ω-3 LCPUFA supplementation during pregnancy and risk of allergic outcomes or sensitisation in offspring: a systematic review and meta-analysis

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Abstract

Background: Allergic diseases have seen a rise worldwide in the last two decades, with children suffering the highest burden of the condition. The ω-3 Long-Chain Poly-Unsaturated fatty Acid (LCPUFA) poses anti-inflammatory properties that could lead to a reduction in inflammatory mediators in allergies.

Objective: A systematic review and meta-analysis of the most recent follow-ups of randomised controlled trials (RCTs) was conducted to assess the effectiveness of ω-3 LCPUFA supplementation started during pregnancy on allergic outcomes in offspring.

Methods: RCTs with a minimum of 1-month follow-up post gestation were eligible for inclusion. The CENTRAL, MEDLINE, SCOPUS, WHO's International Clinical Trials Register, E-theses and Web of Science databases were searched. Study quality was evaluated using the Cochrane Collaboration's risk of bias tool.

Results: Ten RCTs (3,637 children), from nine unique trials examined the effectiveness of ω-3 LCPUFA supplementation started during pregnancy on the development of allergic outcomes in offspring. There were heterogeneities between the trials in terms of their sample, type and duration of intervention and follow-up. Pooled estimates showed a significant reduction in childhood "sensitisation to egg" (Relative Risk (RR)=0.54, 95% Confidence Interval (CI)=0.32-0.90) and "sensitisation to peanut" (RR=0.62, 95% CI=0.40-0.96). No statistical differences were found for other allergic outcomes e.g. any allergies, eczema, asthma/wheeze.

Conclusion: These results suggest intake of ω-3 LCPUFA started during pregnancy can reduce the risk of sensitisation to egg and peanut; however the evidence is limited due to the small number of studies contributed to meta-analyses. The current evidence on the association between supplementation with ω-3 LCPUFA started during pregnancy and allergic outcomes is weak due to the risk of bias and heterogeneities between studies.

Key words: Systematic Review, Meta-analysis, omega-3 Fatty acids, Fish oil, n-3 LCPUFA, Allergies, Childhood allergies, Asthma, Wheeze, Wheezing, Asthma/wheeze, Sensitisation, Food allergy, Eczema

Introduction

In the last two decades allergic diseases have seen a rise worldwide with children suffering the highest burden of the condition[1]. Food allergies, eczema and asthma are the most common allergic disorders in children[1-2]. The increasing burden of allergic conditions is an important public health concern and understanding how to prevent the development of allergic diseases is a vital area of research.

The first 1,000 days of life is key to good health for a lifetime as the developmental model of health and disease states exposures and incidents during this critical period could initiate the susceptibility of many chronic diseases, including allergies[3]. In this context, the role of environmental factors such as diet and lifestyle-related behaviours are key for primary/early prevention of chronic diseases[4-5]. Factors such as maternal diet could directly affect an infant's health and immune system[6-7]. On this basis, interventions that aim to improve nutritional adequacy in pregnant women could, in theory, reduce the incidence of chronic diseases including allergy.

The intake of LCPUFAs, particularly the ω-3/ω-6 ratio, has been proposed as a risk factor for childhood allergies. Over recent decades, Western diet has shifted towards higher intake of ω-6 by increased consumption of vegetable oils and a corresponding decrease in intake of foods rich in ω-3 PUFAs e.g. seafood and oily fish[8-9]. The LCPUFAs family, mostly known by ω-3 and ω-6 products, is involved in immune-regulation and inflammatory pathways in foetuses[10]; with ω-3 fatty acids having anti-inflammatory, and ω-6 fatty acids pro-inflammatory effects[11]. Higher concentrations of ω-6 LCPUFAs promote the production of inflammatory mediators such as prostaglandins (PGE2, F2α) and leukotriene (LTB4), which compete with the synthesis of ω-3 in cellular phospholipids membranes[11]. Subsequently, a diet rich in sources of ω-3 LCPUFAs could lead to a reduction in inflammatory mediators and

also alter the balance of T_H1 to T_H2[12-13], a recognised hallmark of allergic diseases. Furthermore, observational studies summarised in a narrative systematic review[14] have shown an association between lower levels of oily fish intake during pregnancy and the development of allergies in children. Collectively, these data suggest that ω-3 fatty acid supplementation during pregnancy may be a strategy to reduce childhood allergies.

The effect of exposure to an increased supply of ω-3 LCFUFA (or in combination with ω-6) *in utero* and the risk of developing allergic disorders has been studied in various trials; however these have produced inconsistent results. This systematic review and meta-analysis aimed to assess the effectiveness of these interventions, including the longest available follow-ups reported. This will provide an update to the earlier systematic reviews on this topic[15-17] to include recently published trials. It would also allow us to assess the effectiveness of ω-3 LCFUFA supplementation commencing during pregnancy for prevention of childhood allergies, while the focus of earlier systematic reviews on this topic has been on both the prenatal and postnatal ω-3 LCPUFA interventions.

Methods

Criteria for considering studies for this review

Types of studies

Randomised controlled trials (RCT), including cluster randomised controlled trials and quasi-randomised controlled trials with a minimum follow-up of one month postnatally were included. The review considered studies which documented allergic outcome and/or sensitisation data. No language or country restrictions were applied.

Types of participants

Pregnant women and their offspring were considered as the target group for this systematic review. High-risk populations were not excluded.

Types of interventions

Studies that used ω-3 LCPUFA supplementation during pregnancy, irrespective of dose, formulation or mode of delivery and composition e.g. oil, tablet were included. Trials were also included if the intervention(s) had been continued after pregnancy through breast-feeding and/or supplements to the infant.

Types of outcomes measures

Trials were included if they had reported allergic outcomes and/or sensitisation in the offspring, either as a primary or secondary endpoint. Allergic outcomes were defined as: any allergies, asthma, wheeze, rhinitis, eczema, food allergy, positive skin prick test (to any and/or single allergens) or elevated specific IgE. Only outcomes which had utilised a validated method were included (as opposed to, for example, parental report). Data from the latest follow-up of allergic outcomes by the included trials (and cumulative prevalence where possible) were also considered.

