EFFECT OF n-3 LONG CHAIN POLYUNSATURATED FATTY ACIDS DURING THE PERINATAL PERIOD ON LATER BODY COMPOSITION

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Abbreviations: LCPUFA – long chain polyunsaturated fatty acids; LA – linoleic acid; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid; ALA – alpha linolenic acid; AA – araquidonic acid; BMI – body mass index; BF – body fat; FFM – fat free mass; GLA – gamma-linolenic acid; PMA – postmenstrual age; RCT – randomized controlled trial

Abstract

A systematic review to identify studies reporting the effects of n-3 long chain polyunsaturated fatty acids (LCPUFA) intake, during pregnancy and postnatally, on infants and young children's body composition was performed. A structured search strategy was performed in the MEDLINE (PubMed), EMBASE, and LILACS databases. Inclusion and exclusion criteria were defined according to the research question. Only those studies addressing the relationship between n-3 LCPUFA exposure during the perinatal period and later adiposity measured in terms of weight, height, body mass index (BMI), skinfold thickness and/or circumferences were included regardless of the study design. Studies quality was scored and were thereafter categorised into those reporting on maternal intake of n-3 LCPUFA during pregnancy or lactation (6 publications) or on infant's n-3 LCPUFA intake (7 publications). Two studies showed inverse associations between maternal n-3 LCPUFA intake and children's later body composition (lower adiposity, BMI or body weight), two showed direct associations and no effects were observed in the remaining two studies. Among those studies focusing on n-3 LCPUFA intake through enriched infant formulas; three observed no effect on later body composition and two showed higher weight and adiposity with increased amounts of n-3 LCPUFA. Reversely, in two studies weight and fat mass decreased. In conclusion, reported body composition differences in infants and young children were not clearly explained by perinatal n-3 LCPUFA intake via supplemented formulas, breastfeeding or maternal intakes of n-3 LCPUFA during pregnancy and lactation. Associated operational mechanisms including n-3 LCPUFA doses and sources applied are not sufficiently explained and therefore no conclusions could be made.

INTRODUCTION

Both intrauterine and early infancy are periods of rapid growth and development during which insufficient supply of energy and nutrients might result to metabolic or body composition alterations. Its relative impact on the different periods has not yet been elucidated [1] but it appears to be modulating early life outcomes and later risk of chronic disease [1, 2]. Specifically, early life nutrition has been shown to significantly contribute to adiposity development variability [2-4]. The fetal-infant programming hypothesis states that increased risk of adiposity later in life is originated from early exposure to detrimental environments including nutritional aspects; however, the mechanisms are still unclear [1, 2]. Therefore, effective preventive measures of the obesity epidemic require knowledge of the dietary risk factors and their consequences which act during critical life periods [5].

The effects of essential long chain polyunsaturated fatty acids (LCPUFA) supplementation during the perinatal period on neurobehavioral development or visual acuity, infant growth as well as safety monitoring outcomes has been addressed by a number of clinical trials mainly in preterm infant populations [6, 7]. The majority of the studies tested the effect of specific LCPUFA concentrations added to an infant formula on postnatal growth outcomes including body weight and length [8, 9]. Some of the limited reporting effects on body composition and long-term body fat programming, both from animal and human studies, indicate that early availability of LCPUFA might influence development of adipose tissue during fetal life and infancy [10]. Eicosanoids derived from arachidonic acid (AA), n-6 LCPUFA, appear to have an adipogenic effect, by providing a molecular link between fatty acid uptake and preadipocytes differentiation during early hyperplasic growth stages of adipose tissue. In contrast, those derived from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), n-3 LCPUFA, have an antiadipogenic effect [11, 12]. Consequently, fatty acid levels and the ratio between n-6 LCPUFA and n-3 LCPUFA in the maternal diet, during pregnancy and lactation, may play an important role in early adipose tissue development [13].

The present systematic review presents studies addressing the relationship between n-3 LCPUFA intakes, during pregnancy and postnatally, on early and long-term body composition variability.

METHODS

The research question to be answered by the systematic review was if early life n-3 LCPUFA intake (prenatal and early postnatal periods) has any influence on childhood body composition. The flow chart of the process is illustrated in figure 1. The search process was not limited to any language, timeframe or country of publication and was performed in three electronic databases (MEDLINE, EMBASE and LILACS). The general search strategy included terms related to the population under question (infants and children), predictor (n-3 LCPUFA intake) and

dependent variables (obesity and body composition). The shared terms used in MEDLINE and EMBASE search included (pregnant women [MeSH] OR breastfeeding [MeSH] OR age group [MeSH]) AND (Fatty Acids, Unsaturated [MeSH]) AND (body weight [MesH] OR metabolic syndrome X [MesH]). In LILACS, terms used were slightly different: (Risk groups Nutrition [MeSH] OR feeding behaviour [MeSH] OR age groups [MeSH]) AND (Fatty Acids, Unsaturated [MeSH]) AND (body weight [MesH] OR Nutritional and Metabolic Diseases [MesH]).

The initial search yielded 2605 references after exclusion of duplicates. Additional publications were identified from references listed in the identified original papers reviewed. This secondary search added 47 potential relevant papers (total of 2653). Selected studies were then classified into two different groups: 1) maternal n-3 LCPUFA intake during pregnancy and lactation on infants and young children's body composition; and 2) effects of infant's n-3 LCPUFA intake (from birth or early postnatal period) on later body composition.

