmental Score system, the PDI. None of the seven studies

that assessed this outcome found a statistically significant difference between diet groups at different follow-ups.[104,182,203,205,227,265] The endpoints were measured at 6, 12, 18 and 24 months of age.

There were other type of outcomes measured, like the Brunet-Lézine test in an Italian trial,[176] which showed a significantly better result in the LCPUFA supplemented group compared with the control group at 4 months of age (after exclusive formula intake). However, this result was not significant at 24 months of age, possibly due to the potential covariates and confounders after 20 months of lack of intake.[176]

All the studies included healthy term infants, although the sources and type of omega-3 fatty acids supplementation, as well as the duration of the intervention, were different across the studies. Other potential confounders that were not assessed in the analysis were the lack of information regarding the background diet from 4 months of age until the time of assessment, and the absolute and relative amount of omega-3 and omega-6 fatty acids intake that was associated with the infant formulas. This last piece of information was not provided in any of the included trials. Jensen et al. was the only trial that compared the use of LCPUFA precursors such as LA (omega-6) and ALA (omega-3) in different ratios.[203] The remaining studies used DHA and AA as type of LCPUFA, yet from completely different sources (egg lipids, vegetable oils, fish oil).

We did not include the comparisons made with the reference standard group, breastfed infants, given that those infants were not randomized and belonged to a different population. Only one study included human milk as a cointervention of the infant formulas.[227] This study did not find differences between groups in any of the neurological outcomes (i.e., Bayley's PDI, and BRS at 6 and 12 months).[227]

Meta-analysis of the outcome measured with the Bayley's PDI was conducted in three RCTs that compared the use of formula supplemented with DHA+AA with control formula.[104,205,227] The overall effect size at 12 months was nonstatistically significant between groups. No other time points could be combined. These conclusions are consistent with the meta-analysis done by Simmer in 2003.[350]

One cross-sectional study conducted in the United States assessed the *association of maternal LCPUFA content (DHA) in plasma and RBC at delivery and the neurological status of their newborns*.[274] Maternal DHA was negatively associated with active sleep (AS), AS:QS (quiet sleep) and sleep-wake transition, and positively associated with wakefulness (postpartum day 2).[274] The ratio of n-6:n-3 in maternal plasma was positively associated with AS, AS:QS and sleep-wake transition, and negatively associated with wakefulness (day 2). On day 1, the ratio of n-6:n-3 in maternal plasma was negatively associated with QS and positively associated with arousals in QS.[274] These results mean that lower amounts of AS and the greater amounts of QS observed in the infants exposed prenatally to higher DHA concentrations suggest greater CNS maturity. Furthermore, the lower AS:QS observed in the infants in the high-DHA group shows that their sleep organization soon after birth was approaching that of normal, older infants.[338]

When the cohort was analyzed by maternal DHA plasma concentration, the high DHA group (>3.0% by wt of total fatty acids) did not significantly differ from the low DHA group (≤3.0% by wt of total fatty acids) in terms of maternal age, race, parity, duration of gestation, maternal education, infant birth weight and length, infant HC and Apgar score at 1 and 5 minutes.[274]

However, infants from mothers with high plasma DHA concentrations had significantly less AS and had a lower AS:QS compared with infants of mothers with low plasma DHA concentrations. Furthermore, infants in the high DHA group had significantly less sleep-wake transition and more wakefulness than did infants in the low DHA group on postpartum day 2.[274]

The difficulty with the interpretation of these results lies in the research design. Cross-sectional studies are appropriate to measure prevalence, yet not appropriate for measuring the etiological association between two variables, such as maternal biomarkers at delivery and neurological development in the infant. The outcomes assessed in this study are related to sleep patterns rather than other neurological functions such as motor, sensation and brain development, which can be associated with the CNS maturity of the infant at birth.

No studies were identified to answer the question about the association with fetal biomarkers. Four RCTs[176,182,203,205] and one observational study[271] addressed the question regarding the *association of the child content of omega-3 and/or omega-6 and the neurological outcomes*.

Three RCTs[182,203,205] and a prospective cohort study[271] evaluated the association between the infant's plasma and RBC DHA content and the Bayley's PDI score in term infants. All these studies assessed this association in healthy term infants. Two RCTs found a significant positive correlation between the plasma DHA and the PDI score.[203,205] However, the timing of assessment was different for both studies. Makrides et al. measured both the blood content of biomarkers and the PDI at 12 months of age,[205] while Jensen et al. measured the plasma and RBC content of PUFA at 120 days of age and the PDI at 12 months.[203] The formula intake was also different in both trials. Two other studies (including the observational study), did not find a significant correlation between the PDI and the infant content of PUFA in plasma or RBC.[182,271]

Innis et al. did not find a statistically significant relation between the infant RBC DHA or AA status at 2 months of age and the Bayley's PDI score at 6 and 12 months of age.[271] But given the research design of this study, the interpretation of the results is very limited. Bias could have been introduced due to several potential effect modifiers that could underestimate results, such as maternal diet of the breastfed infants, child's background diet after 3 months of age, as well as other environmental factors that can influence the content of LCPUFAs and the neurological development in infants. The results, across the studies, are not consistent enough to draw any conclusions.

Two studies addressed the question of the *influence of omega-3 fatty acids intake during pregnancy and the visual function in term infants*.[235,275] There were no studies identified that included a preterm population.

The first study was a double-blinded RCT that assessed the retinal function of term infants of mothers that were or were not taking DHA during pregnancy.[235] This trial failed to find a significant effect of DHA supplementation during pregnancy on the retinal sensitivity (ERG) measured at birth in term infants. The cross-sectional study was conducted in Cuba and measured the visual function of a cohort of term infants from mothers who had a high intake of high-fat fish during pregnancy and breastfeeding.[275] This study failed to find a statistically significant difference in mean visual function values between the exclusively breastfed group and the infants who were also receiving formula.[275] However, the purpose of this study was to evaluate the correlation between the visual function at 2 month of age and their blood LCPUFA biomarkers; and, no correlations were found.[275] The interpretation of such research design on

the clinical outcomes is very difficult given the lack of an appropriate comparator, randomization, blinding and other variables necessary to produce more accurate results.

These findings suggest that maternal intake of omega-3 fatty acids supplements may not effect visual function outcomes in term infants. Yet, better-conducted studies are required to support this conclusion.

Five studies addressed the question regarding the *influence of human milk content of omega-3 or omega-6/omega-3 fatty acids on the visual function of term infants*.[138,140,248,275,276] Two were RCTs,[138,248] one was a prospective cohort study,[276] and two were cross-sectional studies.[140,275] The RCTs did not detect a statistical difference in the VEP acuity among infants of mother who were or were not receiving DHA at any age (from 12 weeks to 8 months of age).[138,248] No studies were identified in the preterm population.

Two observational studies found a significant association between the DHA content of breast milk and visual function in term infants at 4 months of age,[140] and at 3.5 years old.[276] The Cuban cross-sectional study, on the other hand, did not observe this correlation at 2 months of age.[275]

The correlation between the DHA content in breast milk and visual function was not consistent with the clinical outcomes measured in breastfed term infants of mothers who were or were not taking supplements containing high DHA.

The *influence of omega-3 fatty acids supplementation of infant formula, with or without maternal breast milk, on the visual function in preterm infants* was evaluated in nine RCTs with an average quality score approaching good internal validity (Jadad: 2.9/5).[185,191,198,201,207,212,251,254,272] Five studies used the VEP as the main outcome measure,[198,207,212,254,272] while six trials measured the visual acuity with the Teller's Acuity Card Procedure for binocular vision.[185,191,201,207,251,272] Only two trials measured the ERG to evaluate the retinal function of the infants, and did not detect a significant effect with LCPUFA supplementation compared with control formula.[198,212]

Of the five studies that measured VEP, two did not find a statistical difference between feeding groups at any time point (1, 3, 4, 12 months of CA).[254,272] Three studies found that compared with the unsupplemented group, infants fed with LCPUFA-supplemented formula had a better or faster maturation of visual function, in terms of significantly shorter waves in the VEP.[198,207,212] O'Connor et al., however, only detected this positive effect at 6 months, but not at 4 months of CA.[207] Uauy et al. included VLBW preterm infants (60% Black),[212] whereas Faldella et al.[198] and O'Connor et al.[207] included healthy preterm infants with an appropriate weight for GA.

Among the studies that evaluated the visual acuity using the Teller's Acuity Card test, only two studies found a significant difference between groups.[185,191] Carlson et al. observed a higher acuity in the LCPUFA group compared with the control group at 2 months of CA, but not at 4 and 12 months.[191] The same significant difference favoring the supplemented group was seen in the other Carlson et al. study at 2 and 4 months of CA, but not from 6.5 to 12 months of CA.[185]

A meta-analysis of the relevant visual outcomes was performed, comparing the studies by the type of omega-3 fatty acids used in the supplemented formula (DHA or DHA+AA) and control formula, and by the type of outcome (VEP and Teller's test of visual acuity). For the VEP visual acuity outcomes, only two studies were combined.[207,212] O'Connor et al. found that the use of

formulas with DHA+AA resulted in a better VEP measurements compared with control formula, but only at 6 months of age. At 4 months of CA, none of the interventions showed a significant difference.[207,212]

Regarding the behavioral visual acuity measured with the Teller's Card test, compared with controls, there was no significant effect of DHA-supplementation at 2,4,6 or 9 months of CA,[185,201] or DHA+AA supplementation at 2, 3, 4 or 6 months of CA.[191,201,207,212,272]

Only O'Connor et al. allowed their infants to receive breast milk besides the formula.[207] The results were controlled for the amount of formula taken (>80%) in contrast with the breast milk, and the differences were still not significant for both outcomes (VEP and Teller).[207]

The differences across the trials were mostly related to the intervention characteristics (amount of formula, type of supplementation, duration of intervention) and some population characteristics, such as birth weight (VLBW, AGA), race/ethnicity distribution, and socioeconomic status, among others. These differences could explain the discrepancies in the results. These findings are consistent with the meta-analysis done by Simmer and Patole.[349] However, the conclusions of another meta-analysis conducted by SanGiovanni et al.[351] were somewhat different. Their meta-analysis of four studies showed that at 2 and 4 months of age there was a statistically significant difference between the DHA and control groups in the visual resolution acuity (behavioral test) .[351] They did not observe a significant overall effect after 4 months of age. In SanGiovanni et al., the comparisons used in the meta-analysis were taken from the same trial that included more than two dietary groups (corn oil vs. soy/marine oil, soy vs. soy/marine oil, human milk vs. corn oil and human milk vs. soy oil). We did not use this approach given that we considered more appropriate to combine the dietary groups without omega-3 FA as control group and the intervention groups discriminated by content of DHA+AA or DHA alone. Therefore, their approach to do meta-analysis is different from ours and that could be the result of the discrepancies between them.

Thirteen RCTs, of average good quality (Jadad: 3.61/5), addressed the question of the *influence of the omega-3 fatty acids supplementation of infant formula, with or without breast milk intake, on the visual function outcomes in term infants*.[104,182,203,205,227,263,264,266,269,270,277,352]

The outcomes assessed were the VEP in nine trials,[104,182,203,205,264,266,269,270,352] visual acuity (binocular vision) using the Teller's Card test (behavioral visual function) in five studies,[104,227,263,277] retinal function using the ERG in one study,[182] and stereoacuity using the FPL in three studies.[182,269,270]

Five of nine studies did not find a significant difference between groups in the VEP at any age.[104,203,205,264,266] Whereas, the other four trials did find a significantly better VEP in the LCPUFA-supplemented group compared with the control group at a number of time points, from 1.5 to 13 months of age.[182,262,269,270] The meta-analysis performed on this particular outcome, by LCPUFA content of DHA alone (or with the addition of AA), versus control, showed that the studies that compared DHA supplemented formula with control formula did not have an overall significant effect at any age.[104,182,205] Conversely, in seven studies that compared the use of DHA+AA formula with placebo, there was no difference between groups at any age,[104,182,205,262,264,269,270] with the exception of four studies that found a significant difference at 12 months of age.[104,182,269,270]

One of five studies that evaluated behavioral visual acuity with the Teller's test,[277] found a significantly better acuity in the LCPUFA formula group compared with the control group at 2 months of age, yet not at 4, 6, 9 or 12 months. The remaining four studies did not observe a significant difference between groups in this outcome, at any time point.[104,227,263] The meta-analysis performed on this outcome showed that, in studies comparing the use of DHA+AA with a control intervention, acuity was only significantly better in the DHA+AA group at 2 months of age,[104,182,277] but not at 4, 6, 9 or 12 months of age.

These findings suggest that there are conflicting results across the trials regarding the efficacy of the omega-3 fatty acids supplementation of infant formula on the visual function outcomes. These conclusions are consistent with the meta-analysis done by Simmer in 2003.[350] Another meta-analysis performed by SanGiovanni et al., also showed that there was a significantly better visual acuity (Teller's Card test) in the DHA supplemented group compared with the control group at 2 months of age, yet this effect was not seen at any other age. This result is also consistent with our findings.[353]

One study measured the *association between the maternal content of biomarkers* at 2 months postpartum *and the visual acuity* (Teller's Card Test) in term infants at 2 months of age. This study failed to find a significant correlation.[275] No studies were identified to assess the association of the omega-3 fatty acids content in fetal biomarkers and the visual function outcomes. However, 21 studies assessed the question of the *association between child's omega-3 or omega6/omega-3 fatty acids biomarkers and the visual function outcomes*. Five studies included a preterm population,[185,198,212,278,279] while 16 included term infants. Of the five studies in the preterm group, three were RCTs,[185,198,212] and two were cross-sectional studies.[278,279] Of the 16 term infant studies, nine were RCTs,[138,182,203,248,262-264,269,270] and seven were observational studies.[140,271,275,278,280-282]

In all the preterm RCTs, the results were conflicting. In the study by Birch et al, the LCPUFA content of RBC DHA/DPA ratio correlated with both FPL and VEP at 57 weeks PCA.[212] Based on ANOVA, there was a statistically significant correlation between RBC DHA at 2 months and visual acuity at 2 and 4 months, in the Carlson et al. study.[185] Faldella et al. found a negative correlation between the RBC DHA and the N4 and P4 wave latency of the VEP at 52 weeks PCA.[198]

In two preterm cross-sectional studies, the results also were divergent.[278,279] Birch et al. found that the LogMAR (VEP) acuity was significantly associated with the end-product ratio [DHA n-3/DPA n-6] in total RBC lipids. For FPL acuity, the results were the same for both the breastfed and formula-fed groups.[278] Whereas, Leaf et al. observed a positive correlation between scotopic b wave (ERG) implicit time and percentage composition of DHA in both plasma and RBC PL. A similar relationship was seen with total omega-3 LCPUFA in both plasma and RBC PL. There was a positive correlation between both RBC AA and total omega-6 LCPUFA and scotopic a-b amplitude. No significant relationships were seen between photopic ERGs and either plasma or RBC LCPUFAs.[279]

Given the different designs and interventions (human milk or formula), it is very challenging to draw a conclusion in the preterm population.

In the term population, of the seven RCTs that had an infant intake, four[182,264,269,270] reported associations between milk or blood biomarkers (plasma/RBC DHA and/or AA content) and the

sweep VEP acuity measures. Of these trials, three[182,269,270] found statistically significant negative linear regression coefficients indicating that higher RBC DHA content was associated with a better sweep VEP acuity in infants at different age time points. The remaining study[264] suggested that the RBC DHA content was not associated with the measured sweep VEP acuity at 4 months of age. The results of both trials[182,264] that looked at the RBC EPA and AA content in relation to the measure of sweep VEP acuity, indicated that neither RBC AA nor EPA content was associated with the sweep VEP acuity during the first year of the infants' life. One study,[269] that investigated the relationship between infant's plasma DHA and AA content, found that higher plasma contents of both DHA and AA were associated with better sweep VEP acuity at 4 and 13 months of age.

The relationship between the infants' blood biomarkers and the measures of infant amplitude of VEP acuity were reported in two trials.[203,262] Both trials suggested that RBC DHA correlated negatively with the amplitude of VEP acuity (in log MAR), measured at 4[203,262] and 7.5[262] months of age (i.e., infants at 4 and 7.5 months of age who on average had a higher RBC DHA content, tended to have a lower log MAR or better VEP acuity). The former trial[203] also showed that there was no correlation between either plasma or RBC DHA content at 4 months of age, and the latency measure of VEP acuity obtained at either 4 or 8 months of age. The same trial,[203] however, found a statistically significant negative correlation between plasma-DHA content and the amplitude of VEP acuity both measured at 4 months of age.

One study reported the association(s) of the plasma DHA or RBC DHA content in relation to the measure of Teller's visual acuity.[263] The plasma or RBC DHA content did not correlate with the Teller's acuity, measured at 3 months of age.

Only two trials reported the associations between the infants' RBC DHA content and their stereoacuity (in log seconds) measured at 4[269] and 12[270] months of age. Both trials found that there was no association between the two factors.

The correlation between plasma- and RBC DHA and ERG parameters in infants was reported in one trial.[182] None of the Naka-Rushton parameters except for log k (in scotopic troland seconds) was significantly correlated with plasma or RBC DHA content at either 1.5 or 4 months of age. There was a statistically significant negative correlation between the RBC DHA content and log k in the infants at 1.5 months of age.

None of the RCTs that measured the association of the infant's biomarkers after exclusive breast milk intake and the visual acuity outcomes found any significant correlation.[138,248]

The seven observational studies were very heterogeneous in term of exposure characteristics and population, as well as outcomes. Most of them used breast milk as the main exposure, as well as formula. However, the overall association was that in four cross-sectional studies there was a nonsignificant correlation between infant's biomarkers and the visual acuity at any age.[140,275,281,282] In three studies, there was significant correlation between the biomarkers and the visual acuity.[271,278,280] Yet, the biomarkers and the outcomes were different in each. Birch et al. found that there was a positive correlation between the infant's RBC DHA/DPA ratio and the stereoacuity,[278] whereas, Makrides et al. observed a positive correlation between the RBC DHA and LA and the VEP (logMAR).[280] Finally, Innis et al. also detected a positive association between the RBC DHA at 2 months and the visual acuity (Teller's test) at 2 and 12 months of age, but not at 4 and 6 months of age.[271]

Overall, there was a lack of pattern of correlation between the infant's biomarkers in blood and the visual function outcomes across 21 studies that addressed this issue.

One RCT addressed the question regarding the *influence of maternal intake of omega-3 fatty acids during pregnancy on the cognitive development in infants*.[141] This study measured the cognitive development using the Fagan Test of Infant Intelligence at 6 and 9 months of age in the infants of mother who had taken either cod liver oil (DHA+EPA) or corn oil (LA+ALA) during pregnancy and lactation. There was no differences between groups in the novelty preference at both time points.[141] There was a follow-up study at 4 years of age that measured the Kaufman Assessment Battery for Children (K-ABC), which is a measurement of intelligence and achievement designed for children between 2.5 years and 12.5 years old.[354] The supplemented group (DHA+EPA) had significantly higher scores than children in the corn oil group (mothers) on the Mental Processing Composite of the K-ABC at 4 years old. However, the scores in the Sequential Processing Scale, the Simultaneous Processing Scale and the Nonverbal Scale among children who were born to mothers who were given cod liver oil were non statistically different from the control group.[141]

The latter relationship may be relevant, although the clinical importance of this result has yet to be determined. The potential confounders such as infant's diet after the exclusive breastfeeding, medications, supplements and other variables that could affect the results, were not measured at the time of the outcome (4 years of age).

Three studies were identified to respond to the question of *the influence of maternal content of omega-3 fatty acids in breast milk influences the cognitive development in infants*.[138,141,284] Two were RCTs[138,141] and one was a prospective cohort.[284] The study by Helland et al. was an RCT described above,[141] and the study by Gibson et al. was a double-blind RCT that included mother of term infants who intended to breastfeed.[138] They were randomized to receive five increasing doses of DHA (algal oil) during the first 3 months postpartum. The mean Bayley's MDI score did not differ between groups at 1 or 2 years of age.[138] The environmental factors that were associated with the Bayley's MDI at 1 year of age were the home stimulation test, partner smoking status, length of breastfeeding and the 3-month DHA status of breast milk and infant blood. The only one that was still correlated to the Bayley's MDI at 2 years was the home stimulation test.[138]

This study was underpowered to detect a significant difference between groups in the MDI scores, which makes it very difficult to draw a conclusion. There was no comparator without omega-3 fatty acids. The infants were fed solid foods before the measurements (Bayley's score), which can be a potential effect modifier.

Six average good quality (Jadad: 4.4/5) RCTs addressed the question of the *influence of formula intake, with or without breast milk, on the cognitive development of preterm infants*.[185,193,207,258,272,273] The main outcome measured was the Bayley's MDI score, at different time points in the five RCTs.[193,207,258,273,355] Overall, four of the five trials did not find that the supplementation of infant formula with omega-3 fatty acids had an effect on this particular outcome at 3, 6, 12, 18 and 24 months of age. This remained true even after controlling for potential effect modifiers such as site, gender, birth weight, maternal education, gestational age, and human milk intake, among others.[207] Except for one trial which found that sex was an important covariate, males in the supplemented formula group had a significantly higher score

than those in the control group at 18 months.[258] Only one study, which included preterm and term infants, found that the supplemented groups had greater scores than the control group at 118 weeks PMA, and the term infants had higher scores than the preterm infants.[193]

Regarding the Fagan test of Infant Intelligence outcome, two studies found a significant difference between the omega-3 fatty acids group and the control group.[185,207] Carlson et al. observed that the DHA group had significantly more discrete looks in the novelty test,[185] however, at 12 months the DHA-supplemented group had a significantly lower novelty preference compared with the control group. Whereas, O'Connor found that the DHA+AA (egg-TGL/fish) group had a significantly greater mean novelty preference look compared with the DHA+AA (fish/fungal) formula and the control group at 6 months.[207]

O'Connor et al. also found that there was no significant differences between groups in the Infant version of the MacArthur Communicative Development Inventories (a standardized parent-report instrument) at 9 months CA and 14 months CA.[207]

Meta-analysis was not possible given the heterogeneity across the studies for each of the different outcomes. This heterogeneity was observed in the intervention characteristics (meaning dose, source of omega-3 fatty acids, duration of intervention), cointerventions, and timing of the outcomes measures. Other potential confounding factors can be associated with the discrepancies in the study results such as background diet, breast milk intake, and environmental factors (parental education, stimulus at home, smoking status at home, etc), as well as the use of different assessment tools. It is thought that global measures of cognitive development (Griffith, Bayley, Brunet-Lezine scales) may not be sensitive enough to detect differences in normal infants supplemented with or without DHA. It is likely that specific functional tests (Fagan's test, Means-end problem solving test) would be more sensitive and specific in detecting these differences in assessing the adequacy of DHA intake on optimizing neurocognitive development. The more specific tests used during infancy have been shown to have a better correlation with testing later in childhood than the global infant tests.[356,357]

Overall, most of the studies did not find a significant effect of the omega-3 fatty acids supplementation in preterm infants on the cognitive developmental outcomes using the Bayley's MDI scale. Nonetheless, a question remains as to which would be the best instrument to measure this particular outcome. These conclusions are consistent with the meta-analysis done by Simmer and Patole.[349]

Eight good quality RCTs were identified to address the question of the *influence of omega-3 fatty acids supplementation of infant formula, with or without breast milk intake, on the cognitive development in term infants*.[104,182,203,205,223,227,265] The mean outcome that was measured across seven RCTs was the Bayley's MDI score at different time points.[104,182,203,205,227,265] All but one of the studies did not find a significant difference between groups (supplemented vs. control) in this outcome at 6, 12 and 18 months of age. Only Birch et al. observed that the DHA+AA group had a significantly higher score compared with the control group at 18 months of age.[182]

There were five other different cognitive outcomes measured across the trials. The Knobloch, Passamanik, and Sherrards Development Screening Inventory test, performed at 9 months of age in the study by Lucas et al., and the Fagan Test of Infant Intelligence, performed at 6 and 9 months of age in two other trials by Auestad et al., did not reveal an effect with omega-3 fatty acids supplementation.[227,265] The IQ (Stanford-Binet), Receptive Vocabulary

(PPVT-R), Expressive Vocabulary, and Visual-Motor Index scores, as well as the Problem-Solving scores, did not differ between groups in two studies.[104,223]

Regarding the Infant version of the MacArthur Communicative Development Inventories, Auestad et al. found that the DHA group had a significantly lower vocabulary production score compared with the control group at 14 months of age.[104] Yet, the other Auestad et al. study found that at 14 months, the DHA+AA (fish/fungal) group had a significantly higher vocabulary expression score than those fed with DHA+AA (egg-TG) supplemented formula.[227] Both Auestad et al. studies did not reveal a between-group significant difference at 9 months.[227]

A meta-analysis of the main outcome used across the trials, the Bayley's MDI score at 12 months of age, was performed. Three studies were identified to be appropriately comparable in terms of type of supplementation (DHA+AA) and population characteristics (healthy term infants).[104,205,227] The overall size of the effect was nonstatistically different between study groups.

Overall, it appears that the supplementation with omega-3 fatty acids does not have an effect on the cognitive development outcomes. These conclusions are consistent with the meta-analysis done by Simmer in 2004.[350] Although the design of the studies is very appropriate, they have some limitations. The studies did not measure the total dose of omega-3 or omega-6 fatty acids contained in the formulas, since they failed to account for the total amount of formula intake per day. They also were unsuccessful in controlling for background diet, in the infants (from 4 months of age) and the mothers (breastfed infants). There were also discrepancies in the intervention length and the outcome measures (e.g., formula given until 4 months of age and Bayley's MDI measured at 12 months of age) within each trial and across all the included studies.

An attempt to control for potential confounders was appropriately done in almost all the studies. However, none of them use the omega-3 fatty acids dose as a covariate. Instead, they used the plasma or RBC DHA content, or the type of diet.

Only one study allowed the infants to be breastfed as a cointervention.[227] Nevertheless, the use of both supplemented formula and breast milk, did not show an effect on the cognitive development when compared with breast milk alone (control formula).

No studies were identified to answer the questions of the *association of omega-3 or omega-6/omega-3 fatty acids content of maternal or fetal biomarkers and the cognitive development in term or preterm infants*.

Six studies addressed the question of the *association of omega-3 or omega-6/omega-3 fatty acids content of child biomarkers and the cognitive development in term infants.*[138,182,203,205,271,285] Four of them were good quality RCTs,[138,182,203,205] and two were single prospective cohort studies.[271,285] There were no studies identified to address the same question in preterm children.

Gibson et al found that the infants were exclusively breastfed for 3 months. There was a significant correlation between the Bayley's MDI score at 1 year old and DHA indices in plasma and RBC at 12 weeks of age, yet this correlation was not seen at 2 years of age.[138]

Birch et al. found that the MDI score at 18 months was positively correlated with plasma and RBC DHA at 4 months of age. None of the other plasma biomarkers (LA, AA, ALA, EPA) were

correlated with the MDI at 18 months, however the RBC-LA and RBC ALA were negatively correlated with the MDI at 18 months of age.[182] None of the biomarkers measured at 12 months of age were correlated with the MDI at 18 months of age.[182]

Jensen et al. and Makrides et al. did not observe a significant correlation between the PUFA content in infant's plasma and RBC, and the Bayley's MDI at 1 and 2 years of age.[203,205] However, these studies used a different type of intervention—Jensen et al. used increasing ratios of LA/ALA in four groups,[203] whereas, Makrides et al. used three formulas with LCPUFAs (DHA+AA vs. DHA alone vs. control).[205]

Finally, both observational studies failed to find a significant correlation between the biomarkers and the cognitive outcomes.[271,285] Innis et al. did not find a statistically significant relation between the infant RBC DHA or AA status at 2 months of age and the Bayley's MDI score at 6 and 12 months of age, as well as the Novelty Preference at 6 and 9 months.[271] Ghys et al. did not observe a correlation between the DHA and AA concentration in infant's plasma or RBC and the cognitive development at 4 years of age. Small but significant associations occurred with maternal IQ, birth weight, duration of breast-feeding, maternal smoking during pregnancy, and paternal educational attainment.

Meta-analysis of these associations was not possible given the differences in the intervention characteristics, as well as in the timing of the blood samples and the cognitive outcomes measures. In general, there are discrepancies in the results related to the association between the child's biomarkers and the cognitive developmental outcomes.

Clinical Implications

The intake of omega-3 fatty acids in the present review's collection of interventional studies by maternal and child populations did not appear to be associated with moderate or severe adverse events. Supplementation studies enrolling pregnant women typically utilized controlled, capsule delivery of relatively simple interventions (e.g., fish oil, containing DHA); and, supplementation appeared to be well tolerated, with some mild, mostly gastrointestinal events occurring occasionally. A similar pattern was observed in supplementation studies with child populations. However, a few factors make it very difficult to identify the specific or collective safety profiles of the individual omega-3 fatty acids in studies investigating their influence on child outcomes.

First, there was a wide variety of types of omega-3 fatty acid employed in these studies. Second, more than just a single omega-3 fatty acid was typically employed in these pediatric trials. The latter observation likely has strong implications for what can be understood as the meaningfulness of possible differences or similarities in the adverse event profiles associated with the respective study groups (i.e., "intervention," "control"), even in RCTs considered well-controlled in other ways (e.g., allocation concealment; blinding).

In a study comparing the effects of DHA and an olive oil placebo (i.e., "no-DHA"), for example, typically added to the active and placebo formulations are the exact same constituents (e.g., other omega-3 fatty acids; omega-6 fatty acids; iron; anti-oxidants). However, the possibility that individually or collectively these cointerventional or background elements could

"interact"—metabolically speaking—differently with DHA and olive oil to potentially produce different "synergistic" influences on clinical outcomes suggests that, in these studies: a) what is meant by the "intervention" and "control" is more complicated than a simple distinction between "DHA present" and "DHA absent;" and b) the exact absolute and relative influences of DHA on clinical outcomes in this example cannot be readily isolated. Especially problematic for interpretation are those interventional studies whose specific cointerventional or background constituents included various other omega-3, omega-6 or omega-9 fatty acids, which constitute various metabolites along the metabolic pathway (from the parent EFAs, LA and ALA). In short, the dynamic interplay among these fatty acid contents (e.g., competition for enzymes), and how this interplay may influence outcomes, may differ in important ways depending on whether DHA or olive oil is added to this combination of cointerventional or background constituents.

Thus, the ability to reliably associate the presence, or absence, of specific adverse effects with specific omega-3 fatty acids may be impeded by the inclusion of background constituents within studies of formula supplementation. At best, inferences may be drawn with respect to often very complex combinations of constituents. This research strategy adds considerable "noise" to studies, which precludes the identification of clear "signals" regarding the adverse effects associated with specific omega-3 fatty acids. Moreover, definitions of interventions in the different studies were often diverged, even though they appeared to share the same key active ingredient, such as "DHA." This clinical heterogeneity complicated attempts to compare studies.

The evidence pertaining to the possible impact of supplementation with omega-3 fatty acids on predefined pregnancy outcomes showed either evidence of no effect, or the results were inconclusive. Results suggested the absence of effects with respect to the impact of supplementation on the incidence of GHT, preeclampsia or eclampsia, as well as on infants being born SGA (measured via birth weight and incidence of IUGR). However, regarding evaluations of the duration of gestation, some discrepancies were observed, although most of the studies failed to detect a statistically significant effect.

Regarding the questions of the biomarker content during pregnancy, and its possible association with pregnancy outcomes, nothing conclusive can be asserted. There was considerable heterogeneity in the research designs (i.e., experimental versus observational), the types of biomarker that were evaluated, the timing of these measurements, and the types of intervention given to study participants (i.e., source of omega-3 fatty acids; omega-6 fatty acids; omega-6/omega-3 ratio intake).

Overall, results concerning the impact of the intake of omega-3 fatty acids on the development of infants are primarily, although not uniformly, inconclusive. The inconsistencies in study results may be due to differences in the: a) definitions of the type and source of omega-3 fatty acids; b) omega-6/omega-3 fatty acid intake ratio in the intervention, the background diet, or both; c) absolute and/or daily amounts of formula supplementation received by the children; or, d) duration of the intervention. Most of the studies did not control for the absolute or daily amounts of formula ingested by the child populations, which lessens our ability to draw unequivocal inferences about the value of this supplementation. Moreover, making clear sense of the absolute or relative effects of individual omega-3 fatty acids, or even omega-3 fatty acid combinations, on child outcomes is complicated by the same problem of "noise" described with

respect to the safety evidence in child supplementation studies. It is very difficult to reliably ascribe definite benefits, or the absence thereof, to specific omega-3 fatty acids.

Looking at specific categories of child outcome, growth patterns were not affected by the intake of omega-3 fatty acids via human milk or formula supplementation in either term or preterm infants. With biomarker data obtained exclusively from infant population sources, results across the different studies concerning the association between child biomarker content and growth outcomes were inconsistent, and thus inconclusive.

The neurological development outcomes were influenced somewhat by the omega-3 fatty acids supplementation of infant formula in preterm infants,[193,207] although not all of the studies found evidence for a benefit. Overall, however, the results must be considered inconclusive for preterm offspring. On the other hand, term infants did not receive any benefit from the intervention in the short- or longterm. A reliable association between infant biomarker content and neurological outcomes for both term and preterm infants was not supported, because of the lack of consistency in the results across the studies.

Visual function outcomes provided the most inconsistent data in both the preterm and term infant populations. This suggests an inconclusive response to the question of the value of omega-3 fatty acid intake for visual development. This same observation characterizes the results concerning the association between biomarker content and visual outcomes.

In the preterm population, the only type of clinical outcome that showed a significant favorable effect related to the intake of omega-3 fatty acids was the Fagan test of Infant Intelligence (i.e., "novelty preference looks") at 6 and 12 months of age.[185,207] It assesses cognitive function. However, the scores on the Bayley's Developmental Index (MDI) were not influenced by infant supplementation at any age.[185,207,258,273] In most of these studies, the intervention was stopped months before the final cognitive assessment was performed (i.e., 12 or 18 months). This observation suggests a likely problem in interpreting the results. Between the end of the intervention period and the final cognitive evaluation, dietary intake was not measured and controlled for analytically. This factor may have contributed to what was observed at the final outcome evaluation. Other factors that could have influenced the outcomes included child illnesses, perceptual-cognitive stimulation, smoking, and parental education.

In the term population, while there was some disagreement in results across the trials, most of them reported a lack of effect using the Bayley's MDI. The association between biomarker content and cognitive outcomes has yet to be determined.