Search strategy for identification of studies

A comprehensive search strategy, including all the relevant synonyms for the main concepts, was developed. Trials were identified through systematic searches within three main electronic databases, as advised by the Cochrane collaboration[18]:

a. Cochrane Library (current issue) including:

- Cochrane Database of Systematic Reviews (CDSR)

- CENTRAL (trials)

- DARE

b. MEDLINE (EBSCOhost)

c. SCOPUS

When searching MEDLINE, the subject-specific terms were combined with the Cochrane Highly Sensitive Search Strategy for identifying randomised trials in MEDLINE: sensitivity-maximising version[18]. We adapted the preliminary search strategy for MEDLINE (EBSCOhost) for use in the other databases when relevant. Searches of databases were carried out between November 2017 to January 2018.

The clinical trials registry and WHO platform were searched for ongoing and recently completed trials. Conference proceedings were identified through the ISI Web of Science and the British Library E-Theses Online Service was searched. No language or publication status restrictions were imposed. References of included studies were crosschecked for additional studies.

Data collection

Selection of studies

Two reviewers (MV, HM) screened all the search results against the eligibility criteria and all those which were clearly irrelevant were excluded from further consideration. Thereafter, a tailored eligibility form was used to appraise the retrieved studies, abstract and full text for relevance against the full inclusion criteria. Where there was

uncertainty about inclusion of a particular study, other members of the review team were consulted and a consensus was reached about the study eligibility. All the included studies were discussed and approved by the review team.

Data extraction and management

Data were extracted by two reviewers (MV, HM) using a tailored data extraction form in EPPI Reviewer. Detailed information on study characteristics were recorded. Throughout the data extraction process, any disagreements about the interventions and outcomes were discussed and resolved within the review team. There was no blinding of the authors' name, institutions, journals or the outcomes of the trials during the process.

Assessment of risk of bias in included studies

The risk of bias tool described in the Cochrane Handbook for Systematic Reviews for Interventions was used to appraise the studies[19].

Data synthesis

Calculation of treatment effects

Dichotomous data were analysed as risk ratios or relative risk (RR) with 95% CI, entered as events and the number of participants, and continuous data as mean difference or standardised mean difference, with 95% CI. In trials with more than one intervention arm, multiple pairwise comparisons of intervention groups versus comparator were avoided. Therefore, data from different intervention arms were pooled for an overall comparison with the control or placebo arm. The weight assigned to the control group was considered as the total number of participants in the comparator group versus the total number of participants in the combined intervention arms[20].

All the relevant reported information for the number of missing participants was extracted and if undocumented, this was incorporated into the assessment of risk of bias, incomplete outcome data. No imputed techniques were used for missing data.

Pooling of treatment effects

Assessment of heterogeneity

We used visual inspection of forest plots and also, the Chi^2 test to measure statistical heterogeneity between effect sizes of included studies. I^2 statistics were used to quantify the amount of possible variability in effect estimates that is due to inconsistency rather than chance (I^2<25% low heterogeneity, I^2>50% moderate heterogeneity, I^2≥75% high heterogeneity). All meta-analyses were undertaken using a random effect model and reported where heterogeneity was reported as ≤75% [18].

Assessment of reporting biases

Every effort was made to identify unpublished studies through searching abstracts and ongoing trials databases. Small study effects was not assessed since the number of studies included in meta-analyses were ≤10.

We used Eppi Reviewer version 4.4.3.0. for conducting meta-analyses. We reported relative risk to describe the study effect. Due to the small number of studies and also, large inconsistencies between studies, the reported allergic outcomes were included in meta-analyses irrespective of IgE status.

Subgroup analysis and investigation of heterogeneity

Where possible, sub-group analyses were performed where there was large discrepancies in the duration of follow-up between the included studies e.g. less than and above 6 years old. Additional analyses were also conducted when level of maternal EPA+DHA pre-treatment was reported in the trials.

Sensitivity analysis

Influence analysis as a type of sensitivity analysis was conducted where trials were excluded from the meta-analyses models to examine how deletion of a study affects overall results. This was done for trials that used either a different intervention or control group.

Results

Electronic searches yielded a total of 1,733 results (Figure 1). After removal of duplicates and title/abstract screening, the remaining 79 full text papers were assessed against the eligibility criteria for this systematic review. Ten publications from nine unique RCTs were included in the final analyses, including a total of 3,637 children[21, 28, 30, 32, 34, 38, 43, 47, 48, 50]. In the case of one trial[29], both the reports of an earlier[28] as well as latest follow-up data[47] were included, since some of the allergic outcomes of interest for this systematic review were reported only in the earlier published paper of this trial i.e. any allergic diseases. The characteristics of the included trials, their companion papers and study population are presented in Table 1. Four studies were conducted in Australia and Denmark (two each) and the rest in Mexico, Sweden, Finland, United Kingdom and United States.

Study design

All the included trials were parallel RCTs. Seven trials had two parallel groups while two trials had three parallel groups. The trial by Olsen[30] included intervention, placebo and no oil groups and for the purpose of this review, only the intervention and placebo arms were included in meta-analyses. The other trial by Berman[50] included two intervention groups of EPA, DHA and placebo. For this review, the two intervention arms were combined and compared versus the control group. In addition, the nature of control group in the study by Noakes[34] was different from other trials i.e. standard diet and therefore, this study was not included in meta-analyses and only described narratively.

Participants and sample sizes

Four trials were conducted on high-risk (atopy) populations[21, 32, 34, 38] and the remainder[28, 30, 43, 47, 48, 50] were carried out on sample with low-risk allergies. The

sample size of studies at randomisation were also varied, from 98[21] to 1,094[43] pregnant women.

Intervention

Seven of the included trials used ω-3 LCPUFA (combination of EPA, DHA and/or both)[21, 28, 32, 38, 43, 47, 48, 50], one used oily fish[34] and the other used black currant seed oil (BCSO)[30]. Six[21, 28, 34, 38, 43, 47, 50] trials supplemented women only during pregnancy and in three trials[30, 32, 48], the intervention was continued postnatally (Table 1).

Compliance with the intervention was measured by different methods in trials, including total number of capsules consumed, women's self-report of oily fish intake using food frequency questionnaire during pregnancy, telephone call reminder(s), as well as assessing plasma concentration of LCPUFA either at birth or a few weeks after delivery[21, 28, 32, 34, 38, 47].