The results of the searches were stored in an Endnote XII library. Firstly, references were screened on the basis of title and abstract. Those clearly not meeting the review's criteria were excluded. Selected references in the previous step were all screened based on full text. Reasons for exclusion were registered in the Endnote library. Criteria for inclusion/exclusion are stated in Table 1. References were excluded on the basis of irrelevant health outcome (no adiposity or body composition measurements) and targeted population or dietary exposure (only ALA as n-3 LCPUFA or only n-6 essential fatty acids as LCPUFA supplementations). When in doubt, the team reviewed the papers to ensure alignment and quality control. Only papers fulfilling the inclusion criteria were considered in the present review.

Assessment of risk of bias in included studies

The quality and risk of bias were assessed as indicators of validity for identified observational and intervention studies. The studies included in this review were checked for a minimum quality score system developed by EURopean micronutrient RECommendation Aligned (EURRECA -network of excellence-) which was adapted from The Cochrane Handbook [(14)]. Criteria for intervention studies were based on method of sequence generation (adequate randomization procedure) and allocation concealment, blinding, potential funding bias, number of participants at start, number of dropouts and suggested reasons, dose check, dietary intake data reported, and similarities between the most and least exposed groups at baseline. For longitudinal studies criteria were based on number of dropouts and reasons, potential funding bias, lack of other potential threats to validity, inclusion of confounders, and assessment of adequacy of exposure. Concepts were evaluated as of high, low or of uncertain risk of bias. Overall risk of bias was judged as high if more than one of the following concepts were uncertain or inadequately addressed: confounders, exposure assessment , potential funding bias for observational studies and sequence

generation, allocation concealment, blinding or potential funding bias for intervention studies. Observational and intervention studies were judged of moderate risk of bias, if reviewed studies had one of the above stated criteria judged as high risk. If there was no risk of bias or the risk of bias was present only in other criteria different from those mentioned above, the overall risk of bias was judged as low.

RESULTS

A total of 13 publications [15-27] were eventually selected for inclusion in this systematic review. All studies are summarised in tables 2 and 3 together with extracted information on country where the study was performed, number of participants and age at enrolment, intervention and follow-up duration, intervention details and diet/formula LCPUFA composition, body composition-related outcomes and conclusions. Studies were split into two categories; those reporting data on maternal intake of n-3 LCPUFA during pregnancy or lactation (Table 2) [15, 18, 20, 23, 24, 27] and those assessing infant n-3 LCPUFA intake (beginning during neonatal period) and potential effects on later body composition (Table 3) [16, 17, 19, 21, 22, 25, 26].

Effects of maternal n-3 LCPUFA intake during pregnancy and lactation on body composition during infancy and childhood

A total of six studies assessed maternal intakes of n-3 LCPUFA, one being an observational and five intervention studies (Table 2).

Only one study [18] addressed longitudinally the relationship between prenatal n-3 fatty acid intake and long term adiposity at 3 years of age. This recently US published study was carried out in a cohort of pregnant women where n-3 LCPUFA maternal intake was measured at 29 weeks of pregnancy (mean) and a month before delivery, using a previously validated food frequency questionnaire. LCPUFA intake was reported as total n-3 fatty acids, alpha-linolenic acid (ALA) and DHA + EPA. Blood samples were obtained at mid-pregnancy and after delivery, from the umbilical cord, for quantification of fatty acids from erythrocyte membranes. Children's body composition parameters (height, weight, skinfold thickness) and dietary intakes were measured at the age of 3. The authors concluded that higher maternal prenatal n-3 intake was associated with lower adiposity in early childhood. They observed that a higher DHA + EPA intake during mid-pregnancy was associated with lower subscapular and triceps skinfold thickness, and with reduced odd of obesity at 3 years (OR=0.68, 95% CI=0.50-0.92). Moreover, higher DHA + EPA concentrations in umbilical cord plasma were similarly associated with lower adiposity (skinfold thickness) and obesity (OR=0.09, 95 % CI=0.02-0.52).

Five trials relating infant body composition with pre- and postnatal maternal intake of n-3 LCPUFA were included [15, 20, 23, 24, 27] of which all were double-blinded [15, 20, 23, 24] except one [27] where no information was given. All presented results come from European countries (Denmark,

Norway and Germany), conducted between 2000 and 2009 with the number of participants varying from 175 to 198 mother-term infant pairs. Body mass index (BMI) was reported in all five trials, skinfold thickness in four [15, 23, 27] and waist circumference in two [15, 23]. Two studies [24, 27] measured head circumference and one [27] also calculated weight-for-length and ponderal weight. In order to make possible the comparison of results among intervention studies, sources of n-3 LCPUFA, intervention periods as well as point of time in which the outcomes were measured were considered. In this sense, studies showed some differences (Table 2). Hauner et al. [27] started supplementing pregnant women at 15 weeks of pregnancy till 4 months of lactation and infant's measurements were taken at birth, 6 weeks, 4 months and 12 months postpartum. In Lauritzen et al. [23], the intervention was carried out during the first 16 weeks of lactation and outcome assessment took place at 9 months and at 2.5 years respectively. The trial of Asserhøj et al. [15], a follow-up of the Lauritzen et al. [23] study, described results at 7 years of age. Helland et al. [20], measured BMI as a secondary outcome at 7 years of age, in an intervention trial performed between 18 weeks of pregnancy and 3 months following delivery with either cod liver oil or corn oil differed in the LCPUFA type. In the study by Lucia Bergman et al. [24] pregnant women were supplemented with fish oil DHA (200 mg) from the 21st week of pregnancy until the 3rd month of lactation. Infants were measured at birth and thereafter at 1, 3 and 21 months.