In summary, definitions of the maternal population in studies of pregnancy outcomes varied considerably, yet no conclusive evidence for benefit was identified. Results based on both term and preterm study populations were also inconclusive, although these studies typically entailed interventions of the complex nature discussed earlier. Thus, when it came to the set of child developmental/health questions investigated in our review, it must be asked whether or not the included studies could have been expected to provide unequivocal evidence regarding the value of all, or individual, omega-3 fatty acids in influencing child health? Could these studies have been expected to permit the isolation of the impact of the omega-3 fatty acids in these populations? That said, had the results been conclusive one way or the other, much of the included research studies lacked strong applicability to the North American population.

What, then, are the research implications?

Research Implications and Directions

Questions for which no evidence was identified clearly require empirical studies. The studies enrolling child populations typically exhibited sound quality, defined in terms of Jadad total scores. However, these investigators typically failed to design studies where the specific effects of omega-3 fatty acids could be isolated. While this outcome may have been necessary, given the expectation that all of the constituents were likely important for child health, the results were difficult to interpret. Biomarkers measure the content of specific fatty acids of different lipid fractions in plasma (individual fatty acids or content in triglycerides, cholesterol esters or phospholipids), cell membranes (red blood cells, platelets) or tissues (such as adipose, umbilical cord). These biomarkers are used to reflect dietary intake or as a surrogate measurement of the fatty acid content of various tisses that are not readily available for measurement. The essential n-3 and n-6 fatty acids content of these biomarkers reflect the exogenous intake of these fatty acids within hours to years. The inherent difficulty with using membrane and accessible tissue biomarkers as surrogate measurements of the fatty acid content of for example, the brain or retina, is the difference in preferential deposition of these fatty acids in different membranes and tissues and the rate of turnover. For example, DHA is preferentially accumulated in the brain and retina but not in the red cell membrane. As well, once DHA is deposited in the brain and retina, the amount is relatively resistant to turnover even with subsequent dietary n-3 fatty acid or DHA deficiency, whereas RBC membrane levels would decrease.

During different stages in life there are changes fatty acid metabolism, storage and turnover that affect the fatty acid profile of the various biomarkers. The choice of biomarker is dependent on the intervention and outcome of interest. For example, during pregnancy there are significant changes in lipid metabolism with increased fat storage in the early stages and mobilization in the later stages. If the outcome of interest is the effect of maternal intake of n-3 fatty acids on pregnancy outcomes, then markers that reflect shorter term dietary intake should be used (plasma lipid fractions, RBC membranes). During periods of growth and development in infancy, there is rapid accumulation of n-3 fatty acids that are preferentially deposited in neurologic tissues, which may not be reflected in the available biomarkers. Again, it is likely that RBC membrane fatty acid content more closely reflects the content in neurologic tissue than from plasma or adipose tissue. It is clear that further research is required to establish the predictive value of available biomarkers or the development of new biomarkers of n-3 fatty acid status on clinical outcomes.

One key implication is that the most likely question that the included child outcome studies might have been able to address is whether formula supplementation “cocktails,” which included at least one type of omega-3 fatty acid content, could provide a benefit to child health. The overarching question concerning the role of omega-3 fatty acids in child health that we aimed to address with this review might have been too narrow especially in light of: a) expectations that the omega-6 fatty acids alone (e.g., AA), or possibly in combination with the omega-3 fatty acids, might substantially influence child health; and b) knowledge that the available, relevant studies invariably employed interventions including elements other than the omega-3 fatty acids.

Thus, one key contribution of our review may be that we have now raised an additional question: can questions concerning the possible impact of any of the EFAs on child health be conceived without concurrently considering the (e.g., interactive) roles of both the omega-3 and omega-6 fatty acids?

That said, one possible strategy for research entails defining interventions according to specific omega-6/omega-3 fatty acid intake ratios, which would be achieved via the co-modification of the intake of omega-3 and omega-6 fatty acids. While the ideal design with which to test questions of efficacy is the RCT, pilot work using less complex designs would need to be done first. These would help establish intake ratios with some potential to benefit child outcomes. It might then be observed that different intake ratios positively influence different developmental outcomes, or yield different safety profiles.

Decisions as to the "appropriate" or "reasonable" intake ratios for use as interventions in RCTs could then be made based on what is considered an acceptable benefit/safety profile and/or what are the most important outcomes—and the timing of their assessment—requiring modification. It may turn out that in a preliminary cohort study, exposure to EFAs is most beneficial for early neurodevelopment.

Evidence concerning the metabolic interplay of the fatty acid contents in biomarkers might also help shape the "appropriate" or "reasonable" intake ratio. This preliminary work could demonstrate that certain combinations of fatty acids actually produce antagonistic, rather than synergistic, effects, metabolically speaking. In this way the optimal combinations of EFA (e.g., DHA+AA), and sources thereof (e.g., marine, plant) could be identified, including circumstances where it is an antagonistic metabolic dynamic that is desired, since it appears to produce important clinical effects. Work with biomarker data could thus be helpful in designing studies and not just as a means to predict clinical outcomes, or to make sense of relationships between patterns of EFA intake and clinical outcomes. Nevertheless, to produce readily interpretable results, at least two additional strategies would be helpful.

First, the nutrients obtained via the background diet would also need to be factored into the definition of the intake ratios. Second, to control for the possibility that it is the volume of intake of supplementation that positively influences child outcomes, daily or weekly amounts of intake should be measured, and the corresponding data are entered into covariate analysis. For ethical reasons, this approach would likely be preferable to one whereby a minimum or maximum volume of intake is established.

These strategies would complement the other, typically necessary research-design elements, and maximize the meaningful interpretability of even RCT results (e.g., control for caloric/energy intake across study groups). Data regarding the maternal preconceptional and perinatal diets should be retrieved before a study begins.[353] Data concerning the maternal diet during pregnancy or breastfeeding may help explain (the lack of) beneficial effects with respect to child outcomes. Likewise, data regarding the dietary intake of children following the termination of the intervention period (e.g., at 4 months), yet preceding a longer term followup (e.g., at 12 months), need to be collected to help explain (the lack of) beneficial effects on child outcomes.

Many of these variables were not assessed in the studies focusing on child outcomes in our review. Failure to control for these or other variables, either experimentally or analytically,

complicate or preclude the meaningful interpretation of results. Also very important is the need to take into account the possible influences of key confounders, such as mother's smoking or alcohol consumption. If it is assumed that EFA content in mother's biomarkers may be associated with child outcomes, then these and other factors with the ability to negatively influence the fatty acid content of biomarkers need to be evaluated. These factors are likewise important when trying to make sense of maternal outcome data.

Future child outcome trials will always be faced with the problem of selection bias inherent in appropriately giving women the choice of whether or not to breastfeed, and then excluding those who decide to breastfeed from being randomized to study groups varying in terms of the constituents defining formula supplementation. As we did in this review, data from children of mothers who breastfed can be used as a reference point from which to understand results produced by supplementation. That said, it must also be appreciated that the choice not to breastfeed could also influence child outcomes in ways that are as yet unclear.

The relevance of the instruments chosen by the investigators to measure the neurological development, cognitive development and visual function are perhaps open to debate. Future research might benefit from the work of a panel to establish the most important outcome constructs as well as the most reliable and valid instruments. Candidate outcomes and instruments should include, yet without being restricted to, those instruments utilized in studies included in our review (e.g., Bayley's Developmental Index, Fagan test of Infant Intelligence, EEG).

Regarding pregnancy outcomes, the issue of the length of the omega-3 fatty acid intervention may be an important one. Most of the studies initiated the intervention during the second or third trimester of pregnancy. Almost none provided it before, or at the beginning of, the pregnancy. One empirical question is whether or not ingesting omega-3 fatty acids for a longer period of time might increase their contents in maternal stores, which in turn could have a beneficial impact on maternal or child outcomes.

Most of the interventions given to maternal populations identified in our review were relatively simple, in that they did not contain the myriad constituents such as those received in formula supplementation studies. However, while the problem of "noise" discussed above with respect to child outcome studies typically did not characterize the maternal outcome investigations, studies relating to pregnancy outcomes might consider concurrently modifying the intake of both omega-3 and omega-6 fatty acid contents for the purposes of evaluating the possible beneficial impact of specific omega-6/omega-3 fatty acid intake ratios.

In preparing such intake ratio interventions, the exact source, type and doses of fatty acids will require definition in pilot work. As with interventions given to child populations, the efficacy and safety of those provided to maternal populations needs to be balanced. Moreover, the possible interactions of fatty acid contents and other types of supplementation routinely taken during pregnancy (e.g., vitamins, iron) should likely be fully understood to assure that positive clinical outcomes are afforded.

In the studies of pregnancy outcomes per se, a number of factors need to be controlled either experimentally (e.g., stratification) or analytically, which will permit meaningful inferences to be drawn from results. These variables include the maternal background diet, smoking, alcohol

consumption, obstetric history, other supplementation, medication, and socioeconomic status. Most of the included studies did not control for maternal background diet, for example.

Future studies hoping to investigate the possible role played by biomarker data—obtained from the mother, fetus or child—in understanding the relationship between the intake of specific nutrients and clinical-developmental outcomes should likely be undertaken as an integral part of RCTs evaluating this relationship. Observational studies lack the types of controls required to best minimize bias from known and unknown confounders. The timing of the measurement of biomarker data is also very important. If an argument can be made to conduct followup assessments of clinical-developmental outcomes at specific time points, or according to specific milestones, then it might be reasonable to evaluate the fatty acid content of biomarkers at these same times. If there is no concurrence in the measurement of these two classes of outcomes, then it may be difficult to detect the most meaningful parallels in the respective patterns of results.

Finally, in order to maximize the applicability of the evidence to the reference standard established in our review—the North American population—it would be helpful to conduct more research in North America. Furthermore, evidence concerning otherwise healthy populations should likely be obtained, before attempts are made to understand the interrelationships among intake, biomarkers, and clinical-developmental outcomes in populations with specific disorders or problems (e.g., celiac disease; malnutrition).

Limitations of the Review

One of the main limitations was that we did not investigate studies assessing the possible impact of the intake of omega-3 fatty acids on the fatty acid content of biomarkers. While it might be assumed that omega-3 fatty acids, when ingested, eventually find their way into pertinent biomarkers, it may be the case that it is actually the failure to become incorporated in pertinent biomarkers that prevents (some or all of) the fatty acid contents from positively influencing clinical-developmental outcomes. Thus, problems complicating or preventing their accretion should likely be understood before interpretations can be accepted that omega-3 fatty acids have no effect on clinical-developmental outcomes in various populations.

Another limitation is the difficulties that we were faced to identify studies that addressed some of the questions, specially the association between fetal biomarkers and clinical outcomes and the influence of other sources of omega-3 fatty acids on the child's clinical outcomes.

Safety data obtained from RCTs are typically under-reported. Thus, the exclusive focus on RCT evidence for certain questions in our review may have allowed us to miss key adverse effects data contained in reports of studies employing less inherently rigorous types of study design.

The quality assessment of observational studies was conducted using items we modified from existing instruments. A design-specific, total quality score was then generated for each study, from which a single summary value was derived. (i.e., A, B, C). This simplification permitted the entry of these values into summary matrices. However, the design-specific cutpoints used to assign these values were established without any validational basis, and so their value is likely

extremely limited. The modified instruments themselves were also never subjected to a validational exercise. The applicability indices, while continuing the work we did when we systematically reviewed the evidence for the health effects of omega-3 fatty acids on asthma,[163] likewise did not receive validational support.

We recognize that the issue of investigating the possible impact of the background diet's omega-6/omega-3 fatty acid intake ratio within studies evaluating the health effects of omega-3 fatty acids is a very complex one. There are many ways to produce the same ratio, for example. Ratios of 30:2 and 15:1 are equivalent, yet the absolute amounts may also need to be taken into consideration when appreciating the possible benefits of omega-3 fatty acid supplementation. Moreover, there are multiple definitions of each of these classes of fatty acid (i.e., omega-3 vs omega-6 fatty acids), and the types of dynamic metabolic interaction between fatty acids appears to depend greatly on which fatty acids are involved. One likely needs to distinguish the absolute and relative amounts of the short- versus long-chain fatty acids, for example. EPA and DHA (i.e., long-chain omega-3 fatty acids) have markedly different metabolic properties than ALA (i.e., short-chain omega-3 fatty acid). The same may be said about LA (i.e., short-chain omega-6 fatty acid) when compared with AA (i.e., long-chain omega-6 fatty acid). The interaction of EPA and AA is different from the interaction of DHA and AA. Moreover, AA and DHA do not compete for positions in cell membrane phospholipids: AA may be found in PI, while DHA is contained in PS and PE. That said, future research might end up concluding that especially in the North American diet—where much more omega-6 fatty acid content is consumed when compared with omega-3 fatty acid content—the best way to alter the omega-6/omega-3 fatty acid intake ratio is to focus exclusively on increasing the intake of (especially long-chain) omega-3 fatty acids.

Finally, time constraints made it impossible to perform additional meta-analysis of other time points relating to the neurological and cognitive outcomes.

Conclusion

Studies investigating the influence of omega-3 fatty acids on child and maternal health revealed the absence of a notable safety profile (i.e., moderate-to-severe AEs). Pregnancy outcomes were either unaffected by omega-3 fatty acid supplementation, or the results were inconclusive. Results suggested the absence of effects with respect to the impact of supplementation on the incidence of GHT, preeclampsia or eclampsia, as well as on infants being born small for gestational age. However, regarding evaluations of the duration of gestation, some discrepancies were observed, although most of the studies failed to detect a statistically significant effect. Biomarker data failed to clarify patterns in pregnancy outcome data.

Results concerning the impact of the intake of omega-3 fatty acids on the development of infants are primarily, although not uniformly, inconclusive. The inconsistencies in study results may be attributable to numerous factors.

In addition, making clear sense of the absolute or relative effects of individual omega-3 fatty acids, or even omega-3 fatty acid combinations, on child outcomes is complicated or precluded by the following problem. Studies typically employed interventions that involved various

cointerventional or background constituents (e.g., omega-6 fatty acids), yet whose metabolic interactions with the omega-3 fatty acid(s) were not taken into account in interpreting the results. The dynamic interplay among these fatty acid contents (e.g., competition for enzymes), and how this interplay may influence outcomes, may differ in important ways depending on whether DHA or olive oil is added to this combination of cointerventional or background constituents, particularly in the maternal population. This strategy prevented the isolation of the exact effects relating to the omega-3 fatty acid content. It is thus very difficult to reliably ascribe definite child outcome-related benefits, or the absence thereof, to specific omega-3 fatty acids. Biomarker data failed to clarify patterns in child outcome data.

Future research should likely consider investigating the impact of specific omega-6/omega-3 fatty acid intake ratios, in no small part to control for the possible metabolic interactions involving these types of fatty acid. To produce results that are applicable to the North American population, populations consuming high omega-6/omega-3 fatty acid intake ratios should likely be randomized into trials also exhibiting better control of confounding variables than was observed, especially in the present collection of studies of child outcomes.

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Abbreviations

AA (20:4 n-6)	Arachidonic acid
ACP	Teller Acuity Card Procedure
AE	adverse effects
AGA	Adequate for gestational age
AHRQ	Agency for Healthcare Research Quality
AI	Adequate Intake
ALA (18:3 n-3)	Alpha linolenic acid
ALSPAC	Avon Longitudinal Study of Pregnancy and Childhood
ANCOVA	analysis of co-variance
ANOVA	analysis of variance
AS	active sleep
BAEP	Brainstem auditory evoked potentials
BAEP test	brainstem auditory evoked potential
BMI	body mass index
BP	Blood pressure
BRS	Behavioral Rating Scale
C5a	Complement fragment 5a
CA	corrected age
CAM	complementary alernative medicine
cAMP	Cyclic adenosine monophosphate
CNS	central nervous system
COX	Cyclooxygenase
CSFII	Continuing Survey of Food Intakes by Individuals
DHA (22:6 n-3)	Docosahexaenoic acid
DPA	docosapentaenoic acid
DQ	developmental quotient
DTS	Dense tubular system
EAR	Estimated Average Requirement
EEG	Electroencephalogram
EFA	Essential fatty acid
EPA (20:5 n-3)	Eicosapentaenoic acid
ERG	Electroretinogram
ERG	electroretinogram
FA	fatty acids
FLP	Forced Choice Preferential Looking Procedure
GA	Gestational age
GHT	Gestational hypertension
GLA (18:3 n-6)	Gamma linolenic acid

GM DQ	gross motor developmental quotient
GOS	Groningen Developmental Scale
HC	Head circumference
HDL	High density lipoprotein
HM	Human milk
IFN	Interferon
IgE	Immunoglobulin E
IL	Interleukin
ITT	intention-to-treat
IUGR	Intra-uterine growth retardation
K-ABC	Kaufman Assessment Battery for Children
LA (18:2 n-6)	Linoleic acid
LBW	low birth weight
LC PUFA	Long-chain polyunsaturated fatty acid
LDL	Low density lipoprotein
LGA	large for gestational age
LT	Leukotriene
MAR	Minimal angle of resolution
MDI	Mental developmental index
MDI	Mental Developmental Index (Bayley Scales of Infant Development)
MJ/day	daily energy intake
MLR	multiple linear regression
MLU	Mean length of utterance
MRC	Medical Research Council
NCV	Nerve conduction velocity
NEC	Necrotizing enterocolitis
NEC	Necrotizing enterocolitis
NHANES III	National Health and Nutrition Examination (NHANES III)
NICU	neonatal intensive care unit
NIH	National Institutes of Health
NOS	Newcastle and Ottawa Scale
ODS	Office of Dietary Supplements
OPL	Operant preferential looking
PC	phosphatidyl choline
PCA	post conceptional age
PDI	Psychomotor developmental index
PDI	psychomotor developmental index
PE	phosphatidyl ethanolamine
PG	Prostaglandin

PGI2	antiaggregatory prostacyclin
PL	Phospholipids
PPAR	Peroxisome proliferator activated receptor
PPVT-R	Peabody Picture Vocabulary Test - Receptive Vocabulary
PUFA	Polyunsaturated fatty acid
QS	quiet sleep
QUORUM	Quality of the reporting of meta-analysis
RBC	red blood cells
RCT	Randomized Controlled Trial
RDA	Recommended Dietary Allowances
SCN	special care nursery
SCO	single cell oils
SD	standard deviation
SEMs	standard errors of the means
SGA	Small for gestational age
SIDS	sudden infant death syndrome
Sp	species
SREBP	Sterol regulatory element binding protein
TEP	technical expert panel
Tg	Triglycerides
TGL	triglyceride
TNF	Tumor necrosis factor
TPN	Total parenteral nutrition
Tx	Thromboxane
TXA2	proaggregatory thromboxane
UK	United Kingdom
USDA	United States Department of Agriculture
VEP	Visual evoked potentials
VLBW	Very low birth weight
VLDL	Very low density lipoprotein
VLN-3FA	very long chain n-3 fatty acids
WAIS-R	Wechsler Adult Intelligence Scale - Revised
WMD	weighted mean difference

Appendix A. Search Strategies

Search Strategy 1

Ovid interface for Medline, MEDLINE(R) In-Process & Other Non-Indexed Citations, Embase, Cochrane Central Register of Controlled Trials, CDSR, DARE

1. exp growth/
2. exp child development/
3. Gestational Age/
4. (Gestat$ and (age$ or durat$ or week$)).tw.
5. Infant, Premature/
6. exp Infant, Low Birth Weight/
7. (Prematur$ or preterm or pre-term).mp.
8. ((Infant$ or baby) adj3 (low adj3 (birthweight or weight))).mp.
9. ((Infant$ or baby or birth) adj3 (prematur$ or gestational age)).mp.
10. (newborn or neonatal).mp.
11. Retinopathy of Prematurity/
12. retrolental fibroplasia$.mp.
13. Retinopathy of Prematurity.tw.
14. or/1-13
15. Fetal Growth Retardation/
16. exp "Embryo and Fetal Development"/
17. exp Fetus/
18. ((fetal or fetus or intrauterine) adj3 (growth or develop$)).mp.
19. or/15-18
20. Pre-Eclampsia/
21. Preeclamp$.mp.
22. (Pregnan$ adj10 Toxemia$).mp.
23. ((Gestation$ or pregnan$) and (hypertens$ or toxemia$)).mp.
24. gestat$.mp.
25. 24 and (child$ or newborn$ or infan$ or neonat$ or baby or babies or pediatr$ or paediatr$).tw.
26. or/20-23,25
27. exp fatty acids, omega-3/
28. fatty acids, essential/
29. Dietary Fats, Unsaturated/
30. linolenic acids/
31. exp fish oils/
32. (n 3 fatty acid$ or omega 3).tw.
33. docosahexa?noic.tw,hw,rw.
34. eicosapenta?noic.tw,hw,rw.
35. alpha linolenic.tw,hw,rw.
36. (linolenate or cervonic or timnodonic).tw,hw,rw.
37. menhaden oil$.tw,hw,rw.
38. (mediterranean adj diet$).tw.

39. ((flax or flaxseed or flax seed or linseed or rape seed or rapeseed or canola or soy or soybean or walnut or mustard seed) adj2 oil$).tw.
40. (walnut$ or butternut$ or soybean$ or pumpkin seed$).tw.
41. (fish adj2 oil$).tw.
42. (cod liver oil$ or marine oil$ or marine fat$).tw.
43. (salmon or mackerel or herring or tuna or halibut or seal or seaweed or anchov$).tw.
44. (fish consumption or fish intake or (fish adj2 diet$)).tw.
45. diet$ fatty acid$.tw.
46. or/27-45
47. dietary fats/
48. (randomized controlled trial or clinical trial or controlled clinical trial or evaluation studies or multicenter study).pt.
49. random$.tw.
50. exp clinical trials/ or evaluation studies/
51. follow-up studies/ or prospective studies/
52. or/48-51
53. 47 and 52
54. (Ropufa or MaxEPA or Omacor or Efamed or ResQ or Epagis or Almarin or Coromega).tw.
55. (omega 3 or n 3).mp.
56. (polyunsaturated fat$ or pufa or dha or epa or long chain or longchain or lc$).mp.
57. 55 and 56
58. 46 or 53 or 54 or 57
59. 14 and 58
60. limit 59 to all child <0 to 18 years>
61. 19 and 58
62. limit 61 to human
63. 26 and 58
64. limit 63 to human
65. or/60,62,64

Search Strategy 2

CAB Health on Silverplatter

#1 growth in SU
#2 "postnatal-development" in SU
#3 "cognitive-development" in SU
#4 child* develop* in ti,ab,id
#5 psychomotor develop* in ti,ab,id
#6 (Gestat* and (age* or durat* or week*)) in ti,ab,id
#7 premature infants in SU
#8 low birth weight infants in SU
#9 (Prematur* or preterm or pre-term) in ti,ab,id
#10 (newborn* or neonatal*) in ti,ab,id
#11 ((Infant* or baby or babies or birth*) near3 (prematur* or gestational age)) in ti,ab,id

#12 ((Infant* or baby or babies) near3 (low near3 (birthweight or weight))) in ti,ab,id
#13 retinopathy in SU
#14 Retinopathy of Prematurity in ti,ab,id
#15 retrolental fibroplasia* in ti,ab,id
#16 #1 or #2 or #3 or #4 or #5 or #6 or #7 or #8 or #9 or #10 or #11 or #12 or #13 or #14 or #1535419
#17 fetal growth in SU
#18 gestation period in SU
#19 explode embryonic development in SU
#20 explode fetus in SU
#21 ((fetal or fetus or intrauterine) near3 (growth or develop*)) in ti,ab,id
#22 #17 or #18 or #19 or #20 or #214221
#23 Preeclampsia in SU
#24 pregnancy toxaemia in SU
#25 Preeclamp* in ti,ab,id
#26 Pre-eclamp* in ti,ab,id
#27 Toxemia*in ti,ab,id
#28 ((Gestation* or pregnan*) and hypertens*) in ti,ab,id
#29 pregnancy-induced hypertens* in ti,ab,id
#30 (gestat* in ti,ab,id) and ((child* or newborn* or infan* or neonat* or baby or babies or pediatr* or paediatr* or human) in ti,ab,id)+A71
#31 #23 or #24 or #25 or #26 or #27 or #28 or #29 or #304754
#32 baby* or babies* or newborn* or infan* or neonat* or preschool* or pre-school* or child*115761
#33 (#22 or #31 or (#16 and #32)) and (man in od)16359
#34 omega 31043
#35 ("essential-fatty-acids" in SU) or ("linolenic-acid" in SU)1895
#36 ("docosahexaenoic-acid" in SU) or ("eicosapentaenoic-acid" in SU)1440
#37 explode "plant-oils" in SU
#38 explode "fish-oils" in SU
#39 "fish-consumption" in SU
#40 "polyenoic-fatty-acids" in SU
#41 "polyunsaturated-fats" in SU
#42 "dietary-fat" in SU
#43 (n 3 fatty acid* or omega 3) in ti,ab,id
#44 (docosahexanoic or docosahexaenoic) in ti,ab,id
#45 (eicosapentanoic or eicosapentaenoic) in ti,ab,id
#46 (alpha linolenic)in ti,ab,id
#47 (linolenate or cervonic or timnodonic) in ti,ab,id
#48 (mediterranean diet) in ti,ab,id
#49 ((flax or flaxseed or flax seed or linseed or rape seed or rapeseed or canola or soy or soybean or walnut or mustard seed or menhaden) and oil*) in ti,ab,id
#50 (walnut* or butternut* or soybean* or pumpkin seed*) in ti,ab,id
#51 (fish oil* or cod liver oil* or marine oil* or marine fat*) in ti,ab,id
#52 (salmon or mackerel or herring or tuna or halibut or seal or seaweed or anchov*) in ti,ab,id
#53 (fish consumption or fish intake) in ti,ab,id

#54 (diet* fatty acid*) in ti,ab,id
#55 (ropufa or maxepa or omacor or efamed or resq or epagis or almarin or coromega) in ti,ab,id
#56 ((omega 3 or n 3) and (polyunsaturated fat* or pufa or dha or epa or long chain or longchain or lc*)) in ti,ab,id
#57 "long-chain-fatty-acids" in SU
#58 (fish and diet) in ti,ab,id
#59 (explode "essential-oils" in SU) or (explode "olive-oil" in SU) or (explode "palm-oils" in SU) or (explode "plant-oils" in SU) or (explode "seed-oils" in SU)7742
#60 explode "fish-liver-oils" in SU
#61 ("long-chain-fatty-acids" in SU) or (((omega 3 or n 3) and (polyunsaturated fat* or pufa or dha or epa or long chain or longchain or lc*)) in ti,ab,id) or ((ropufa or maxepa or omacor or efamed or resq or epagis or almarin or coromega) in ti,ab,id) or ((diet* fatty acid*) in ti,ab,id) or ((n 3 fatty acid* or omega 3) in ti,ab,id) or ("dietary-fat" in SU) or ("polyunsaturated-fats" in SU) or ("polyenoic-fatty-acids" in SU) or ("fish-consumption" in SU) or (explode "fish-oils" in SU) or (explode "plant-oils" in SU) or (("docosahexaenoic-acid" in SU) or ("eicosapentaenoic-acid" in SU)) or (("essential-fatty-acids" in SU) or ("linolenic-acid" in SU)) or (omega 3) or ((fish consumption or fish intake) in ti,ab,id) or ((salmon or mackerel or herring or tuna or halibut or seal or seaweed or anchov*) in ti,ab,id) or ((fish oil* or cod liver oil* or marine oil* or marine fat*) in ti,ab,id) or ((walnut* or butternut* or soybean* or pumpkin seed*) in ti,ab,id) or (((flax or flaxseed or flax seed or linseed or rape seed or rapeseed or canola or soy or soybean or walnut or mustard seed or menhaden) and oil*) in ti,ab,id) or ((mediterranean diet) in ti,ab,id) or ((linolenate or cervonic or timnodonic) in ti,ab,id) or ((alpha linolenic)in ti,ab,id) or ((eicosapentanoic or eicosapentaenoic) in ti,ab,id) or ((docosahexanoic or docosahexaenoic) in ti,ab,id) or (explode "fish-liver-oils" in SU) or ((explode "essential-oils" in SU) or (explode "olive-oil" in SU) or (explode "palm-oils" in SU) or (explode "plant-oils" in SU) or (explode "seed-oils" in SU)) or ((fish and diet) in ti,ab,id)
#62 ((explode "almond-oil" in SU) or (explode "castor-oil" in SU) or (explode "coconut-oil" in SU) or (explode "cottonseed-oil" in SU) or (explode "groundnut-oil" in SU) or (explode "jojoba-oil" in SU) or (explode "linseed-oil" in SU) or (explode "maize-oil" in SU) or (explode "melon-seed-oil" in SU) or (explode "mustard-oil" in SU) or (explode "palm-kernel-oil" in SU) or (explode "rapeseed-oil" in SU) or (explode "rice-oil" in SU) or (explode "safflower-oil" in SU) or (explode "sesame-oil" in SU) or (explode "soyabean-oil" in SU) or (explode "sunflower-oil" in SU) or (explode "tung-oil" in SU) or (explode "wheat-germ-oil" in SU)) or (("cod-liver-oil" in SU) or ("menhaden-oil" in SU))
#63 #61 or #62
#64 #33 and #63

Appendix B. Letter to Industry Representatives

Letter to Industry Representatives from the Three EPCs Investigating the Health Benefits of Omega-3 Fatty Acids

May 2, 2003

Dear _____,

I am writing on behalf of the Evidence Based Practice Centers at RAND, New England Medical Center and the University of Ottawa. We are conducting a systematic review of the efficacy and toxicity of omega-3 fatty acids in the prevention and treatment of a number of different diseases/conditions. This review is being conducted under a contract from the Agency for Healthcare Research and Quality (AHRQ).

We are contacting you to see if there is any evidence, including unpublished evidence, that you want considered. Our focus is on clinical trials of omega-3 fatty acids in humans, so animal and chemical studies are not necessary.

The specific questions that all the EPCs will address are detailed in the attachment to this letter.

Please contact me with any information that you might have. I will be out of town next week and will respond to any questions when I get back. If you have any questions that you would like addressed before I return, please contact Donna Mead at the address above.

Best regards,

Catherine MacLean, M.D., Ph.D.
RAND1700 Main Street, M 23-C
Santa Monica, CA 90407-2138
Voice: 310 393-0411, x6364
Fax: 310-451-6930
maclean@rand.org

Appendix C. Data Assessment and Data Abstraction Forms

Relevance Assessment Form

Please respond to each question.* Use the comments box to identify duplicate reports, a key review whose references should be checked, anomalies, etc.

a. **Inclusion criteria**:

1. Does this report describe a study involving human participants?
   YES Can't Tell NO

2. Does this study evaluate the role of: a. omega-3 fatty acid intake (diet and/or supplementation) as an intervention/exposure; or b. omega-3 or omega-6/omega-3 fatty acid content of biomarkers?
   YES Can't Tell NO

3. Is the purpose of the study to investigate the effect (e.g., efficacy, effectiveness, adverse events) of maternal or child intake (via diet, supplementation, or human milk) of omega-3 fatty acids *on*, or the association of the omega-3 or omega-6/omega-3 fatty acid content of maternal, child or placental biomarkers *with*: a. visual function, cognitive development, neurological development, or growth patterns in (preterm* or term) infants; b. the incidence of retinopathy of prematurity in preterm infants; c. the duration of gestation in women with or without a history of preterm birth; d. the incidence of preeclampsia (gestational hypertension); or e. the incidence of births of infants small for gestational age?
   YES Can't Tell NO

b. **Exclusion criterion**:

5. If this is a narrative or systematic review, opinion piece or editorial, letter, guideline or policy paper, etc., does it *exclusively* describe studies already reported elsewhere (i.e., it does not present any empirical evidence published for the first time)?
   YES Can't Tell NO

c. **Context**:

6. The study appears to *also or instead* concern omega-3 fatty acids as an intervention/exposure associated with the following human health/disease domains *(select at least one option; click on all that apply)*:

__transplantation
__cancer
__mental health
__neurology
__eye health
__none of the above

7. Is this report written in English?
   YES NO

8. Comments (write "only biomarkers" if it exclusively investigates "the omega-3 or omega-6/omega-3 fatty acid content in biomarkers"): BOX

Note: *Preterm = gestational duration less than 37 weeks

Level of Evidence Assessment

1. Is this a Randomized Controlled Trial (for efficacy questions only) or an observational study (i.e., prospective cohort, case-control study, cross-sectional) for Biomarkers association questions?
   YES NO

Data Abstraction Form

Instructions: ***Please answer each question.*** Selecting response options means clicking on them. A text box ("BOX") requires that you provide specific data, and allows you to provide clarification, as needed (e.g., when the available data are not straightforward). When data are not reported (= NR), the question does not apply (= N/A), you cannot tell what/where the data are in the report (= CT), the data are not broken down (= NBD) to permit the required abstraction (e.g., by study group), or you have no comment to make (= NC), type the code in the BOX.

'Participants' refers to study participants. 'Group' refers to a study group, arm or cohort or, in a crossover design, a study phase. Often, you will be asked to abstract 'full' sample data as well as by group. If requested group data are not available, abstract full sample data and label it as such.

If more than one report describes this study, draw on each to abstract study data. This means that, for question 2, record all of the relevant report Refid#s, and for question 3, record all of the relevant reports' data. When you are abstracting data from multiple reports for a given study, point out any inconsistencies.

If the research report describes more than one unique study, answer in this eForm all the questions for the *first reported study* while immediately notifying the review manager that another data abstraction form is required.

BOX = single box at end of list
All abstractors access each level, for verification possibilities.
Each abstractor assigned level(s), and Refids

1. Initials of reviewer: **BOX**

2. Reference identification #s (Refid#s) of all report(s) referring to this study, including duplicate reports, data-splitting reports, additional follow-ups, re-analyses, etc.: **BOX**

3. First author's last name, year of publication, country(s) in which study conducted (*from each relevant report*), [# study sites] (e.g., Smith, 1988, Canada [1 site]): **BOX**

4. Number of unique, review-relevant studies that this report describes (*if more than one, notify review manager*): **BOX**

Publication status, per report/Refid# referring to this study (e.g., Refid 3000=journal publication, Refid 6=conference abstract):

Peer-reviewed journal publication
Journal publication
Conference abstract/poster
Book
Book chapter
HTA/technical report
Thesis
Unpublished document
Study sponsor's internal report
Internet document/material
Other
BOX

Identity of funding source(s), including category per source (e.g., government, industry, private/non-industry, hospital), and what each provided: **BOX**

Question(s) addressed (*select all that apply*):

Pregnancy question(s), with clinical outcomes, investigated (*select all that apply*):

What is the evidence that intake of omega-3 fatty acids influences the duration of gestation in women with or without a history of a previous preterm birth (gestational duration less than 37 weeks)?

What is the evidence that maternal intake of omega-3 fatty acids influences the incidence of preeclampsia, eclampsia or gestational hypertension?

What is the evidence that maternal intake of omega-3 fatty acids influences the incidence of births of human infants small for gestational age?