Reported allergic outcomes and follow-up duration

Allergic outcomes were diagnosed by a physician or nurse in 6 trials[21, 30, 32, 34, 38, 48] and the remainder[28, 43, 50] used validated or semi-validated questionnaires. The definitions and diagnosis method for each outcome included in meta-analyses are presented in Table 2. The duration of follow-up in trials ranged from 6months[34] to 24 years[47].

The most frequently reported allergic outcomes were eczema, asthma, wheeze or asthma/wheeze together. Also, while there was a later publication from a study published in 2010[31], data were extracted from the 2010 report since the follow-up paper[30] only reported pre-clinical outcomes. Furthermore, the register-based outcomes in the study by Hansen 2016 were considered in this review. In addition, two trials[28, 50] were primarily conducted to measure other outcomes in pregnant

women and, using secondary analyses, the occurrence of allergic outcomes in the offspring were also reported. Olsen and colleagues[29] principally assessed the effect of LCPUFA on the duration of pregnancy and reported allergic outcomes in the offspring in the longer-term follow-ups. The trial conducted by Berman[50] initially measured the effectiveness of ω-3 LCPUFA for prevention of antenatal and postpartum depressive symptoms in pregnant women and reported the occurrence of allergic outcomes in their secondary analysis.

Quality of RCTs

The methodological quality of the included trials varied as shown in Figure 2. The risk of bias judgment is presented in Appendix 1. Only four trials[30, 34, 38, 43] had a low risk regarding random sequence generation. Most trials had adequate allocation concealment[21, 28, 38, 43, 47], blinding of both participants and staff[21, 30, 32, 43, 47, 48], and outcome assessor[21, 28, 30, 32, 34, 38, 43, 47]. Half of the trials were rated as high risk of bias for the completeness of data since there was a high rate of loss to follow-up. All trials were deemed to have a low risk of bias for the reported outcomes[21, 32, 30, 34, 50]. With the exception of one trial rated as unclear for other sources of bias[48], there were no other potential sources of bias in other trials.

Pooled effect of interventions

The definition of the outcomes in each study, as included in meta-analyses, and their diagnosis method are presented in Table 2.

Any allergies as outcome measure

Three trials[28, 32, 38] reported any allergies in children characterised by different phenotypes of allergy. Moderate statistical heterogeneity was observed between the trials (χ^2=5.54, P=0.06, I^2=63.9%) and the pooled results did not show an association between prenatal supplementation with ω-3 LCPUFA and the development of any

allergies in the offspring (RR=0.74, 95% CI=0.44-1.25) (Forest plot not shown). The definitions of any allergies included in meta-analysis are shown in Table 2.

In the sub-group analysis, the Olsen study[28] with the longest follow-up duration was excluded. No statistical heterogeneity was found between the included trials (I^2=0%) and the results did not significantly change (RR=0.97, 95% CI=0.79-1.19) (Forest plot not shown).

Asthma/wheeze as outcome measure

Trials reported the outcome differently, as either asthma only, wheezing or asthma/wheeze together. When pooling the results, we considered the outcome as "asthma and/or wheeze" together in the meta-analysis. Moderate heterogeneity was observed between the seven included trials (χ^2=10.5; P=0.10; I^2=43.1%). Meta-analysis did not show a preventative effect of prenatal intake of ω-3 LCPUFA on childhood asthma/recurrent wheeze (RR=0.87, 95% CI=0.70-1.07) (Figure 3).

In the sub-group analysis, the study by Hansen[47] with the longest follow-up duration was excluded. Low heterogeneity was found between the studies (I^2=21.9%) and the results did not significantly change (RR=0.93, 95% CI=0.78- 1.11) (Forest plot not shown). Also in the study by Bisgaard[48], prevalence of asthma/wheezing in children was also reported based on maternal lowest third of EPA+DHA pre-treatment. The meta-analysis, including this outcome in the model showed higher heterogeneity between trials (I^2=63.8%) and the results did not significantly change (RR=0.83, 95% CI=0.64-1.08).

Eczema as outcome measure

The outcome was reported in six trials and pooled results showed moderate statistical heterogeneity between studies (χ^2=11.2; P=0.046, I^2=55.5%). The results of meta-

analysis did not show an association between prenatal intake of ω-3 LCPUFA and the development of childhood eczema (RR=1.01, 95% CI=0.76-1.34) (Figure 4).

Sensitivity analysis excluding the study conducted by Linnamaa[30], as the only trial to use BCSO as the intervention, also showed moderate statistical heterogeneity between studies (χ^2= 9.08; P= 0.059; I^2=56%) with no significant change in the pooled results (RR=1.1, 95% CI=0.78-1.54) (Forest plot not shown). Two more sensitivity analyses were conducted. In the first, the study undertaken by Berman[50] was excluded since it reported higher rates of eczema in the intervention arms and in the second, both Linnamaa[30] and Berman[50] studies were excluded. In both meta-analyses, moderate heterogeneities were observed between studies and the results did not significantly change (RR=0.952, 95% CI=0.767-1.18; RR=1.01, 95% CI=0.786-1.29, respectively) (Forest plots not shown).

Allergic rhinitis as outcome measure

The outcome was reported in two trials[38, 47] and in the two other trials, "rhinoconjunctivitis" was reported[32, 50]. We pooled the results from trials that only reported allergic rhinitis where there was a moderate heterogeneity between trials (χ^2=1.37, P=0.243, I^2=27%). The results of meta-analysis did not show an association between prenatal intake of ω-3 LCPUFA and development of allergic rhinitis in children (RR=084, 95% CI=0.65-1.1) (Forest plot not shown).

Food allergy as outcome measure

Three trials reported the outcome[21, 32, 50] and in the pooled analysis, low heterogeneity was found between the studies (χ^2=2.32, P=0.31, I^2=13.8%). The results of meta-analysis did not show an association between prenatal intake of ω-3 LCPUFA and development of food allergy in children (RR=0.67, 95% CI=0.34-1.35) (Forest plot not shown).