Results on the effect of maternal n-3 supplementation on infant's body composition were inconsistent and did no enable the authors' to underpin any relevant conclusion (Table 2). Additionally, the fact that outcomes were assessed at different age points increased difficulties in result's comparison. Lauritzen et al. [23] observed significantly higher BMI, waist circumference and adipose tissue in the supplemented group at 30 months compared to the control group. These differences, however, disappeared at 7 years in the Asserhøj study [15]. Helland et al. [20], reported no significant effect on BMI at 7 years, in the supplemented group however, concentrations of ALA in breast milk 3 months after birth were positively correlated with BMI at 7 years. On the contrary, Donahue et al. [18] concluded that higher maternal prenatal n-3 intake was associated with lower adiposity in early childhood and Lucia Bergman et al. [24] showed that DHA supplements during pregnancy and lactation may reduce BMI in late infancy. More specifically, lower weight and BMI at 21 months were observed in infants whose mothers were supplemented with DHA, whereas no effects were found for height and head circumference. Hauner et al. [27] however, reported no effect on infants fat mass and growth at ≤ 1 year of life between the intervention and the control groups.

Effects of infant's n-3 LCPUFA intake on body composition

A total of seven studies are presented in table 3 [16, 17, 19, 21, 22, 25, 26]. One was an observational cohort study [26] and six RCTs [16, 17, 19, 21, 22, 25] four of which performed in North America (mainly

in the US) [16, 19, 21, 25] and three in Europe: two in The Netherlands [17, 26], and one in the UK [22]. One was single-blind [25] and five were double-blind [16, 17, 19, 21, 22].

Preterm infants. Four out of seven studies included preterm newborns and varied in sample size (60 to 194 infants) [19, 21, 22, 25], intervention periods (4 weeks to 12 months) as well as in formula composition with n-3 fatty acid. All used DHA as the main n-3 differential fatty acid with contents ranging from 0.2% to 0.5% of total fatty acid weight. The composition of the control formulas differed to that of supplemented formulas in different terms: in two studies LA and ALA but no DHA and AA were added [19, 21], in one study the control formula was not [25], and in another the control formula was supplemented with smaller amounts of LA and ALA compared to the supplemented formula [22]. Three trials [19, 22, 25] accurately measured body compartments as body fat (BF) and fat-free mass (FFM) although methods of assessment and indicators of body composition varied i.e., skinfolds were assessed in two studies [22, 25], one measured total body conductivity [25], dual X-ray absorptiometry (DEXA) was used in another one [19] and both bioelectrical impedance and deuterium dilution in another study [22].

Results among trials varied in use of supplemented LCPUFA formulas, age and gender groups. Innis et al. [21] reported higher weight, length and weight-to-length ratio in infants fed with DHA and AA from algal /fungal oils (0.33% DHA and 0.60% AA) supplemented formula for 4 weeks to the controls (formula 21-22% LA, 3- 3.1% ALA) and those given single-cell algal oil DHA supplemented formula at 40-57 weeks post-menstrual age (PMA). Infants supplemented with DHA and AA formula during 12 months had significantly more FFM and less BF than controls (16-19% LA and 2.5% ALA) at 1 year of age; however, no differences were reported for weight, height, and head circumference [19]. In preterm infants fed with supplemented formula containing DHA and EPA until 9 [22] and 59 weeks postpartum of PMA [25], lower FFM and BF in males [25] and increased weight and adiposity at 9-11 years among females [23] was observed.

***Term infants*.** Three out of seven studies were RCTs with a sample size of 79 [16] and 341 [17] infants respectively. One study was a cohort including 244 mother-infant pairs [26]. The intervention periods with LCPUFA supplemented formulas varied from 2 months [17] to 4 months [16]. DHA was used as the main n-3 differential fatty acid for supplementation [16, 17]. No long-term changes in body composition were observed at 1 [16] or 9 years of age [17]. Reported results in term infants are similarly inconsistent those of preterm infants. No clear relationship between any of the used LCPUFA supplemented formulas and later body composition variability was found. The findings of the cohort study which measured the composition of maternal milk in fatty acids suggested that the n-3 and n-6 LCPUFA content did not have an effect on weight gain or BMI during the first year of life in this group of breast-fed infants [26].

Quality of included studies

Table 4 summarizes the method used to assess the quality of the included studies. Different levels of risk of bias among the studies were observed. For intervention studies involving infants' mothers, four studies had a high risk of bias [15, 20, 23, 27] and one had moderate risk [24]. Repeated reasons for having risk of bias included an inadequate or unclear blinding procedure, inadequate explanation on dropouts, and an inadequate funder and other potential threats of validity. The only observational study included [18] was defined to be of low risk of bias due to insufficient description of dropouts classified as "unclear". Regarding the studies focused exclusively on children, the observational study [26] had a moderate risk of bias because of several threats to validity. Out of the seven RCTs [16, 17, 19, 21, 22, 25], two [17, 25] were classified at high risk of bias and four [16, 19, 21, 22] at moderate risk of bias. Insufficient description of funding and other potential threats to validity were some of the reasons.

DISCUSSION

The aim of this systematic review was to identify and summarize evidence on studies assessing the relationship between n-3 LCPUFA intake on infancy and early childhood body composition across consecutive life stage. A total of 13 studies [15-27] met the inclusion criteria and were included in this review. A very sensitive search was performed, considering limited availability of literature addressing the topic. References from excluded papers were also reviewed to avoid the omission of any relevant report. Appraised studies were categorised into two groups; those of mothers and infants and to those of infants. It should be taken into consideration that studies carried out in newborns are less solid due to the ethical implications.

The most important strength of the present study is that the review has been performed systematically. On the other hand, the limited number of articles relating intake of n-3 LCPUFA supplemented formulas and short and long-term body composition variability in infants represents the main study weakness. It is difficult to obtain conclusive and comparable results from the scarce existing information. In addition, there is a major discrepancy between the results obtained across the available studies.