None of the above

Pregnancy question(s), with biomarker outcomes, investigated (*select all that apply*):

What is the evidence that the duration of gestation in women with or without a history of a previous preterm birth is associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?

What is the evidence that the incidence of preeclampsia, eclampsia or gestational hypertension is associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?

What is the evidence that the incidence of births of human infants small for gestational age is associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?

None of the above

Growth patterns question(s), with clinical outcomes, investigated (*select all that apply*):

What is the evidence that maternal intake of omega-3 fatty acids during pregnancy influences growth patterns in term or preterm human infants?

What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences growth patterns in term or preterm human infants?

What is the evidence that the omega-3 fatty acid content of infant formula influences growth patterns in term or preterm human infants?

What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, and together with the omega-3 fatty acid content of infant formula, influences growth patterns in term or preterm human infants?

What is the evidence that intake of omega-3 fatty acids from sources other than maternal breast milk or infant formula supplemented with omega-3 fatty acids, by term or preterm human infants, influences growth patterns?

None of the above

Growth patterns question(s), with biomarker outcomes, investigated (*select all that apply*):

What is the evidence that term or preterm human infants' growth patterns are associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?

What is the evidence that term or preterm human infants' growth patterns are associated with the omega-3 or omega-6/omega-3 fatty acid content of fetal biomarkers during pregnancy?

What is the evidence that term or preterm human infants' growth patterns are associated with the omega-3 or omega-6/omega-3 fatty acid content of child biomarkers?

None of the above

Neurological development question(s), with clinical outcomes, investigated (*select all that apply*):

What is the evidence that maternal intake of omega-3 fatty acids during pregnancy influences neurological development in term or preterm human infants?

What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences neurological development in term or preterm human infants?

What is the evidence that the omega-3 fatty acid content of infant formula influences neurological development in term or preterm human infants?

What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, and together with the omega-3 fatty acid content of infant formula, influences neurological development in term or preterm human infants?

What is the evidence that intake of omega-3 fatty acids from sources other than maternal breast milk or infant formula supplemented with omega-3 fatty acids, by term or preterm human infants, influences neurological development?

None of the above

Neurological development question(s), with biomarker outcomes, investigated (*select all that apply*):

What is the evidence that term or preterm human infants' neurological development is associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?

What is the evidence that term or preterm human infants' neurological development is associated with the omega-3 or omega-6/omega-3 fatty acid content of fetal biomarkers?

What is the evidence that term or preterm human infants' neurological development is associated with the omega-3 or omega-6/omega-3 fatty acid content of child biomarkers?

None of the above

Visual function question(s), with clinical outcomes, investigated (*select all that apply*):

What is the evidence that maternal intake of omega-3 fatty acids during pregnancy influences visual function in term or preterm human infants?

What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences visual function in term or preterm human infants?

What is the evidence that the omega-3 fatty acid content of infant formula influences visual function in term or preterm human infants?

What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, and together with the omega-3 fatty acid content of infant formula, influences visual function in term or preterm human infants?

What is the evidence that intake of omega-3 fatty acids from sources other than maternal breast milk or infant formula supplemented with omega-3 fatty acids, by term or preterm human infants, influences visual function?

None of the above

Visual function question(s), with biomarker outcomes, investigated (*select all that apply*):

What is the evidence that term or preterm human infants' visual function is associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?

What is the evidence that term or preterm human infants' visual function is associated with the omega-3 or omega-6/omega-3 fatty acid content of fetal biomarkers?

What is the evidence that term or preterm human infants' visual function is associated with the omega-3 or omega-6/omega-3 fatty acid content of child biomarkers?

None of the above

Cognitive development question(s), with clinical outcomes, investigated (*select all that apply*):

What is the evidence that maternal intake of omega-3 fatty acids during pregnancy influences cognitive development in term or preterm human infants?

What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, influences cognitive development in term or preterm human infants?

What is the evidence that the omega-3 fatty acid content of infant formula influences cognitive development in term or preterm human infants?

What is the evidence that the omega-3 fatty acid content of maternal breast milk, with or without known maternal intake of omega-3 fatty acids, and together with the omega-3 fatty acid content of infant formula, influences cognitive development in term or preterm human infants?

What is the evidence that intake of omega-3 fatty acids from sources other than maternal breast milk or infant formula supplemented with omega-3 fatty acids, by term or preterm human infants, influences cognitive development?

None of the above

Cognitive development question(s), with biomarker outcomes, investigated (*select all that apply*):

What is the evidence that term or preterm human infants' cognitive development is associated with the omega-3 or omega-6/omega-3 fatty acid content of maternal biomarkers during pregnancy?

What is the evidence that term or preterm human infants' cognitive development is associated with the omega-3 or omega-6/omega-3 fatty acid content of fetal biomarkers?

What is the evidence that term or preterm human infants' cognitive development is associated with the omega-3 or omega-6/omega-3 fatty acid content of child biomarkers?

None of the above

Adverse events question(s) investigated (*select all that apply*):

What is the evidence for the risk, in pregnant women, of short and long-term adverse events related to their intake of omega-3 fatty acids?

What is the evidence for the risk, in breastfeeding women, of short and long-term adverse events related to their intake of omega-3 fatty acids?

What is the evidence for the risk, in term or preterm human infants, of short and long-term adverse events related to maternal intake of omega-3 fatty acids during pregnancy?

What is the evidence for the risk, in term or preterm human infants, of short and long-term adverse events related to their intake of omega-3 fatty acids after birth (e.g., maternal breast milk, infant formula supplemented with omega-3 fatty acids)?

What is the evidence that these adverse events, or any contraindications, are associated with the intake of specific sources (e.g., marine, plant), types (e.g., EPA, DHA, ALA) or doses of omega-3 fatty acids, including in specific populations such as diabetics?

None of the above

Study design (*select one*):

a. RCT parallel design
b. RCT crossover design
c. RCT factorial design
d. Controlled clinical trial (non-RCT)
e. Multiple prospective cohorts
f. At least one prospective cohort and one retrospective cohort
g. Case-control
h. Cross-sectional
i. Before-after (pre-post)
j. Single prospective cohort
k. Single retrospective cohort
l. Case series (noncomparative)
m. Case study
n. Sequential
o. Cross-national ecological analysis
p. Other: **BOX**

Any notable details (e.g., restricted randomization; blocking size) or problems (i.e., no or inappropriate run-in or washout procedures or durations; study stopped prematurely): **BOX**

Full sample eligibility criteria (e.g., population [e.g., pregnant women and/or children; permitted vs mandated/required characteristics/experiences/histories, including health status, complications, medications, etc.) (*complete both*):

Inclusion criteria: **BOX**
Exclusion criteria: **BOX**

Were the same eligibility criteria employed with reference to each study group? (*select one*)

a. Yes

b. No
c. Unclear
d. Not reported
e. Not applicable (e.g., a single group study)

Adequacy of reporting of eligibility criteria (*select one*):
a. Likely adequate (= not inadequate)
b. Likely inadequate (= missing, incomplete or conflicting data)

Adequacy of eligibility criteria:
a. Likely adequate (= not inadequate)
b. Likely inadequate (e.g., the inclusion criteria will not lead to the study of the target population the investigators intend to study; populations with characteristics/experiences/histories outside the investigators' intended scope, yet who show the same characteristics/experiences/histories as the target population, have not been identified as requiring exclusion)

Sample sizes (by population, if appropriate) (*complete all*):
Total # individuals screened: **BOX**
selected/allocated participants (full [e.g., n=12]; by group [e.g., group 1 n=5; group 2 n=7]): **BOX**
completers (= final followup)/total (full; per group) (e.g., group 1: n=4/5; group 2: n=6/7): **BOX**

Settings (*complete both*):
Type(s) of setting (e.g., tertiary care hospital vs. community facility) (full; by group): **BOX**
Proportion of participants in relatively controlled (e.g., inpatients) settings during study (full; by group): **BOX**

Study period (*complete all*):
Intervention length (d, wk, mo, y) (*by group only if it varies*): **BOX**
Timing of intervention (e.g., beginning the 3rd day of life, for 4 mo; beginning the 5th wk of pregnancy, until delivery): **BOX**
Study duration, including units (h, d, wk, mo) (includes intervention length + run-in period duration, washout duration[s], etc.): **BOX**
Run-in duration/protocol: **BOX**
Washout duration/protocol: **BOX**

Did participants in each study group receive the intervention/exposure for the same length of time? (*select one*)
a. Yes
b. No
c. Unclear
d. Not reported
e. Not applicable (e.g., a cross-sectional survey)

Was the same study procedure employed with reference to each study group? (*select one*)

a. Yes
b. No
c. Unclear
d. Not reported
e. Not applicable

Were participants in each study group assessed at the same number of followups, and with the same timing, during the study (*select one*)?

a. Yes
b. No
c. Unclear
d. Not reported
e. Not applicable (e.g., a cross-sectional survey)

Number and timing of followups (i.e., *specify* corrected age [mos] and/or actual mos of age), and any definition of the 'length of followup required to observe an/no impact of the exposure/intervention:' **BOX**

Adverse events, and losses to followup (*complete both*):

withdrawals vs. # dropouts, with reasons (full; by group): **BOX**
Adverse events/side effects and contraindications (full; by group): **BOX**

Basic population characteristics (maternal and/or children) (*complete all*):

Mean age (mean (range) y) of all relevant participants at study onset (full; by group, by population): **BOX**
Percentage of male children (full; by group): **BOX**
Maternal racial composition (proportions: full; by group) (e.g., Caucasian 50%, Asian 50% per group) **BOX**
Children's racial composition (proportions: full; by group) (e.g., Caucasian 50%, Asian 50% per group) **BOX**
Maternal socioeconomic status (i.e., employment status, income, marital status, education) (full; by group): **BOX**

Maternal health history *prior to current pregnancy* (*complete all*) (*if this study does not specifically investigate a maternal population, click* here):

Gynecologic history (e.g., STD, uterine anomalies, cervical incompetence) (full, by group): **BOX**
Obstetric history (i.e., n gestations, deliveries, abortions, live births, premature births, multiple gestations; complications, pre/eclampsia, gestational hypertension or gestational diabetes in previous pregnancies) (full, by group): **BOX**
Medical conditions (full, by group): **BOX**
Medications/treatments (full, by group): **BOX**
Breastfeeding history, including difficulties (full; by group): **BOX**

Alcohol (ab)use, especially during previous pregnancies/breastfeeding (full, by group): **BOX**
Smoking tobacco use or exposure, especially during previous pregnancies/breastfeeding (full, by group): **BOX**
Illicit drug use, especially during previous pregnancies/breastfeeding (full, by group): **BOX**
Other (e.g., domestic violence**)** (full, by group): **BOX**

Maternal health status of current pregnancy/breastfeeding (*complete all*):
Age at conception AND at delivery (*specify*) (full, by group): **BOX**
Obstetric history of current pregnancy (full, by group): **BOX**
Medical conditions, including psychiatric conditions (full, by group): **BOX**
All medication/treatments (e.g., prescription and non-prescription) (dose/frequency) (full, by group): **BOX**
Supplement use (vitamins, minerals) and/or CAM therapies prior to study onset: **BOX**
Alcohol (ab)use (full, by group): **BOX**
Smoking tobacco use or exposure (full, by group): **BOX**
Illicit drug use (full, by group): **BOX**
Other (e.g., domestic violence) (full, by group): **BOX**

Child's pre-study health history (*complete all*) (*if this study does not specifically investigate a child population, click* here):
Prenatal history (i.e., GA, complications during pregnancy, delivery and/or labor anomalies, etc) (full, by group): **BOX**
Neonatal history (e.g., asphyxia, intracranial hemorrhage, kernicterus, TORCH, hydrocephalus, congenital cataracts, coriorretinitis) (full, by group): **BOX**
Pediatric history (i.e., medical conditions, immunizations, etc.) (full, by group): **BOX**
Weight (W), height (H) and head circumference (HC) at birth, with percentiles (Pc) (full; by group): **BOX**
Medications/treatments (with dose/frequency) (full, by group): **BOX**
Other (e.g., exposure to toxic material) (full, by group): **BOX**

Child's health status at study baseline (*complete all*):
Current weight (Pc) (full, by group): **BOX**
Current height (Pc) (full, by group): **BOX**
Current head circumference (Pc) (full, by group): **BOX**
Growth patterns (percentile pattern, to study baseline) (full, by group): **BOX**
Visual function (full, by group): **BOX**
Cognitive developmental status (e.g., language) (full, by group): **BOX**
Neurodevelopmental status (full, by group): **BOX**
Medical conditions (full, by group): **BOX**
Medications/treatments (e.g., prescription and non-prescription drugs), with dose/frequency (full, by group): **BOX**
Supplement use (vitamins, minerals) and/or CAM therapies prior to study onset: **BOX**
Other (full, by group): **BOX**

Describe the method(s)/test(s) used to assess the child's visual development (full, by group; *at each evaluation*) (e.g., visual acuity, electroretinogram, etc): **BOX**

Describe the method(s)/test(s) used to assess the child's cognitive developmental status (full, by group; *at each evaluation*) [e.g., Bayley's mental developmental index (<2 years of age), Weschler (WPPSI, > 2 years of age), and WISC (> 7 years of age)]: **BOX**

Describe the method(s)/test(s) used to assess the child's neurological development status (full, by group; *at each evaluation*) [e.g., Bayley's motor developmental index (< 2 years of age), Peabody (>2 years of age)]: **BOX**

Maternal n-3 intake (pre-study/baseline) (complete all):
Pre-study/baseline total **maternal** (daily, weekly or monthly) n-3 intake *via diet and/or supplementation*, with amount per n-3 type (EPA, DHA, ALA), and source (e.g., fish servings; walnuts; flaxseed oil) (*by group*) (e.g., group 1: 1.8g/d EPA, 1.2g/d DHA, from 3 fish oil capsules/d; and, NR [likely EPA &/or DHA], from 1-2 fish servings/wk; group 2: 0g/d EPA, 0g/d DHA, water placebo; and, NR, 0 fish servings/wk): **BOX**

Pre-study/baseline total (daily, weekly or monthly) **maternal** dietary n-6/n-3 intake (*by group*) (e.g., group 1: 15/1; group 2: 10/1): **BOX**

Pre-study/baseline % (daily, weekly or monthly) **maternal** caloric/energy intake from fat (*by group*): **BOX**

Absolute and *relative* n-3 fatty acid content of the pre-study/baseline **maternal** diet (full; by group): **BOX**

Types of pre-study/baseline **maternal** diet (*proportion of participants on each diet: in full; by group*):

High fish diet
Fish-vegetarian diet
Low fish diet
Low fat diet
High fat diet
Mediterranean diet
Other
Unclear
Not reported
BOX

How was the pre-study **maternal** dietary intake of n-3, n-6 and n-6/n-3 evaluated/estimated (*select all that apply*)?

Nutritionist-administered quantitative food-frequency survey(s)
Nutritionist-administered semi-quantitative food-frequency survey(s)
Self-administered quantitative food-frequency survey(s)
Self-administered semi-quantitative food-frequency survey(s)

Parent-administered quantitative food-frequency survey(s)
Parent-administered semi-quantitative food-frequency survey(s)
Direct measurement(s) of food intake
Survey(s) (e.g., 24-hour recall): **BOX**
Survey(s), yet no details provided
Other: **BOX**
Unclear
Not reported
Not applicable

Maternal total amount of dietary n-3 intake **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Total amount of **maternal** n-3 intake from supplementation **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Total amount of **maternal** n-3 intake from diet and supplementation **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal dietary n-6/n-3 intake **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

% **Maternal** caloric/energy intake **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's n-3 intake (pre-study/baseline) (complete all):

Pre-study/baseline **child's** total (daily, weekly or monthly) n-3 intake *via breast milk, diet (source) and/or formula/supplementation,* with amount per n-3 type (EPA, DHA, ALA) (*by group*): **BOX**

Pre-study/baseline **child's** total (daily, weekly or monthly) dietary n-6/n-3 intake (*by group*) (e.g., group 1: 15/1; group 2: 10/1): **BOX**

Pre-study/baseline % (daily, weekly or monthly) **child's** caloric/energy intake from fat (*by group*): **BOX**

Child's types of pre-study/baseline diet (*proportion of participants on each diet: in full; by group*):

High fish diet
Fish-vegetarian diet
Low fish diet
Low fat diet
High fat diet
Mediterranean diet
Other
Unclear
Not reported
BOX

Absolute and *relative* n-3 fatty acid content of the pre-study/baseline **child's** diet (full; by group): **BOX**

How was the pre-study **child's** dietary intake of n-3, n-6 and n-6/n-3 evaluated/estimated (*select all that apply*)?

Nutritionist-administered quantitative food-frequency survey(s)
Nutritionist-administered semi-quantitative food-frequency survey(s)

Self-administered quantitative food-frequency survey(s)
Self-administered semi-quantitative food-frequency survey(s)
Parent-administered quantitative food-frequency survey(s)
Parent-administered semi-quantitative food-frequency survey(s)
Direct measurement(s) of food intake
Survey(s) (e.g., 24-hour recall): **BOX**
Survey(s), yet no details provided
Other: **BOX**
Unclear
Not reported
Not applicable
BOX

Total amount of **child's** n-3 intake via breast milk **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's total amount of dietary n-3 intake **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's total amount of n-3 intake from supplementation/formula **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Total amount of **child's** n-3 intake from breast milk, diet and supplementation/formula **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's total dietary n-6/n-3 intake **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's (daily, weekly or monthly) % caloric/energy intake from fat **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Pre-study/baseline **maternal** biomarkers data (by biomarker: e.g., breast milk, placental blood, RBCs; for DHA, EPA, AA, AA/EPA, AA/DHA, AA/EPA+DHA levels, with units (e.g., % total fatty acids; absolute amount) (full; by group): **BOX**

Maternal DHA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal EPA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal EPA+DHA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal AA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal AA/DHA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal AA/EPA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal AA/EPA+DHA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Pre-study/baseline **child's** biomarkers data (by biomarker: e.g., cord blood, RBCs; for DHA, EPA, AA, AA/EPA, AA/DHA, AA/EPA+DHA levels, with units (e.g., % total fatty acids; absolute amount) (full; by group): **BOX**

Child's DHA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's EPA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's EPA+DHA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's AA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's AA/DHA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's AA/EPA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's AA/EPA+DHA status (per biomarker) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

ON-STUDY

How was **maternal** on-study dietary intake of n-3 or n-6/n-3 evaluated/estimated (*select all that apply*)?

Nutritionist-administered quantitative food-frequency survey(s)
Nutritionist-administered semi-quantitative food-frequency survey(s)
Self-administered quantitative food-frequency survey(s)
Self-administered semi-quantitative food-frequency survey(s)
Parent-administered quantitative food-frequency survey(s)
Parent-administered semi-quantitative food-frequency survey(s)
Direct measurement(s) of food intake
Survey(s) (e.g., 24-hour recall): **BOX**
Survey(s), yet no details provided
Other: **BOX**
Unclear
Not reported
Not applicable

How was **child's** on-study dietary intake of n-3 or n-6/n-3 evaluated/estimated (*select all that apply*)?

Nutritionist-administered quantitative food-frequency survey(s)
Nutritionist-administered semi-quantitative food-frequency survey(s)

Self-administered quantitative food-frequency survey(s)
Self-administered semi-quantitative food-frequency survey(s)
Parent-administered quantitative food-frequency survey(s)
Parent-administered semi-quantitative food-frequency survey(s)
Direct measurement(s) of food intake
Survey(s) (e.g., 24-hour recall): **BOX**
Survey(s), yet no details provided
Other: **BOX**
Unclear
Not reported
Not applicable

On-study GROUP 1 (highest dose n-3 [or lowest n-6/n-3 ratio if both n-3 and n-6 are modified] study first, active comparator next (e.g., n-6), placebo control last) (*complete all*):

total (daily, weekly or monthly) **maternal** n-3 dose *via supplementation* during pregnancy and/or breastfeeding, with amount per n-3 type; source, frequency and timing of delivery; other active ingredients (e.g., pregnancy = 1.8g/d EPA, 1.2g/d DHA, from 3 [1g vegan outer] fish oil capsules/d, with breakfast, NR): **BOX**

total (daily, weekly or monthly) **maternal** n-3 exposure *via diet* during pregnancy and/or breastfeeding, with amount per n-3 type, source, frequency and timing of delivery (e.g., breastfeeding = NR, likely EPA or DHA, from 2 0.5oz oily fish servings/wk, as dinner; e.g., pregnancy = NR, likely ALA, from 8-10oz/wk flaxseed oil as salad dressing, NR): **BOX**

total (daily, weekly or monthly) **maternal** n-3 exposure *via all sources* (diet+supplementation) during pregnancy and/or breastfeeding, per n-3 type (e.g., pregnancy = NR, at least 3g/d EPA+DHA): **BOX**

total (daily, weekly or monthly) **maternal** n-6 and/or n-6/n-3 intake from diet+supplementation during pregnancy and/or breastfeeding: **BOX**

type, source and total (daily, weekly or monthly) **maternal** intake of other mandated or permitted exposures during pregnancy and/or breastfeeding (e.g., vitamins, minerals), with dose/serving/frequency: **BOX**

n allocated-selected/ n completed **(maternal)** (e.g., n=24/21): **BOX**

total (daily, weekly or monthly) **child's** n-3 **intake amount** *via breast milk*, with amount per n-3 type; source, frequency and timing of delivery: **BOX**

total (daily, weekly or monthly) **child's** n-3 dose *via supplementation/formula*, with amount per n-3 type; source, frequency and timing of delivery; other active ingredients: **BOX**

total (daily, weekly or monthly) **child's** n-3 exposure *via diet*, with amount per n-3 type, source, frequency and timing of delivery (e.g., NR, likely EPA or DHA, from 2 0.5oz oily fish

servings/wk, as dinner; e.g., NR, likely ALA, from 8-10oz/wk flaxseed oil as salad dressing, NR): **BOX**

total (daily, weekly or monthly) **child's** n-3 exposure *via all sources* (diet+supplementation), per n-3 type (e.g., NR, at least 3g/d EPA+DHA): **BOX**

total (daily, weekly or monthly) **child's** n-6 and/or n-6/n-3 intake from diet+supplementation: **BOX**

type, source and total (daily, weekly or monthly) **child's** intake of other mandated or permitted exposures (e.g., vitamins, minerals), with dose/serving/frequency: **BOX**

n allocated-selected/ n completed (**child)** (e.g., n=24/21): **BOX**

On-study GROUP 2 (next highest dose n-3 [or lowest n-6/n-3 ratio if both n-3 and n-6 are modified] study first, active comparator next (e.g., n-6), placebo control last) (*complete all; click* here *if there are no more study groups*):

total (daily, weekly or monthly) **maternal** n-3 dose *via supplementation* during pregnancy and/or breastfeeding, with amount per n-3 type; source, frequency and timing of delivery; other active ingredients (e.g., pregnancy = 1.8g/d EPA, 1.2g/d DHA, from 3 [1g vegan outer] fish oil capsules/d, with breakfast, NR): **BOX**

total (daily, weekly or monthly) **maternal** n-3 exposure *via diet* during pregnancy and/or breastfeeding, with amount per n-3 type, source, frequency and timing of delivery (e.g., breastfeeding = NR, likely EPA or DHA, from 2 0.5oz oily fish servings/wk, as dinner; e.g., pregnancy = NR, likely ALA, from 8-10oz/wk flaxseed oil as salad dressing, NR): **BOX**

total (daily, weekly or monthly) **maternal** n-3 exposure *via all sources* (diet+supplementation) during pregnancy and/or breastfeeding, per n-3 type (e.g., pregnancy = NR, at least 3g/d EPA+DHA): **BOX**

total (daily, weekly or monthly) **maternal** n-6 and/or n-6/n-3 intake from diet+supplementation during pregnancy and/or breastfeeding: **BOX**

type, source and total (daily, weekly or monthly) **maternal** intake of other mandated or permitted exposures during pregnancy and/or breastfeeding (e.g., vitamins, minerals), with dose/serving/frequency: **BOX**

n allocated-selected/ n completed **(maternal)** (e.g., n=24/21): **BOX**

total (daily, weekly or monthly) **child's** n-3 **intake amount** *via breast milk*, with amount per n-3 type; source, frequency and timing of delivery: **BOX**

total (daily, weekly or monthly) **child's** n-3 dose *via supplementation/formula*, with amount per n-3 type; source, frequency and timing of delivery; other active ingredients: **BOX**

total (daily, weekly or monthly) **child's** n-3 exposure *via diet*, with amount per n-3 type, source, frequency and timing of delivery (e.g., NR, likely EPA or DHA, from 2 0.5oz oily fish servings/wk, as dinner; e.g., NR, likely ALA, from 8-10oz/wk flaxseed oil as salad dressing, NR): **BOX**

total (daily, weekly or monthly) **child's** n-3 exposure *via all sources* (diet+supplementation), per n-3 type (e.g., NR, at least 3g/d EPA+DHA): **BOX**

total (daily, weekly or monthly) **child's** n-6 and/or n-6/n-3 intake from diet+supplementation: **BOX**

type, source and total (daily, weekly or monthly) **child's** intake of other mandated or permitted exposures (e.g., vitamins, minerals), with dose/serving/frequency: **BOX**

n allocated-selected/ n completed (**child)** (e.g., n=24/21): **BOX**

On-study GROUP 3 (next highest dose n-3 [or lowest n-6/n-3 ratio if both n-3 and n-6 are modified] study first, active comparator next (e.g., n-6), placebo control last) (*complete all; click* here *if there are no more study groups*):

total (daily, weekly or monthly) **maternal** n-3 dose *via supplementation* during pregnancy and/or breastfeeding, with amount per n-3 type; source, frequency and timing of delivery; other active ingredients (e.g., pregnancy = 1.8g/d EPA, 1.2g/d DHA, from 3 [1g vegan outer] fish oil capsules/d, with breakfast, NR): **BOX**

total (daily, weekly or monthly) **maternal** n-3 exposure *via diet* during pregnancy and/or breastfeeding, with amount per n-3 type, source, frequency and timing of delivery (e.g., breastfeeding = NR, likely EPA or DHA, from 2 0.5oz oily fish servings/wk, as dinner; e.g., pregnancy = NR, likely ALA, from 8-10oz/wk flaxseed oil as salad dressing, NR): **BOX**

total (daily, weekly or monthly) **maternal** n-3 exposure *via all sources* (diet+supplementation) during pregnancy and/or breastfeeding, per n-3 type (e.g., pregnancy = NR, at least 3g/d EPA+DHA): **BOX**

total (daily, weekly or monthly) **maternal** n-6 and/or n-6/n-3 intake from diet+supplementation during pregnancy and/or breastfeeding: **BOX**

type, source and total (daily, weekly or monthly) **maternal** intake of other mandated or permitted exposures during pregnancy and/or breastfeeding (e.g., vitamins, minerals), with dose/serving/frequency: **BOX**

n allocated-selected/ n completed **(maternal)** (e.g., n=24/21): **BOX**

total (daily, weekly or monthly) **child's** n-3 **intake amount** *via breast milk*, with amount per n-3 type; source, frequency and timing of delivery: **BOX**

total (daily, weekly or monthly) **child's** n-3 dose *via supplementation/formula*, with amount per n-3 type; source, frequency and timing of delivery; other active ingredients: **BOX**

total (daily, weekly or monthly) **child's** n-3 exposure *via diet*, with amount per n-3 type, source, frequency and timing of delivery (e.g., NR, likely EPA or DHA, from 2 0.5oz oily fish servings/wk, as dinner; e.g., NR, likely ALA, from 8-10oz/wk flaxseed oil as salad dressing, NR): **BOX**

total (daily, weekly or monthly) **child's** n-3 exposure *via all sources* (diet+supplementation), per n-3 type (e.g., NR, at least 3g/d EPA+DHA): **BOX**

total (daily, weekly or monthly) **child's** n-6 and/or n-6/n-3 intake from diet+supplementation: **BOX**

type, source and total (daily, weekly or monthly) **child's** intake of other mandated or permitted exposures (e.g., vitamins, minerals), with dose/serving/frequency: **BOX**

n allocated-selected/ n completed (**child)** (e.g., n=24/21): **BOX**

On-study GROUP 4 (next highest dose n-3 [or lowest n-6/n-3 ratio if both n-3 and n-6 are modified] study first, active comparator next (e.g., n-6), placebo control last) (*complete all; click* here *if there are no more study groups*):

total (daily, weekly or monthly) **maternal** n-3 dose *via supplementation* during pregnancy and/or breastfeeding, with amount per n-3 type; source, frequency and timing of delivery; other active ingredients (e.g., pregnancy = 1.8g/d EPA, 1.2g/d DHA, from 3 [1g vegan outer] fish oil capsules/d, with breakfast, NR): **BOX**

total (daily, weekly or monthly) **maternal** n-3 exposure *via diet* during pregnancy and/or breastfeeding, with amount per n-3 type, source, frequency and timing of delivery (e.g., breastfeeding = NR, likely EPA or DHA, from 2 0.5oz oily fish servings/wk, as dinner; e.g., pregnancy = NR, likely ALA, from 8-10oz/wk flaxseed oil as salad dressing, NR): **BOX**

total (daily, weekly or monthly) **maternal** n-3 exposure *via all sources* (diet+supplementation) during pregnancy and/or breastfeeding, per n-3 type (e.g., pregnancy = NR, at least 3g/d EPA+DHA): **BOX**

total (daily, weekly or monthly) **maternal** n-6 and/or n-6/n-3 intake from diet+supplementation during pregnancy and/or breastfeeding: **BOX**

type, source and total (daily, weekly or monthly) **maternal** intake of other mandated or permitted exposures during pregnancy and/or breastfeeding (e.g., vitamins, minerals), with dose/serving/frequency: **BOX**

n allocated-selected/ n completed **(maternal)** (e.g., n=24/21): **BOX**

total (daily, weekly or monthly) **child's** n-3 **intake amount** *via breast milk*, with amount per n-3 type; source, frequency and timing of delivery: **BOX**

total (daily, weekly or monthly) **child's** n-3 dose *via supplementation/formula*, with amount per n-3 type; source, frequency and timing of delivery; other active ingredients: **BOX**

total (daily, weekly or monthly) **child's** n-3 exposure *via diet*, with amount per n-3 type, source, frequency and timing of delivery (e.g., NR, likely EPA or DHA, from 2 0.5oz oily fish servings/wk, as dinner; e.g., NR, likely ALA, from 8-10oz/wk flaxseed oil as salad dressing, NR): **BOX**

total (daily, weekly or monthly) **child's** n-3 exposure *via all sources* (diet+supplementation), per n-3 type (e.g., NR, at least 3g/d EPA+DHA): **BOX**

total (daily, weekly or monthly) **child's** n-6 and/or n-6/n-3 intake from diet+supplementation: **BOX**

type, source and total (daily, weekly or monthly) **child's** intake of other mandated or permitted exposures (e.g., vitamins, minerals), with dose/serving/frequency: **BOX**

n allocated-selected/ n completed (**child)** (e.g., n=24/21): **BOX**

On-study GROUP 5 (next highest dose n-3 [or lowest n-6/n-3 ratio if both n-3 and n-6 are modified] study first, active comparator next (e.g., n-6), placebo control last) (*complete all; click* here *if there are no more study groups*):

total (daily, weekly or monthly) **maternal** n-3 dose *via supplementation* during pregnancy and/or breastfeeding, with amount per n-3 type; source, frequency and timing of delivery; other active ingredients (e.g., pregnancy = 1.8g/d EPA, 1.2g/d DHA, from 3 [1g vegan outer] fish oil capsules/d, with breakfast, NR): **BOX**

total (daily, weekly or monthly) **maternal** n-3 exposure *via diet* during pregnancy and/or breastfeeding, with amount per n-3 type, source, frequency and timing of delivery (e.g., breastfeeding = NR, likely EPA or DHA, from 2 0.5oz oily fish servings/wk, as dinner; e.g., pregnancy = NR, likely ALA, from 8-10oz/wk flaxseed oil as salad dressing, NR): **BOX**

total (daily, weekly or monthly) **maternal** n-3 exposure *via all sources* (diet+supplementation) during pregnancy and/or breastfeeding, per n-3 type (e.g., pregnancy = NR, at least 3g/d EPA+DHA): **BOX**

total (daily, weekly or monthly) **maternal** n-6 and/or n-6/n-3 intake from diet+supplementation during pregnancy and/or breastfeeding: **BOX**

type, source and total (daily, weekly or monthly) **maternal** intake of other mandated or permitted exposures during pregnancy and/or breastfeeding (e.g., vitamins, minerals), with dose/serving/frequency: **BOX**

n allocated-selected/ n completed **(maternal)** (e.g., n=24/21): **BOX**

total (daily, weekly or monthly) **child's** n-3 **intake amount** *via breast milk*, with amount per n-3 type; source, frequency and timing of delivery: **BOX**

total (daily, weekly or monthly) **child's** n-3 dose *via supplementation/formula*, with amount per n-3 type; source, frequency and timing of delivery; other active ingredients: **BOX**

total (daily, weekly or monthly) **child's** n-3 exposure *via diet*, with amount per n-3 type, source, frequency and timing of delivery (e.g., NR, likely EPA or DHA, from 2 0.5oz oily fish servings/wk, as dinner; e.g., NR, likely ALA, from 8-10oz/wk flaxseed oil as salad dressing, NR): **BOX**

total (daily, weekly or monthly) **child's** n-3 exposure *via all sources* (diet+supplementation), per n-3 type (e.g., NR, at least 3g/d EPA+DHA): **BOX**

total (daily, weekly or monthly) **child's** n-6 and/or n-6/n-3 intake from diet+supplementation: **BOX**

type, source and total (daily, weekly or monthly) **child's** intake of other mandated or permitted exposures (e.g., vitamins, minerals), with dose/serving/frequency: **BOX**

n allocated-selected/ n completed (**child)** (e.g., n=24/21): **BOX**

On-study GROUP 6 (next highest dose n-3 [or lowest n-6/n-3 ratio if both n-3 and n-6 are modified] study first, active comparator next (e.g., n-6), placebo control last) (*complete all; click* here *if there are no more study groups*):

total (daily, weekly or monthly) **maternal** n-3 dose *via supplementation* during pregnancy and/or breastfeeding, with amount per n-3 type; source, frequency and timing of delivery; other active ingredients (e.g., pregnancy = 1.8g/d EPA, 1.2g/d DHA, from 3 [1g vegan outer] fish oil capsules/d, with breakfast, NR): **BOX**

total (daily, weekly or monthly) **maternal** n-3 exposure *via diet* during pregnancy and/or breastfeeding, with amount per n-3 type, source, frequency and timing of delivery (e.g., breastfeeding = NR, likely EPA or DHA, from 2 0.5oz oily fish servings/wk, as dinner; e.g., pregnancy = NR, likely ALA, from 8-10oz/wk flaxseed oil as salad dressing, NR): **BOX**

total (daily, weekly or monthly) **maternal** n-3 exposure *via all sources* (diet+supplementation) during pregnancy and/or breastfeeding, per n-3 type (e.g., pregnancy = NR, at least 3g/d EPA+DHA): **BOX**

total (daily, weekly or monthly) **maternal** n-6 and/or n-6/n-3 intake from diet+supplementation during pregnancy and/or breastfeeding: **BOX**

type, source and total (daily, weekly or monthly) **maternal** intake of other mandated or permitted exposures during pregnancy and/or breastfeeding (e.g., vitamins, minerals), with dose/serving/frequency: **BOX**

n allocated-selected/ n completed **(maternal)** (e.g., n=24/21): **BOX**

total (daily, weekly or monthly) **child's** n-3 **intake amount** *via breast milk*, with amount per n-3 type; source, frequency and timing of delivery: **BOX**

total (daily, weekly or monthly) **child's** n-3 dose *via supplementation/formula*, with amount per n-3 type; source, frequency and timing of delivery; other active ingredients: **BOX**

total (daily, weekly or monthly) **child's** n-3 exposure *via diet*, with amount per n-3 type, source, frequency and timing of delivery (e.g., NR, likely EPA or DHA, from 2 0.5oz oily fish servings/wk, as dinner; e.g., NR, likely ALA, from 8-10oz/wk flaxseed oil as salad dressing, NR): **BOX**

total (daily, weekly or monthly) **child's** n-3 exposure *via all sources* (diet+supplementation), per n-3 type (e.g., NR, at least 3g/d EPA+DHA): **BOX**

total (daily, weekly or monthly) **child's** n-6 and/or n-6/n-3 intake from diet+supplementation: **BOX**

type, source and total (daily, weekly or monthly) **child's** intake of other mandated or permitted exposures (e.g., vitamins, minerals), with dose/serving/frequency: **BOX**

n allocated-selected/ n completed (**child)** (e.g., n=24/21): **BOX**

protocol (e.g., what is mandated vs. permitted), with method and target values, to modify daily, weekly or monthly n-6 or n-6/n-3 **maternal** intake (e.g., increase daily n-3 intake to Y% of total daily fat intake, decrease daily n-6 intake to X% of total daily fat intake; e.g., none, participants told to maintain background diet) (by population, by group): **BOX**

protocol (e.g., what is mandated vs. permitted), with method and target values, to modify daily, weekly or monthly **child's** n-6 or n-6/n-3 intake (e.g., increase daily n-3 intake to Y% of total daily fat intake, decrease daily n-6 intake to X% of total daily fat intake; e.g., none, participants told to maintain background diet) (by population, by group): **BOX**