Sensitisation to any allergens as outcome measure:

Sensitisation to any allergens, measured by Skin Prick Test (SPT), was reported in four trials[21, 32, 38, 48] and there was moderate heterogeneity between studies (χ^2=6.03, P=0.11, I^2=50.3%). The results of meta-analysis did not show an association between prenatal intake of ω-3 LCPUFA and childhood sensitisation to any allergens (RR=0.91, 95% CI=0.66-1.27) (Forest plot not shown).

Sensitisation to specific individual allergens as outcome measure

The meta-analyses of sensitisation to individual allergens, measured by SPT showed that prenatal intake of ω-3 LCPUFA significantly reduced the risk of "sensitisation to egg" (RR=0.54, 95% CI=0.32-0.90) (Figure 5) and "sensitisation to peanut" (RR=0.62, 95% CI=0.40-0.96) (Figure 6). No significant associations were found for sensitisation to "cows milk" and "cat" in the conducted meta-analyses (Forest plots not shown).

Of note, we conducted a meta-analysis on raised specific immunoglobulin E (sIgE), reported in three studies[32, 47, 48] and the results yielded a substantial heterogeneity (I^2=77.5%) (Forest plot not shown).

Description of the outcomes reported in Noakes study[34]

This study used 'farmed salmon portion' (as opposed to LCPUFA supplements, as utilised in all the other included studies) compared to 'standard diet' during pregnancy for prevention of allergic disorders in offspring, and was therefore not included in any meta-analyses. The results suggest that the use of salmon portions in pregnant women in comparison to standard diet did not have an influence for the prevention of atopic dermatitis (12 vs. 7, p=0.46) and wheeze (11 vs. 7; p=0.58) in the offspring by 6 months age; however, the small sample size limits confidence in these findings.

Discussion

Ten RCTs were identified, with a total number of 3,637 children that started supplementation with ω-3 LCPUFA during pregnancy and followed-up the development of allergic diseases during childhood. Trials were heterogeneous in their sample, reported outcomes and duration of follow-up. Random sequence generation was deemed adequate in four of the included trials. Three trials were also judged to have a high risk of bias for their performance bias and five trials had a high attrition bias. The findings from this systematic review and meta-analysis did not provide evidence that intake of ω-3 LCPUFA starting during pregnancy could protect against the subsequent development of a number of childhood allergic diseases i.e. eczema, asthma/wheeze, food allergy, sensitisation. However, the pooled results showed that prenatal intake of ω-3 LCPUFA starting during pregnancy might have a protective effect on developing sensitisation to egg and also peanuts in the offspring, as measured by SPT. Furthermore, while no effect was observed for childhood asthma/wheezing following ω-3 LCPUFA intake starting during pregnancy, the upper bound was very close to 1 and could be considered borderline (RR=0.87, 95% CI=0.70-1.07, 7 trials, 2,951 children). Overall, the results from this review need to be considered with caution because of the heterogeneity observed between studies and also risk of bias.

Overall completeness and applicability of evidence

There was no evidence that ω-3 LCPUFA supplementation starting during pregnancy is effective for prevention of several allergic manifestations in the offspring. There were no statistical heterogeneity between the included trials for the outcome of "sensitisation to egg and peanuts, measured by SPT" (I^2=0%) and however; the number of included studies were small; the meta-analyses included 665 and 775

children respectively. Nevertheless, these are potentially important findings since food allergies are common in children[1] and well-documented research indicates that childhood food allergies, including to egg and peanuts, are a key risk factor for developing sensitisation to aero-allergens and allergic respiratory diseases later in life[54-57].

The heterogeneity between the trials limit the findings and random effect models were used to pool the results. Heterogeneity between the included trials could have resulted from differences in the choice of ω-3 LCPUFA and the dose, mode of administration, timing and duration of interventions as well as duration of follow-up and small sample size in most studies. Overall, a few trials were well-powered and high quality[38, 43, 48] and contrasting results on childhood allergic outcomes were reported across trials. One reason for the inconsistent results between trials could be because of the differences in baseline ω-3 LCPUFA levels that were only considered in the Bisgaard study[48]. Children of mothers with the lowest third level of ω-3 LCPUFA status (EPA and DHA) gained the most protection from ω-3 LCPUFA intervention. Higher doses of ω-3 LCPUFA were also used in the trial by Bisgaard that consisted predominantly of EPA versus DHA. It is also worth noting that in this study, a subgroup of pregnant women concomitantly took a high dose of 2400 IU of Vitamin D3 on a daily basis during the third trimester of pregnancy. The authors reported that there was no significant interaction between ω-3 LCPUFA and Vitamin D3 and the prevalence of persistent wheeze ($p=0.065$); however further analysis showed that the effects of ω-3 LCPUFA was less evident in children randomised to the Vitamin D3 group[48]. Furthermore, in the study by Berman[50], higher numbers of eczema cases were reported in both intervention arms, EPA and DHA as opposed to soy oil as the control. The authors reasoned that ω-6 LCPUFA as the predominant component in the

soy oil might have exerted a protective effect on developing eczema in the control group. It is important to add that none of the high quality and large trials reported a beneficial effect for childhood eczema in their longer-term follow-up[38, 43, 47, 48]. Again heterogeneities in the type and dose of ω-3 LCPUFA plus differences in the duration of follow-up could be a plausible explanation for the inconsistent results between trials.

Quality of evidence

Overall, the trials were at moderate to high risk of bias (Figure 2). Randomisation and allocation concealment were deemed as unclear for 50% of included studies in each domain. High loss to follow-up was also a major concern where 50% of studies showed a high attrition bias which might have largely biased the effect of interventions within these trials. In addition, a limited number of trials fulfilled the inclusion criteria for this systematic review and consequently, a small number of studies contributed in the conducted meta-analyses for most allergic outcomes. Most trials also used medical diagnosis of allergy, although a few tested for IgE-mediated status of the reported allergic outcomes[38]. Furthermore, since the diagnosis of asthma is difficult at a young age, most trials reported wheeze or asthma/wheeze together and consequently; either of these reported outcomes were included in meta-analyses. It is acknowledged that phenotype expression of allergic diseases in children varies by age, as more eczema and food allergy would be expected in children under 2 years of age and more wheeze/asthma at older age. Where possible, sub-group meta-analyses were conducted to address the age differences for the reported allergic outcomes.