Effects of maternal n-3 LCPUFA intake during pregnancy and lactation on body composition during infancy and childhood

All intervention studies had an RCT design. Points which should be considered when interpreting the results of the findings include: geographical location, patterns of intake regarding cold water fish, source of n-3 LCPUFA, and comparability of outcome assessment.

Other important points affecting comparisons and which should be considered include the type of LCPUFA supplemented, formula composition, the different sources of n-3 LCPUFA, the duration of the intervention and the timeframe in which the outcome was measured. The results of this review showed wide variability. The duration of intervention, which ranged from 16 weeks [23]

to 36 weeks [20], as well as the point in time when the outcome was assessed may have an influence on the outcomes evaluated. While two studies measured body composition at 7 years [15, 20], in one trial measurements were taken at 1 year [27]. Three studies [20, 24, 27] comprised both pregnancy and lactation periods; Donahue et al. [18] only considered pregnancy and two studies evaluated the intake of n-3 LCPUFA exclusively during lactation [15, 23]. That variability on methodology could explain the different findings observed across trials. Two studies [20, 27] did not observe any effect on body composition in terms of fat mass, growth or BMI, although in Hauner et al. [27] outcome measures were obtained much earlier (12 months after birth) than in the other studies. One study [24] positively associated n-3 LCPUFA intake with body composition at 2.5y but further measurements at 7y of age [15] did not confirm that association suggesting a time-dependent effect on later body composition. Lucia Bergman et al. [24], however, associated maternal supplementation with DHA with a decrease in children weight and BMI, although no effect was observed on length and head circumference. It is clear that included articles are heterogeneous regarding the interventions as well as the type, sources and doses of n-3 LCPUFA used (see Tables 2 and 3). It is important to remark that exposure time to LCPUFA and EPA/DHA or n-3/n-6 fatty acid ratios might contribute to differential outcome effects.

Literature availability addressing this topic longitudinally is scarce and did not enable conclusion drawing. This is reflected by the nearly non-existent identified literature, since only one paper showing results on the effects of n-3 LCPUFA intake in early periods of life on later adiposity met the inclusion criteria and is presented in this review.

Effects of infant n-3 LCPUFA intake on body composition

Limited literature availability similarly to available evidence on maternal intake should be stressed. Only one paper presented results on the effects of n-3 LCPUFA intake at early life on later body composition in term newborns [26]. In this study, neither n-3 nor n-6 LCPUFA breast milk content influences weight gain or BMI.

All intervention studies were performed in developed countries. Differences in applied protocols in terms of LCPUFA formula composition or the source of n-3 LCPUFA, target sample, sample size, supplementation design of intervention / control groups intervention and monitoring period duration, outcomes measures and implications of accuracy of body composition assessment should be noted.

Preterm infants supplemented with DHA and AA formula in two studies [19, 21] had significantly higher weight, length, weight-to-length ratio [21] and FFM but less BF [19] compared with controls at 1 year of age. Although n-3 LCPUFAs may have antiadipogenic effects inhibiting fat development, AA appears to have an adipogenic effect [9, 11]. Recent systematic reviews however, showed that global growth of both term and preterm infants assessed in terms of weight,

length and head circumference appears was unaffected by LCPUFA intake [8]. Despite the lack of evidence on the relationship between changes on body composition and LCPUFA intake, n-3/n-6 LCPUFA ratio as well as doses applied and fatty acid sources are still taken into consideration by the studies [22, 25]. In addition, preterm infants fed supplemented formulas fish oil containing DHA and EPA (without AA supplementation) during the first months of life it was observed lower FFM and BF among males [25], and higher weight and adiposity at 9-11 years among females [22]. These findings are in concordance to those observed by Donahue et al. [18] where higher prenatal fish intake and exposure to n-3 LCPUFAs were associated with lower adiposity in early childhood. A programming effect could be involved in the modulation of pre-adolescent body composition in subjects with low early adiposity having a different effect in each gender. It should be noted however, that there is a number of studies which failed to show any body composition variations later in life [16, 17, 26].

Conclusions and final comments

Two studies [18, 24)] showed positive effects of n-3 LCPUFA (only DHA or DHA+EPA) maternal intake during pregnancy and lactation on infant and young children's later body composition i.e., decreasing adiposity or BMI. Breastfed infants whose mothers were supplemented with fish oil during lactation had higher BMI, adipose tissue and waist circumference at 2.5 and at 7 years of age [15, 23]. Breast milk n-3 LCPUFA content but not umbilical cord levels were associated with BMI at 7 years of age [20]. One study did not found any related effect [27]. Focusing on the perinatal period, three out of seven studies did not observed any effects on later body composition among infants supplemented with n-3 LCPUFA enriched formulas [16, 17, 26]. On the other hand, two studies [21, 22] reported higher weight and adiposity when consuming increased amounts of n-3 LCPUFA (DHA+AA), whereas two [19, 25] showed a decrease in weight and fat mass (DHA+EPA and high ALA intake).

In summary, evidence on the potential relationship between maternal n-3 LCPUFA intake and infant growth or later body composition are not conclusive. In addition, contradictory findings among trials on use of varied supplemented n-3 LCPUFA formulas and on short and long-term effects on body composition or body fat were observed. Results derived from the studies included in this systematic review suggest that mechanisms are not understood and data synthesis is inconclusive. Differences in n-3 LCPUFA formula composition due to the heterogeneity in the type, sources and doses of LCPUFA as well as the timeframe of exposure prevent conclusive findings. Therefore, the association between early n-3 LCPUFA exposure during perinatal period and long-term body composition remains unclear. More studies addressing this relationship are needed.

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Table 1. Inclusion and exclusion criteria.

Inclusion criteria

1. Studies on n-3 LCPUFA intake around the perinatal period involving pregnant women and infants, considering n-3 LCPUFA exposure during pregnancy and/or neonatal period (first four weeks of life) and infant body composition or adiposity measurements as outcome measures (those meeting the objectives of the current systematic review).