Briefly describe whether there was a clearly planned and instituted difference, between study groups, in their (daily, weekly or monthly) total-gram n-3 and/or n-6/n-3 intake (by population): **BOX**

Briefly describe whether there was a clearly planned and instituted equivalence, across study groups, of (daily, weekly or monthly) caloric/energy intake from study-relevant exposures/interventions (by population): **BOX**

Briefly describe any problems with compliance whereby notable deviations (e.g., decreases) from the planned amounts of intake (e.g., frequency of breastfeeding, formula, servings) in one or more of the study groups violated the difference(s) established *a priori* between study groups for n-3 and/or n-6/n-3 intake or the equivalence established *a priori* across study groups for caloric/energy intake (full; by group; by population): **BOX**

Briefly describe whether, and which, study groups/participants were asked to maintain their (pre-study/baseline) background diet while on-study (full; by group; by population): **BOX**

Briefly describe whether, and how, without specific instruction to do so, or with specific instruction *not* to do so, participants' (pre-study/baseline) background diet was altered while on-study (full; by group): **BOX**

Briefly describe whether, and which, study groups/participants were asked to maintain their (pre-study/baseline) therapies/medications while on-study (full; by group): **BOX**

n-3: Briefly describe whether, and how, without specific instruction to do so, or with specific instruction *not* to do so, participants' (pre-study/baseline) therapies/medication were altered while on-study (full; by group): **BOX**

Briefly describe any evidence of selection bias: **BOX**

Child's prenatal history **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's neonatal history (up to 28 days of age) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's pediatric history **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's weight (Pc) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's height (Pc) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's head circumference (Pc) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's growth patterns (percentile's profile) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's medications/treatments: **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's visual function **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's cognitive developmental history and/or status **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's neurodevelopmental history and/or status **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Child's use of other licit (prescription and non-prescription) drugs, supplements (e.g., vitamins, minerals) and/or complementary/alternative therapies (*specify*) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal age at conception and at delivery **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline were taken into consideration in the study analysis: **BOX**

Maternal gynaecologic history **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Number of premature deliveries: **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

History of eclampsia/preeclampsia **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

History of gestational hypertension **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal obstetric history **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline were taken into consideration in the study analysis: **BOX**

Maternal obstetric history of current pregnancy **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal medical status **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal medication and/or treatment types and doses during pregnancy and/or breast-feeding (*clarify*) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) **baseline** between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal use of other licit (prescription and non-prescription) drugs, supplements (e.g., vitamins, minerals) and/or complementary/alternative therapies during current pregnancy and/or breastfeeding **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal (ab)use of alcohol, illicit drugs and/or use or exposure to smoking tobacco during current pregnancy and/or breastfeeding **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Maternal socioeconomic status (i.e., employment status, income, marital status, and education) (*specify*) **(*complete all*):**

Significant (e.g., p-value; stated result of statistical test) pre-study/baseline between-group difference(s) (specify direction of difference(s): e.g., n-3 > pb): **BOX**

Significant (e.g., p-value; stated result of statistical test) between-group difference(s) in within-group changes from baseline, to each followup (specify nature of change(s), by followup: e.g., final followup = n-3 > pb): **BOX**

Specify whether any significant between-group differences at pre-study/baseline or regarding within-group changes from baseline, to each followup, were taken into consideration in the study analysis: **BOX**

Name of n-3(-containing) product (e.g., Almarin, Coromega, Eiconol; Efamed, Epagis, MaxEPA, Menhaden oil, ResQ, Omacor, Ropufa, Similac, Enfalac, Isomil, etc.): **BOX**

Manufacturer (*per product*): **BOX**

Purity data (*per product*): **BOX**

Presence of other, potentially active agents in n-3 product (*per product*): **BOX**

n-3 composition (%) of the exposure (e.g., 18% EPA, 12% DHA in each fish oil capsule) (*per product*): **BOX**

Reported method(s) to maintain the freshness (i.e., preclude rancidity) of n-3 exposures/interventions (e.g., added anti-oxidants to capsules, with fish oil exposure, to minimize oxidation): **BOX**

Reported method(s) to eliminate methylmercury from fish or its products/derivatives: **BOX**

Note any descriptions of inappropriate methods of lipid extraction/preparation (e.g., failure to extract blood after a [overnight] fasting period; failure to collect blood in EDTA- or EGTA-containing vials): **BOX**

Note any descriptions of inappropriate methods of lipid storage (e.g., failure to store samples at –70 to –80 degrees C if not analyzed immediately): **BOX**

Note any descriptions of inappropriate methods of lipid analysis (e.g., failure to conduct lab measurements on coded samples by technicians blinded to participants' identity and allocation; failure to use a standard protocol [e.g., Bligh & Dyer] requiring, for example, purging samples with nitrogen, or using thin-layer chromatography or gas liquid chromatography): **BOX**

Adequacy of method to deodorize smell of especially fish oil exposure (*select one*):

Adequate = reported that study participants could not reliably guess which exposure they received

Inadequate = reported that participants could reliably guess which exposure they received

Unclear = incomplete or conflicting data reported

Not reported = no method reported, or method reported but no data reported

Not applicable = did not use an exposure requiring or permitting such a method (e.g., flaxseed; full fish servings)

If this is a controlled study, briefly describe whether clinical outcome data from all study groups (e.g., active vs placebo) were simultaneously entered into data analysis: **BOX**

If this is a controlled study, briefly describe whether biomarker data from all study groups (e.g., active vs placebo) were simultaneously entered into data analysis: **BOX**

Data were analyzed according to which criterion (*select one*)?

Intention-to-treat (all randomized/enrolled)

Those receiving at least one dose/serving

Those completing the study (i.e., with final follow-up data)

Unclear

Other: **BOX**

Was the study adequately powered to detect a difference? **BOX**

Any further comments about the study: **BOX**

Quality Assessment Form—Randomized Controlled Trials

1. Randomization: Was the study described as randomized (i.e. including words such as randomly, random, randomization)? **Yes = 1** **No = 0** **=___**

A trial reporting that it is 'randomized' is to *receive one point.* Trials describing an appropriate method of randomization (table of random numbers, computer generated*) receive an additional point.* **Appropriate = 1** **Not appropriate = 0** **= ___**

However, if the report describes the trial as randomized and uses an inappropriate method of randomization (e.g. date of birth, hospital numbers), *a point is deducted.*

TOTAL POINTS: **0** **1** **2** **SCORE = __**

2. Double-blinding: Was the study described as double-blind? **Yes = 1** **No = 0 =___**

A trial reporting that it is 'double-blind' is to *receive one point.* Trials that describe an appropriate method of double-blinding (identical placebo: color, shape, taste) are to *receive an additional point.* **Yes = 1** **No = 0** **= ___**

However, if the report describes the trial as double-blind and uses an inappropriate method (e.g. comparison of tablets vs. injection with no dummy), *a point is deducted.*

TOTAL POINTS: **0** **1** **2** **SCORE = ___**

3. Withdrawals and dropouts: Was there a description of withdrawals and dropouts?
Yes = 1 **No = 0** **SCORE = ___**

A trial reporting the number of and reasons for withdrawals or dropouts is to *receive one point.* If there is no description, *no point is given.*

JADAD TOTAL SCORE = ___

4. Adequacy of Allocation Concealment: (*select one*):

-Central randomization; numbered or coded bottles or containers; drugs prepared by a pharmacy, serially numbered, opaque, sealed envelopes,
etc……. **ADEQUATE**

-Alternation; reference to case record # or date of birth,
etc……. **INADEQUATE**

-Allocation concealment is not reported, or, fits neither
category…….. ……... **UNCLEAR**

Quality Assessment (Internal Validity) Forms—Designs Other than an RCT

Controlled Study Designs

DESIGN: CASE-CONTROL STUDY (Newcastle-Ottawa, with assessment of an additional confounder)

1. Is the case definition adequate?

a. yes, with independent validation (e.g., clinical/research diagnostic criteria) (1 point)
b. yes: e.g., record linkage or based on reports
c. no description

2. Representativeness of the cases

a. consecutive or obviously representative series of cases (1 point)
b. potential for selection biases, or not stated

3. Selection of controls

a. community controls (1 point)
b. hospital controls
c. no description

4. Definition of controls

a. no history of disease (requires clinical/research diagnostic criteria to determine this) (1 point)
b. no description of source

5. Comparability of cases and controls on the basis of the design or analysis:

a. study controls for maternal background diet (omega-6/omega-3 fatty acid intake) at baseline and in possible changes during "intervening period" (1 point)
b. study fails to control for this confounding influence

6. Comparability of cases and controls on the basis of the design or analysis:

a. study controls for age and *sex* (only child) at baseline (1 point)
b. study fails to control for this confounding influence

7. Comparability of cases and controls on the basis of the design or analysis:

a. study controls for maternal obstetric history (e.g., Gestational age) or child's health status (at birth) at baseline and in possible changes during "intervening period" (1 point)
b. study fails to control for this confounding influence

8. Comparability of cases and controls on the basis of the design or analysis:

a. study controls for child's pre/term status and/or maternal socieconomic status at baseline (1 point)
b. study fails to control for this confounding influence

9. Comparability of cases and controls on the basis of the design or analysis:

a. study controls for maternal smoking status at baseline and in possible changes during "intervening period" (1 point)
b. study fails to control for this confounding influence

10. Ascertainment of exposure

a. validated method used to extract, prepare, store and analyze lipid data where appropriate in cases/controls (1 point)
b. Inappropriate method
c. no description

11. Same method of ascertainment for cases and controls

a. yes (1 point)
b. no

12. Non-response rate (blood samples analyzed)

a. same rate for both groups (1 point)
b. non respondents described
c. rate different and no designation

DESIGN: (MULTIPLE-GROUP) CROSS-SECTIONAL STUDY

1. Control for selection bias

a. Yes = 1
b. No = 0
c. Unable to determine = 0

2. Description of the same validated method to distinguish the study populations (i.e., to confirm pre/term status, presence or absence of pre-eclampsia, etc.)

a. Yes = 1
b. No = 0
c. Unable to determine = 0

3. Homogeneity of the target population

a. Yes = 1
b. No = 0
c. Unable to determine = 0

4. Comparability of study groups on the basis of the design or analysis: age and *sex* (only child)

a. Yes = 1
b. No = 0
c. Unable to determine = 0

5. Comparability of study groups on the basis of the design or analysis: omega-3 fatty acid intake in recent (last 6 months) background diet (maternal)

a. Yes = 1
b. No = 0
c. Unable to determine = 0

6. Comparability of study groups on the basis of the design or analysis: omega-6 fatty acid intake, or omega-6/omega-3 fatty acid intake ratio, in recent (last 6 months) background diet (maternal)

a. Yes = 1
b. No = 0
c. Unable to determine = 0

7. Comparability of study groups on the basis of the design or analysis: current smoker status (maternal)

a. Yes = 1
b. No = 0
c. Unable to determine = 0

8. Comparability of study groups on the basis of the design or analysis: Gestational age (maternal)

a. Yes = 1
b. No = 0
c. Unable to determine = 0

9. Comparability of study groups on the basis of the design or analysis: pre/term status (child)

a. Yes = 1
b. No = 0
c. Unable to determine = 0

10. Description of a validated primary clinical outcome measure(s)

a. Yes = 1
b. No = 0
c. Unable to determine = 0

11. Description of the same appropriate methods used to extract, prepare, store and analyze lipid data from all study populations

a. No inappropriate descriptions = 1
b. At least one inappropriate description = 0
c. Different methods used for different study groups = 0
d. Unable to determine for one or more of the methods = 0

Uncontrolled Study Designs

DESIGN: SINGLE PROSPECTIVE COHORT STUDY (Modified Newcastle-Ottawa)

1. Representativeness of the exposed cohort

a. Truly or somewhat representative of the average individual at no (or elevated) risk/potential for a given outcome (defined by the report) in the community = 1
b. Selected group of users e.g., nurses, volunteers = 0
c. No description of the derivation of the cohort = 0

2. Ascertainment of exposure

a. Validated dietary assessment questionnaire or structured interview = 1
b. Written self-report = 0
c. No description = 0

3. Demonstration that outcome of interest was not present at start of study

a. Yes = 1
b. No = 0
c. Unable to determine = 0

4. Description of a validated method to quantify the amount, per type, of omega-3 fatty acids

a. Yes = 1
b. No = 0
c. Unable to determine = 0

5. Assessment of outcome

a. Independent blind assessment = 1
b. Record linkage = 1
c. Self-report = 0
d. No description = 0

6. Was followup long enough for outcomes to occur?

a. Yes (maternal: until delivery) = 1
b. No = 0

c. Unable to determine = 0

7. Adequacy of followup of cohort

a. Complete followup, all subjects accounted for = 1
b. Subjects lost to followup unlikely to introduce bias, small number lost, at least 90% followup, or description provided of those lost = 1
c. Followup rate of less than 90% and no description of those lost = 0

8. Analytic control for confounding: age and sex (child)

a. Yes = 1
b. No = 0
c. Unable to determine = 0

9. Analytic control for confounding: omega-6 fatty acid intake or omega-6/omega-3 fatty acid intake ratio

a. Yes = 1
b. No = 0
c. Unable to determine = 0

10. Analytic control for confounding: smoking history (maternal)

a. Yes = 1
b. No = 0
c. Unable to determine = 0

Applicability Indices

For studies involving at least one target maternal population.

Assign 'I' to a target study population of otherwise "healthy" North American (or similar) pregnant women or mothers of preterm or term infants representing a somewhat broad socio-demographic spectrum (i.e., age, race), and eating a diet "typical" of a broad spectrum North American population (e.g., with an estimated omega-6/omega-3 intake ratio of at least 15).

Assign 'II' to a target study population of otherwise 'healthy' North American (or similar) of pregnant women or mothers of preterm or term infants, *yet* representing a more circumscribed socio-demographic picture (e.g., Asian-American/Canadian), and likely eating a diet "somewhat different" from that of a broad spectrum North American population (e.g., with an estimated omega-6/omega-3 intake ratio notably less than 15, yet likely not reaching a value of 4, such as observed in Japan).

Assign 'III' to a target study population of pregnant women or mothers of preterm or term infants representing a population whose socio-demographic characteristics are notably "atypical" of a broad spectrum North American population, and eating a diet that is "notably different" from that of a broad spectrum North American population (e.g., with an estimated omega-6/omega-3 intake ratio perhaps reaching a value of 4, such as observed in Japan, or 38-50, as observed in urban India).

Assign 'X' when applicability cannot be ascertained due to incomplete or conflicting reporting of the details concerning the target study population, particularly relating to the background diet.

For studies involving a target population with or without a known elevated risk for a particular pregnancy and/or infant outcome

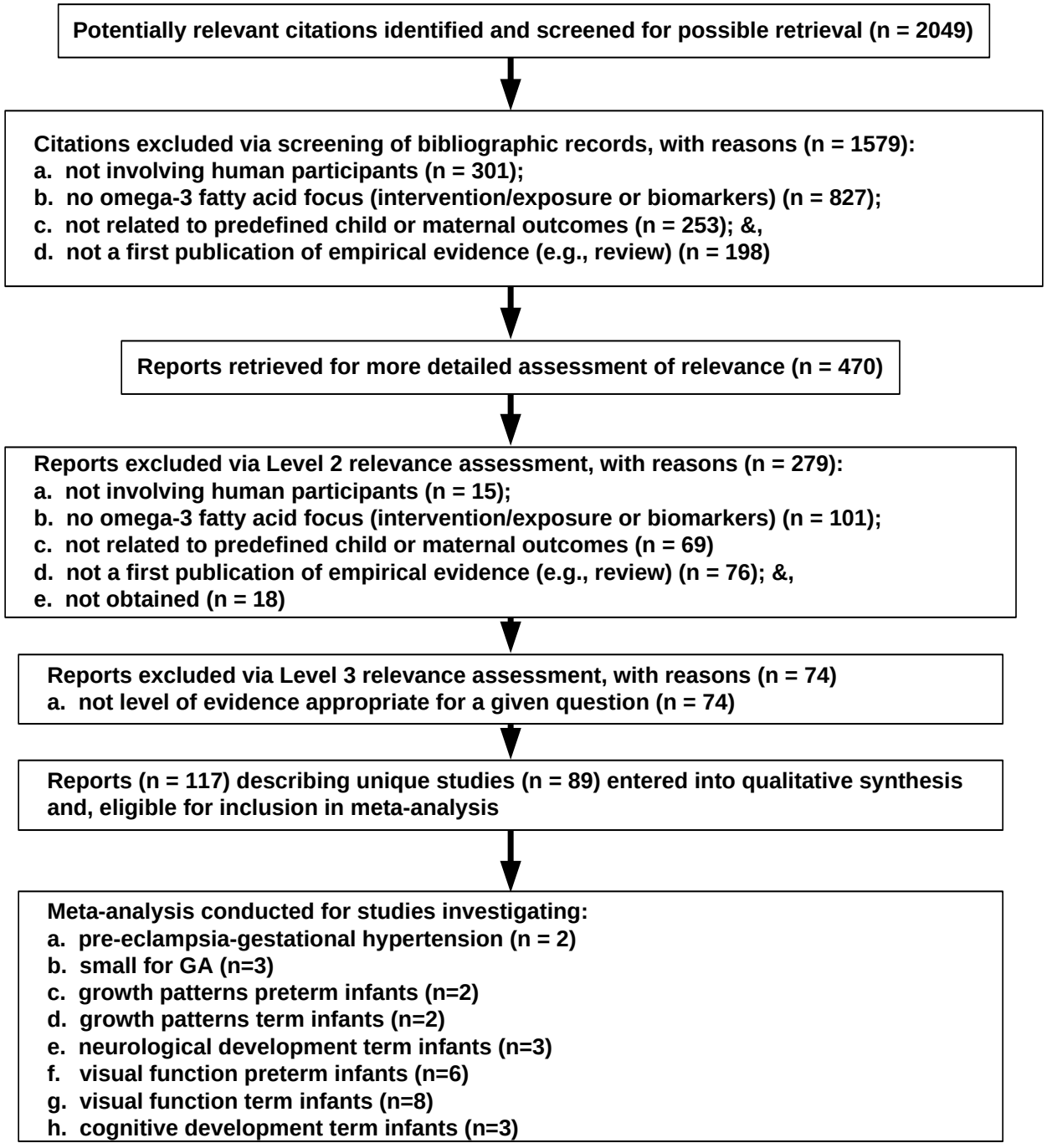
Assign 'I' to a target study population of otherwise "healthy" North American (or similar) of pregnant women or mothers of preterm or term infants, with or without a known elevated risk for pregnancy or child's outcomes (e.g., IUGR), representing a somewhat broad socio-demographic spectrum (i.e., age, race), and eating a diet "typical" of a broad spectrum North American population (e.g., with an omega-6/omega-3 intake ratio of at least 15).

Assign 'II' to a target study population of otherwise "healthy" North American (or similar) of pregnant women or mothers of preterm or term infants, with or without a known elevated risk for pregnancy or child's outcomes (e.g., IUGR), *yet* representing a more circumscribed socio-demographic picture (e.g., Asian-American/Canadian), and likely eating a diet "somewhat different" from that of a broad spectrum North American population (e.g., with an omega-6/omega-3 intake ratio notably less than 15, yet likely not reaching a value of 4, as observed in Japan).

Assign 'III' to a target study population of otherwise "healthy" of pregnant women or mothers of preterm or term infants, with or without a known elevated risk for pregnancy or child's outcomes (e.g., IUGR), *yet* representing a very circumscribed population whose socio-demographic characteristics are "notably atypical" of a broad spectrum North American population, and eating a diet that is "notably different" from that of a broad spectrum North American population (e.g., with an omega-6/omega-3 intake ratio perhaps reaching a value of 4, such as observed in Japan, or 38-50, as observed in urban India).

Assign 'X' when applicability cannot be ascertained due to incomplete or conflicting reporting of the details concerning the target study population, particularly relating to the background diet.

Appendix D. Modified QUOROM Flow Chart



Appendix E. Evidence Tables

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Agostoni, 1995, Italy {2940,359,467}	▪ RCT Parallel Double-blind ▪ Jadad total: 4 [Grade: A] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy term infants of both sexes born between Sept. 1992-Aug. 1993; GA end of 37 wk-42 wk, AGA; Apgar >7 @ 5min & absence of disease **Exclusion criteria:** NR **Enrolled/Completed:** n=90/86 **Mean Age:** • Child: F1 39.0 (1.3) wks GA; F2: 39.4 (1.4); BF 39.0 (1.1)	LCPUFA formula (palm, coconut, soybean, sunflower, evening primrose oils, egg-lipids) (n=29) vs. crtl formula (n=31) vs. HM (RS) (n=30)	LCPUFA formula (0.44 wt% AA+ 0.05 wt% EPA + 0.30 wt% DHA) within d 3 of life to 4 mo of age	• Brunet-Lézine's psychomotor developmental test: S better score in DHA+EPA in Brunet-Lezine test (DQ) at 4; NS at 24 mo • PUFA in RBC PC & PE (n=20): RBC DHA at 4 mo S (+) correlation with DQ at 4 mo; NS at 24 mo	NR
Auestad, 1997, US {380,298,6}	▪ RCT Parallel ▪ Jadad total: 3 [Grade: B] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy term infants ≥ 37 wk AGA. **Exclusion criteria:** Apgar <7 @ 5 min, physical or metabolic defects, receipt of lipid or blood transfusion, infants born to mothers with hx diabetes, hyperlipidemia, or perinatal infection **Enrolled/Completed:** n=274/173 **Mean Age:** • Child: (d) HM: 3.6 ± 2.3; F crtl: 3.2 ± 2.5; F DHA+AA: 2.8 ± 1.9; F DHA n= 2.6 ± 1.9	DHA+AA formula (from egg yolk phospholipids) (n=68) vs. DHA+EPA formula (from high-DHA, low-EPA tuna oil) (n=65) vs. Crtl formula (n=65) vs. HM (RS) (n=76)	Dose NR; all formulas at least 4 mo	• Growth patterns: S↓ wt in F4 than in F1 at 4 mo; NS in L, HC, TST, & SST at 4 & 8 mo • Bayley's PDI & MDI: S better in crtl gp vs. DHA+AA in PDI at 12 mo; NS among 3 gps • Sweep VEP: NS acuity thresholds at 2, 4, 6, 9 or 12 mo • Acuity card procedure: NS at 2,4,6,9, 12 or 39 mo of age	Ross Products Division, Abbott Laboratories; US Maternal & Child Health Bureau

d = day(s); ERG = electroretinogram; g = gram(s); HM = human milk; mo = month; n = number of participants; NR = not reported; RCT = randomized control trial; RS = reference standard; TG = triglycerides; UK = United Kingdom; US = United States; VEP = visual evoked potential; wk(s) = week(s); LCPUFA = long-chain polyunsaturated fatty acids; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; GA = gestational age; AGA = weight appropriate for gestational age; F = feeding formula; GP = growth parameters; TST = triceps skinfold thickness; SST = subscapular skinfold thickness; PDI = psychomotor developmental index; MDI = Mental developmental index; S = statistically significant difference; NS = nonsignificant statistical difference; DQ = developmental quotien; RBC = red blood cells; PE = phosphatidyl ethanolamine; HC = head circumference; wt = weight; L = length; gp(s) = group(s); ctrl(s) = control(s)

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Auestad, 2001a US {125}	▪ RCT Parallel Double-blind ▪ Jadad total: 5 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** Healthy term infants fed formula (GA 37-42 wk) **Exclusion criteria:** Evidence of S cardiac, respiratory, ophthalmologic, gastrointestinal, hematologic, or metabolic disease; milk-protein allergy; or maternal medical hx with proven adverse effects on the fetus, tuberculosis, human immunodeficiency virus infection, prenatal infections or substance abuse **Enrolled/Completed:** n=239/165 **Mean Age:** • Maternal: NR • Child: FF crtl = 39.4 ± 1.2 wk; FF egg-DTG = 39.0 ±1.3 wk, FF fish/fungal = 39.3 ± 1.2 wk	DHA+AA formula (derived from egg-TG) (n=80) vs. DHA+EPA+AA formula (LCPUFAs derived from fish and fungal oils) (n=82) vs. Crtl formula (n=77) vs. HM (n=165)	LCPUFA formulas (0.13-0.14 wt% DHA & 0.45 wt% AA & <0.04 wt% EPA) for 12 mo	• Teller Acuity Card Procedure: NS at 2, 4, 6 & 12 mo of age • Growth patterns: NS in wt, L, HC at 1, 2, 4, 6, 9, & 12 mo; S↑ wt gain in males in DHA+AA (egg) at 4 mo • Fagan Test: NS at 6, 9 mo • Bayley Scale: NS in PDI & MDI at 6 & 12 mo • MacArthur Communicative Development Inventories: NS at 9 mo; S ↑ vocabulary expressions score in DHA+AA (fish/fungal) vs. DHA+AA (egg-TG) at 14 mo	Ross Products Division

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; ERG = electroretinogram; RCT = randomized control trial; RS = reference standard; TG = triglycerides; UK = United Kingdom; US = United States; LCPUFA = long chain poly unsaturated fatty acids; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); PDI = psychomotor developmental index; MDI = Mental developmental index; HC = head circumference; wt = weight; L = length; ctrl(s) = control(s)

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Auestad, 2001b US {125}	▪ RCT Parallel Double-blind ▪ Jadad total: 5 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** Healthy term infants breast-fed (gestational age 37-42 wk) **Exclusion criteria:** Evidence of significant cardiac, respiratory, ophthalmologic, gastrointestinal, hematologic, or metabolic disease; milk-protein allergy; or maternal medical hx with proven adverse effects on the fetus, tuberculosis, human immunodeficiency virus infection, prenatal infections or substance abuse **Enrolled/Completed:** NR **Mean Age:** • Maternal: NR • Child: NR	HM/DHA+AA formula (derived from egg-TG) (n=83) vs. HM/Crtl formula (n=82)	LCPUFA formulas (0.13-0.14 wt% DHA & 0.45 wt% AA) from 3 mo of age to 12 mo	• Teller Acuity Card Procedure: NS at 2, 4, 6 & 12 mo of age • Growth patterns: NS in wt, L, HC at 1, 2, 4, 6, 9, & 12 mo or in wt, L, HC gain • Fagan Test: NS at 6, 9 mo • Bayley Scale: NS in PDI & MDI at 6 & 12 mo • MacArthur Communicative Development Inventories: NS at 9 mo; S ↑ vocabulary expressions score in DHA+AA (fish/fungal) vs. DHA+AA (egg-TG) at 14 mo	Ross Products Division

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; RCT = randomized control trial; RS = reference standard; TG = triglycerides; UK = United Kingdom; US = United States; LCPUFA = long chain polyunsaturated fatty acids; NIH = National Institute of health; BAEP = brainstem auditory evoked potentials; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); PDI = psychomotor developmental index; MDI = Mental developmental index; HC = head circumference; wt = weight; L = length; ctrl(s) = control(s)

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Birch, 1992, US {603,672,598,235,534,561}	▪ RCT Parallel ▪ Jadad: total: 2 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** VLBW infants with birth weight of 1000-1500 g appropriate for GA, able to receive enteral feedings (70-120 kcal/g) & free of major neonatal morbidity by d 10 **Exclusion criteria:** S respirator tx > 7 d, congenital infection, gross congenital malformation, Grade III or IV intracranial hemorrhage, & > Grade 2 retinopathy of prematurity **Enrolled/Completed:** n=81/52 **Mean Age:** • Materanl: NR • Child: Intrauterine = 35.0 wk (0); VLBW gp = 30.4 wk (1.5)	Soy/marine oil (DHA+EPA+AA) formula (n=26) vs. soy oil formula (n=22) vs. corn oil formula (low n-3) (n=18) vs. HM (RS) (n=10)	LCPUFA formula (1.4 g AA, 0.65 g EPA, 0.35 g DHA in 100 ml) for 6 mo	• VEP: S↓ in VEP for all grps at 57 wks; S ↓ VEP in DHA+EPA vs. grps 2-3 at 36-57 wks • Full-field ERG: NS b-Rod ERG at 36-57 wks • FPL acuity: DHA+EPA gp had a better FPL acuity (of borderline statistical significance) vs. corn oil at 57 wks • Growth parameters: NS in wt, L, HC, TST, SST at 3, 9, 17, 26 wks • BMK: S correlation (-) between RBC AA at 57 wks & length z score at 57 wks PCA; S correlation between RBC-DHA/DPA & VEP; RBC-DHA/DPA & FPL at 57 wks	National Eye Institute, National Institute of Child Health & Development, United Cerebral Palsy Research Foundation, Pediatric Subunit US Public Health Service

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; ERG = electroretinogram; RCT = randomized control trial; RS = reference standard; TG = triglycerides; US = United States; VEP = visual evoked potential; LCPUFA = long chain polyunsaturated fatty acids; NIH = National Institute of health; VLBW = very low birth weight; FPL = forced-choice preferential looking; BMK = biomarkers in blood or other tissues; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; TST = triceps skinfold thickness; SST = subscapular skinfold thickness; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); HC = head circumference; wt = weight; L = length; PCA = postconceptional age; RBC = red blood cells; ctrl(s) = control(s)

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Birch, 1998, US {2301,198,164}	▪ RCT Parallel Double-blind ▪ Jadad total: 5 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** Term infants (37-40 wk) singleton, appropriate weight for GA **Exclusion criteria:** Family hx of mild protein allergy, genetic or familial eye disease, vegetarian or vegan maternal dietary patterns, maternal metabolic disease, anemia, or infection, presence of congenital malformation or infection, jaundice, perinatal asphyxiameconium aspiraton, & any perinatal event leading to NICU admission **Enrolled/Completed:** n=108/80 **Mean Age:** • Maternal: 29.1 (4.8) y • Child: NR	DHA+AA formula (derived from SCO) (n=27) vs. DHA formula (derived from SCO) (n=26) vs. Crtl formula (n=26) vs. HM (n=29)	DHA+AA formula (0.36 wt% DHA, 0.72wt% AA, 14.9wt% LA, 1.53 wt% ALA); DHA formula (0.35 wt% DHA, 0.02 wt% AA, 15.1 wt% LA, 1.54 wt% ALA); Crtl formula (14.6 wt% LA, 1.49 wt% ALA) for 17 wk	• Sweep VEP: S poorer sweep VEP acuity in crtl than DHA or DHA+AA at 6 wks; DHA or DHA+AA at 17 wks; DHA or DHA+AA at 52 wks • ERG: S better ERG & DHA or DHA+AA at 6 wks • FPL acuity: NS diet on FPL acuity • Growth pattern: NS in wt, L, HC, TST, SST at 17 wks • Neurological development: NS in PDI at 18 mo; NS in BRS at 18 mo • Cognitive: MDI S better in n-3 formulas vs. crtl at 18 mo • BMK: NS correlation of PDI & BRS at 18 mo and plasma & RBC LA, ALA, AA, EPA, or DHA at 4 mo & 12 mo; MDI score at 18 mo correlated (+) with plasma & RBC DHA at 4 mo; RBC-LA & ALA correlated (-) with MDI at 18 mo	NIH

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; ERG = electroretinogram; RCT = randomized control trial; RS = reference standard; TG = triglycerides; US = United States; VEP = visual evoked potential; LCPUFA = long chain polyunsaturated fatty acids; NIH = National Institute of health; FPL = forced-choice preferential looking; BMK = biomarkers in blood or other tissues; NICU = neonatal intensive care unit; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; BRS = behavioral rating scales; ERG = electroretinogram; MDI = mental developmental index; PDI = psychomotor developmental index; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s)