Strengths and limitations

This systematic review only included trials that started intake of ω-3 LCPUFA during pregnancy, and thus crucially, allowed the effect of prenatal intake of the

supplementation for prevention of childhood allergies to be investigated. Following an a priori published protocol[58], a comprehensive search strategy allowed for a complete coverage of all the relevant literature through citation databases, trial registries and conference proceedings with no limitation by language or publication status. In addition, a range of allergic outcomes were considered and the most up-to-date results from the trials, reported as the longest available follow-up data, were included in the meta-analyses. As a post hoc decision, sub-group analyses were only conducted for the duration of follow-up and not for the dose/timing/type of ω-3 LCPUFA because of the limited number of studies that could contribute in the meta-analyses. A potential limitation is also that there were a small number of studies that met the inclusion criteria for this systematic review.

Consistency with other reviews

The two reviews published in 2011[15, 59] included studies that administered ω-3 LCPUFA either prenatally or postnatally with the former[15] being a narrative review. The latter[59] conducted meta-analysis on selected allergic outcomes and did not include some trials in their review[34, 40, 43].

The two recent reviews by Gunaratne[15] and Best[16] had different approach, with first including both prenatal and postnatal administration of ω-3 fatty acids[15] and the other only on prenatal supplementation, but with small number of trials[16]. Both reviews have conducted their meta-analyses based on IgE status of allergic outcomes, including point prevalence of allergies and earlier follow-up data of included trials. Also, the trial by Linnamaa[30] that used BCSO was only included in the present review since the composition of BCSO corresponds to the recommended optimal dietary intake ω-3 and ω-3 fatty acids with ω-3/ω-6 ratio from 1.3 to 1.4. Due to the differences in focus between these systematic reviews and the current review, the

ability to make meaningful comparisons between the findings is to some extent limited. Both reviews[15 & 16] showed a beneficial effect for atopic eczema for some age strata; however, these analyses only included the earlier follow-up data from included trials.

The most recent review[17] has also included both prenatal and postnatal ω-3 LCPUFA interventions and has not identified the latest report of one trial[43] and full paper of another trial[50]. There are also differences between the current systematic review and Garcia-Larsen in term of the approach for inclusion of trials in the conducted meta-analyses. For example, we did not include the Noakes[34] trial in the meta-analyses since it had a different control while this study has been included in meta-analyses by Garcia-Larsen. Also, the trial by Linnamaa has been considered as ω-6 fatty acid intervention in the review by Garcia-Larsen and additionally, they have included different reports of Makrides 2009 trial in the meta-analyses conducted for food allergy e.g. Palmer 2012 in "egg sensitisation" and Best 2016 in "Peanut sensitisation". Nevertheless, the results from the current systematic review are comparable with the recent review[18] where there was evidence that ω-3 LCPUFA supplementation has been beneficial for prevention of sensitisation to egg and peanut in children. Since the current review included ω-3 LCPUFA trials that were only started during pregnancy, it can be concluded that the preventive effect of the intervention on sensitisation to egg and peanut in children is related to this very early stage in life i.e. pregnancy. We also did not find evidence that ω-3 LCPUFA could protect against other allergic outcomes in offspring which is similar to the recent review[17].

Authors' conclusion

Implications for practice

The evidence on ω-3 LCPUFA intake starting during pregnancy and prevention of allergic outcomes in children is limited, although there appears to be an association with reduction of childhood sensitisation to egg and peanuts. There is a need to interpret these findings with caution as a result of risk of bias between studies, different methods for reporting the outcome measures and variability of the ω-3 LCPUFA supplementations. Furthermore the included trials differed greatly in the dose of ω-3 supplementation, ranging from 0.8 to 2.4g per day and contained various percentages of EPA and DHA. The current guidelines advise an intake of 200mg of DHA per day in pregnancy[60] and differences in the dose/ratio of DHA in the ω-3 supplementation in trials could have consequently had an impact on the overall effectiveness of intervention.

Implications for research

Taking the quantity and quality of the available evidence into account, the effect of ω-3 LCPUFA intake starting during pregnancy for prevention of childhood allergies needs to be further investigated in well-designed and executed RCTs.

The timing of interventions have been varied in the conducted trials and the optimal timing of ω-3 LCPUFA intervention is an essential uncertainty that needs to be addressed more clearly in further trials. Given the anti-inflammatory properties of ω-3 LCPUFAs, it could be hypothesised that early introduction of ω-3 LCPUFA during pregnancy could promote a beneficial immunomodulatory effect in foetuses as early as possible by programming the immune system. Furthermore, future trials could consider a differentiation between DHA to EPA ratios in LCPUFA supplementations as well as baseline LCPUFA status in their sample. The dose and duration of intervention as well as the choice of control should also be addressed in future studies. Large multi-centre RCTs with coherent methodology and standardised measures for

assessing the outcomes could provide stronger inferences for the efficacy of ω-3 LCPUFA intervention in pregnant women as a preventive approach for childhood allergies. In addition, studies should aim to minimise the attrition bias in their extended follow-ups. There is also a need for well-designed epidemiological studies from under-reported populations, since the current evidence originates dominantly from the developed regions.