Exclusion criteria

1. Infants or mothers affected by an intermediate disease affecting the research question.
2. Irrelevant health outcomes and no adiposity or body composition measurements (i.e., paper was excluded if only body weight and body length were reported)
3. Animal studies.
4. Studies in adults or in children without perinatal EPA or DHA n-3 LCPUFA exposure (a paper was excluded if only ALA was used as n-3 LCPUFA or only n-6 essential fatty acids as LCPUFA supplementations).
5. Studies with insufficient sample size (< 50).
6. Studies in which plasma n-3 LCPUFA was considered as n-3 LCPUFA biomarker of long-term exposure.
7. Studies in which outcome measure could not be related to n-3 LCPUFA intake (i.e., combination with other micronutrients….).

Table 2. Included studies on maternal n-3 polyunsaturated fatty acid intake during pregnancy and lactation and possible effects on infant body composition.

Author Year Country	Study design	Participants Age group	Intervention / follow up duration	Description of intervention/Mean intake	Outcome Measurements	Results
Lauritzen et al [23] 2005 Denmark	RCT double-blinded	175 pregnant women and their infants. Pregnant women age: 27.8-36 y	Intervention: 4 first months of lactation in each mother (exclusively breastfeeding). Follow up of children until 2.5 years of age	Three groups: - 2 intervention groups (122 lactating women in the below-median quartile of fish intake randomly supplemented with fish or olive oil) 1.5 g/d of n-3 LCPUFA on fish oil capsules vs capsules of olive oil - Control group (53 lactating women in the highest quartile of fish intake, not supplemented).	Skinfold thickness, waist circumference and BMI during infancy and early childhood until 2.5 years of age.	Across the follow-up period, differences were only found at 2.5 years in body composition (children in the fish oil group had statistically significant larger waist circumference, BMI and adipose tissue compared with those in the olive oil group)
Lucia Bergmann et al. [24] 2007 Germany	RCT double-blinded	144 pregnant women and their infants. Pregnant women age: >18 y	Intervention: 21st weeks' pregnancy-the end of third month of lactation. Follow up of children until 21 months of age	Two groups: 1 intervention group supplemented with a basic supplement containing vitamins and minerals, 4.5 g of fructooligosaccharide, and fish oil DHA (200 mg). Control group: two groups, one receiving a basic supplement containing vitamins and minerals, and the other group received a basic supplement containing vitamins and minerals plus 4.5 g of fructooligosaccharide.	Height, weight, BMI, head circumference.	A significant time effect was observed for the DHA group on the development of the BMI and of weight, but no effect on the development of length, or of head circumference. At 21 months, weight of the DHA group was lower by -601 g (95% CI -171; -1030 g) and BMI was lower by -0.76 kg/m2 (95% CI -0.07; -1.46) compared to controls.
Helland et al.[20] 2008 Norway	RCT double-blinded	143 mother-child pairs Pregnant women 19-35 y	Intervention: 18th week of pregnancy-3rd month after delivery. Follow up until 7 years of age.	Supplementation with cod liver oil (containing 1183 mg/10 mL of DHA, 803 mg/10 mL of EPA, and a total of 2494 mg/10 mL of *n*-3PUFAs per day) or corn oil (containing 4747 mg/10 mL of LA and 92 mg/10 mL of ALA per day).	BMI till 7 years of age.	Umbilical fatty acid status does not seem to have any influence on BMI at 7 years of age. It was not the case of ALA concentration in breast milk at four weeks and three months after delivery. ALA in breast milk at 3rd month after birth positively correlated with BMI at 7 years of age.
Asserhøj et al. [15] 2008 Denmark	RCT double-blinded	98 pregnant women and their infants. Pregnant women age: 27.8-36 y	Intervention: 4 first months of lactation in each mother (exclusively breastfeeding).	Three groups: - 2 intervention groups (64 lactating women in the below-median quartile of fish intake randomly supplemented with fish or olive oil) 1.5 g/d of n-3	Skinfold thickness, waist circumference and BMI during infancy and early childhood until 7 years of age.	None of the anthropometric measures differed between the randomized groups. Body composition variables at 2.5 y (Lauritzen results) and 7 y of age were correlated (BMI, r =0.63 and P<0.001; waist: height ratio, r

			Follow up of children until 7 years of age	LCPUFA on fish oil capsules vs capsules of olive oil - Control group (34 lactating women in the highest quartile of fish intake, not supplemented).		=0.49 and P<0.001).
Donahue et al. [18] 2011 USA	Cohort study	1250 mother-child pairs Pregnant women age: 32.4 ± 5.1 y	Follow-up period until 4 years of age.	Midpregnancy intake: total n-3 intake = 1.16 ± 0.42 g/d; ALA intake= 0.99 ± 0.40 g/d; DHA+EPA intake = 0.15 ± 0.14 g/d; total n-6 fatty acids = 12.27 ± 3.18 g/d Intake a month before delivery: DHA+EPA intake = 0.11 ± 0.11 g/d	Height, weight, BMI, subscapular and tricipital skinfolds at 3 years of age	Higher prenatal fish intake and exposure to n-3 LCPUFAs were associated with lower adiposity in early childhood.
Hauner et al. [27] 2011 Germany	RCT	170 mother-child pairs. Pregnant and lactating women: 18-43 y	15th week of pregnancy or before-4th month after delivery	Intervention group: healthy diet containing 1200 mg n-3 LCPUFA/day (1020 mg DHA+180 mg EPA) and a concomitant reduction in AA intake Control group: healthy diet with a reduction in AA intake	Weight, length, head circumference, fat mass (skinfold thickness), BMI, weight-for-length, and ponderal weight at birth, 6 week, 4 month, and 12 month.	Infants did not differ in the sum of their skinfold thickness at ≤1 y of life.

ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; AA, araquidonic acid; RCT, randomized controlled trial; LCPUFA, polyunsatured fatty acid; BMI, body mass index.

Table 3. Included studies on infant's n-3 polyunsaturated fatty acid intake and possible effects on body composition

Author Year Country	Study design	Participants Age group	Intervention / follow up duration	Description of intervention	Outcome Measurements	Results
Birch et al. [16] 1998 USA	RCT double-blinded	79 term infants. Gestational age 37-40 weeks Singleton and weight appropriate for gestational age	Intervention: from birth (between 1-5 days of life) to 17 weeks of age. Follow up period until 12 months of age.	Three groups (LCPUFAs and % of total fatty acids): - LCPUFA (DHA 0.36% and AA 0.72%) supplemented formula (N=27) - LCPUFA (DHA alone 0.36%) supplemented formula (N= 26) - Control formula without addition of DHA and AA: N = 26	Length, weight, weight-for-length, head circumference, subscapular and triceps skinfolds. Outcomes were measured at birth, 1, 4, 6 and 12 months of age	Infants in all diet groups had similar rates of growth and anthropometric measurements. Supplementation of term infant formula with DHA or with DHA and AA does not affect body composition at 12 months of age
Ryan et al. [25] 1999 USA	RCT blinded	63 healthy low birth weight infants (32 males and 31 females). Gestational age at birth 32 weeks aprox. 35 weeks at enrolment. Birth weight 940-2250 g.	Intervention and follow up period from the first week of life to 59 weeks of PMA	Two groups: - Supplemented formula with fish oil containing DHA (0.20% of total fatty acids) and EPA (0.04%) in a 5:1 ratio - Control group: without addition of DHA and EPA. Preterm formula with or without DHA was fed from the first week of life though 43 weeks PMA. Then from 43 to 59 weeks PMA infants were fed with a term infant formula with or without DHA depending of the study group.	Length, weight, head circumference. Subscapular, triceps and suprailiac skinfolds. Arm, abdominal and chest circumferences. FFM, TBF, and TOBEC. Anthropometric outcomes were measured at birth, enrolment, and at 37, 39, 43, 47, 51 and 59 weeks PMA. TOBEC measurements at 43, 51 and 59 weeks PMA.	Infant formula with fish oil containing DHA and EPA in a 5:1 ratio had a significant negative effect on growth and body composition in low-birth-weight premature males during the first 6 months of life. Only in males fed the DHA formula, weight, length, FFM and TBF were all significant lower at 51 and 59 weeks PMA; even when energy and protein intake were included as covariates. There were no body composition differences between the feeding groups when body compartments were expressed relative to body weight (i.e. %FFM, %TBF)
Innis et al. [21] 2002 Canada and USA	RCT double-blinded	194 healthy preterm infants. Birth weight 846-1560g. Weight appropriate for gestational age, with full enteral feeds tolerated before 24 days of life, and no disease or malformation that may impair growth.	Intervention for at least 28 days, until hospital discharge. Follow up period until 57 weeks PMA.	Three groups: - Supplemented formula with enriched single-cell algal oil DHA (0.34% of total fatty acids) - Supplemented formula with DHA and AA from algal /fungal oils (0.33% DHA and 0.60% AA). - Control (21-22% LA, 3- 3.1% ALA) Infant formulas neither had EPA. Preterm formulas were fed for at least 28 days (until discharge) after an enteral intake of 50kcal/kg/d was tolerated. Term formula without DHA and	Length, weight, weight-for-length and head circumference. Outcomes were measured at birth and at 40, 48 and 57 weeks PMA after discharge.	Weight, length and weight-to-length ratio of infants fed the formula with DHA+AA were consistently higher from 40 to 57 weeks PMA than those of infants fed the control formula or DHA formula.

				AA was fed after discharge in all participants.		
Groh-Wargo et al. [19] 2005 USA	RCT double-blinded	60 preterm infants. Birth weight 750-1800 g. Gestational age at birth <33 weeks. Infants without serious congenital malformations and no disease that may impair growth.	Intervention and follow up period from birth to 12 months of age	Three groups randomized and stratified by gender and birth weight (750-1250g/1251-1800g) in permuted blocks - Supplemented formula with DHA and AA from fungal/fish oil - Supplemented formula with DHA and AA from egg-triglycerides/fish oil - Control group formula contained 16-19% LA and 2.5% ALA. Supplemented preterm infant formulas contained 0.42% AA and 0.27% DHA of total fatty acids. After term age (40 weeks PMA) the supplemented and enriched formula post-discharge contained 0.42% AA and 0.16% DHA until 12 months.	Length, weight, head circumference at birth, weekly before discharge and postdischarge at 35 and 40 weeks, and at 4 and 12 months of corrected age. Body mineral content, body mineral density, body fat and lean mass were measured by DEXA at 35 and 40 weeks, and at 4 and 12 months of corrected age.	There were no significant differences among the three study groups at any time point in weight, length, or head circumference. Bone mineral content and bone mineral density did not differ among groups. Infants who were fed with DHA and AA supplemented formulas had significantly greater lean body mass and less fat mass than controls by 1 y of age.
Scholtens et al. [26] 2009 The Netherlands	Cohort study	244 mothers and their infants, all born at term and still breastfed at 3 months of age.	Follow up period from birth to 12 months of age.	NA	Weight gain per week, length gain per week and BMI gain per week from birth to 1 year of age. These outcomes were analyzed in relation with fatty acid composition of breast milk (LA, ALA, AA, EPA, DHA, total n-3, total n-6 and n-3/n-6 ratio) collected between 9 to 26 weeks of age (15.1±3.4 weeks)	The n-3 and n-6 LCPUFA content in breast milk did not affect weight or BMI gain in the first year of life in breast-fed term infants
Kennedy et al. [22] 2010 United Kingdom	RCT double-blinded until 18 months of age	107 children aged 9-11 years who were born preterm (<35 weeks and birth weight < 2000 g) and participated in the original RCT.	Intervention since randomization time (supplemented group 14.3 ± 9.6 days; control group13.9 ± 10.4 days) until 9 months post-term. Follow up study at	Two groups: - Supplement infant formula: 12.3 % LA, 1.5 % ALA, 0.5 % DHA, 0.9% C18:3 n-6 GLA, 0.04 % AA and 0.1 % EPA from borage/fish oil. - Control formula: 11.5 % LA and 1.6 % ALA. Formula was given from enrollment	At 9-11 years of age. Weight, height, head circumference, arm circumference and BMI. Skinfolds in four sites. Body fat and fat free mass by two-component models (bioelectrical impedance + deuterium dilution + skinfold equations).	Girls born preterm and randomized to LCPUFA-supplemented formula showed increased weight and adiposity at 9-11 years of age. Weight SD score, height and the sum of skinfolds (*LogN*) were all higher after confounder adjustments No effects were seen in boys.