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Birch, 2002, US {87}	▪ RCT Parallel ▪ Jadad total: 5 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** Healthy term infants 6 wks age born at 37-40 wk PMA, singleton births, BW appropriate for GA. **Exclusion criteria:** Family hx of milk protein allergy; genetic or familial eye disease; vegetarian or vegan maternal dietary patterns; maternal metabolic disease, anemia or infection; presence of a congenital malformation or infection; jaundice; perinatal asphyxia; meconiumaspiration; & any perinatal event that resulted in NICU admission **Enrolled/Completed:** n=65/58 **Mean Age** • Maternal: NR • Child: 5.1± 1.2 wks	DHA+AA formula (derived from SCO) (n=32) vs. Crtl formula (n=33)	DHA+AA formula (0.36 wt% DHA, 0.72wt% AA, 14.9wt% LA, 1.53 wt% ALA); Crtl formula (14.6 wt% LA, 1.49 wt% ALA) from 7 to 52 wk	• Cortical VEP: NS DHA+AA on sweep VEP at 6 wks; S DHA+AA & better sweep VEP 17, 26 & 52 wks • FPL: S DHA+AA & better FPL at 17 wks • Growth patterns: NS in wt, L, HC, TST & SST at 0,6,17,26 & 52 wks • BMK: S better sweep VEP & plasma AA at 17, 52 wks & plasma DHA at 17, 52 wks; S better sweep VEP & RBC AA at 52 wks & RBC DHA at 17 & 52 wks; NS sweep VEP & plasma or RBC LA or ALA at 17 or 52 wks; S better FPL & plasma DHA at 17 wks or RBC LA at 17 wks; NS FPL & plasma or RBC ALA, AA, plasma LA, or RBC DHA	NIH

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; PMA = postmenstrual age; BW = birth weight; US = United States; VEP = visual evoked potential; LCPUFA = long chain polyunsaturated fatty acids; NIH = National Institute of health; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; TST = triceps skinfold thickness; SST = subscapular skinfold thickness; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); SCO = single cell oil; NICU = neonatal intensive unit care; HC = head circumference; wt = weight; L = length; FPL = forced-choice preferential looking

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Bougle, 1999, France {233}	▪ RCT Parallel Double-blind ▪ Jadad total: 3 [Grade: B] ▪ Schulz: Unclear	**Inclusion criteria:** Premature, healthy, appropriate for GA infants (<34 wk postmenstrual age); free of respiratory, metabilic or neurological disease, of malformation, & of infection; fed by digestive route within the first 7 d of life, no hx of intrauterine asphyxia **Exclusion criteria:** Stop or change of study diet for > 2 d; any neurological event; haemorrhage of > 2 in cerebral ultrasound **Enrolled/Completed:** 40/33 **Mean Age:** • Maternal: NR • Child: BF GA = 33.1 (1.5) wk; FF gp A GA = 32.2 (1.1) wk; FF gp B GA = 33.9 (1.0) wk	LCPUFA-enriched formula (DHA, EPA, ALA) (n=14) vs. Crtl formula (n=11)/ HM: DHA 0.5%; EPA 0.5%; ALA 0.4% (n=15)	Dose: NR during 30 d (from 1st d of enteral feeding)	• Growth: NS in wt, L, HC, Δ L, & Δ HC at 1 mo • Electrophysiologic studies of peripheral nerves: NS LAEP between d 0 & 30d; S↑ Δ motor NCT (m/s) in DHA/EPA/AA supplemented formula & HM from d0-30; NS Δ sensory (m/s) test • VEP: NS in VEP (N1 wave latency) at 30 d	NR

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported;; hx = history; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; VEP = visual evoked potential; LCPUFA = long chain polyunsaturated fatty acids; LAEP = latency auditory evoked potentials; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); NCT = nerve conduction tests; HC = head circumference; wt = weight; L = length

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Bulstra-Ramakers, 1994, Netherlands {481}	RCT Parallel Double-blind Jadad total: 5 [Grade: A]; Schulz: Adequate	**Inclusion criteria:** BW < the 10th PC corrected for gestational age, parity & sex in association with GHT; BW < the 10th PC in association with chronic renal disease; BW < the 10th PC & placental abnormalities suggestive of impaired uterplacental circulation **Exclusion criteria:** Women with diabetes, systemic lupus erythematosus or other connective tissue disease; women on low dose aspirin for tx with obstetric hx **Enrolled/Completed:** n=68/63 **Mean Age:** • Maternal: NR • Child: NR	EPA + DHA capsules (n=32) vs. placebo (coconut oil) (n=31)	4 capsules, 025 mg EPA +DHA, 3 x d (3g/d); from 12-14 wks PMA unitl delivery	• Incidence of IUGR: NS in IUGR recurrence rate (grp 1 vs. grp 2) • Incidence GHT: NS rate of GHT (grp 1 vs. grp 2) • Duration of gestation: NS in % premature deliveries	NR
Carlson, 1987, US {736}	RCT parallel Double-blind Jadad total: 2 [Grade: C]; Schulz: Unclear	**Inclusion criteria:** Healthy LBW infants < 1,500g **Exclusion criteria:** Free of major congenital malformations & had no major disease process such as bronchopulmonary dysplasia **Enrolled/Completed:** n=61/39 **Mean Age:** • Maternal: NR • Child: crtl = 28 ± 14 d, fish oil = 26 ± 10 d; age for subgroup of n=19 (completing 6 wk f/u) crtl = 29 ± 18 d, fish oil = 27 ± 11 d	MaxEPA preterm formula (fish oil) (n=30) vs. preterm formula (n=31)	MaxEPA 750 mg/kg/d 1/d by orogastric tube during 4 wks	• Weight gain: NS in ∆ wt at 4 wks	Ross Laboratories

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; ERG = electroretinogram; RCT = randomized control trial; RS = reference standard; TG = triglycerides; US = United States; LCPUFA = long chain polyunsaturated fatty acids; NIH = National Institute of health; V(LBW) = very (low birth weight); PC = percentile; GHT = gestational hypertension; f/u = follow-up; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); IUGR = intrauterine growth retardation; wt = weight

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics (enrolled/evaluated)	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Carlson, 1992, US {581,555,423,634,573,2950}	▪ RCT Parallel ▪ Jadad total: 4 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** VLBW premature infants, tolerating enteral intakes > 462 kJ.kg body wt -1 for 5-7 d if at that time they did not require 1) mechanical ventilation, 2) have intraventricular hemorrhage > grade 2, 3) have ROP > stage 2, 4) require surgical intervention for necrotizing enterocolitis, 5) have severe intrauterine growth retardation defined as BW < 5th PC for GA, or 6) have a hx of maternal substance abuse **Exclusion criteria:** Risk factors for poor growth other than prematuritly, severely growth retarded in utero (weight < the 5th PC for their GA), long tx of mechanical ventilation or GI surgery; risk factors for cognitive & visual development: intraventricular/ periventricular hemorrhage > grade 2, ROP > stage 2, hx of maternal cocaine use **Enrolled/Completed:** n=79/65 **Mean Age:** • Maternal: NR • Child: crtl = 25 (10) d; marine FF = 22 (8) d	Supplemented formula (marine oil) (n=31) vs. crtl formula (n=34)	Preterm formula (0.3 g EPA, 0.2 g DHA) until discharge (1,800g), then term formula until 79 wks	• Growth patterns: S↓ wt, L, HC in marine oil at 40, 48, 57, 68, 79, 93 wks PCA • Teller Acuity Card: S ↑ resolution acuity in DHA + EPA vs. crtl at 2 & 4 mo • Fagan Test of Infant Intelligence: DHA-supplemented infants had a S ↓ novelty preference vs. crtl gp • BMK: wt & L z-scores correlated + with plasma & RBC AA at 2,4,5,6,9, 12 mo; HC correlated (+) plasma & RBC AA at 2, 4 mo; S correlation (+) RBC DHA at 2 mo with visual acuity at 2 & 4 mo	National Eye Institute Ross Laboratories

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; ERG = electroretinogram; RCT = randomized control trial; RS = reference standard; TG = triglycerides; US = United States; VEP = visual evoked potential; LCPUFA = long chain polyunsaturated fatty acids; NIH = National Institute of health; V(LBW) = very (low birth weight); FPL = forced-choice preferential looking; BMK = biomarkers in blood or other tissues; GI = gastrointestinal; PC = percentile; ROP = retinopathy of prematurity; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); PCA = postconceptional age; HC = head circumference; wt = weight; L = length

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics (enrolled/evaluated)	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Carlson, 1996, US {415}	▪ RCT Parallel Double-blind ▪ Jadad total: 3 [Grade: B] ▪ Schulz: unclear	**Inclusion criteria:** Full term healthy infants (37-43 wk PMA) **Exclusion criteria:** IUGR; medical problems that may influence long-term growth & development **Enrolled/Completed:** n=94/58 **Mean Age:** • Maternal: NR • Child: BF GA = 39.5 ± 1.3 wk; Crtl FF GA = 40.3 ± 0.9 wk; Experimental FF GA = 39.8 ± 1.2 wk	DHA+AA formula (LCPUFAs derived from egg-TG) (n=28) vs. Crtl formula (n=31) vs. HM (n=35)	LCPUFA formula (0.1 wt% DHA, 0.43 wt% AA) for at least 4 mo	• Binocular visual acuity: S better visual acuity with DHA+AA at 2 mo of age	National Institute of Child Health & Human Development
Carlson, 1996, US {434,424}	▪ RCT Parallel Double-blind ▪ Jadad total: 3 [Grade: B] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy preterm infants who acheived full enteral feeding of 418 kJ (200 kcal). kg-1 by 6 wk of age & tolerated enteral feeding thereafter were allowed to remain in the study **Exclusion criteria:** Intraventricular or perventricular hemorrhage > grade 2, hx of maternal cocaine or alcohol abuse, congenital anomalies affecting long-term growth & development, or intrauterine growth retardation (wt < the 5th PC for GA) **Enrolled/Completed:** n=94/59 **Mean Age:** • Maternal: NR • Child: Ctrl no BPD = 28.6 (1.3) wk, BPD =27.5 (1.6) wk; FF (marine) no BPD = 28.5 (1.2) wk, BPD = 27.0 (1.1) wk	LCPUFA formula (marine oil) (n=18) vs. crtl formula (n=18)	Similac Special Care 0.2%DHA, 0.06%EPA, vitamin E from 3-5 d age to 2 mo CA	• Teller Acuity Card: S↑ higher acuity in DHA+EPA vs. crtl at 2 mo; NS at 4-12 mo • Growth patterns: S↓ wt, L, HC in LCPUFA at 6 & 9 mo • BMK: S (-) correlation between wt-for-L & RBC PE DHA at 5 mo; S (+) correlation between L & RBC PC AA at 5 mo	National Eye Institute National Institute of Child Health &Human Development Ross Products Division Abbott Laboratories

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; RCT = randomized control trial; TG = triglycerides; US = United States; LCPUFA = long chain polyunsaturated fatty acids; PC = percentile; CA = corrected age; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); IUGR = intrauterine growth retardation; wt-for-L = weight for length; PE = phosphatidyl ethanolamine; BPD = bronchopulmonary dysplasia; PC = phosphatidylcholyne; HC = head circumference; wt = weight; L = length

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Clandinin, 2002, Canada {1553,1552, 1565}	▪ RCT Parallel Double Blind ▪ Abstract	**Inclusion criteria:** VLBW term & preterm infants **Exclusion criteria:** NR **Enrolled/Completed:** n=361/NR **Mean Age:** • Maternal: NR • Child: NR	DAS (DHA+AA from SCO) (n=72) vs. DAF (DHA from fish oils+AA from SCO) (n=90) vs. Crtl formula (n=83) vs. HM (RS) (n=105)	Preterm, discharge & term formulas until 57 wks PMA, then beikost until 92 wks PMA	• Bayley's MDI, PDI: MDI: DAS & DAF formulas had S > scores than crtl formula (118 wks PMA); S↑ PDI score formula; (DAS, DAF) vs. crtl gp • Growth patterns: NS in GP at 40, 57 wks PMA; S↑ wt in DHA+AA (SCO) than in crtl at 66-118 wks PMA; S↑ L in DHA+AA (SCO) than in other 2 formulas at 79, 92 wks PMA	Mead Johnson & Co.
D'Almeida, 1992, South Africa {580}	▪ RCT parallel Partially double-blind ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Inadequate	**Inclusion criteria:** Primiparous & multiparous women in the first 4 mo of pregnancy **Exclusion criteria:** NR **Enrolled/Completed:** n=150/NR **Mean Age:** • Maternal: 14-40 y	GLA+EPA+DHA (primrose + fish oil) (n=50) vs. magnesium oxide (n=50) vs. placebo (olive oil + vitamin E) (n=50)	GLA+EPA+DHA & placebo: 240 capsules (8 x d); Magnesium oxide: 60 tablets x mo (2 tablets x d; 500 mg)	• Incidence of GHT, preeclampsia & eclampsia during pregnancy: Rate of GHT ↑ in grps 1-3 vs. grp 2 (p = NR); rate of preeclampsia/eclampsia ↑ in grp 3 vs. grps 1-2	Efamol Ltd.

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; ERG = electroretinogram; RCT = randomized control trial; RS = reference standard; TG = triglycerides; LCPUFA = long chain polyunsaturated fatty acids; V(LBW) = very (low birth weight); GHT = gestational hypertension; ↑ = increase/greater; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); MDI & PDI = Bayley's Mental and Psychomotor Indexes; SCO = single cell oil; GP = growth patterns

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
de Groot, 2003, Netherlands {2907,2935}	▪ RCT parallel Double-blind ▪ Jadad total: 3 [Grade: B] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy white pregnant women GA < 14 wk; normal health; fish consumption of < 2 /wk **Exclusion criteria:** Diastolic BP >90mmHg, multiple pregnancy, use of medication, use of LCPUFA rich supplements **Enrolled/Completed:** n=79/58 **Mean Age:** • Maternal: Crtl = 29.2 ± 3.8 y; Experimental = 30.0 ± 3.3 y • Child: Crtl GA = 276.5 ± 12.2 d; Experimental GA = 281.0 ± 7.4 d	ALA enriched, ↑ LA margarine (n=29) vs. crtl gp (n=29)	25 g margarine/d: 45.4% LA + 14.2% ALA (n-3) of total FA from wk 14 GA until delivery	• Duration of gestation: NS in GA • Birth weight: S ↑ in ALA+LA vs. LA • BMK: S (+) correlation maternal plasma & RBC DHA & birth wt; S (+) correlation DHA intake & BW	Unilever Research & Development

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; ERG = electroretinogram; RCT = randomized control trialTG = triglycerides; LCPUFA = long chain polyunsaturated fatty acids; GHT = gestational hypertension;; ↑ = increase/greater; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; LA = linoleic acid; ALA = α-linolenic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); BW = birth weight

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Decsi, 1995, Hungary {460}	▪ RCT Parallel ▪ Jadad total: 1 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** FF full term infants appropriate for GA **Exclusion criteria:** NR **Enrolled/Completed:** n=22/NR **Mean Age:** • Maternal: NR • Child: FF gp = 39.4 ± 1.3 wk; LCPUFA-F gp = 38.9 ± 1.1 wk	Pre-Aptamil with Milupan (egg lipids, primrose oil) (n=12) vs. Pre-Aptamil without LCPUFA (n=10)	120-150 ml/kg/d 0.5% AA + 0.03% EPA + 0.3% DHA + vitamin E formula ad libitum for 4 mo	• Growth patterns: NS in wt, L, HC at 6, 16, 30 wks • BMK: NS correlation of RBC LCPUFA & GP	Deutsche Forschungsgemeinschaft, Bonn Germany; scholarship Milupa Austria
Dunstan, 2004, Australia {2917}	▪ RCT Parallel Double-blind ▪ Jadad total: 3 [Grade: B] ▪ Schulz: Unclear	**Inclusion criteria:** Pregnant women with hx of allergic rhinitis or asthma but otherwise healthy **Exclusion criteria:** Smokers; high risk pregnancy; ate fish more than once/week **Enrolled/Completed:** n=98/83 **Mean Age:** • Maternal: crtl: 32.4 ±0.5 y; fish Oil: 31.1 ± 0.6 y • Child: NR	Capsules with LCPUFAs (derived from fish oil, treatment gp) (n=40*) vs. Capsules with olive oil (Crtl grp) (n=43*)	2.2 g/day DHA, 1.1 g/day EPA in capsules for 19 wk	• Duration of gestation: NS in GA • Growth patterns at birth: NS in L, wt, & HC at birth	NH & MRC and Raine Medical Research Foundation

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Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Faldella, 1996, Italy {390,2375}	▪ RCT Parallel ▪ Jadad total: 1 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy preterm infants < 33 wk GA, of appropriate weight, no malformation interfering with somatic &/ or psychomotor development, no neurological, visual, acoustic or gastroenterological illnesses, no hx of perinatal asphyxia, normal fundus oculi, & by 10 d age all received at least 50% of calorie requirement through enteral feeding **Exclusion criteria:** NR **Enrolled/Completed:** n=66/58 **Mean Age** • Maternal: NR • Child: BF = 31.8 wk, FF LCPUFA = 31.1 wk, FF Crtl = 31.3 wk	LCPUFA-enriched formula (Milupan) (n=23) vs. Crtl formula (n=26)/ HM (RS) (n=17)	LCPUFA formula (DHA 0.23%; EPA 0.08%;ALA 0.40%) until 52 wks PCA	• VEP: S shorter wave (N4 & P4) latencies VEP in DHA+EPA vs. crtl at 52 wks PCA • BAEP test: NS in BAEP across grps1-3 • ERG: NS in ERG (a & b) latencies across grps1-3 • Growth patterns: NS in ∆ wt, ∆ L, ∆HC at 52 wks PCA • BMK: at 52 wks PCA, inverse correlation between: RBC-DHA & N4 wave latency; RBC-DHA & P4 wave latency	NR

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month(s); y = year(s); n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; ERG = electroretinogram; RCT = randomized control trial; RS = reference standard; TG = triglycerides; VEP = visual evoked potential; LCPUFA = long chain polyunsaturated fatty acids; BAEP = brainstem auditory evoked potentials; BMK = biomarkers in blood or other tissues; PCA = postconceptional age; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Fewtrell, 2002, UK {2129}	▪ RCT Parallel Double-blind ▪ Jadad total: 5 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** Preterm infants < 1,750 g; GA < 37 wk; free of congenital malformations known to affect neurodevelopment; mothers decided not to BF by 10 d of age; tolerant of enteral feeding at the time of enrolment **Exclusion criteria:** NR **Enrolled/Completed:** n=283/240 **Mean Age** • Maternal: NR • Child: BF crtl = 30.3 (2.4) wk; FF Crtl = 30.3 (2.0) wk; FF LCPUFA = 30.4 (2.3) wk	Supplemented preterm formula (egg-lipids) (n=95) vs. crtl preterm formula (n=95) vs. HM (RS) (n=88)	NR dose; mean 33 (SD=17) d in crtl gp vs. mean 31 (SD=21) d in supplemented formula	• Bayley's MDI & PDI: NS PDI & MDI between formula gps at 18 mo • Knobloch, Passamanick & Sherrard's Developmental Screening Inventory: NS between formula gps at 9 mo • Growth: S↓ wt, L in LCPUFA than in pb at 9 & 18 mo CA; NS in HC at 9, 18 mo CA	Numico Research

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; RCT = randomized control trial; RS = reference standard; LCPUFA = long chain polyunsaturated fatty acids; VLBW = very low birth weight; UK = United Kingdom; ↑ = increase/greater; PCA = postconceptional age; MDI & PDI = Bayley's Mental and Psychomotor Indexes; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; SD = standard deviation; CA = corrected age

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Fewtrell, 2004, UK {2938}	▪ RCT Parallel Double-blind ▪ Jadad total: 5 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** Healthy preterm infants with BW ≤ 2,500g, GA < 35 wks, receiving at least some of their enteral feeds as formula milk during NICU stay **Exclusion criteria:** Congenital malformations known to affect growth or neurodevelopment **Enrolled/Completed:** n=238/199 **Mean Age:** • Maternal: crtl = 28.5 (5.7) y; LCPUFA= 29.0 (4.6) y • Child: crtl= 13.9 (10.4) d; LCPUFA 14.3 (9.6) d	↑ DHA/EPA (borage, tuna fish oil) formula (n=122) vs. Crtl formula (n=116)	Preterm formula until 2 kg or discharge, then postdischarge formula until 9 mo after term	• Bayley's MDI & PDI: NS formula gps in MDI & PDI at 18 mo • Growth patterns: S↑ Δ wt, Δ L in LCPUFA than in crtl at 9 mo; NS in HC at 9 mo; NS in GP at 18 mo	H.J. Heinz Company, Ltd
Field, 2000, Canada, {2191}	▪ RCT Parallel ▪ Jadad total: 1 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria** Preterm infants GAs between 27 & 36 wks size appropriate for GA & receive 100% of daily fluid & energy requirements enterally by d 14 of life **Exclusion criteria:** Infants with major congenital malformation, documented systemic or congenital infection, significant neonatal morbidity or acute illness that precludes oral feeding; mixed feeding; corticosteroid use; RBC & plasma transfusion; or IV lipid emulsion beyond d 8 **Enrolled/Completed:** n=44/44 **Mean Age:** • Maternal: NR • Child: BF = 32 ± 2wk; Ctrl F = 31 ± 2 wk; F + LCPUFA = 32 ± 2 wk	Supplemented preterm formula (DHA+AA derived from SCO) (n=15) vs. Crtl preterm formula (n=12) vs. HM (RS) (n=17)	LCPUFA formula (0.35 wt% DHA, 0.49 wt% AA) from 8 to 42 d of age;	• Growth patterns: S↓ Δ wt in HM than in LCPUFA & pb at 28 d; NS in L, HC at 35 d	Wyeth Nutrionals; Natural Sciences & Engineering Research Council of Canada; Medical Research Council of Canada

g = gram(s); h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; RCT = randomized control trial; RS = reference standard; LCPUFA = long chain polyunsaturated fatty acids; UK = United Kingdom; MDI & PDI = Bayley's Mental and Psychomotor Indexes; NICU = neonatal intensive care unit; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; GP = growth patterns

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Ghebremeskel, 1999, UK {2262}	▪ RCT Parallel ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy preterm infants **Exclusion criteria:** Congenital malformations; metabolic disorders **Enrolled/Completed:** n=61/35 **Mean Age:** • Maternal: NR • Child: GA = 29.5 wks ± 2.4 wks	LCPUFA preterm formula (DHA+AA derived from egg-TG) (n=7) vs. LCPUFA formula+HM (n=14) vs. Crtl preterm formula (n=8) vs. Crtl formula+ HM (n=12) vs. HM (RS) (n=20)	LCPUFA formula+HM (0.85+-0.25wt% DHA); Crtl formula+HM 0.55+-0.25wt% DHA); LCPUFA formula (0.30wt% DHA) for a mean of 11 wk (range 7-15 wk)	• Growth patterns: NS in wt, L, HC at ≈11 wk among 5 grps	The Christopher H.R. Reeves Charitable Trust; Milupa
Gibson, 1997, Australia {2959}	▪ RCT Parallel Double-blind ▪ Jadad total: 3 [Grade: B] ▪ Schulz: Unclear	**Inclusion criteria:** Mothers of term infants (> 37 wks GA), who intended to BF for ≥ 12 wks; infants were healthy, AGA, apgar > 7 @ 5 min **Exclusion criteria: NR** **Enrolled/Completed:** n=52/50 **Mean Age:** • Maternal: 30 ± 4 y • Child: GA = 39 – 40 ± 1 wks	DHASCO (DHA-rich algal oil) 5 different doses (n=12 vs. n=10 vs. n=12 vs. n=10 vs. n=8)	Maternal DHA doses: 0 gvs. 0.2 g vs. 0.4 gvs. 0.9 g vs. 1.3 g). HM (DHA content: 0.21% vs. 0.35% vs. 0.46% vs. 0.86% vs. 1.13% of FA)	• VEP: NS VEP at 12 & 16 wks • Bayley's MDI & PDI: NS in PDI at 12 mo & 24 mo; S correlation between MDI & DHA in infants's diet at 1 y; NS at 2 y • BMK: No correlation VEP & DHA HM, infant plasma or RBC LCPUFA; S correlation between MDI & DHA status (RBC & plasma at 12 wks) at 1 y	Martek Biosciences, NH & MRC

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; RCT = randomized control trial; RS = reference standard; TG = triglycerides; VEP = visual evoked potential; LCPUFA = long chain polyunsaturated fatty acids; NIH = National Institute of health; BAEP = brainstem auditory evoked potentials; VLBW = very low birth weight; BMK = biomarkers in blood or other tissues; UK = United Kingdom; MDI & PDI = Bayley's Mental and Psychomotor Indexes; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Gobel, 2003 Germany {1516}	▪ RCT Parallel ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy preterm infants, GA between 28 wk + 0 d & 36 wk + 6 d, admission to the intensive care nursery of the study centers within 24 h of birth, & expected requirment for parental nutrition providing at least 80% of total energy intake from the duration of study **Exclusion criteria:** Severe malformation of visceral organs, kidneys, lung, or brain or inborn errors of metabolism **Enrolled/Completed:** n=45/33 **Mean Age:** • Maternal: NR • Child: gp O GA = 220 d ±16.5 d, gp S GA = 224 d ±12.8 d	Olive/soybean oil emulsion (DHA + ALA) (n=24) vs. Soybean oil emulsion (DHA + ALA) (n=21)	IV lipid infusion olive/soybean oil emulsion (DHA 0.23% + ALA 2.0%); IV lipid infusion soybean oil emulsion (DHA 0.34% & ALA 6.99%) for 7 d	• Safety	Deutsche Forschungsemeinschaft, Bonn, Germany; Baxter SA, Maurepas, France
Groh-Wargo, 2002, US, Canada {1538}	▪ RCT Parallel ▪ Abstract	**Inclusion criteria:** FF preterm infants, 750-1,800g < 33 wk gestation **Exclusion criteria:** NR **Enrolled/Completed:** n=57/NR **Mean Age:** • Maternal: NR • Child: NR	Supplemented preterm formula (DHA+AA derived from egg-TG) (n=18) vs. Supplemented preterm formula (DHA+AA derived from fish oil) (n=18) vs. Crtl preterm formula (n=21)	Preterm formula (0.26 wt% DHA, 0.42 wt% AA) until term, then postdischarge formula until 1 y CA	• Growth patterns: NS in GP at 12 mo CA	Abbott Laboratories, GCRC NIH

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; ERG = electroretinogram; RCT = randomized control trial; RS = reference standard; TG = triglycerides; LCPUFA = long chain polyunsaturated fatty acids; NIH = National Institute of health; US = United States; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); IV = intravenous; GP = growth patterns; CA = corrected age

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Guesnet, 1999, France {1650}	▪ RCT Parallel ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** Singleton healthy term infants (between 37-42 weeks gestation), appropriate weight for GA after a healthy pregnancy **Exclusion criteria:** NR **Enrolled/Completed:** n=98/83 **Mean Age:** • Maternal: NR • Child: NR	Supplemented formula (DHA+high EPA) (n=23) vs. supplemented (DHA +low EPA) (n=24) vs. crtl formula (n=22) vs. HM (RS) (n=15)	DHA 0.45%, EPA 0.35%, AA 0.05% vs. DHA 0.45% vs. EPA 0.10%, AA 0.05% vs. no DHA or EPA or AA for 6 wks	• RBC & plasma PUFAs correlation with growth: S (-) correlation between ∆ L & plasma & RBC EPA at birth	Bledina-sa, Gpe Danon Paris, French Ministry of Cooperation in Mauritius & the University of Mauritius
Helland, 2001 Norway {111,39}	▪ RCT Parallel Double-blind ▪ Jadad total: 4 [Grade: A] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy women with single pregnancies between 19 to 35 y of age, & nulli or primipara who intended to BF their infants & none have taken any supplements of n-3 fatty acids earlier during the pregnancy **Exclusion criteria:** Premature births, birth asphyxia, infections & anomalies in the infants requiring special attention **Enrolled/Completed:** n=590/341 **Mean Age** • Maternal: cod liver oil SD = 28.6 (3.4) y, corn oil SD = 27.6 (3.2) y; • Child: cod liver oil GA SD = 279.6 (9.2) d; corn oil SD = 279.2 (9.3) d	Cod liver oil (n=301) vs. corn oil (n=289)	10 mL/d oil (1183 mg DHA, 803 mg EPA, 27.5 mg AA /10 ml) from entry to 3 mo after delivery	• Duration of gestation: NS in GA • Birth weight, L, HC: NS in birth wt, birth L, & HC (grp 1 vs. grp 2) • Growth patterns: NS between gps in wt, L & HC at 6 wks & 3, 6, 9 & 12 mo • Fagan test: NS novelty preference (Fagan test) at 6 & 9 mo • EEG: NS EEGs scores between grps (3 mo) • K-ABC: Cod liver oil > Mental Processing K-ABC score than corn oil (4 y)	Peter Møller, Avd. Orkla ASA & "Adtieselskabet Freia Chocoladefabriks Medicinske Fond."

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Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Hoffman, 2003, US {2958}	▪ RCT Parallel ▪ Jadad total: 3 [Grade:B] ▪ Schulz: Adequate	**Inclusion criteria:** Singleton term infants & infants with BW appropriate for GA **Exclusion criteria:** Family hx of milk-protein allergy, genetic or familial eye disease, vegetarian or vegan maternal diet, maternal metabolic disease, maternal anemia, maternal infection, congenital malformation or infection, & any perinatal event that resulted in NICU **Enrolled/Completed:** n=68/61 **Mean Age:** • Maternal: NR • Child: 4-6 mo	Supplemented formula (DHA+AA derived from SCO) (n=33) vs. crtl formula (n=35)	LCPUFA formula (0.36 wt% DHA, 0.72 wt% AA) after weaning at 4-6 mo to 12 mo of age	• VEP: S better sweep VEP & DHA+AA at 12 mo • Acuity card procedure: NS DHA+AA & FPL at 4,6,9, & 12 mo • Growth patterns: NS in wt, L, HC, wt-for-L at 4, 6, 9 & 12 mo • BMK: S better sweep VEP at 12 mo & RBC DHA; Σ n-3, n-3/n-6, DHA/DPA, n-6 unsaturation index; S poorer sweep VEP at 12 mo & RBC LA, AA; NS FPL & RBC n-3 or n-6 FA	NIH
Innis, 1997, US, Canada {374}	▪ RCT Parallel ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** Full term healthy infants (37-41 wk), with a BW > 2,500g to< 4500g, < 14 d old, mother had chosen to either exclusively BF or FF for 3 mo **Exclusion criteria:** BF infants recieving formula later than 6 d after birth; infants with congenital problems or disease considered likely to interfere with normal feeding or nutrient metabolism; with feeding intolerance; poor milk or formula intake; or with abnormal eye exam (as judged by infant's physician) **Enrolled/Completed:** n=238/191 **Mean Age:** • Maternal: NR • Child: NR	Formula 1 (cow milk-protein based) (n=69) vs. Formula 2 (cow milk-protein based) (n=70) vs. HM (n=99)	Formula 1 (18.0% LA, 1.9% ALA, with LA/ALA ratio of 9.5:1) & Formula 2 (34.2% LA, 4.7% ALA, with an LA/ALA ratio of 7.3:1) for 3 mo	• Acuity card procedur: NS FPL at 90 d of age • Growth patterns: NS in wt, L, & HC at 3 mo • BMK: NS visual acuity & plasma & RBC CPG DHA	Mead Johnson Research Center

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; ERG = electroretinogram; RCT = randomized control trial; RS = reference standard; TG = triglycerides; VEP = visual evoked potential; LCPUFA = long chain polyunsaturated fatty acids; NIH = National Institute of health; FPL = forced-choice preferential looking; BMK = biomarkers in blood or other tissues; US = United States; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; SCO = single cell oil; NIC = neonatal intensive care; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; NICU = neonatal intensive unit care; BW = birth weight

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Innis, 2002, Canada, US {80,2279}	▪ RCT Parallel Double-blind ▪ Jadad total: 3 [Grade: B] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy VLBW (846-1,560g) FF preterm infants **Exclusion criteria:** Preterm infants SGA, >24 days postnatal age when full enteral feeds ≥375 kJ/kg/day achieved, had necrotizing enterocolitis or other gastrointestinal disease, impaired visual or ocular status, or a hx of underlying disease or congenital malformation that could interfere with growth **Enrolled/Completed:** n=194/121 **Mean Age:** • Maternal: NR • Child: NR	DHA+AA formula (SCO) (n=66) vs. DHA formula (n=66) vs. crtl fornula (n=62)	LCPUFA formula (0.14% DHA + 0.27%AA or 0.15% DHA) for at least 28 d, then unsupplemented term formula to 57 wks PMA	• Growth patterns: S↑ Δ wt in DHA+AA than in crtl at 40 wks PMA; S↑ wt, L, wt-to-L in DHA+AA than in DHA at 48 wks PMA; S↑ wt, wt-to-L in DHA+AA than in crtl at 48 wk PMA; NS in HC at 48, 57 wks PMA • Teller acuity card procedure: NS in FPL visual acuity at 48 & 57 wks PCA • BMK: S (+) correlation between Δ wt & RBC PE AA at 8 wks; S (+) correlation between wt, L & RBC PE AA at 8 wks	Mead Johnson Nutritionals

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; VLBW = very low birth weight; US = United States; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; SCO = single cell oil; PMA = postmenstrual age; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; SGA = small for gestational age; PE = Phosphatidylethanolamine; FPL = forced-choice preferential looking

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Jensen, 1997, US {350,82}	▪ RCT Parallel ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy full term infants whose mothers had elected not to breast feed **Exclusion criteria:** NR **Enrolled/Completed:** n=80/63 **Mean Age:** • Maternal: NR • Child: Formula 1 GA = 39.8 ± 1.4 wk; Formula 2 GA = 39.6 ± 1.8 wk; Formula 3 GA = 39.6 ± 2.0 wk; Formula 4 GA = 39.6 ± 1.5 wk; BF GA = 40.2 ± 1.2 wk	Formula 1 (CSHPCo) (n=20) vs. Formula 2 (CSHPCo) (n=20) vs. Formula 3 (CSHPCo) (n=20) vs. Formula 4 (CSHPCo) (n=20)	Formula 1 (15.6% -17.6% LA, 0.4% ALA); Formula 2 (15.6% -17.6% LA, 1% ALA); Formula 3 (15.6% -17.6% LA, 1.7% ALA); Formula 4 (15.6% -17.6% LA, 3.2% ALA) for 4 mo	• Growth patterns: S↓ wt in F4 than in F1 at 4 mo; NS in L, HC, TST, & SST at 4 & 8 mo • VEP: NS latency VEP among gps at 120 & 240 d; NS amplitude VEP among gps at 120 & 240 d • BMK: S (+) correlation between wt at 4 mo & plasma AA at 120d; NS correlations between wt & plasma n-3 at 4 mo; S correlation between plasma DHA & PDI; NS correlation between RBC DHA & PDI; NS plasma & RBC PL DHA & amplitude at 120 & 240 d • Bayley's: NS in PDI & MDI at 12 mo	US dept of Agriculture, Agriculture Research Services; Mead-Johnson Nutritional Group, Foundation Fighting Blindness, Research to Prevent Blindness, Inc. & Retina Research Foundation