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│ Records identified through database │     │ Additional records identified   │
│ searches (n=1732)                   │     │ through other sources           │
│ Medline: n=138, SCOPUS: n=1213,     │     │ (n=1)                           │
│ Cochrane (trials only): n=381       │     │                                 │
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         ┌──────────────────────────────┐        ┌─────────────────────────┐
         │ Titles and abstracts screened│───────▶│ Duplicates removed      │
         │ (1733)                       │        │ (n=1308)                │
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         │ Abstracts selected for       │───────▶│ Abstracts excluded      │
         │ further screening            │        │ (n=346):                │
         │ (n=425)                      │        │ Reviews/different       │
         │                              │        │ intervention /different │
         │                              │        │ outcome/after birth     │
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         │ Full-text articles assessed  │───────▶│ Full-text papers        │
         │ for eligibility              │        │ excluded (n=48)         │
         │ (n=79)                       │        │  Non-randomised: 17     │
         │                              │        │ Different outcome: 31   │
         └──────────────────────────────┘        └─────────────────────────┘
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         ┌──────────────────────────────────────┐
         │ Citations included: 31               │
         │ Primary articles included in         │
         │ quantitative syntheses               │
         │ (meta-analyses): n=10                │
         │ Companion papers: 21                 │
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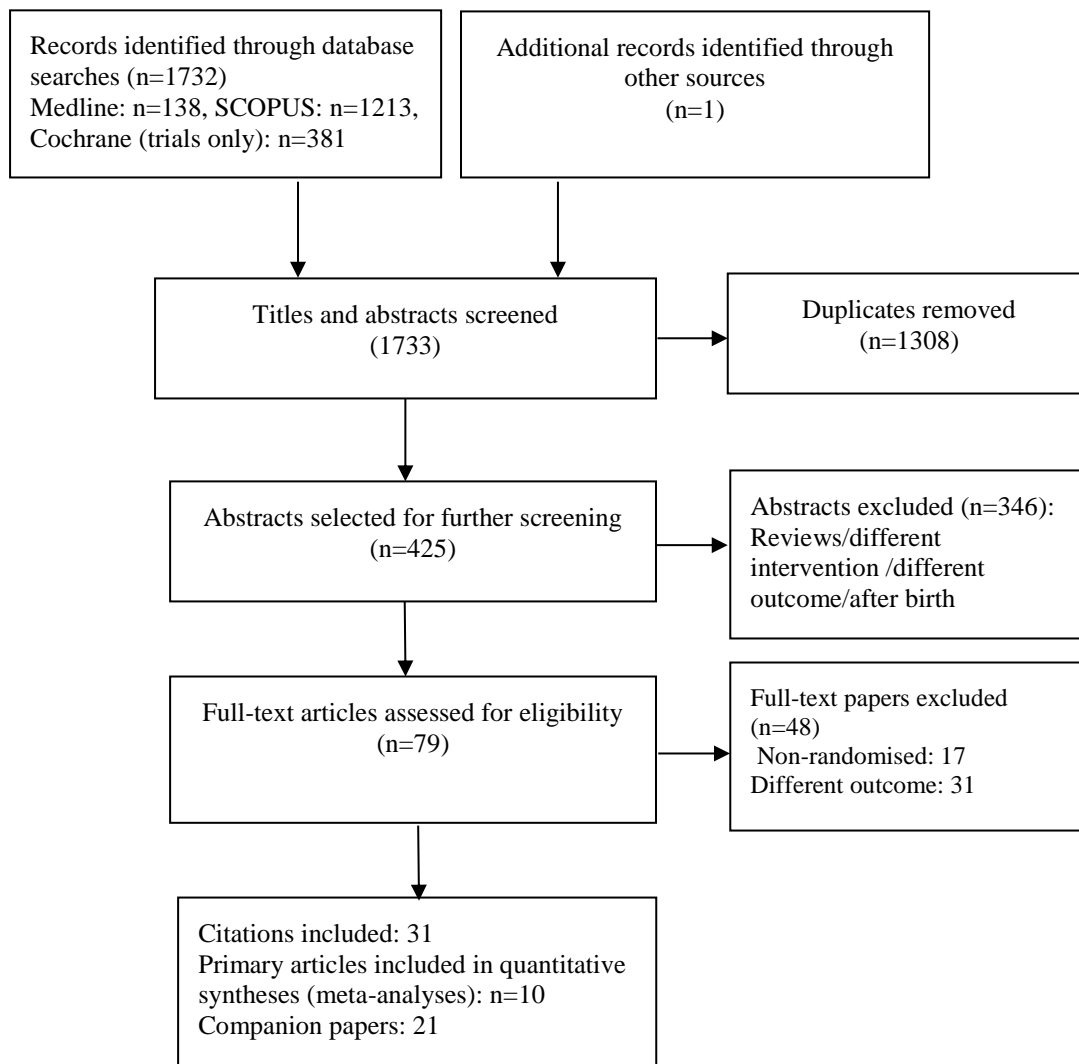
Figure 1: Study flow diagram, following PRISMA criteria

Table 1. Characteristics of included trials and study population for fatty acids and prevention of allergic and/or respiratory outcomes in offspring

	Companion articles[*]	Country, enrolment period	Participants receiving intervention	Age at last F-U	Sample: high risk of Atopy	Intake of Int. From/until	Duration of Int. (months)	No. at randomis ation[**]	No. at last Follow-up	Fatty acid product	Placebo	Total daily dose	Outcomes reported
Dunstan, et al., 2003a (21)	References 22-27	Australia 1999-2001	Prenatally in mothers	1yr	Yes	20wks to delivery	4.5-5	98	83 (40 vs. 43)	ω-3 PUFA	Olive oil	1-g, 4 a day	-Asthma -Recurrent wheeze -Eczema -SPT (any+); (egg) (peanut) (HDM) (cows milk) (CAT) -Food Allergy -Anaphylaxis -Angioedema -Chronic cough -SCORAD >=25
Olsen et al., 2008 (28)	Reference 29	Denmark 1989-90	Prenatally in mothers	16yrs	No	30wks to delivery	2-2.5	533: 266 vs. 131 vs. 136	528 (263 vs. 129 vs. 136)	Fish oil	Olive oil & no oil	1-g, 4 a day	-Any Allergic disease(s) -Asthma
Linnamaa et al., 2010 (30)	Reference 31	Finland 2004-08	Prenatally in mothers & postnatally in mothers & infants (2-yrs after birth)	2yrs.	No	8-16wks to 2yrs. postnatal	30-32	322: 151 vs. 162	177 (85 vs. 92)	Blackcu rrant seed oil	Olive oil	3 g/day, 6 capsules, oil drops in infants	-Eczema -SPT (egg) -SCORAD -Any IgE (mean)
Furuhjelm et al., 2011 (32)	Reference 33	Sweden 2003-05	Pre & postnatally in mothers (3.5 months after birth)	2yrs	Yes	25wks to 3.5months postnatal	7 to 7.5	145: 70 vs. 75	116 (53 vs. 63)	ω-3 group	Mainly the omega-6 PUFA LA	500-mg, nine a day	-Eczema -Asthma -SPT (any+) (egg) (milk) (food) (wheat) -Specific IgE -Food Allergy -Allergic Rhinoconjunctivitis -SCORAD -IgE (any+) (egg) (milk) (wheat) (food)
Noakes et	References	UK.	Prenatally in	6mont	Yes	20wks to	4.5-5	123: 62	86 (48 vs.	Salmon	Standar	2	-Wheeze