			9-11 years of age.	to 9 months post term. Infants were fed with preterm formula until the infant reached 2 kg or was discharged. After this point, post-discharge (nutrient-enriched) formula was given.		
De Jong C et al. [17] 2011 The Netherlands	RCT double-blinded	341 term infants enrolled in the neonatal period. Gestational age 37-42 weeks at birth. 91 in the LCPUFA group, 123 in the control group and 127 in the breastfed group.	Intervention since the first 1-5 days of life until the end of the 2nd postnatal month. Follow up study at 9 years of age.	Three groups: - Standard formula control group - LCPUFA-supplemented group (0.45% (by wt) AA from egg yolk and a single cell oil produced by a common soil fungus, and 0.30% (by wt) DHA from egg yolk and tuna oil). - Breastfed group served as reference. Supplementation lasted till the end of the second postnatal months. In case breastfeeding stopped prior to 2 months, the infant received LCPUFA supplemented formula till the full age of 2 months. All formula-fed infants received control formula from two completed months until the age of 6 months.	Weight, body length, head circumference and BMI were recorded at birth and at 3 and 18 months during the RCT, and at 9 years of age. Covariates included into the multivariate analyses: gender, maternal level of education, smoking during pregnancy, birth weight, and pre-pregnancy maternal BMI, among others.	At 9 years of age, weight, height, BMI and head circumference of the breastfed group were similar to those of the two formula groups. The lack of difference in these outcome parameters was confirmed in the multivariate analyses. Relative risk analysis demonstrated no difference these outcomes between the feeding groups neither for high blood pressure, formula fed nor for being overweight.

RCT, randomized controlled trial; LA, linoleic acid; ALA, alpha-linolenic acid; LCPUFA, polyunsatured fatty acids; DHA, docosahexaenoic acid; AA, araquidonic acid; PMA, postmenstrual age; EPA, eicosapentaenoic acid; FFM, fat free mass, TBF, total body fat; TOBEC, total body electrical conductivity; DEXA, X-ray absorptiometry; GLA, gamma-linolenic acid; BMI, body mass index

Table 4. Assessment of methodological quality of included RCTs and longitudinal studies.

Study (Author, year)	Adequate sequence generation	Allocation concealment adequate	Blinding adequate	Dropouts adequate and outcome data complete	Funder adequate	Lack of other potential threats to validity	Confounders	Assessment of exposure adequate	Overall risk of bias
Donahue et al, [18] 2011	NA	NA	NA	Unclear	Yes	Yes	Yes	Yes	Low
Lauritzen et al, [23] 2005	Unclear	Unclear	Yes	Yes	Yes	No	NA	NA	High
Lucia Bergmann et al, [24] 2007	Yes	Yes	Yes	Unclear	Unclear	No	NA	NA	Moderate
Helland et al, [20] 2008	Unclear	Unclear	Unclear	No	No	No	NA	NA	High
Asserhøj et al, [15] 2008	Unclear	Unclear	Unclear	No	No	No	NA	NA	High
Hauner et al, [27] 2012	Yes	Yes	No	Yes	No	Yes	NA	NA	High
Birch et al,[16] 1998	Yes	Yes	Unclear	Yes	Yes	No	NA	NA	Moderate
Ryan et al, [25] 1999	Yes	Yes	Unclear	Yes	Unclear	Yes	NA	NA	High
Innis et al, [21] 2002	Yes	Yes	Yes	Unclear	No	Yes	NA	NA	Moderate
Groh-Wargo et al, [19] 2005	Yes	Yes	Yes	Yes	No	No	NA	NA	Moderate
Scholtens et al, [26] 2009	NA	NA	NA	Yes	Yes	No	Yes	Yes	Moderate
Kennedy et al, [22] 2010	Yes	Yes	Yes	No	No	Yes	NA	NA	Moderate
De Jong et al, [17] 2011	Unclear	Unclear	Yes	Yes	Yes	No	NA	NA	High

NA, not applicable

Donahue: Insufficient information on drop-outs.

Lauritzen: Insufficient information on the sequence generation and allocation concealment. Group sizes were based on power calculation for infant visual acuity instead of infant growth which is our main outcome.

Lucia Bergmann: Insufficient information on drop-outs. No explanation on differences between those who completed the study and drop-outs.