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; hx = history; tx = treatment; GA = gestational age; BF = breast fed; HM = human milk ; S = significant; RCT = randomized control trial; VEP = visual evoked potential; LCPUFA = long chain polyunsaturated fatty acids; VLBW = very low birth weight; US = United States; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; PMA = postmenstrual age; CSHPCo = Canola, Safflower, High oleic sunflower, Palm starin, Coconut oils; LA = linoleic acid; ALA = α-linolenic acid; GI = gastrointestinal; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; MDI = mental developmental index; PDI = psychomotor developmental index

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Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Jensen, 1999, US {240}	▪ RCT Parallel ▪ Abstract	**Inclusion criteria:** Healthy full term infants **Exclusion criteria:** NR **Enrolled/Completed:** n=126/NR **Mean Age:** • Maternal: NR • Child: NR	algal DHA (n=42) vs. fish oil derived DHA (n=42) vs. Crtl grp. (n=42)	Breast-feeding maternal intake of 200-250 mg DHA/d for 4 mo after delivery	• Transient VEP (120 & 240 d post delivery): NS in VEP latency & sweep VEP acuity • Teller Acuity Card Procedure: NS • Growth patterns: NS in wt, L & HC at 4-8 mo • BMK: NS correlation visual function & infant plasma PL DHA at 120 d	Mead Johnson Nutritionals & NRICGP
Jorgensen, 1996, 1998, Denmark {1159}	▪ RCT Parallel ▪ Jadad total: 2 [Grade: C] ▪ Schulz: unclear	**Inclusion criteria:** For FF & BF infants; uncomplicated pregnancy, term delivery (GA 37-42 weeks); BW between 2700 & 4500g; Apgar score >7 after 5 min & no neonatal diseases. For FF infants, termination of BF prior to 30 days of age without using a DHA supplemented formula. **Exclusion criteria:** Infant hospitalization; serious illness during the study period; formula intolerance (vomiting/diarrhea) **Enrolled/Completed:** n=39/37 **Mean Age:** • Maternal: NR • Child: Gp 1 (DHAGF) = 25.8 d; Gp 2 (DHAF) = 23.8 d; gp 3 (STF) = 22.5 d	Formula 1 (DHA+EPA -fish oil) (n=15) vs. Formula 2 (DHA+EPA, -fish oil, & GLA - borage oil) (n=13) vs. Crtl formula (n=11) vs. HM (RS) (n=17)	Formula 1 (0.3wt% DHA, 0.4wt% EPA); Formula 2 (0.3wt% DHA, 0.4wt% EPA, 0.5wt% GLA) for 3 mo	• Sweep VEP: NS effect of DHA on visual acuity at 4 mo • Growth patterns: NS in wt, L, HC, GV at 1, 2, & 4 mo • BMK: NS visual acuity at 4 mo & RBC DHA, EPA, or AA; S (-) correlation visual acuity & RBC CPG LA	Food Technology Research & Development Program; DanoChemo AS; BASF Health & Nutrition, Swedish Medical Research Council

NRICGP = National Research Initiative Competitive Grants Program; h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; RCT = randomized control trial; VEP = visual evoked potential; LCPUFA = long chain polyunsaturated fatty acids; BW = birth weight; US = United States; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; SCO = single cell oil; PMA = postmenstrual age; LA = linoleic acid; ALA = α-linolenic acid; GI = gastrointestinal; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; BMK = biomarkers correlations

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Koletzko, 1995, Germany {455}	▪ RCT Parallel Double-blind ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy preterm infants with BW ≤1850 a /= 130 ml milk/ Kg /day **Exclusion criteria:** Need for artificial ventilation or an oxygen supply with FiO2 > 0.30 at the time of enrollment or during the study; apparent GI, hepatic, & metabolic abnormalities; & septicemia **Enrolled/Completed:** n=27/27 **Mean Age:** • Maternal: NR • Child: BF SD GA = 32.6 wk (1.9); FF no LCPUFA SD GA = 34.2 wk (2.3); FF+ LCPUFA SD GA = 33.8 wk (1.9)	LCPUFA Prematil (Milupa) formula (egg-lipid, evening primrose oil) (n=9) vs. Crtl formula (n=10) vs. HM (RS) (n=8)	LCPUFA formula (0.5 % AA, 0.03% EPA, 0.3% DHA, vitamin E 20 mg/L) for 3 wks	• Growth patterns: NS in wt, L, HC at 3 wks • Visual acuity Teller's test: NS difference in visual acuity across at 3 wks	Deutsche Forschungs-gemeinschaft, & Milupa AG
Koletzko, 2003, Germany {940}	▪ RCT Parallel Double-blind ▪ Jadad total: 3 [Grade: B] ▪ Schulz: Unclear	**Inclusion criteria:** Preterm infants in stable condition with BW < 1800g **Exclusion criteria:** Artificial ventilation or oxygen supply with FiO2 > 0.3 at time of enrollment & presence of genetic GI or metabolic disorders. **Enrolled/Completed:** n=49/33 **Mean Age:** • Maternal: NR • Child: full PCA = 35 ± 2 wk; crtl BF = 26±14 d; FF 39 ± 22 d; F + LCPUFA-F = 39 ± 24 d	LCPUFA formula (egg, black currant seed oil, low EPA fish oil) (n=15) vs. crtl formula (n=15) vs. HM (RS) (n=19)	0.57 mol DHA+ 0.1 mol AA formula + vitamin E during 28 d	• Growth patterns: NS wt, L, HC at 28 d	Deutsche Forschungsgemeinschaft, Bonn, Germany; Nestec S.A; Vevey, Switzerland; & NestleAlete Gmb, Munich, Germany

NRICGP = National Research Initiative Competitive Grants Program; h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; BW = birth weight; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; PMA = postmenstrual age; LA = linoleic acid; GI = gastrointestinal; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; RS = reference standard; PCA = postconceptional age

Table 1: Randomized controlled trials evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Laivuori, 1993, Finland {547}	▪ RCT parallel design ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Adequate	**Inclusion criteria:** Preeclamptic women admitted to hospital between 26 & 37 wks of gestation **Exclusion criteria:** NR **Enrolled/Completed:** n=18/12 **Mean Age:** • Maternal: Primerose oil = 32 y (23-40), Fish oil = 30.3 y (24-40), Placebo = 30.2 y (26-32) • Child: NR	MaxEPA (fish oil) (n=3) vs. Preglandin (primrose oil) (n=4) vs. placebo (maize oil, olive oil) (n=5)	Max EPA (180 mg EPA, 120 mg DHA, 680 mg fish oils), Preglandin (375 mg LA, 45 mg GLA), placebo (500 mg each oil); 10 capsules	• Effect on BP, proteinuria & edema: NS (grp 1 vs. grps 2-3)	NR
Lapillone, 1997, France {1760}	▪ RCT Parallel ▪ Abstract	**Inclusion criteria:** Preterm infants appropriate for GA, (29.3 wk) **Exclusion criteria:** NR **Enrolled/Completed:** n=33/NR **Mean Age:** • Maternal: NR • Child: GA = 29.3 ±1.6 wk	LCPUFA formula (DHA+AA derived from fish oil) (n=16) vs. Crtl formula (n=17)	LCPUFA preterm formula (0.37wt% DHA, 0.05wt% EPA) until 40 wk CA, then LCPUFA term formula (0.45wt% DHA, 0.09wt% EPA) until 4 mo CA	• Growth patterns: NS in GP at 4 mo CA	NR
Lapillonne, 2000, France {1621}	▪ RCT Parallel ▪ Jadad total: 1 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** Term infants appropriate for GA & born with a BW of > 2800g; free of neonatal morbidity **Exclusion criteria:** Hx of maternal cocaine or alcohol abuse, or born to mothers with a hx of diabetes, hyperlipidaemia, abnormal dietary pattern (strict vegetarian or vegan) **Enrolled/Completed:** n=NR/24 **Mean Age** • Maternal: NR • Child: GA = 40.1 ± 0.8 wk;	LCPUFA formula (DHA+EPA+AA derived from fish oil) (n=12) vs. Crtl formula (n=12)	LCPUFA formula (0.31wt% DHA, 0.08wt% EPA, 0.03wt% AA) from 3 d to 4 mo of age	• Growth patterns: S↑ HC in crtl than in LCPUFA & HM at 4mo; NS in wt, L, at 2, 4 mo	Blédina-sa

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; BW = birth weight; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; CA = corrected age; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; GP = growth patterns

Table 1: Randomized controlled trials evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Lucas, 1999, UK & Australia {270}	▪ RCT Parallel Double-blind ▪ Jadad total: 5 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** FF gp: women giving birth to healthy singletons of appropriate size for GA & of at least 37 wk gestation, mothers who decided on FF after birth; BF crtl gp: plan to BF for at least 6 wk **Exclusion criteria:** Congenital abnormalities affecting development; BF pts were excluded from analysis if BF< 6 mo **Enrolled/Completed:** n=447/354 **Mean Age:** • Maternal: FF Crtl = 27.5 ± 5.23 y; FF LCPUFA = 27.0 ± 5.12 y; BF=30.6 ± 4.34 y • Child: NR	LCPUFA (egg lipids) formula (n=154) vs. crtl formula (n=155) vs. HM (RS) (n=138)	0.30% AA + 0.32% DHA formula from 1st wk age until 6 mo	• Bayley's MDI & PDI: NS at 18 mo • Knobloch, Passamanick & Sherrard's test: NS in KPS at 9 mo • Growth patterns: NS in wt, L, HC, MAC, SST at 6, 9, 18 mo	Nestec Ltd (Switzerland)
Makrides, 1995, Australia {477}	▪ RCT Parallel ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy infants of 37-42 weeks gestation, appropriate weight for gestation **Exclusion criteria:** Mothers with hx of lipid metabolism disorders, IDDM, drug or ETOH abuse **Enrolled/Completed:** n=89/79 **Mean Age:** • Maternal: NR • Child: fully BF GA = 39.8 wk; Partially BF GA = 39.7 wk; pb FF GA = 39.6 wk; Supplemented FF GA = 39.1wk	Supplemented formula (DHA+ EPA, derived from fish oil, and AA derived from primrose oil) (n=13*) vs. Crtl formula (n=19*) vs. HM (n=47*)	LCPUFA formula (0.36 wt% DHA+0.58 wt% EPA+0.01 wt% AA);	• Growth patterns: NS in wt, L, HC at 6, 16, 30 wks • VEP: S improved visual acuity of DHA+GLA at 16 & 30 wk • BMK: NS correlation of RBC LCPUFA & GP; S correlation RBC DHA & VEP acuity at 16 & 30 wks of age	Children's Medical Research Foundation, Nestle Australia, Scotia Pharmaceuticals UK & Flinders Medical Research Foundation

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; RCT = randomized control trial; VEP = visual evoked potential; LCPUFA = long chain polyunsaturated fatty acids; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; MDI = mental developmental index; PDI = psychomotor developmental index; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; MAC = mid arm circumference; KPS = Knobloch, Passmark, and Sherrard's test; ETOH = alcohol abuse

Table 1: Randomized controlled trials evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Makrides, 1999, Australia {229,213}	▪ RCT Parallel double-blind ▪ Jadad total: 5 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** Healthy white term infants **Exclusion criteria:** SGA, evidence of congenital disease, mother had IDDM or hx of drug or ETOH abuse **Enrolled/Completed:** n=146/114 • **Mean Age:** • Maternal: NR • Child: PB formula GA = 39.6 ± 1.5 wk; DHA formula GA = 39.6 ± 1.1 wk; DHA+ AA formula GA = 39.8 ± 1.3 wk; BF GA = 39.3 ± 1.4 wk	DHA + AA formula (tuna oil, egg-PL) (n=28) vs. DHA formula (n=27) vs. crtl formula (n=28) vs. HM (RS) (n=63)	DHA+AA formula (0.34%DHA + 0.34%); DHA formula (0.35% DHA) during 12 mo;	• Growth patterns: NS in wt, L, HC at 6, 16, 34 wk, 12 & 24 mo • BMK: S (-) correlation of plasma DHA at 16 wks & wt at 12 mo & 24 mo; S correlation between PDI at 12 mo & plasma AA levels at 12 mo; NS with MDI • VEP: NS VEP acuity at 16 or 34 wk • Bayley's: NS in MDI & PDI at 12 & 24 mo	Nestec Ltd. Switzerland; Australian National Health & Medical Research Council
Makrides, 2000, Australia {220,109}	▪ RCT Parallel ▪ Jadad total: 5 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** White term infants **Exclusion criteria:** SGA; evidence of congenital disease; mother had diabetes requiring insulin; or a hx of drug or ETOH abuse **Enrolled/Completed:** n=176/145 **Mean Age:** • Maternal: NR • Child: full NBD (formula LA : ALA) 10:1 GA = 39.4 ± 1.2 wks; 5:1 GA = 39.2 ± 1.3 wk; BF GA = 39.5 ± 1.1 wk	Formula 10:1 (FAs from CSHPCo) (n=36) vs. Formula 5:1 (FAs from CSHPCo) (n=37) vs. HM (n=103)	Formula 10:1 (16.9 wt% LA, 1.7 wt% ALA); Formula 5:1 (16.6 wt% LA, 3.3 wt% ALA) from 4-6 d to 34 wk of age;	• Growth patterns: NS in Δ wt, Δ L, Δ HC between 10:1-F & 5:1-F at 6, 16, 34 wks; S↑ wt at 6 wks & L at 16 wks in 5:1 F • VEP: NS VEP acuity at 16 & 34 wk	Wyeth Nutritionals International; Australian National Health & Medical Research Council; MS McLeod Research Trust

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; GA = gestational age; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; RCT = randomized control trial; VEP = visual evoked potential; LCPUFA = long chain polyunsaturated fatty acids; DHA = docosahexaenoic acid; AA = arachidonic acid; LA = linoleic acid; ALA = α-linolenic acid; PL = phospholipids; CSHPCo = Canola, Safflower, High oleic sunflower, Palm starin, Coconut oils; MDI = mental developmental index; PDI = psychomotor developmental index; IDDM = insulin dependant diabetes mellitus; ETOH = alcohol abuse; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); HC = head circumference; wt = weight; L = length

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Malcolm, 2003, UK {12}	▪ RCT Parallel Double-blind ▪ Jadad total: 3 [Grade: B] ▪ Schulz: Unclear	**Inclusion criteria:** Mothers: women at approximately 15 wk of pregnancy; infants: healthy born > 36 wk gestation, with Apgar score of >7 at 5 m &with no visual, medical or developmental disorders **Exclusion criteria:** Mothers: diabetes, twin pregnancies, k pre-eclampsic toxemia, hx of abruption or postpartum hemorrhage, allergy to fish products, or a thrombophilic tendency or those receiving drugs affecting thrombocyte function **Enrolled/Completed:** • Mothers: n=100/63 • Child: n=60/56 **Mean Age:** • Maternal: NR • Child: fish oil = 279.7 (9.5) d, pb = 279.6 (8.5) d	Maternal LCPUFA supplementation capsules (from fish oil) (n=50) vs. Placebo capsules (n=50)	LCPUFA capsules (40.4 wt% DHA, 7.2 wt% EPA) from 15 wk of pregnancy until delivery	• Duration of gestation: NS in GA • Growth patternss: NS in birth wt, L & HC • ERG (24 h): NS in b wave implicit time; NS in Naka-Rushton function; NS in log δ; NS in maximium combined ERG • BMK: NS correlation of max combined ERG & cord blood DHA; NS (-) correlation of log δ & cord blood AA; S (+) correlation of log δ & cord RBC proportion DHA & total n-3 FA, n-6/n-3; S correlation of log δ & cord RBC quartiles of DHA, AA, total n-3 LCPUFAs	Scottish Office Health Department

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; ERG = electroretinogram; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; UK = United Kingdom; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; GI = gastrointestinal; PL = phospholipids; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Martinez, 1999, Brazil {2258}	▪ RCT Parallel ▪ Jadad total: 1 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** GA betweeen 28 & 34 weeks BW between 900g- 1500g, on enteral feeding for 2 days before the beginning of the study **Exclusion criteria:** Congenital anomalies; requirements of special care such as sepsis, hyaline membrane disease, patent ductus arteriosus, need for ventilatory support or O2 supplementation **Enrolled/Completed:** n=58/NR **Mean Age:** • Maternal: NR • Child: BF = 15.8 ± 1.2 d; FF = 18.0 ± 2.1 d; FF+ LCPUFA = 12.8 ± 1.0 d	LCPUFA formula (Egg-TG & primrose oil) (n=20) vs. crtl formula (n=20) vs. HM (n=18)	LCPUFA formula (NR) for 1 mo	• Growth patterns: NS in wt, L, HC at 30 d	Brazilian Research Council; Milupa GmbH & Co.
McClead, 1985, US {2550}	▪ RCT Parallel ▪ Jadad total: 3 [Grade: B] ▪ Schulz: Unclear	**Inclusion criteria:** Infants in NICU requiring TPN for at least 7 days **Exclusion criteria:** Medical conditions that precluded IV fat therapy (e.g. severe hyperbilirubinemia, respiratory distress, thrombocytopenia) **Enrolled/Completed:** n=23/20 **Mean Age:** • Marternal: NR • Child: NR	Modified Liposyn 20% (high ALA) (n=10) vs. Liposyn 20% (low ALA) (n=10)	IV ALA 3 (SD: 1.5)% safflower oil emulsion vs. IV ALA 0.1% safflower oil emulsion for 13 d	• Safety	Abbott Laboratories, Chicago, Illinois

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; PMA = postmenstrual age; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; BW = birth weight; US = United States; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; IV = intravenous; NICU = neonatal intensive care unit; TPN = total parental nutrition; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; SD = standard deviation

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Morris, 2000, UK {2231}	▪ RCT Parallel Double-blind ▪ Jadad total: 3 [Grade: B] ▪ Schulz: Unclear	**Inclusion criteria:** Term infants whose mothers had decided to bottle feed with BW between 2.5-4.5 kg up to age 72 h **Exclusion criteria:** Major congenital abnormalities & infants from multiple pregnancies **Enrolled/Completed:** n=140/109 **Mean Age:** • Maternal: NR • Child: NR	LCPUFA formula (DHA+AA, from Egg-TG) (n=55*) vs. Crtl formula (n=54*)	LCPUFA formula (0.2 wt% DHA, 0.4 wt% AA) for 12 wk	• Growth patterns: S↑ SST in DHA at 6 wk & 3 mo+ NS at 6 mo & 12 mo; NS in wt, L, HC, MAC, TST at 6 & 12 wk, 6 & 12 mo	Cow & Gate Nutricia Ltd

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; UK = United Kingdom; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; NICU = neonatal intensive care unit; * = completers; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; MAC = mid arm circumference; SST = subscapular skinfold thickness; TG = triglycerids

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Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
O'Connor, 2001, 2003, US, UK, Chile {126,1507}	▪ RCT Parallel Double-masked ▪ Jadad total: 3 [Grade: B]; ▪ Schulz: Unclear	**Inclusion criteria:** Initiation of enteral feeding by 28th d of life; singleton & twin births, SGA **Exclusion criteria:** Serious congenial abnormalities affecting growth & development; major surgery before randomization; perivenricular/ intraventricular hemorrhage > Grade II; maternal incapacity; liquid ventilation' asphyxia resulting in severe & permanent neurologic damage, or uncrtlled systemic infection at the time of enrollment **Enrolled/Completed:** n=470/376 **Mean Age:** • Maternal: FF-crtl = 27.2 y, FF-fish/fungal = 27.0 y, FF-egg-TG/fish = 27.0 y, HM = 29.7 y • Child: GA wk (postnatal age d): FF-crtl = 29.6 wk (5.5 d), FF-fish/fungal = 29.8 wk (5.0 d), FF-egg-TG/fish = 29.7 (4.6 d), HM = 29.7 (5.5 d)	DHA+AA (fish/fungal) (n=140)/ vs. DHA+AA (egg-TG/fish) (n=143)/ vs. Crtl formula (n=144) vs. HM (RS) (n=43)	NR dose, Inhospital preterm formula until discharge, then postdischarge formula until 12 mo CA	• Growth patterns: NS Δ wt, Δ L, Δ HC at 8 wk, 4 mo, 12 mo CA • Teller Acuity Card Procedure: NS in FPL acuity at 4 mo CA • VEP: S ↑VEP acuity in grps1-2 vs. grp3 at 6 mo CA; NS VEP acuity across both DHA+AA grps • Bayley's: S↑ PDI score in <1,250 g birth wt fed AA+DHA (egg-TG/fish) than crtl infants; NS score crtl or AA+DHA (fish/fungal) gps; NS Bayley's MDI (12 mo) • Fagan: M novelty preference look (Fagan test) AA+DHA (egg-TG/fish) > crtl & AA+DHA (fish/fungal) (6 mo) • MacArthur Communicative Development Inventories (9, 14 mo): NS • BMK: S (+) correlation rate wt gain & RBC PE AA at 28 d; wt & L S correlated RBC PE AA at 28 d	Ross Products Division, Abbott Lab, US

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; UK = United Kingdom; DHA = docosahexaenoic acid; AA = arachidonic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; MDI = mental developmental index; PDI = psychomotor developmental index; RS = reference standard

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Olsen, 1992, Dalby Salvig, 1996, Denmark {614,425,531}	▪ RCT Parallel Double-blind ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Inadequate	**Inclusion criteria:** All women scheduled to attend for a routine wk 30 GA midwife assessment **Exclusion criteria:** Hx of placental abruption in previous pregnancy; seroius bleeding episode in the present pregnancy; regular use of prostaglandin inhibitors; multiple pregnancy; allergy to fish & regular intake of fish oil **Enrolled/Completed:** n=533/402 **Mean Age:** • Maternal: fish oil=29.4 y (4.4); olive oil= 29.7 y (4.3); ctrl=29.1 y (4.1) • Chid: NR	Fish oil (n=266) vs. placebo (olive oil) (n=136) vs. no oil (n=131)	4 capsules/d of 1 g gelatine capsules with fish oil (Pikasol fish oil: 32% EPA, 23% DHA, 2 mg vit E); 2.7 g n-3 FA/d until delivery	• Duration of gestation: S↑ GA in fish oil grp • Birth weight: NS birth wt • BP (baseline; wks 33, 37, 39 & wkly until delivery): NS in BP or rates of GHT & preeclampsia (grp 1 vs. grps 2-3) NS in BP (grp 1 vs. grps 2-3)	Danish Medical Research Council, Sygekassernes Helsefond, Weiman's Legat & Michaelsen Fonden

GCRC = General Clinical Research Centers; h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; BP = blood pressure; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s)

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Olsen, 2000, Denmark* {66}	▪ RCT Parallel Design ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Adequate	**Inclusion criteria:** Women > 16 wk of gestation with an uncomplicated pregnancy, hx preterm delivery (< 259 d of gestation) **Exclusion criteria:** Diabetes mellitus in or before pregnancy; diagnosed severe fetal malformation or hydrops in current pregnancy; suspicion in current pregnancy, or occurrence in an earlier pregnancy, of placental abruption; drug or alcohol abuse; regular intake of fish oil or of nonsteroidal anti-inflammatory agents or other drugs affecting thrombocyte function or eicosanoid metabolism; allergy to fish products. In the therapeutic trials also high probability of delivering soon after randomization (estimated within one wk) **Enrolled/Completed:** n=232/228 **Mean Age:** • Maternal: fish oil = 29.3 y (4.87); olive oil = 30.0 y (6.22) • Child: GA = 131.8 d (24.6); GA = 130.5 d (27.7)	Fish oil (Pikasol) (n=110) vs. placebo (Olive oil) (n=122)	4 gelatine capsules/d, 32% EPA, 23% DHA, 2mg vit E; 2.7 g of LCPUFA/d	• Preterm delivery: S↑ GA in fish oil gp; S↓ % premature deliveries in fish oil gp • Birth weight: S↑ birth wt in fish oil; NS % IUGR	Concerted Action & PECO programmes of European Commission, Danish National Research Foundation, Lube Ltd

GCRC = General Clinical Research Centers; h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; BP = blood pressure; * Scotland, Sweden, UK, Italy, The Netherlands, Norway, Belgium & Russia; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); IUGR = intrauterine growth retardation

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Olsen, 2000, Denmark* {66}	▪ RCT Parallel Design ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Adequate	**Inclusion criteria:** Women > 16 wk of gestation with an uncomplicated pregnancy, hx IUGR (<5th PC) **Exclusion criteria:** NR **Enrolled/Completed:** n=280/263 **Mean Age:** • Maternal: NR • Child: NR	Fish oil (Pikasol) (n=141) vs. placebo (Olive oil) (n=139)	4 gelatine capsules/d, 32% EPA, 23% DHA, 2mg vit E; 2.7 g of LCPUFA/d	• Duration of gestation: S↑ GA in fish oil gp • Recurrence of IUGR-birth weight: S↑ birth wt in olive oil; NS % IUGR	Concerted Action & PECO programmes of European Commission, Danish National Research Foundation, Lube Ltd
Olsen, 2000, Denmark* {66}	▪ RCT Parallel Design ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Adequate	**Inclusion criteria:** Women > 16 wk of gestation with hx GHT **Exclusion criteria:** NR **Enrolled/Completed:** n=386/350 **Mean Age:** • Maternal: NR • Child: NR	Fish oil (Pikasol) (n=184) vs. placebo (Olive oil) (n=202)	4 gelatine capsules/d, 32% EPA, 23% DHA, 2mg vit E; 2.7 g of LCPUFA/d	• Duration of gestation: NS in GA • Recurrence GHT, preeclampsia: NS in rates of GHT & preeclampsia (grp 1 vs. grp 2); NS in BP (grp 1 vs. grp 2)	Concerted Action & PECO programmes of European Commission, Danish National Research Foundation, Lube Ltd
Olsen, 2000, Denmark* {66}	▪ RCT Parallel Design ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Adequate	**Inclusion criteria:** Women > 16 wk of gestation with current twin pregnancy **Exclusion criteria:** NR **Enrolled/Completed:** n=579/569 **Mean Age:** • Maternal: NR • Child: NR	Fish oil (Pikasol) (n=289) vs. placebo (Olive oil) (n=290)	4 gelatine capsules/d, 32% EPA, 23% DHA, 2mg vit E; 2.7 g of LCPUFA/d	• Duration of gestation: NS in GA • GHT, preeclampsia: NS in rates of GHT & preeclampsia (grp 1 vs. grp 2); NS BP (grp 1 vs. grp 2) • IUGR: NS in birth wt & % IUGR	Concerted Action & PECO programmes of European Commission, Danish National Research Foundation, Lube Ltd

GCRC = General Clinical Research Centers; h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; BP = blood pressure; * Scotland, Sweden, UK, Italy, The Netherlands, Norway, Belgium & Russia; PC = percentile; IUGR = intrauterine growth retardation; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); GHT = gestational hypertension

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics (enrolled/evaluated)	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Olsen, 2000, Denmark* {66}	▪ RCT Prallel Design ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Adequate	**Inclusion criteria:** Women > 16 wk of gestation, threatening preeclampsia current pregnancy **Exclusion criteria:** NR **Enrolled/Completed:** n=79/NR **Mean Age:** • Maternal: NR • Child: NR	Fish oil (Pikasol) (n=44) vs. placebo (Olive oil) (n=35)	9 gelatine capsules/d, 32% EPA, 23% DHA, 2mg vit E; 6.1 g of LCPUFA/d	• Duration of gestation: NS in GA	Concerted Action & PECO programmes of European Commission, Danish National Research Foundation, Lube Ltd
Olsen, 2000, Denmark* {66}	▪ RCT Parallel Design ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Adequate	**Inclusion criteria:** Women > 16 wk of gestation, suspected IUGR (<10th PC in U/S) **Exclusion criteria:** NR **Enrolled/Completed:** n=63/NR **Mean Age:** • Maternal: NR • Child: NR	Fish oil (Pikasol) (n=36) vs. placebo (Olive oil) (n=27)	9 gelatine capsules/d, 32% EPA, 23% DHA, 2mg vit E; 6.1 g of LCPUFA/d	• Duration of gestation: S↑ GA in fish oil gp • IUGR, birth weight: NS in birth wt & % IUGR	Concerted Action & PECO programmes of European Commission, Danish National Research Foundation, Lube Ltd
Onwude, 1995, UK {480}	▪ RCT Prallel Double-blind ▪ Jadad total: 5 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** Primigravida with abnormal Doppler at 24 wks GA; multigravida with hx of small babies (<PC 3), proteinuric or non-poteinuric GHT or unexplained stillbirth **Exclusion criteria:** Hx of diabetes, chronic hypertension, asthma, use of anticoagulants **Enrolled/Completed:** n=233/230 **Mean Age:** • Maternal: Fish Oil =26.8 y; pb =26.1 y • Child: NR	Max EPA (EPA+ DHA form fish oil) (n=113) vs. placebo (olive oil)(n=119)	2.7 g/d (EPA 180 mg, DHA 120 mg), 9 capsules/d until 38 wk GA	• Duration of gestation: NS in GA; NS in % premature deliveries • Proteinuric or non-poteinuric GHT: NS rate of GHT (grp 1 vs. grp 2) • IUGR, birth weight: NS in birth wt & IUGR recurrence rate (grp 1 vs. grp 2)	Yorkshire Region Locally Organised Research, GLAXO & Seven Seas

GCRC = General Clinical Research Centers; h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; BP = blood pressure; * Scotland, Sweden, UK, Italy, The Netherlands, Norway, Belgium & Russia; PC = percentile; pb = placebo; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); IUGR = intrauterine growth retardation

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Ponder, 1992, US {1354}	▪ RCT Parallel ▪ Jadad total: 1 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy term infants, 37-42 wk gestation, wt, L & HC btw 5-95th PC **Exclusion criteria:** NR **Enrolled/Completed:** n=NR/43 **Mean Age:** • Maternal: NR • Child: 3 d	Similac (soy) formula (n=11) vs. Similac (corn) formula (n=14) vs. HM (RS) (n=18)	101-125 kcal/kg/d Soy: 4.8g ALA (n-3) vs. Corn: 0.8g ALA during 8 wks	• Growth patterns: NS in wt, L, HC at 3d, 4wk, 8 wks	Ross Laboratories
Smuts, 2003, US {2896}	▪ RCT Parallel ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** Between 24-28 wk pregnant; between ages of 16-35 yr at time of enrollment; were accessible by phone & planned to deliver at study hospital **Exclusion criteria:** Chronic illness, pregnancy induced hypertension, pre-eclampsia, pregnancy induced diabetes, or more than 4 prior pregnancies **Enrolled/Completeed:** n=73/53 **Mean Age:** • Maternal: low = 21.3 ± 4.8 y; regular = 24.8 ± 7.8 y; high 19.9 ± 4.1 y • Child: NR	DHA-enriched eggs (n=18) vs. ordinary eggs (n=19) vs. placebo	DHA-enriched eggs (135mg DHA/egg); ordinary eggs (18mg DHA/egg) from wk 24-28 until delivery	• Duration or gestation: NS in GA; high-DHA eggs ↓ premature delivery than crtl (no p-value) • Birthweight, SGA: Wt, L, & HC at birth ↑ in grp 1 vs. grp 2 (p-value: NR); LBW ↓ in grp 1 vs. grp 2 (p-value: NR)	Martek Biosciences Boulder Corporation, Boulder, Colorado

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; BP = blood pressure; PC = percentile; pb = placebo; HC = head circumference; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); LBW = low birth weight

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Smuts, 2003, US {31}	▪ RCT Parallel Double-blind Jadad total: 3 [Grade: B] ▪ Schulz: Inadequate	**Inclusion criteria:** Pregnant women 16-36 y of age, 24-28 wk of gestation at enrollment, able & willing to consume eggs, access to refrigeration, plan to deliver at Truman Medical Center, singleton gestation **Exclusion criteria:** <16 or >36 y of age, weight > 240 lb at baseline, serious illness such as cancer, lupus, hepatitis, serious infectious disease, diabetes or gestational diabetes at baseline, high BP attributed to any cause **Enrolled/Completed:** n=350/291 **Mean Age:** • Maternal: Ordinary eggs = 21.6 y (4.2); High-DHA eggs = 21.y Y(4.3) • Child: Ordinary eggs = 271.6 d (15.6); DHA eggs = 274.1 d (13.5)	DHA-enriched eggs (n=176) vs. ordinary eggs (n=174)	12 DHA-eggs (133 mg DHA) per wk until birth	• Duration of Gestation: S↑ in GA in High-DHA vs Regular-DHA; NS in premature delivery rate • Birth wt, L, HC at birth: NS, NS rate of LBW • Incidence of preeclampsia: NS (grp 1 vs. grp 2) • BMK: S (+) correlation between infant RBC DHA & GA; NS correlation between maternal RBC DHA & GA	Omega Tech Inc.