al., 2012 (34)	35-37	No reported	mothers	hs		delivery		vs. 61	38)	portions	d diet	portions per week	-Eczema -SPT (any+) (egg) (cows milk) (CAT) (Cod) (grass) (Dog) -Sensitisation -Chest infection -Pneumonia/ bronchiolitis -SCORAD -SPT (Derp-1) -Any IgE -Itchy skin -Dry skin
Best et al., 2016 (38)	References 39-42	Australia 2006-08	Prenatally in mothers	6yrs	Yes	21wks to delivery	4-5	700: 363 vs. 337	603 (313 vs. 290) Included in the ITT analysis: 367 vs. 336	ω-3 LC-PUFA	Vegetable oil	500 mg, 3 per day	-Allergic disease(s) with sensitisation -Eczema with sensitisation -Wheeze with sensitisation -Rhinitis with sensitisation -Rhino-conjunctivitis with sensitisation -Sensitisation (any) -Sensitisation (any+) (hen's egg) (peanut) (cashew) (ryegrass) (olive tree pollen) (D. pteronyssinus) (D.farinae) (Cat hair) (Alternaria tenius) (dog hair)
Escamilla-Nuñez et al., 2014 (43)	References 44-46	Mexico 2005-07	Prenatally in mothers	1.5yrs.	No	18-22wks until delivery	4.5-5.5	1094: 547 vs. 547	869 (429 vs. 440)	Docosahexaenoic acid (DHA)	A mixture of corn and soy oil	400 mg, twice per day	-Wheezing -Cough -Breathing Difficulty -Phlegm & Nasal -Discharge -Fever
Hansen	References	Denmark	Prenatally in	24yrs	No	30wks to	2-2.5	533: 266	396 (262	Fish oil	Olive	1-g,	-Asthma, discharge

2017 (47)	28-29	1989-90	mothers			delivery		vs. 131 vs. 136	vs. 134)		oil	4 a day	-Asthma medication -Allergic rhinitis -sIgE
Bisgaard 2017 (48)	Reference 49	Denmark Copenhagen & Zealand 2008-2010	Prenatally and postnatally in mothers (1-week after birth)	5yrs.	No	at week 24 of pregnancy to one week after delivery	15-17weeks	738: not specified per group	695 (346 vs. 349)	ω-3 LC-PUFA (55% EPA and 37% DHA) in triacylglycerol form	Olive oil	2.4 g per day	-Asthma/persistent wheeze (3-5yrs, birth to 5yrs), -Eczema -Sensitisation (SPT, sIgE) at 6 or 18months -Risk of lower respiratory infection -Risk of asthma exacerbations
Berman 2016 (50)	References 51-53	US, South-eastern Michigan 2008-2011	Prenatally in mothers	3yrs	No	Between 12-20 gestation wks.	20-28 wks.	118: 39 vs. 38 vs. 41	84 (31 vs. 26 vs. 27)	EPA-rich fish oil and DHA-rich fish oil	Soy oil	1060mg EPA + 274mg DHA and 900mg DHA plus 180 mg EPA	-Eczema -Food allergies Asthma/wheezing

* Published data and conference presentations, no unique data were extracted from conference abstracts   ** Indicates the number at randomisation, where recruitment has occurred prenatally

Table 2. Definition of outcomes included in the meta-analyses and their diagnostic criteria in individual studies

Any allergies	Diagnosis
Berman 2016, 3 years follow-up (PPA)[*] Asthma/persistent wheezing (at 3 years) Eczema/Skin (at 3 years) Food allergies (at 3 years)	Maternal report in phone interviews, using questions validated from the 2011 NHIS: -Chronic long-lasting inflammation of the airways -Redness, itching, outbreak of lesions -Abnormal response to food resulting in itching or swelling in mouth, vomiting, diarrhea, abdominal pain
Best 2016, 6 years follow-up (ITT)[**] Allergic diseases symptoms with sensitisation Wheeze with sensitisation Eczema with sensitisation Rhinitis with sensitisation Sensitisation (any) Hens' egg sensitisation Peanut sensitisation	Allergic symptoms were recorded using ISAAC questionnaire by nurses at clinic. Sensitisation was assessed to at least one of the aeroallergens tested -Eczema, wheeze, rhinitis, rhino-conjunctivitis with a positive SPT to ≥1 allergen extracts (sensitisation) -History of wheezing and/or whistling in the chest within the past 12 months with sensitisation -Chronic, itchy rash distributed to the facial, flexural or extensor surface of the skin -History of sneezing or a runny or blocked nose (in the absence of cold or flu) in the past 12 months -Positive SPT with a wheal size ≥3mm to foods (hen's egg, peanut, cashew), airborne allergens (ryegrass pollen, olive tree pollen, alternaria tenius, cat, dog and 2 species of house dust mite -Positive SPT with a wheal size ≥3mm to hen's egg -Positive SPT with a wheal size ≥3mm to peanut
Bisgaard 2016, 3, 5, 0-5 years follow-up (ITT) Persistent wheeze/asthma (0-5 years) Eczema (0-5 years) Sensitisation (6 and 18 months of age), SPT Sensitisation (6 and 18 months of age), sIgE	Clinical visits by pediatricians, additional visits for acute care (lung symptoms, any allergy, skin) -Diary recordings of five episodes of troublesome lung symptoms, within the preceding 6months, each lasting for at least 3 consecutive days, symptoms typical of asthma, the rescue use of inhaled beta^2-agonist, response to a 3months course of inhaled glucocorticoids following by relapse after the end of treatment. The diagnosis termed as persistent wheeze by child 3yrs old and asthma afterwards. -Based on the criteria defined by Hanifin and Rajka -A wheal >2mm in response to any SPT to milk, egg, dog or cat allergens -Specific IgE level of ≥0.35 ku/lit to milk, egg, dog or cat allergens
Dunstan 2003, 1 year follow-up (PPA) Recurrent wheeze Atopic eczema Food allergy	Clinical evaluations by pediatricians, taking a detailed history and examination: -More than 2 episodes of wheezing, at least 1 confirmed by a pediatrician or general practitioner -Typical skin lesions or physician-diagnosed eczema responsive to topical steroids -Undefined