Helland: Insufficient information on sequence generation, allocation concealment and blinding procedure. Maternal age and education significantly differed among those mothers included in the study and those who were excluded. The study was co-financed by an enterprise which also provided the supplements. Power calculation of group sizes was based on an infant intelligence questionnaire instead of using infant growth which is our main outcome. Moreover, main outcomes were eventually related to umbilical cord or breast milk concentrations and not to n-3 LCPUFAs intake or supplementation.

Asserhøj: Insufficient information on sequence generation, allocation concealment and blinding procedure. They were not aware of the group allocation at 7y. Maternal age and education significantly differed among those mothers included in the study and those who were excluded. The study was co-financed by an enterprise which also provided the supplements. Power calculation of group sizes was based on an infant intelligence questionnaire instead of using infant growth which is our main outcome.

Hauner: The blinding procedure did not exclude potential bias. Moreover, the study was co-financed by private enterprises.

Birch: The sample size was calculated based on outcome (visual evoked potential) other to the ones examined in this review. Moreover, the supplemented formulas were provided by a private enterprise.

Ryan: No description of blinding method or funding source.

Innis: No description on dropouts. Moreover, supplemented formulas were provided by a private enterprise.

Groh-Wargo: The funder cannot be considered as adequate because the study was partially supported by private enterprise. The fact that subjects were allowed to take supplements but they did not register that information risks study validity.

Scholtens: Mother allocated in the intervention group were highly educated and smoked less often during pregnancy compared to those of the general study population. Moreover, children weight was self-reported.

Kennedy: Gestational age and birth-weight SD scores of dropouts were significantly different from the final included sample. Not controlled intakes of other n-3 LCPUFA. The study was funded by a private enterprise.

DeJong: Sequence generation and allocation concealment were not insufficiently described. The sample size was calculated based on health outcome other to the ones examined in this review. Supplementation period was too short.

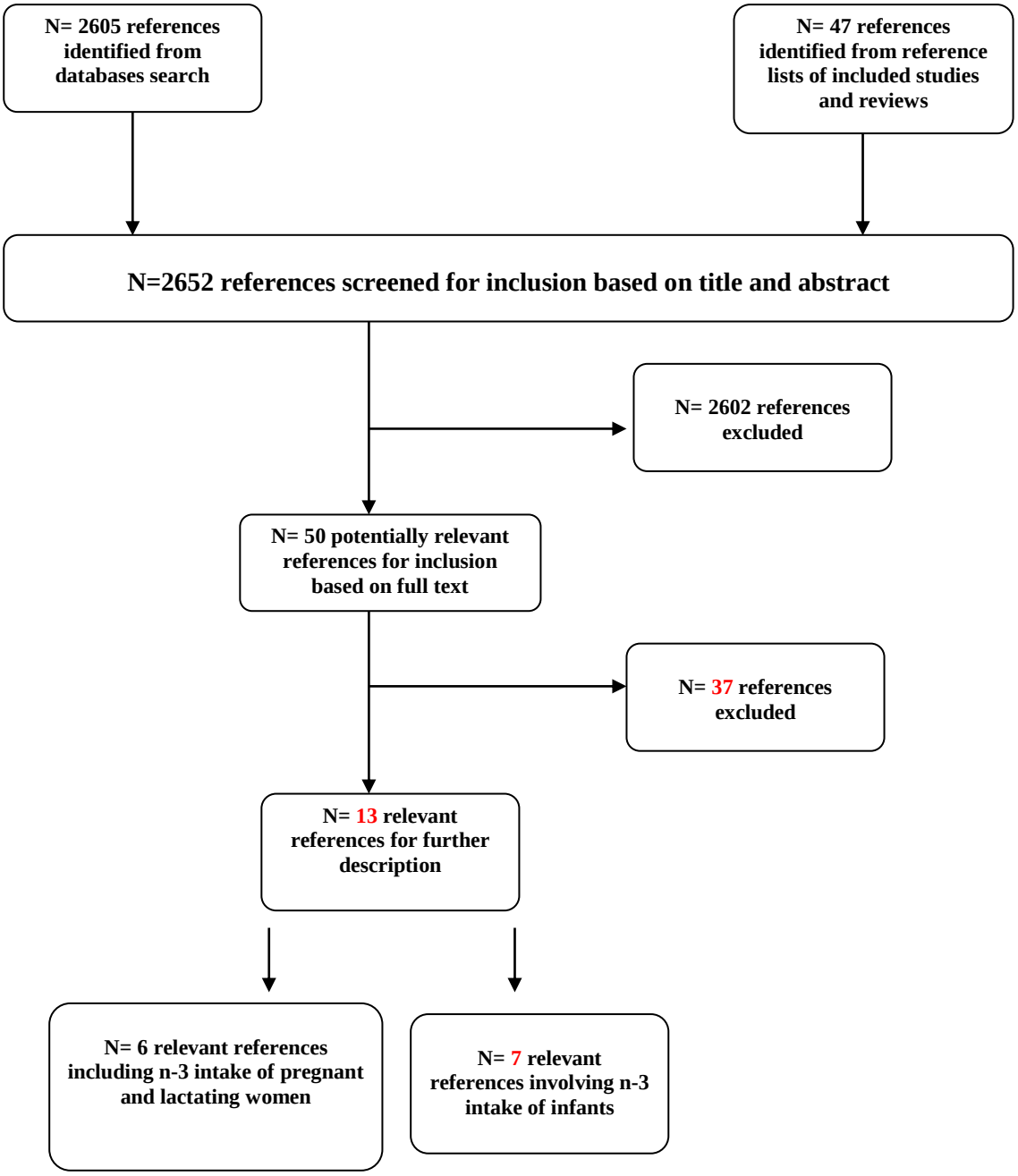

Figure 1. Main stages of the systematic review process

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