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; BP = blood pressure; pb = placebo; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; LBW = low birth weight

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Vanderhoof, 2000, US {2143,182,1752}	▪ RCT Parallel Double-Blind ▪ Jadad total: 4 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** Premature infants 0-28 d of age, medically stable, BW between 750-2,000g appropriate for GA, had received enteral feedings < 24 h **Exclusion criteria:** Significant acute or chronic illnesses, systemic infections, documented major congenital infections, intraventricular hemorrhage more than grade 2, periventricular leukomalacia, neonatal seizures, neonatal meningitis, or maternal substance abuse, BF infants whose mothers were vegans or had hx of metabolic disease that would affect essential fatty acid status were excluded **Enrolled/Completed:** n=288/153 **Mean Age:** • Maternal: NR • Child: PCA at enrollment: LCPUFA formula = 31.2 ± 2.3 wk; Ctrl Formula = 30.9 ± 2.6 wk; HM = 30.5 ± 2.4 wk	LCPUFA formula (microbial fermentation) (n=77) vs. crtl formula (n=78) vs. HM (RS) (n=133)	Preterm formula 0.5% AA + 0.35% DHA until 48 wks PCA, then term formula until 92 wks PCA, ad libitum	• Growth patterns: S↑ wt, L, HC, MAC in LCPUFA & crtl than in HM at 40 wk PCA; NS in L, HC at 48 wks PCA; S↑ L, MAC in LCPUFA than in HM at 48 wks PCA; NS in wt, L, HC at 92 wks PCA	Wyeth Nutritionals International

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; DHA = docosahexaenoic acid; AA = arachidonic acid; PCA = postconceptual age; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; MAC = mid arm circumference; BW = birth weight

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
van Wezel-Meijler, 2002, Netherlands {40}	▪ RCT Parallel Double-blind ▪ Jadad total: 5 [Grade: A] ▪ Schulz: Adequate	**Inclusion criteria:** Premature infants with gestational age < 34 wk, BW of < 1750 g, normal neurological examination throughout the neonatal period; normal repeated brain ultrasound or showing minor abnormalities such as isolated subependymal haemorrhage & subventricle, with no ventricular dilation; transient periventricular echodensities, without evolution into cysts; any combination of previous findings **Exclusion criteria:** Abnormalities of the CNS (excluding items on inclusion criteria), either congenital or acquired; abnormal neurologic examination; seizure; any systemic disease with potential negative influence on future growth or development (chronic lung disease, congenital abnormalities of other organs than the brain; metabolic disease; congenital infections & endocrine dysfunction; serious nutritional or GI problems preventing initiation of enteral feeding after the first wk of life or complete enteral feeding after the 3rd wk of life **Enrolled/Completed:** n=55/42 **Mean Age:** • Maternal: • Child: FF control = 30.4 wks	LC PUFA supplemented formula (microalgae, fungi) (n=22) vs. Control formula (n=20)	Preterm formula: from 2-3 wks of age to 3,000g wt, then Term formula until 6 mo CA	• Bayley's PDI & MDI: S↑ PDI unsupplemented gp vs. supplemented formula at 3, 6, 12 & 24 mo; NS Bayley's MDI at 3, 6, 12 & 24 mo • VEP: NS in VEP (P200 & N300) wave latencies at 3 & 12 mo CA • Teller card test: NS mean visual acuity at 3,6,12 mo CA	Numico Research

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; CNS = central nervous system; GI = gastrointestinal; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s); BW = birth weight; MDI = mental developmental index; PDI = psychomotor developmental index; VEP = visual evoked potentials; CA = corrected age

Table 1: Randomized controlled trial evidence for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Intervention/ comparators	Timing & Dose of intervention (if appropriate)	Clinical Outcomes Results	Funding Source
Willatts, 1998, UK {2307,2293}	▪ RCT Parallel ▪ Jadad total: 3 [Grade: B] ▪ Schulz: Unclear	**Inclusion criteria:** Healthy term infants weight 2,500-4,000 g; gestation 37-42 wk **Exclusion criteria:** NR **Enrolled/Completed:** n=58/40 **Mean Age:** • Maternal: Gp 1= 26.2 ± 4.2 y; Gp 2 = 27.7 ± 4.6 y • Child: Gp 1 = 274.2 ± 2.7 d, Gp 2 = 275.2 ± 5.0 d	LCPUFA formula (DHA+AA derived from Egg-TG) (n=27) vs. Crtl formula (n=31)	LCPUFA formula (0.15-0.25 wt% DHA, 0.30-0.40 wt% AA) for 4 mo;	• Growth patterns: NS wt, L, HC at 3 mo • Cognitive function assessment (3 mo): NS • Problem solving assessment (9 mo): NS	Milupa Ltd.
Woltil, 1999, Netherlands {275,329}	▪ RCT Parallel ▪ Jadad total: 2 [Grade: C] ▪ Schulz: Unclear	**Inclusion criteria:** LBW (< 2500g) either solely BF or solely FF **Exclusion criteria:** Blood transfusions; blood products; or parenteral lipids. **Enrolled/Completed:** n=143/128 **Mean Age:** • Maternal: NR • Child: Formula without LCPUFA = 36 wk; Formula with LCPUFA = 37 wk; HM = 35 wk	LCPUFA preterm formula ↑ n-3 fish oil (n=13) vs. LCPUFA formula ↓ n-3 fish oil (n=13) vs. crtl formula (n=75) vs. HM (RS) (n=27)	↑fish oil (EPA 0.34 mol; DPA 0.03 mol; DHA 0.43 mol vs.↓fish oil (per 100 mol: EPA 0.17 mol; DPA 0.02 mol; DHA 0.20 mol) until d 42 life	• Growth patterns: NS in Δ wt, ΔL, & ΔHC between LCPUFA-1, LCPUFA-2 & pb at 1 mo; S↑ Δ wt, Δ L, Δ brain wt, Δ HC in pb-1 than in pb-2 & pb-3 at 1mo • BMK: S (+) correlation between Δwt, ΔL, ΔHC & plasma - RBC DHA at 1 mo	Friesland Nutrition

h = hour(s); d = day(s); wk(s) = week(s); mo = month; y = year; n = number of participants; NR = not reported; hx = history; tx = treatment; GA = gestational age; FF = formula fed; BF = breast fed; HM = human milk ; S = significant; RCT = randomized control trial; LCPUFA = long chain polyunsaturated fatty acids; GLA = gammalinolenic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; EPA = eicosapentanoic acid; LA = linoleic acid; ALA = α-linolenic acid; BP = blood pressure CA = corrected age; UK = United Kingdom, ↑ = increase; ↓ = decrease; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length; BMK = biomarkers correlations;

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health

Author, Year, Location	Study Design	Population Characteristics	Exposure	Timing & Dose of exposure (if appropriate)	Clinical Outcomes Results	Funding Source
Agostini, 2001, Italy {98}	▪ Single prospective cohort ▪ Quality score: 8 [Grade A]	**Inclusion criteria:** Healthy term infants, exclusively BF for at least 3 mo **Exclusion criteria:** NR **Enrolled:** n=44 **Mean Age:** ▪ Maternal: NR ▪ Child: NR	HM	HM (FA composition NR) for ≥6 mo vs. HM for <6 mo	▪ Bayley's PDI & MDI at 12 mo: NS correlation between Bayley's PDI & length of BF; NS correlation between Bayley's PDI & milk FA content; S correlation between Bayley's MDI & milk total fat content at 6 mo, but NS at 12 mo; NS AA, DHA milk content correlation with MDI at 12 mo	NR
Al, 1995, The Netherlands {55,504}	▪ Nested case-control study ▪ Quality score: 11 [Grade A]	**Inclusion criteria:** Pregnant women < 16 wk gestation, cardiovascular, neurologic, renal or metabolic disease at the beginning of pregnancy; women with no hypertension (controls),or with pregnancy induced hypertension (cases) , matched for parity and hospital with three crtls **Exclusion criteria:** Multiple pregnancy **Enrolled:** n=208 **Mean Age:** ▪ Maternal: NR ▪ Child: NP GA =279.9 d (0.59), PIH GA = 273.3 d (2.18)	N/A	N/A	▪ BMK: NS in absolute FA composition (mg/L) of maternal plasma PL (before 16, at 22 & 32 wks GA); severe GHT women (n=17) mean GA & mean birth wt of their babies were S ↓ than mild GHT; during gestation & after delivery NS in maternal FA composition of the severe GHT vs. mild GHT	Nutricia B.V, Zoetermeer, The Netherlands

N/A = not applicable; US = United States; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); hr (s) = hour (s); NR = not reported; (A)GA = (appropriate for) gestational age; GA = gestational age; IUGR = intrauterine growth retardation; PIH = pregnancy induced hypertension; HM = human milk; PT = preterm; FT = full term; RBC = red blood cells; FA = fatty acids; PL = phospholipids; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; LA = linoleic Acid; ALA = alpha-linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; DGLA = dihomo-gama-linolenic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s)

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health

Author, Year, Location	Study Design	Population Characteristics	Exposure	Timing & Dose of exposure (if appropriate)	Clinical Outcomes Results	Funding Source
Birch, 1993a, US {567}	▪ Cross-sectional ▪ Quality score: 4 [Grade B]	**Inclusion criteria:** Healthy pre-term infants born at 27-33 wk postconception with wirth BW of 1000-1500 g; AGA. **Exclusion criteria:** Inability to accept enteral feeds by d 10 of life, respiratory tx > 7 d, congenital infection or malformation, retinopathy of prematurity, or grade 3 or 4 intraventricular hemorrhage **Enrolled:** n=30 **Mean Age:** ▪ Maternal: NR ▪ Child: 27-33 wk	HM/corn-oil based formula	NR	▪ BMK-visual: LogMAR acuity was S correlated with the ratio [DHA n-3/DPA n-6] in total RBC lipids; FPL acuity LogMAR was S correlated with the ratio DHA n-3/DPA n-6; RBC ratio was S ↑ in HM than in formula fed	NIH; Delta Gamma Foundation of Dallas; Pediatric Subunit; & United Cerebral Palsy Foundation
Birch, 1993b, US {567}	▪ Cross-sectional ▪ Quality score: 4 [Grade B]	**Inclusion criteria:** Healthy term infants, AGA **Exclusion criteria:** Inability to accept enteral feeds by d 10 of life, respiratory tx > 7 d, congenital infection or malformation, retinopathy of prematurity, or grade 3 or 4 intraventricular hemorrhage **Enrolled:** Gp 1, n=30; Gp 2, n=43 **Mean Age:** • Maternal: NR • Child: 27-33 wk	HM/corn-oil based formula	NR	▪ BMK-visual: Mean VEP & FPL acuities better in HM than in formula (4 mo); mean RBC DHA/DPA in total RBC lipids was S ↑ HM than in formula gp & stereo acuity was S correlated with the end-product ratio; letter matching (36 mo) was S correlated with ratio, RBC DHA/DPA (4 mo)	NIH; Delta Gamma Foundation of Dallas; Pediatric Subunit; & United Cerebral Palsy Foundation

N/A = not applicable; US = United States; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); hr (s) = hour (s); NR = not reported; (A)GA = (appropriate for) gestational age; GA = gestational age; IUGR = intrauterine growth retardation; PIH = pregnancy induced hypertension; HM = human milk; PT = preterm; FT = full term; RBC = red blood cells; FA = fatty acids; PL = phospholipids; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; LA = linoleic Acid; ALA = alpha-linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; DGLA = dihomo-gama-linolenic acid; BF = breast fed; tx = treatment; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s)

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Exposure	Timing & Dose of exposure (if appropriate)	Outcomes Results	Funding Source
Cetin, 2002, Italy {33}	▪ Case-control ▪ Quality score: 5 [Grade B]	**Inclusion criteria:** Pregnancies with AGA & IUGR fetuses **Exclusion criteria:** Gestational diabetes; pregnancy-induced hypertension **Enrolled:** n=21 **Mean Age:** ▪ Maternal: gp 1 AGA = 28.2 y; gp 2: IUGR = 29.6 y ▪ Child: NR	N/A	N/A	▪ Maternal plasma EPA, DHA & AA, (19-39 wk of gestation): S↑ maternal plasma EPA in IUGR grp than in pb at ≈28.2(8.0) wk GA; NS in maternal plasma DHA & AA at ≈28.2 (8.0) wk GA	European Economic Community ; Italian Ministry of University & Scientific & Technologic Research (MURST) & CNR
Cheruku, 2002, US {73}	▪ Cross sectional ▪ Quality score: 6 [Grade B]	**Inclusion criteria:** Healthy pregnant women & infants (n=17) **Exclusion criteria:** Hx of chronic hypertension, hyperlipidemia, renal or liver, heart, thyroid disease, multiple gestations, or pregnancy-induced complications, pts under tx with drugs during labor affecting respiration of new borns such as magnesium sulfate, & butorphanal, any infants with <4 h of crib time in the 1st & 2nd d postpartum **Enrolled:** n=17 **Mean Age** ▪ Maternal: High-DHA = 29.20 (5.2) y; low-DH A= 24.28 (5.12) y ▪ Child: High-DHA = 40.4 (0.96) wk; low-DHA = 39.0 (1.86) wk	N/A	N/A	▪ Infant sleep-state pattern –maternal BMK: (postpartum d 1 & 2): Maternal DHA was (-) associated with AS, AS:QS & sleep-wake transition (d 2); maternal DHA (+) associated with wakefulness (D2); n-6:n-3 ratio in maternal plasma was (+) associated with AS, AS:QS &sleep-wake transition (d 1); n-6:n-3 ratio in maternal plasma was (-) associated to wakefulness (d 1)	NIH, US Department of Agriculture, the Donaghue Medical Research Foundation, & the University of Connecticut Research Foundation

N/A = not applicable; US = United States; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); g = grams; NR = not reported; N/A = not applicable; AGW = infants with appropriate gestational weight; GA = gestational age; HM = Human milk; (B)W = (birth) weight; (B)L = (birth) length; RBC = red blood cells; (LC)PUFA = (long chain) polyunsaturated fatty acids; PE = phosphatidylethanolamine; FA = fatty acids; LA = linoleic Acid; ALA = alpha-linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; DGLA = dihomo-gama-linolenic acid; tx = treatment; IUGR = intrauterine growth retardation; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s)

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Exposure	Timing & Dose of exposure (if appropriate)	Outcomes Results	Funding Source
Craig-Schmidt, 1994, US {503}	▪ Cross sectional ▪ Quality score: 2 [Grade C]	**Inclusion criteria:** Healthy nulliparous women **Exclusion criteria:** NR **Enrolled:** n=36 **Mean Age:** ▪ Maternal: 21 ± 6 y ▪ Child: NR	N/A	N/A	▪ BMK: NS among gps in plasma saturated, monosaturated & PUFAs; NS in n-6 or n-3 FA between normal pregnancies & GHT, preeclamsia or CHT; CHT S ↑ AA in plasma PL vs. other gps; NS in plasma PL EPA among the gps; NS in AA/EPA ratio & n-6/n-3 ratio	NR
Elias, 2000, Canada {143}	▪ Single prospective cohort ▪ Quality score: 6 [Grade B]	**Inclusion criteria:** Healthy pregnant women (from 22-24 wk gestation until delivery) & infants **Exclusion criteria:** Medical or surgical problems influencing lipid metabolism or fetal growth, communicable disease; > 1 fetus, hypermesis, psychological or social problems, illicit drug or alcohol use, cardiac or renal disease, diabetes, epilepsy, respiratory or rheumatoid conditions, cholestasis, hx of high blood cholesterol or tricylglycerol concentrations before pregnancy, HIV infection or AIDS, hepatitis, or tuberculosis **Enrolled:** n=84 **Mean Age:** ▪ Maternal: NR ▪ Child: GA = 40.0 wk	Maternal intake of LCPUFAs during pregnancy	13.6+-0.9 g/d LCPUFAs at 28 wk of gestation; 12.1±0.6 g/d LCPUFAs at 35 wk gestation;	▪ BMK: Maternal plasma TGL AA, S (+) correlated to infant birth wt & L	Molly Towell Perinatal Research Foundation & the National Science & Engineering Research Council of Canada

N/A = not applicable; US = United States; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); g = grams; NR = not reported; N/A = not applicable; AGW = infants with appropriate gestational weight; GA = gestational age; HM = Human milk; (B)W = (birth) weight; (B)L = (birth) length; (LC)PUFA = (long chain) polyunsaturated fatty acids; PE = phosphatidylethanolamine; FA = fatty acids; LA = linoleic Acid; ALA = alpha-linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; DGLA = dihomo-gama-linolenic acid; hx = history; HIV = human immuno-deficiency virus; AIDS = acquired immune deficiency syndrome; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s)

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Exposure	Timing & Dose of exposure (if appropriate)	Outcomes Results	Funding Source
Ghys, 2002 Netherlands {38}	▪ Prospective single cohort ▪ Quality score: 8 [Grade A]	**Inclusion criteria:** Full-term neonates **Exclusion criteria:** NR **Enrolled:** n=128 **Mean Age:** ▪ Maternal: NR ▪ Chid: 47 (1.3) mo	N/A	N/A	▪ BMK: No correlation between plasma or RBC DHA & AA & cognitive development (4 y)	NR
Hofmann, 1998, Germany {1145}	▪ Cross sectional ▪ Quality score: 6 [Grade B]	**Inclusion criteria:** Pregnant women with preeclampsia (BP at rest > 140/90 beyond the 20th wk gestation) & healthy pregant women **Exclusion criteria:** Endocrionological sx affecting the lipide metabolism **Enrolled:** PE n=14; ctrl n=16 **Mean Age:** ▪ Maternal: PE = 27 y (17-38); ctrl = 28 y (20-39) ▪ Child: PE GA = 36 wk (32-40); ctrl GA = 37 wk (34-40)	N/A	N/A	▪ BMK: Total FA in plasma TGL during pregnancy were S > in preeclamptic gp vs. crtl; NS between gps in AA plasma TGL during pregnancy; LA (n-6) & DHA (n-3) content in plasma TGL were S ↓ in preeclamptic pts vs. crtls; NS between gps LA & AA (n-6) in plasma PL; DHA plasma PL content was S ↓ in preeclamptic women	NR
Innis, 1994, Canada {521}	▪ Cross sectional ▪ Quality score: 5 [Grade B]	**Inclusion criteria:** Full term infants (> 37 wk GA at birth), AGA, & if mother decided to BF or FF for >/= 3 mo **Exclusion criteria:** NR **Enrolled:** n=35 **Mean Age:** ▪ Maternal: NR ▪ Child: BF GA = 39.5 ± 1.0 wk, FF GA = 39.1 ± 1.0 wk	HM (n=17) vs. CF (n=18)	HM (0.2+-0.02 wt% DHA, 0.1± 0.01 wt% EPA, 0.5± 0.03 wt% AA) from 14± 2 d to ≥3 mo	▪ Visual acuity: NS between gps in visual acuity test (14 d & 3 mo) ▪ BMK: Visual acuity NS to diet or plasma PL, RBC PC or PE concentrations of DHA on entire gp of infants or within the breastfed or formula-fed gp of infants	British Columbia Children's Hospital Investigatorship (SMI)

N/A = not applicable; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); hr (s) = hour (s); g = grams; NR = not reported; (A)GA = (appropriate for) gestational age; GA = gestational age; PCA = post-conceptual age; HM = human milk; PT = preterm; BF = breast fed; FF = formula fed; CF = control formula; FT = full term; CA = corrected age; RBC = red blood cells; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; (LC)PUFA = (long chain) polyunsaturated fatty acids; FA = fatty acids; DHA = docosahexaenoic acid; AA = arachidonic acid; DPA = docosapentaenoic acid; HC = head circumference; BP = blood pressure; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); AGA = adequate for gestational age

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Exposure	Timing & Dose of exposure (if appropriate)	Outcomes Results	Funding Source
Innis, 2001, Canada {112}	▪ Prospective single cohort ▪ Quality score: 8 [Grade A]	**Inclusion criteria:** Mothers who committed to only BF heatlhy term infants (no formula or cow's milk) from at least 3 mo, no solid food for at least 1st 4 mo after birth **Exclusion criteria:** Mothers with substance abuse, metabolic or physiologic problems, infections likely to influence fetal growth , or multiple births & infants with evidence of metabolic or physical abnormality **Enrolled:** n=83 **Mean Age:** ▪ Maternal: 32.2 y ▪ Child: NR	HM	HM for at least 3 mo	▪ BMK: RBC PE DHA (2 mo) was S (+) correlated to visual acuity at 2 & 12 mo, NS at 4 & 6 mo; Infants with RBC PE DHA <8.53g/100g had S ↓ visual acuity at 2 & 12 mo than infants with > 10.78g/100g FA; No correlation between plasma or RBC DHA & AA & cognitive development (4 y)	Medical Research Council (MRC) of Canada & Ross Laboratories, Columbus, Ohio
Jorgensen, 1996, Sweden {422}	▪ Cross sectional ▪ Quality score: 5 [Grade B]	**Inclusion criteria:** Healthy term AGA BF & FF infants; age: 37-42 wk (n=33) **Exclusion criteria:** Major congenital anomaly, severe intra/peri venticular haemorrhage or 5-min APGAR score < 5 **Enrolled:** n=33 **Mean Age:** ▪ Maternal: NR ▪ Child: LBM gp = 28.4 wk; HBM = 28.5 wk	HM (n=17) vs. CF (n=16)	HM (0.49+-0.20 to 0.53+-0.56 wt% DHA; 0.13+-0.07 to 0.23+-0.35 wt% EPA; 0.56+-0.12 to 0.44+-0.09 wt% AA) for 4 mo	▪ BMK: NS correlation between RBC DHA & visual between gps (4 mo); NS correlation between AA levels & visual acuity ▪ Visual acuity at 2, 4 mo: Visual acuity S ↑ overtime in both feeding gps, S ↑ increase in HM grp	Food Technology Research & Development Program (FOTEK), DanoChemo A/S Swedish Medical Research Council

N/A = not applicable; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); hr (s) = hour (s); g = grams; NR = not reported; (A)GA = (appropriate for) gestational age; HM = breast milk; BF = breast fed; FF = formula fed; (B)W = (birth) weight; L = length; HC = head circumference; PT = preterm; RBC = red blood cells; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; PC = phosphatidylcholine; PE = phosphatidylethanolamine; (LC)PUFA = (long chain) polyunsaturated fatty acids; FA = fatty acids; PL = phospholipids; DHA = docosahexaenoic acid; LA = linoleic Acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; DGLA = dihomo-gama-linolenic acid; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s)

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Exposure	Timing & Dose of exposure (if appropriate)	Outcomes Results	Funding Source
Jorgensen, 2001, Denmark {2207}	▪ Cross-sectional study ▪ Quality score: 9 [Grade A]	**Inclusion criteria:** Term delivery (37-42 wk); normal BW for GA; uncomplicated pregnancy, delivery, & neonatal period; Apgar score > 8 after 5 min; & fully BF at time of examination (no energy drinks & < 100 mL fromula /d) **Exclusion criteria:** SGA (< 10th PC of BW); strabismus, operation of pyloric stenosis **Enrolled:** n=39 **Mean Age:** ▪ Maternal: 30.5 (3.9) y ▪ Child: 39.8 (1.2) wk	HM	HM (0.35+-0.20 wt% DHA, 0.39+-0.07 wt% EPA, 0.30+-0.07 wt% AA) for ≥14 wk;	▪ BMK: NS association between AA, EPA, LA & ALA (n-3) with visual acuity ▪ HM LA, ALA, AA, EPA & DHA & correlation with visual acuity: S association between visual acuity (VEP) at 4 mo & mother's milk DHA	Food Technology Research & Development Program (FOTEK) BASF Health and Nutrition A/S
Krasevec, 2002, Cuba {72}	▪ Cross sectional ▪ Quality score: 7 [Grade B]	**Inclusion criteria:** Normal pregnancy, with no medical risks affecting fatty acid metabolism, including heart disease, kidney disease, gestational or other diabetes, hypertension, gallbladder disease, or thyroid disease; resident of Central or Old Havana; ages or 17 to 36 y **Exclusion criteria:** NR **Enrolled:** n=56 **Mean Age:** ▪ Maternal: f/u gp = 26.8 (4.0) y; ot-f/u gp = 26.9 (5.3) y ▪ Child: f/u gp GA = 40.4 (1.5) wk; not-f/u gp GA = 40.2 (1.1) wk	High-fat fish maternal intake during pregnancy; HM (n=31), Formula+HM (n=22), Formula (n=3)	454 g/wk maternal fish intake	▪ Visual acuity scores 99% prediction for 2.5 mo old infants; NS Mean values for visual acuity between HM vs. HM + formula infants ▪ BMK: NS correlation visual acuity & any PUFA concentration, ratio of PUFA or gps of PUFAs in infant tissues; NS correlation for full sample & each feeding gp (i.e., exclusively breast milk vs. not exclusively breastfed); NS correlation between PUFA profiles of maternal tissues for exclusively breastfed infants & visual acuity	Canadian Bureau of International Education Vistech Consultants, Dayton Ohio

N/A = not applicable; US = United States; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); hr (s) = hour (s); g = grams; SEC = socioeconomic class; NR = not reported; (A)GA = (appropriate for) gestational age; IUGR = intrauterine growth retardation; CF = control formula; PIH = pregnancy induced hypertension; HM = breast milk; BF = breast fed; FF = formula fed; (B)W = (birth) weight; L = length; HC = head circumference; PT = preterm; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; PC = phosphatidylcholine; PE = phosphatidylethanolamine; (LC)PUFA = (long chain) polyunsaturated fatty acids; FA = fatty acids; PL = phospholipids; DHA = docosahexaenoic acid; LA = linoleic Acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; DGLA = dihomo-gama-linolenic acid; PC = percentile; f/u = follow-up; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s)

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Exposure	Timing & Dose of exposure (if appropriate)	Outcomes Results	Funding Source
Leaf, 1996, Australia {402}	▪ Cross sectional ▪ Quality score: 6 [Grade B]	**Inclusion criteria:** Healthy preterm infants < 32 wk GA **Exclusion criteria:** Major congenital anomaly, severe intra/peri venticular haemorrhage or 5-min apgar score < 5 **Enrolled:** n=18 **Mean Age:** ▪ Maternal: NR ▪ Child: LBM = 28.4 wk; HBM = 28.5 wk	HHM (n=9) vs. LHM (n=9)	HM (32 mg/kg/d AA, 17 mg/kg/d DHA) ± "Intralipid 20%" (6.4 mg/kg/d AA, 5.8 mg/kg/d DHA) from birth up to 40 wk PCA	▪ BMK: S (+) correlation between scotopic b wave implicit time & % DHA in plasma & RBC PL, total n-3 in plasma & RBC PL; S (+) correlation between RBC AA & total n-6 FA & scotopic a-b amplitude; NS relationships were seen between photopic ERGs & plasma or RBC LCPUFAs	NR
Makrides, 1993, Australia {560}	▪ Cross sectional ▪ Quality score: 4 [Grade B]	**Inclusion criteria:** Healthy infants born at term with appropriate weight for GA & were approximately 5 mos of age. **Exclusion criteria:** NR **Enrolled:** n=16 **Mean Age:** ▪ Maternal: NR ▪ Child: BF = 22.4 (±3.7) wk; FF= 22.3 (± 4.3) wk	HM (n=8) vs. CF (>70% nutrition from formula)+HM (n=8)	NR	▪ Visual acuity: HM gp S ↓ logMAR (i.e., better VEP acuity) than formula-fed (5 mo) ▪ BMK: S correlation between logMAR (VEP acuity) & % DHA & LA in RBC PL	Scotia Pharmaceuticals & Nestle Australia

N/A = not applicable; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); hr (s) = hour (s); g = grams; NR = not reported; HM = human milk; BF = breast fed; FF = formula fed; (B)W = (birth) weight; (B)L = (birth) length; HC = head circumference; Δ = change; RBC = red blood cells; FA = fatty acids; LA = linoleic Acid; ALA = alpha-linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; PL = phospholipids; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); ERG = electroretinogram; VEP = visual evoked potential; HHM = high intake of human milk; LHM = low intake of human milk

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Exposure	Timing & Dose of exposure (if appropriate)	Outcomes Results	Funding Source
Matorras, 1994, Spain {494}	▪ Case-control ▪ Quality score: 9 [Grade A]	**Inclusion criteria:** Healthy women at labor with term IUGR & their singleton infants; no malformations or chromosomal abnormalities; no antepartum death; accordancy of GA & pediatric evaluation by means of Dubowitz test & neonatal weight < 10th PC for GA for geographic area (cases); healthy women at labor with term AGA births, neonatal weight > 10th PC (control) **Exclusion criteria:** NR **Enrolled:** Mother n=69; infants n=51 **Mean Age:** ▪ Maternal: IUGR = 28.4 ±6.4 y Ctrl = 26.2 ± 6.2 yr ▪ Child: IUGR GA = 39.1 ±1.4 wk; Ctrl GA = 39.4 ±1.3 wk	N/A	N/A	▪ BMK: S↑ maternal plasma EPA in IUGR grp than in pb at delivery; NS in maternal plasma DHA & AA at delivery	Basque Country Government

N/A = not applicable; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); hr (s) = hour (s); g = grams; NR = not reported; (A)GA = (appropriate for) gestational age; IUGR = intrauterine growth retardation; RBC = red blood cells; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; PL = phospholipids; PC = percentile; IUGR = intrauterine growth retardation; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s)

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics	Exposure	Timing & Dose of exposure (if appropriate)	Outcomes Results	Funding Source
Reece, 1997 US {53}	▪ Case-control ▪ Quality score: 4 [Grade C]	**Inclusion criteria:** Healthy preterm infants; mean GA 33.9±0.6 wk, (cases); term infants; mean GA 40.2±0.2 wk (control) **Exclusion criteria:** Cases (preterm): recognised causes of preterm birth, including uterine abnormalities, intrauterine infection, substance abuse, multiple gestations, pregnancy-onset hypertension, or other medical disorders; Controls (term): recognized medical problems, multiple gestations, multiple parity, pregnancy-onset hypertension, recognized substance abuse **Enrolled:** n=71 **Mean Age:** ▪ Maternal: Cases = 22 y; Controls = 24 y ▪ Child: Cases GA = 40.2 wk; Controls GA = 33.9 wk	N/A	N/A	▪ Maternal BMK: RBC LA, AA, DHA S ↑ in preterm vs. 34-wk control+ & term; RBC EPA S ↑in term controls vs. both preterm & 34-wk control; RBC & plasma n-3/n-6 ratio was S↑ in term controls vs. preterm; NS RBC n-3/n6 between preterm & 34-wk control; plasma LA S ↑ in preterm & 34-wk crtl vs. term crtl; plasma LA, AA, EPA S↑ in preterm vs. term crtls	Colorado Agricultural Experiment Station
Rocquelin, 2003, Congo, Burkina Faso {3}	▪ Cross-national ▪ Quality score: 5 [Grade B]	**Inclusion criteria:** Healthy term infants from Congo & healthy term infants from Burkina faso **Exclusion criteria:** NR **Enrolled:** Congo n=102; Burkina faso n=101 **Mean Age:** ▪ Maternal: NR ▪ Child: Congo = 4.9 (± 0.3) mo; Burkina faso = 5.1 (± 0.2) mo	HM	HM from Congo (0.15+-0.07 wt% DHA, 0.12+-0.06 wt% AA) vs. HM from Burkina Faso (0.08+-0.05 wt% DHA, 0.21+-0.08 wt% AA) for 5 mo	▪ Growth patterns: S↓ wt-for-age & wt-for height z-scores & wt gain (g) in Burkina Faso than in Congo; NS birth wt, age, wt gain of predominantly breastfed to complementary fed infants in Burkina Faso	Institut National de la Recherche Agronomique

N/A = not applicable; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); hr (s) = hour (s); g = grams; NR = not reported; (A)GA = (appropriate for) gestational age; SGA = small for gestational age; IUGR = intrauterine growth retardation; HM = human milk; BF = breast fed; FF = formula fed; (B)W = (birth) weight; (B)L = (birth) length; HC = head circumference; BRW = brain weight; ∆ = change; GP = growth parameters; RBC = red blood cells; PDI = psychomotor developmental index; MDI = mental developmental index; FA = fatty acids; GLA = gammalinolenic acid; LA = linoleic Acid; ALA = alpha-linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; PL = phospholipids; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s)

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics)	Exposure	Timing & Dose of exposure (if appropriate)	Outcomes Results	Funding Source
Rump, 2001, Netherlands {144}	▪ Cross-sectional ▪ Quality score: 9 [Grade A]	**Inclusion criteria:** Healthy singleton term infants, GA of< 16 wk at entry, a diastolic BP < 90mm Hg, & no signs of cardiovascular, neurologic, renal or metabolic disorders at the time of recruitment **Exclusion criteria:** Infants with unknown gestational age or BW, born prematurely, or who died & of mothers with diabetes or pregnancy-induced hypertension. **Enrolled:** n=627 **Mean Age:** ▪ Maternal: SGA: 28.9 (± 4.1) y; AGA 10-25 PC: 28.9 (± 4.6) y; 25-75 PC: 29.5 (± 4.2); 75-90 PC: 29.3 (± 4.2y); LGA: 29.4 (± 3.9) y ▪ Child: SGA: 40.1 (± 1.3) wk; AGA 10-25 PC: 40.0 (±1.0) wk; AGA 25-75 PC: 40.1 (± 1.2) wk; AGA 75-90 PC: 40.6 (± 1.2) wk; LGA: 40.4 (± 1.3) wk	N/A	N/A	▪ BMK: NS correlation between maternal plasma FA at 11 (8) wk GA & at delivery & GA	Dutch Organization for Scientific Research; University Hospital of Maatricht. FA analysis by Nutricia Research, Zoetemeer, Netherlands
Shouk, 1999, Egypt {243}	▪ Case-control ▪ Quality score: 7 [Grade B]	**Inclusion criteria:** Pregnant women with severe preeclampsia in 3rd T; healthy pregnant women without proteinuria or hx of renal disease, not on medications & no hx of obstetric complications **Exclusion criteria:** NR **Enrolled:** n=45 **Mean Age:** ▪ Maternal: Preeclampsia gp: 29 (20-40) y	N/A	N/A	▪ BMK: AA in plasma was S > in preeclamptic women vs. crtl; NS between gps LA & ALA (n-3) content	NR

N/A = not applicable; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); hr (s) = hour (s); g = grams; WG = weight gain; WFA Z-score = weight-for-age Z-score; WFH Z-score = weight-for-height Z-score; HFA Z-score = height-for-age Z-score; GA = gestational age; NR = not reported; (A)GA = (appropriate for) gestational age; GP = growth parameters; IUGR = intrauterine growth retardation; HM = human milk; CF = control formula; RBC = red blood cells; FA = fatty acids; LA = linoleic Acid; DHA = docosahexaenoic acid; AA = arachidonic acid; BP = blood pressure; hx = history; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s)

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics)	Exposure	Timing & Dose of exposure (if appropriate)	Outcomes Results	Funding Source
Vilbergsson, 1991, Sweden {633,505}	▪ Cross sectional ▪ Quality score: 7 [Grade B]	**Inclusion criteria:** Healthy pregnant women at risk of IUGR (cases); healthy pregnant women at no risk of IUGR (crtl) **Exclusion criteria:** Diabetics **Enrolled:** n=48 **Mean Age:** ▪ Maternal: NR	N/A	N/A	▪ BMK: S↓ maternal plasma DHA & AA in SGA grp than in crtl at 34 weeks GA & at delivery	Gothenburg Medical Society; Gothenburg Masonic Order Orphanage Foundation; Faculty of Medicine, Gothenburg University
Wang, 1991, US {59}	▪ Cross sectional ▪ Quality score: 5 [Grade B]	**Inclusion criteria:** Healthy normal & preeclamptic pregnant women (not on a regimen of aspirin tx) at term & nonpregnant women **Exclusion criteria:** NR **Enrolled:** n=30 **Mean Age:** ▪ Maternal: NR	N/A	N/A	▪ BMK: Total PUFA, LA (n-6), ALA (n-3) & EPA plasma of normal pregnant women was S > preeclamptic pts; NS between gps plasma AA & DHA; S > EPA & DHA in normal pregnant women vs. nonpregnant	Glaxo, Inc., Research Triangle Park, North Carolina

N/A = not applicable; US = United States; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); hr (s) = hour (s); NR = not reported; N/A = not applicable; HM = human milk; PT = preterm; FT = full term; RBC = red blood cells; HM = human milk; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; (LU)PUFA = (long chain) polyunsaturated fatty acids; LA = linoleic Acid; ALA = alpha-linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; DGLA = dihomo-gama-linolenic acid; GLA = gamma-liolenic acid; BRW = brain weight; tx = treatment; IUGR = intrauterine growth retardation; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); ctrl(s) = control(s)

Table 2: Evidence from observational studies for omega-3 fatty acids in child and maternal health (cont'd)

Author, Year, Location	Study Design	Population Characteristics)	Exposure	Timing & Dose of exposure (if appropriate)	Outcomes Results	Funding Source
Williams, 2001, UK {153}	▪ Prospective cohort ▪ Quality score: 9 [Grade A]	**Inclusion criteria:** Healthy full-term BF infants; term infants never BF **Exclusion criteria:** Strabilsmus, reduced vision, high refractive error, missing dietary data, GA < 37 wk **Enrolled:** BF n=101; non-BF n=101 **Mean Age:** • Maternal: NR • Child: NR	N/A	N/A	▪ Stereoacuity (3.5 y): BF was S correlated to foveal (adult) stereacuity; maternal oily fish intake during pregnancy was S correlated with foveal stereoacuity ▪ BMK: S correlation between child's stereoacuity at 3.5 y & antenatal mother's RBC DHA content	Medical Research Council, Wellcome Trust, Ministry of Agriculture, Food & Fisheries, Departments of Health & Enviroment, Milupa, National Eye Research Centre
Xiang, 2000, Sweden {202}	▪ Single prospective cohort ▪ Quality score: 5 [Grade B]	**Inclusion criteria:** Healthy mother-infant pairs **Exclusion criteria:** NR **Enrolled:** n=19 **Mean Age:** ▪ Maternal: 29.5 y ▪ Child: 40.1 wk	HM	NR	▪ BMK: LA, ALA in maternal milk S↑ during 3 mo; DHA in maternal milk S↓ during 3 mo; AA/DHA in maternal milk S correlated with infants' rate ↑ HC at 1 & 3 mo; AA/DHA in maternal milk S correlated with infants' brain wt gain at 1 & 3 mo	Wenner-Gren Centre Foundation

N/A = not applicable; US = United States; n = number of participants; y = years; mo= month(s); wk(s) = week (s); d= day (s); hr (s) = hour (s); NR = not reported; N/A = not applicable; HM = human milk; PT = preterm; FT = full term; RBC = red blood cells; HM = human milk; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; (LU)PUFA = (long chain) polyunsaturated fatty acids; LA = linoleic Acid; ALA = alpha-linolenic acid; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; AA = arachidonic acid; DGLA = dihomo-gama-linolenic acid; GLA = gamma-liolenic acid; BRW = brain weight; S = statistically significant difference; NS = nonsignificant statistical difference; gp(s) = group(s); RBC = red blood cells; ctrl(s) = control(s); HC = head circumference; wt = weight; L = length

Safety Profile Tables

Preterm Infants

Summary Table 1: Studies reporting adverse events (e.g., side effects) and contraindications in relation to dietary intake of omega-3 fatty acids

Author, Year, Location: Length & Design	Study groups[1]		Safety data
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	
Preterm infants			
McClead, 1985, US: 1-3 wks parallel RCT [287]	Safflower oil emulsion 'high ALA' (n=10)ϕ	Safflower oil emulsion 'low ALA' (n=10)	ALA 3 (SD: 1.5)% safflower oil emulsion (high ALA) No adverse events/effects ALA 0.1% safflower oil emulsion (low ALA) Tachycardia: n=1, tachycardia and tachypnea (secondary to presumed sepsis): n=1
Birch, 1992 US: 6 mo parallel RCT [212]	n-3 FA-enriched F soy/marine oil (n=22)/ Non-randomized HM(n=10)	Control F soy oil (n=20)/ Control F corn oil (n=18)	NS diet-induced differences in neonatal morbidity, bleeding time, growth of the LBW infants, or other AE
Koletzko, 1995, Germany: 3 wks parallel RCT [251]	n-3 FA-enriched F primrose oil (n=9)	Control F (n=10)/Non-randomized HM (n=8)	NS between-arm differences in gastric residuals, spitting & abdominal distention (rare occurrence), or other adverse events ascribable to feeding

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; $^{+}p<.05$ or significant with 95% confidence interval; $^{++}p<.01$; $^{+++}p<.001$; $^{++++}p<.0001$; FA = fatty acids; PD = preterm delivery; LBW = low birth weight; ICU = intensive care unit; SCN = special care nursery; GD = gestational diabetes; HM = human milk; NEC = necrotizing enterocolitis; F = formula ; IVH = Intra-ventricular haemorrhage; PCA: post-conception age; SIDS = sudden infant death syndrome; TG = triglyceride; ϕ = completed (otherwise enrolled); AE = adverse events

Summary Table 2: Studies reporting adverse events (e.g., side effects) and contraindications in relation to dietary intake of omega-3 fatty acids

Author, Year, Location: Length & Design	Study groups[1]		Safety data
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	
Preterm infants			
Vanderhoof, 1999, US, Canada: 17 wks parallel RCT[218]	n-3 FA-enriched F triglycerides derived from microbial fermentation (n=77)	Control F (source NR) (n=78)/Non-randomized HM: (n=133)	<u>F (DHA 0.35%) vs. F(control) vs. HM (grp 1 vs. grp 2 vs. grp 3)</u> <u>Events at 48 wks PCA (17 wks of feeding)</u> Death (due to SIDS & NEC): n=1 vs. n=1 vs. n=0 (NS) Diarrhea: n=10 vs. n=8 vs. n=4; S (grp 1 vs. grp 3)[+] Flatulence: n=12 vs. n=3 vs. n=7; S (grp 1 vs. grps 2-3)[+] Jaundice: n=5 vs. n=1 vs. n=13; S (grp 2 vs. grp 3)[+] Milk intolerance: n=0 vs. n=3 vs. n=0; S (grp 2 vs. grps 1,3)[+] Anemia: n=12 vs. n=25 vs. n=28; S (grp 1 vs. grps 2-3)[+] <u>Events leading to discontinuation at 48 wks PCA</u> All: n=11 vs. n=11 vs. n=8 (NS) Diarrhea: n=1 (grp 1 vs. grp 2 vs. grp 3) Vomiting n=1 (grps 1-2) vs. n=3 NEC: n=2 (grps 1-2) vs. vs. n=0 Abdominal pain: n=1 vs. n=0 vs. n=1 Ileus: n=2 vs. n=0 vs. n=1 Infections: n=0 vs. n=1 (grps 2-3) Milk intolerance: n=2 vs. n=5 vs. n=1 Cerebral necrosis or hemorrhage: n=0 (grps 1-2) vs. n=1 Rash: n=0 vs. n=1 vs. n=0 Constipation: n=1 vs. n=0 (grps 2-3) Esophageal reflux: n=1 vs. n=0 (grps 2-3) NS between-arm differences in respiratory, cardiovascular, gastrointestinal, hemic, lymphatic, or urogenital system events; n=2 deaths due to SIDS & NEC not diet related <u>At 92 wks PCA (60 weeks of feeding)</u> ≥ 1 AE: 96.1% vs. 93.6% vs. 86.5% Bradycardia: 40.3% vs. 33.3% vs. 37.6% Apnea: 36.4% vs. 24.4% vs. 32.3% Infection: 32.5% vs. 35.9% vs. 23.3% Pharyngitis: 23.4% vs. 20.5% vs. 17.3% Otitis media: 23.4% vs. 19.2% vs. 12.0% Bilirubinemia: 22.1% vs. 11.5% vs. 13.5% Anemia: 19.5% vs. 32.1% vs. 21.8% Flatulence: 16.9% vs. 5.1% vs. 5.3% Vomiting: 15.6% vs. 16.7% vs. 6.8% Hypoxia: 15.6% vs. 11.5% vs. 13.5% Bronchiolitis: 15.6% vs. 7.7% vs. 7.5% Ileus: 14.3% vs. 10.3% vs. 9.8% Oral moniliasis: 14.3% vs. 7.7% vs. 6.0% Diarrhea: 13.0% vs. 15.4% vs. 5.3% Rhinitis: 13.0% vs. 12.8% vs. 6.0% Rash: 13.0% vs. 10.3% vs. 11.3% Cardiovascular event: 11.7% vs. 16.7% vs. 7.5% Enlarged abdomen: 11.7% vs. 10.3% vs. 13.5% Irritability: 11.7% vs. 10.3% vs. 9.8% Increased cough: 11.7% vs. 1.3% vs. 4.5% Pneumonia: 9.1% vs. 14.1% vs. 7.5%

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; $^{+}p<.05$ or significant with 95% confidence interval; $^{++}p<.01$; $^{+++}p<.001$; $^{++++}p<.0001$; FA = fatty acids; PD = preterm delivery; LBW = low birth weight; ICU = intensive care unit; SCN = special care nursery; GD = gestational diabetes; HM = human milk; NEC = necrotizing enterocolitis; F = formula ; IVH = Intra-ventricular haemorrhage; PCA: post-conception age; SIDS = sudden infant death syndrome; TG = triglyceride; ϕ = completed (otherwise enrolled); AE = adverse events

Summary Table 3: Studies reporting adverse events (e.g., side effects) and contraindications in relation to dietary intake of omega-3 fatty acids

Author, Year, Location: Length & Design	Study groups[1]		Safety data
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	
Preterm infants			
O'Connor, 2001, US, UK, Chile: 14 mo parallel RCT [207]	n-3 FA-enriched F fish/fungal oil (n=140)	n-3 FA-enriched F egg-TG/fish oil (n=143)/ Control F coconut/safflower oil (n=144)	Between-arm differences in death, chronic lung disease, systemic infection, hospital readmission, and feeding intolerance: NS F with fish/fungal oil (DHA 0.27% + EPA 0.08%) Symptoms of feeding intolerance leading to withdrawal: 14%,died: n=3, serious adverse event (n ≥ 1): 46%, hospital readmission (n ≥ 1): 39% F with egg-TG/fish oil (DHA 0.24% + EPA 0%) Symptoms of feeding intolerance leading to withdrawal: 8%, died: n=6, serious adverse event (n ≥ 1): 47%, hospital readmission (n ≥ 1): 43% Control F with coconut/safflower oil (no DHA or EPA) Symptoms of feeding intolerance leading to withdrawal: 13%, died: n=6, serious adverse event (n ≥ 1): 44%, hospital readmission (n ≥ 1): 38%
Innis, 2002, US, Canada: 4 wks parallel RCT [201]	n-3 FA-enriched F alga/fungal oil (n=66)	n-3 FA-enriched F alga oil (n=66)/ Control F (source NR) (n=62)	Between-arm differences in SAE, retinopathy of prematurity, IVH, NEC, or sepsis: NS F with alga/fungal oil (DHA 0.33% + AA 0.60%) NEC: n=0, sepsis: n=24, SAE: n=4 F with alga oil (DHA 0.34%) NEC: n=2, sepsis: n=31, SAE: n=3, death: n=1 (due to SIDS) Control F (source: NR; no DHA, EPA, or AA) NEC: n=1, sepsis: n=24, SAE: n=4, death: n=1 (due to SIDS)
Clandinin, 2002, Canada, US: 20 wks parallel RCT [193]	n-3 FA-enriched F fish/single-cell oil (n=130)	n-3 FA-enriched F single-cell oil (n=112)/ Control F (source NR) (n=119)	NS between-arm differences in adverse events or concomitant medical conditions Well tolerated, although > infants had gas in grp 2 vs. grp 3 at 40-44 wks PCA, but not at 48-57 wks PCA

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; FA = fatty acids; PD = preterm delivery; LBW = low birth weight; ICU = intensive care unit; SCN = special care nursery; GD = gestational diabetes; HM = human milk; TG = triglyceride; NEC = necrotizing enterocolitis; F = formula ; IVH = Intra-ventricular haemorrhage; PCA: post-conception age; SIDS = sudden infant death syndrome; AE = adverse events; SAE = serious adverse events
ϕ = completed (otherwise enrolled)

Summary Table 4: Studies reporting adverse events (e.g., side effects) and contraindications in relation to dietary intake of omega-3 fatty acids

Author, Year, Location: Length & Design	Study groups[1]		Safety data
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	
Preterm infants			
Fewtrell, 2002, UK: 4 wks parallel RCT [273]	n-3 FA enriched F primrose oil/egg lipids (n=95)	Control F (source NR) (n=100)/Non-randomized HM (n=88)	<u>n-3 FA-enriched F vs. control F vs. HM</u> Death: 4.2% vs. 0% vs. 2.3% (NS) NEC: 5.3% vs. 2% vs. 0% (NS; withdrew before 3 wks) Systemic infection: 5.3% vs. 7% vs. 2.3% (NS) Skin sepsis: 13% vs. 8% vs. 8% (NS) IVH: 8.4% vs. 3% vs. 9.9% (NS) Pulmonary haemorrhage: 2.1% vs. 1% vs. 0% (NS) N ventilated: 51% vs. 50% vs. 48% (NS) Periventricular leukomalacia: 3.1% vs. 4% vs. 3.7% (NS) Patent ductus arteriosus: 6.3% vs. 7% vs. 2.5% (NS) Retrolental fibroplasia: 2.1% vs. 3% vs. 0% (NS) Retinopathy of prematurity: NR (NS) Mean n of d abdominal distension: NR (NS) Mean n of d nappy rash reported: NR (NS) Mean n of stools per d (grp 1 vs. grp 2): 1.96 vs. 2.12; S^{+} <u>4 deaths in n-3 FA-enriched F</u> n=1 early death due to NEC(d 9), and n=3 late deaths (d 46-135) due to chronic lung disease <u>Follow-up data on AE</u> NS Between-arm differences in the incidence of respiratory tract infections and eczema, n of doctor visits and hospital admissions, between discharge and 18 mo follow-up: NR
Koletzko, 2003, Germany: 4 wks parallel RCT [257]	n-3 FA enriched F black currant seed oil/fish oil/egg lipids (n=15)	Control F (source NR) (n=15)/Non-randomized HM (n=19)	Frequency of gastric residuals, vomiting, or stools: NR (NS)

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; FA = fatty acids; PD = preterm delivery; LBW = low birth weight; ICU = intensive care unit; SCN = special care nursery; GD = gestational diabetes; HM = human milk; NEC = necrotizing enterocolitis; F = formula ; IVH = Intra-ventricular haemorrhage; PCA: post-conception age; SIDS = sudden infant death syndrome; TG = triglyceride; AE = adverse events; SAE = serious adverse events
ϕ = completed (otherwise enrolled)

Summary Table 5: Studies reporting adverse events (e.g., side effects) and contraindications in relation to dietary intake of omega-3 fatty acids

Author, Year, Location: Length & Design	Study groups[1]		Safety data
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	
Preterm infants			
Gobel, 2003, Germany: 1 wk parallel RCT [286]	Olive/soybean oil emulsion (n=24)	Soybean oil emulsion (n=21)	No SAE NS between-arm differences in AE <u>Olive/soybean oil emulsion (DHA 0.23% + ALA 2.0%)</u> Bradycardia: n = 7 (29%) Gastroesophageal reflux: n = 7 (29%) Hyperbilirubinemia: n = 5 (20.8%) Apnea: n = 4 (16.7%) <u>Soybean oil emulsion (DHA 0.34% and ALA 6.99%)</u> Bradycardia: n = 6 (28.6%) Gastroesophageal reflux: n = 5 (23.8%) Hyperbilirubinemia: n = 3 (14.3%) Apnea: n = 2 (9.6%)
Fewtrell, 2004, UK: 42 wks parallel RCT [258]	n-3 FA enriched F starflower and tuna fish oil (n=122)	Control F Sunflower/canola oil (n=116)	<u>n-3 FA-enriched F (grp 1) vs. control F (grp 2)</u> Death: 0% vs. 1%; NS NEC: 4% vs. 2%; NS Systemic infection: 9% vs. 7%; NS Skin infections: NR; NS IVH: 7% vs. 8%; NS Pulmonary haemorrhage: 0% vs. 1%; NS n ventilated: 38% vs. 38%; NS Median d ventilated: 4 (3-8) vs. 2 (2-5); S^{+} Periventricular leukomalacia: NR; NS Patent ductus arteriosus: NR; NS Retinopathy of prematurity: NR; NS Required respiratory assistance: 8% vs. 5%; NS Median d with umbilical catheters: 4 (3-6) vs. 3 (2-5); S^{+} Mean n of stools per d: 3 vs. 3; NS Mean n of d abdominal distension reported: NR; NS <u>Follow-up data on AE</u> Between-arm differences in the incidence of respiratory tract infections & eczema, n of doctor visits & hospital admissions, between discharge & 18 mo follow-up: NR (NS) Stool frequency & consistency between the arms were similar

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; FA = fatty acids; PD = preterm delivery; LBW = low birth weight; ICU = intensive care unit; SCN = special care nursery; GD = gestational diabetes; HM = human milk; NEC = necrotizing enterocolitis; F = formula ; IVH = Intra-ventricular haemorrhage; PCA: post-conception age; SIDS = sudden infant death syndrome; TG = triglyceride; ϕ = completed (otherwise enrolled); AE = adverse events; SAE = serious adverse events

Term Infants

Summary Table 6: Studies reporting adverse events (e.g., side effects) and contraindications in relation to dietary intake of omega-3 fatty acids

Author, Year, Location: Length & Design	Study groups[1]		Safety data
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	
Term infants			
McClead, 1985, US: 1-3 wks parallel RCT [287]	Safflower oil emulsion 'high ALA' (n=10)ϕ	Safflower oil emulsion 'low ALA' (n=10)	ALA 3 (SD: 1.5)% safflower oil emulsion (high ALA) No AE ALA 0.1% safflower oil emulsion (low ALA) Tachycardia & tachypnea (2nd to fluid overload): n=1
Decsi, 1995, Germany: 12 wks parallel RCT [261]	n-3 FA enriched F egg lipids evening primrose oil (n=12)ϕ	Control F (n=10)	F-s well tolerated & no serious adverse events reported except for minor dermatological symptoms such as seborrhoeic & diaper dermatitis
Auestad, 1997, US: 16-48 wks parallel RCT [104]	n-3 FA enriched F fish oil (n=43)/Non-randomized HM (n=63)ϕ	n-3 FA enriched F egg lipids (n=46)/ Control F Oil blend: coconut safflower & soy (n=45)	aT 12 mo (cataracts, viral meningitis, pyloric stenosis, phenylketonuria, anisometropia) were not related to F intake At 39 mo, NS between-arm differences in the % of those with ≥ 1 hospitalization, pressure equalization tubes for chronic otitis media, and ≥ 3 prescriptions for antibiotics F (fish oil: DHA 0.23%) SIDS: n=1 (unrelated to study participation), F-intolerance: n=4, ≥ 3 prescriptions for antibiotics: 57%, pressure equalization tubes for chronic otitis media: 6%, ≥1 hospitalization: 12% F (egg lipids: DHA 0.12% + AA 0.43%) Cataracts: n=1, F-intolerance: n=9, ≥3 prescriptions for antibiotics: 46%, pressure equalization tubes for chronic otitis media: 11%, ≥1 hospitalization: 29% F (coconut, safflower, & soy oils; no DHA or AA) Viral meningitis: n=1, pyloric stenosis: n=1, F-intolerance: n=2, ≥3 prescriptions for antibiotics: 62%, pressure equalization tubes for chronic otitis media: 8%, ≥ 1 hospitalization: 19% HM Phenylketonuria: n=1, anisometropia: n=1, ≥3 prescriptions for antibiotics: 66%, pressure equalization tubes for chronic otitis media: 4%, ≥ 1 hospitalization: 14%

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; $^{+}$p<.05 or significant with 95% confidence interval; $^{++}$p<.01; $^{+++}$p<.001; $^{++++}$p<.0001; FA = fatty acids; PD = preterm delivery; LBW = low birth weight; ICU = intensive care unit; SCN = special care nursery; GD = gestational diabetes; HM = human milk; NEC = necrotizing enterocolitis; F = formula; IVH = intra-ventricular haemorrhage; PL = phospholipid; TG = triglyceride; SIDS = sudden infant death syndrome; AE = adverse events; SAE = serious adverse events; ϕ = completed (otherwise enrolled)

Summary Table 7: Studies reporting adverse events (e.g., side effects) and contraindications in relation to dietary intake of omega-3 fatty acids

Author, Year, Location: Length & Design	Study groups[1]		Safety data
	Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	
Term infants			
Birch, 1998, US: 17 wks parallel RCT [182]	n-3 FA enriched F single-cell oils (n=27)/Non-randomized HM (n=29)	n-3 FA enriched F single-cell oils (n=26)/Control F (n=26)	F (grp 1) vs. F (grp 2) vs. F (grp 3) vs. HM (grp 4) Illness unrelated to protocol: At 6 wks: n=0 vs. n=1 vs. n=0 vs. n=1 At 17 wks: n=1 vs. n=0 (grps 2-4) At 52 wks: n=2 vs. n=0 (grps 2-4) Signs of lactose intolerance At 6 wks: n=1 vs. n=2 vs. n=3 vs. n=2
Lucas, 1999, UK: 6-24 wks parallel RCT [265]	n-3 FA enriched F egg PL-TG fractions (n=154)	Control F (source NR) (n=155)/Non-randomized HM (n=138)	F (DHA 0.32% + AA 0.30%) vs. F (control; no DHA or EPA) By 9 mo of follow-up Withdrawals due to AE: n=17 vs. n=19; NS Mild AE: n=5 vs. n=8; NS Moderate AE: n=12 vs. n=8; NS Severe AE: n=0 vs. n=3; NS Constipation: n=1 vs. n=0; NS Gastroenteritis: n=1 vs. n=0; NS Pyloric stenosis: n=1 vs. n=0; NS Vomiting: n=7 vs. n=7; NS Median crying time (min/day): 53 vs. 40; NS Odds of having an event (grp 1 relative to grp 2) by 9 mo Prescribed antibiotics: OR=1.3, 95% CI: 0.8, 2.2 (NS) Respiratory infections: OR=1.1, 95% CI: 0.5, 2.4 (NS) Gastroenteritis: OR=0.8, 95% CI: 0.5, 1.5 (NS) Visit to medical practitioner: OR=1.8, 95% CI: 0.8, 4.2 (NS) Eczema: OR=1.2, 95% CI: 0.7, 2.1 (NS) Asthma: OR=0.8, 95% CI: 0.3, 2.5 (NS) Wheeze: OR=1.1, 95% CI: 0.6, 1.8 (NS)
Makrides, 1999, Australia: 16 wks parallel RCT [205]	n-3 FA enriched F tuna oil (n=27)/Non-randomized HM (n=63)	n-3 FA enriched F egg-PL fraction (n=28)/ Control F (n=28)	At 6 &16 wks of feeding: NS between-arm % of infants with restlessness, rash, vomiting, diarrhea, & constipation NR (NS) Of the 32 withdrawn infants (formula-fed: 15 and HM: 17), n=2 AE; n=11 cataracts (HM) & n=1 (DHA 0.35% or grp 1) - unrelated unspecified medical problem

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; $^{+}p<.05$ or significant with 95% confidence interval; $^{++}p<.01$; $^{+++}p<.001$; $^{++++}p<.0001$; FA = fatty acids; PD = preterm delivery; LBW = low birth weight; ICU = intensive care unit; SCN = special care nursery; GD = gestational diabetes; HM = human milk; NEC = necrotizing enterocolitis; F = formula; IVH = intra-ventricular haemorrhage; PL = phospholipid; TG = triglyceride; ϕ = completed (otherwise enrolled); AE = adverse events; SAE = serious adverse events

Summary Table 8: Studies reporting adverse events (e.g., side effects) and contraindications in relation to dietary intake of omega-3 fatty acids

Author, Year, Location: Length & Design	Study groups[1] Group 1 (n)/ Group 4 (n)	Group 2 (n)/ Group 3 (n)	Safety data
Term infants			
Morris, 2000, UK: 12 wks parallel RCT [268]	n-3 FA enriched F TG-form (n=54)ϕ	Control F (source NR) (n=55)	At 6 wks n-3 FA enriched F (DHA 0.20%) n=2 ('vomiting') n=2 ('slow to feed' and 'hungry') Control F n=3 ('hungry', 'not satisfied', and 'erratic') At 12 wks n-3 FA enriched F (DHA 0.20%) n=1 ('not satisifed') Control F n=1 ('colic') NS between-arm difference in stool consistency NS between-arm difference in frequency of consultations with primary care team, hospital admissions, gastrointestinal disturbances, stools, upper respiratory infections, & allergic reactions
Makrides, 2000, Australia: 34 wks parallel RCT [266]	High 'ALA' F Palm, canola, coconut, & soy oils (n=37)	Low 'ALA' F Oleic, coconut, soy, & safflower oils (n=36)/Non-randomized HM (n=103)	At 6 & 16 wks of age NS in reported frequency of infant restlessness, rash, vomiting, diarrhea, or constipation In HM: n=4 infants had recurrent illnesses unrelated to the trial & withdrew
Auestad, 2001, US: 48 wks 2 parallel RCT [227]	n-3 FA-enriched F fish/fungal (n=82)/Non-randomized HM (n=165)	n-3 FA-enriched F egg-TG (n=80)/ Control F coconut, soy, & safflower oils (n=77)	F intolerance by 48 wks of age NS frequency of spitting up, vomiting, &consistency of stools F intolerance leading to withdrawals: n=14 (fish/fungal F arm) n=13 (egg-TG F arm) n=16 (control F)
Jensen, 2002, US: 16 wks parallel RCT [203]	F1 canola, palm, coconut oils (n=20)/F4 palm, coconut, safflower oils (n=20)	F2 palm, coconut, canola oils (n=20)/ F3 sunflower, palm, coconut oil (n=20)	Dietary protein hypersensitivity n=2 (F3: ALA 0.95% arm)

[1]Proceeding from highest omega-3, or lowest omega-6/omega-3, fatty acid content of intervention/exposure; n-3 = omega-3 fatty acids; n-6 = omega-6 fatty acids; ALA = alpha linolenic acid; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AA = arachidonic acid; E-EPA = ethyl eicosapentaenoate; Length = intervention length; Design = research design; n = sample size; pts = study participants; NR = not reported; NS = nonsignificant statistical difference; n/a = not applicable; pb = placebo; grp = group; wk = week(s); mo = month; wt = weight; $^{+}p<.05$ or significant with 95% confidence interval; $^{++}p<.01$; $^{+++}p<.001$; $^{++++}p<.0001$; FA = fatty acids; PD = preterm delivery; LBW = low birth weight; ICU = intensive care unit; SCN = special care nursery; GD = gestational diabetes; HM = human milk; NEC = necrotizing enterocolitis; F = formula; IVH = intra-ventricular haemorrhage; ; PL = phospholipid; TG = triglyceride; ϕ = completed (otherwise enrolled); AE = adverse events; SAE = serious adverse events

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Appendix F. List of excluded studies (no RCTs)

1.. Pregnancy outcomes

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3. Neurological outcomes

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4. Cognitive Outcomes

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5. Visual outcomes

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Appendix G. Interventional Formula's Content

Interventional Study Formula Content

Formula	DHA	EPA	ALA	LA	AA	GLA	DPA	DGLA	Mono	n-6/ n-3	References
Algal + DHA (%)	0.37										{240}
Algal oil (%)	48.								18.0		{2959}
Aptamil + Milupan	0.15-0.25		0.60-0.65	11.5-12.8	0.30-0.40						{2293}{2307}{359}{2940}[467}
Borage oil (%)	0.32	0.37	1.17	12.67	0.06	0.54	0.10	0.16		10.8	{1159}
Borage oil (g/100g)	0.5	0.1	1.5	12.3		0.9			43		{2938}
Cod liver oil (mg/10ml)	1183	803	75	160	27.5		112				{111}
Corn oil (g/100g)			0.8	31.4					17.1	39:1	{1354}
Corn oil (mg/10ml)	8.3		92	4747							{111}
Egg phospholipids (%)	.34		1.0	16.6	.34				31.0		{213}
Egg phospholipids (%)	0.34		1.02	16.56	0.34	0.13			31.03		{229}
Egg yolk (g/100g)	0.12		1.9	21.7	0.43				42.1	11.1	{380}
Egg yolk Lecithin (g/100g)	0.1		2.0	21.8	0.43						{415}
Egg-derived triglyceride/fish oil (%)	0.26				0.42						{1538}
Egg-DTG (g/100g)	0.14			22.4	0.45	2.5			41.0		{125}
Egg-TG/Fish* (g/100g)	0.24		2.5	17.5	0.41				9.8		{126}
Egg-TG/Fish (g/100g)**	0.15		2.4	20.3	0.41				29.8		{126}
Enfamil + LCPUFA(%)	0.36		1.53	14.9	0.72			0.05	29.2	8.3	{2958}

Enfamil - 0.4% ALA			0.4	17.6						44.0	{350}
Enfamil - 1% ALA			0.95	17.3						18.2	{350}
Enfamil (%)			1.9	18							{374}
Enfamil (%)			4.7	34.2							{374}
Enfamil + DHA (%)	0.34		3.1	22.0							{80}
Enfamil + DHA +ARA (%)	0.33		3.0	21.0	0.60						{80}
Enfamil + LCPUFA (g/L)	0.21		0.86	8.37	0.42			0.01	16.42	8.3	{87}
Enfamil -1.7% ALA			1.7	16.5						9.7	{350}
Enfamil -3.2% ALA			3.2	15.6						4.8	{350}
Enfamil iron DHA+AA (%)	0.36		1.53	14.9	0.72				29.2	8.3	{2301}
Enfamil iron+ DHA (%)	0.35		1.54	15.1	0.02				30.3	7.9	{2301}
EPA (g)		3.0									{481}
Fish oil	0.31	0.08	1.07	17.62	0.03						{1621}
Fish oil – DD (mol/100ml)	0.43	0.34	1.05	11.40	0.03	0.32	0.01	0.01			{275}
Fish oil - high EPA (%)	0.45	0.35	0.85	17.8	0.05				33.8		{1650}
Fish oil - low EPA (%)	0.45	0.10	1.10	17.7	0.05				34.0		{1650}
Fish oil – PT (%)	0.37	0.05									{1760}
Fish oil – SD (mol/100ml)	0.20	0.17	1.06	11.22	0.02	0.31	0.02				{275}

Fish oil – term (%)	0.45	0.09									{1760}
Fish oil (%)	0.36	0.58	1.52	17.44	0.01	0.27	0.07		30.75		{477}
Fish oil (%)	40.4	7.2	0.8	1.2			4.1				{12}
Fish oil (%)	56.0	27.7		0.3	1.8		7.1				{2917}
Fish oil (%)	0.32	0.39	1.20	11.95	0.06		0.07	0.16		10.0	{1159}
Fish oil (%)	0.39										{240}
Fish oil (%)	23.0	32.0									{614}
Fish oil (%)	23.0	32.0									{66}
Fish oil (g)	1.08	1.62									{480}
Fish oil (mg/100kcal)	17				34						{1553}
Fish/Fungal (g/100g)	0.13	<0.04		21.0	0.46	2.4			40.0		{125}
Fish/fungal ** (g/100g)	0.16		2.4	19.5	0.43				27.9		{126}
Fish/fungal oil (%)	0.26				0.42						{1538}
Fish/fungal* (g/100g)	0.27	0.08	2.6	16.8	0.43				8.4		{126}
Fish oil + GLA (mg)	10	18				37					{580}
Formula + LCPUFA (%)	0.32	0.01	1.4	15.9	0.30					9.47	{270}
Formula A (%)			1.3	14.1							{233}
Formula B (%)	0.6	0.1	1.2	17.7	0.1	0.4					{233}
Formula LCPUFA-F (%)	0.57	0.13	1.2	17.7	0.1	0.4			26.9		{940}

Formula+LCPUFA (%)	0.2		2.3	11.6	0.4	0.2					{2231}
High-DHA eggs (g/100g)	5.45										{31}
LA:ALA 10:1 (%)			1.7	16.9					35.8		{220}
LA:ALA 5:1 (%)			3.3	16.6					36.7		{220}
Margarine + ALA			14.18	45.36					17.41		{2907}
Marine oil - PT (g/100g)	0.2	0.3	3.1	18.7						6.0	{581}
Marine oil - term (g/100g)	0.2	0.3	4.9	32.6						6.6	{581}
Marine oil (%)	0.20	0.06	2.4	21.2							{434}
MaxEPA (mg)	120	180									{547}
Microalgae & fungi	0.34				0.70						{40}
Pre-Aptamil Milupan LCPUFA-F (%)	0.3	0.03	1.0	13.8	0.5	0.2			34.4		{460}
Preemie SMA + LCPUFA (%)	0.35		1.5	12.1	0.50						{2143}
Preemie SMA+LCPUFA (%)	0.35		1.5	12.1	0.49						{2191}
Preglandin (mg)				375.		45.					{547}
Prematil + LCPUFA (%)	0.3	0.03	0.8	13.8	0.5	0.2		0.1			{455}
Prematil Milupan (g/100g)	0.17	0.04	0.6	12.0	0.31	0.4					{2129}
Prematil Milupan + LCPUFA (%)	0.30	0.05	0.73	10.85	0.44	0.30	0.07	0.12			{2262}
Single cell oils (mg/100kcal)	17				34						{1553}
Soy oil (g/100g)			4.8	34.2					17.3	7:1	{1354}
Soy/Marine oil (g/100g)	0.35	0.65	1.4	20.4	0.1				10.7	8.5	{603}

Tuna fish oil (g/100g)	0.5	0.1	1.5	12.3		0.9			43		{2938}
Tuna oil (%)	.35	.10	1.2	16.8					31.9		{213}
Tuna oil (%)	0.35	0.10	1.22	16.76		0.12			31.85		{229}
Tuna oil (g/100g)	0.23	0.07	1.9	20.7					40.2	9.4	{380}
* = in-hospital; ** = post discharge; PT = preterm; SD = single dose; DD = double dose; DHA = docosahexaenoic acid; EPA = eicosapentanoic acid; ALA = α-linolenic acid; LA = linoleic acid; AA = arachidonic acid; GLA = gammalinolenic acid; DGLA = dihomo-gama-linolenic acid											

Appendix H. Listing of Excluded Studies at Level 2 and 3 Screening

Level 2

Adair C D, Sanchez-Ramos L, Briones D L et al. The effect of high dietary n-3 fatty acid supplementation on angiotensin II pressor response in human pregnancy. American Journal of Obstetrics & Gynecology 1996;175(3 Pt 1):688-691. Not related to predefined child or maternal health outcomes.

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Appendix I. Additional Acknowledgements

The UO-EPC gratefully acknowledges the following individuals who served on our Technical Expert Panel (TEP). Acknowledgement does not reflect endorsement of this report.

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The UO-EPC gratefully acknowledges the following individuals who reviewed the initial draft of this evidence report, and provided constructive feedback. Acknowledgement does not reflect endorsement of this report.

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