Sensitisation Sensitisation to egg Sensitisation to peanut	-Using a standardised technique ($\geq$2mm) to allergens as egg, milk, peanut, house dust mite, cat -Positive SPT with a wheal size $\geq$2mm to egg -Positive SPT with a wheal size $\geq$2mm to peanut
Escamila-Nunez 2014, 1.5 years follow-up (PPA) Wheezing (maternal atopy and non-atopy)	Regular follow-ups using a clinical questionnaire, including ISAAC: -Symptomatic episode of wheeze as a sign lasting $\geq$3 days and at least one respiratory symptom
Furuhjelm 2011, 2 years follow-up (PPA) Any allergic disease Any asthma (cum. incidence 0–24months) Any eczema (cum. incidence 0-24 months) Any food reactions Sensitisation Sensitisation to egg	Examination by pediatrician at 24mon and validated allergy questionnaires completed by families -Symptoms of eczema, food reaction, asthma or rhinoconjunctivitis -Doctor diagnosed wheezing at least three times during the first 2 years of life -Reoccurring and itching eczematous, lichenified or nummular dermatitis (Seymour criteria 1987) -Gastrointestinal symptoms, hives, aggravated eczema/wheezing following the ingestion of a certain food with recovery after food elimination, reoccurrence of symptoms after re-ingestion -Wheal diameter $\geq$3 mm to milk, egg, wheat and cat, timothy and birch -Wheal diameter $\geq$3 mm to egg
Hansen 2016, 24 years follow-up (ITT) Asthma medication Allergic rhinitis Sensitisation, measured by sIgE (at 18-19yrs follow-up, in 243(46%) subjects)	Information collected from the national prescription register -Using a modified validated definition: filled $\geq$2 prescription for B_2-agonist or steroids, $\geq$1 prescriptions for leukotriene receptor antagonist -Filled $\geq$2 prescriptions including: eye drops, nasal decongestants, oral antihistamines -Positive test result of $\geq$0.35 ku/l to at least 1 of the 12 allergens
Linnamaa 2011, 1 year follow-up (PPA) Atopic eczema	Clinical evaluation of skin by dermatologist at each visit -Chronic or relapsing itchy dermatitis with a characteristic morphology and distribution, based on the Hanifin criteria
Olsen 2008, 16 years follow-up (PPA) Any allergies	Information extracted from National Patient Registry, a mandatory national hospital discharge -Allergic asthma, atopic dermatitis or allergic rhinitis

*Per-Protocol Analysis   **Intention-to-treat analysis

Figure 2. Risk of bias assessment figure in the included trials

Short Title	Random Sequence Generation	Allocation Concealment	Double Blinding	Blinding of Outcome Assessment	Incomplete Outcome Data	Selective Outcome Reporting	Other Sources of Bias
Berman 2016	-	?	?	?	-	+	+
Best 2016	+	+	-	+	+	+	+
Bisgaard 2016	?	?	+	?	+	+	?
Dunstan 2003	?	+	+	+	-	+	+
Escamilla-Nuñez 2014	+	+	+	+	+	+	+
Furuhjelm 2011	?	?	+	+	-	+	+
Hansen 2017	?	+	+	+	+	+	+
Linnamaa 2010	+	?	+	+	-	+	+
Noakes 2012	+	?	-	+	-	+	+
Olsen 2008	?	+	-	+	+	+	+

Random Sequence Generation: 40% Low, 50% Unclear, 10% High

Allocation Concealment: 50% Low, 50% Unclear

Double Blinding: 60% Low, 10% Unclear, 30% High

Blinding of Outcome Assessment: 80% Low, 20% Unclear

Incomplete Outcome Data: 50% Low, 50% High

Selective Outcome Reporting: 100% Low

Other Sources of Bias: 90% Low, 10% Unclear

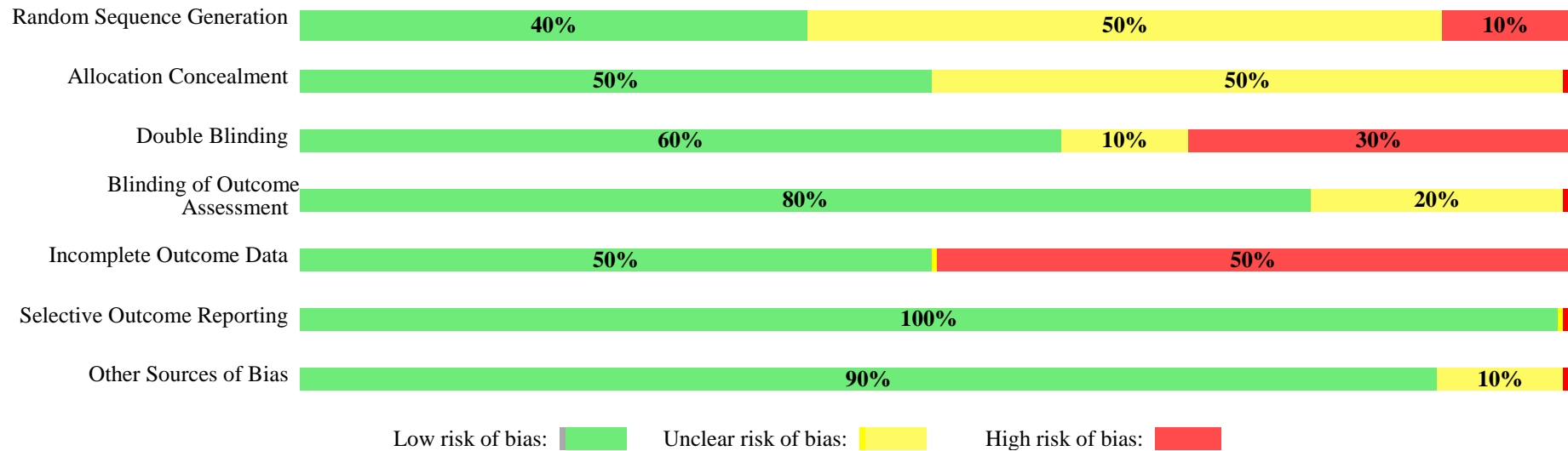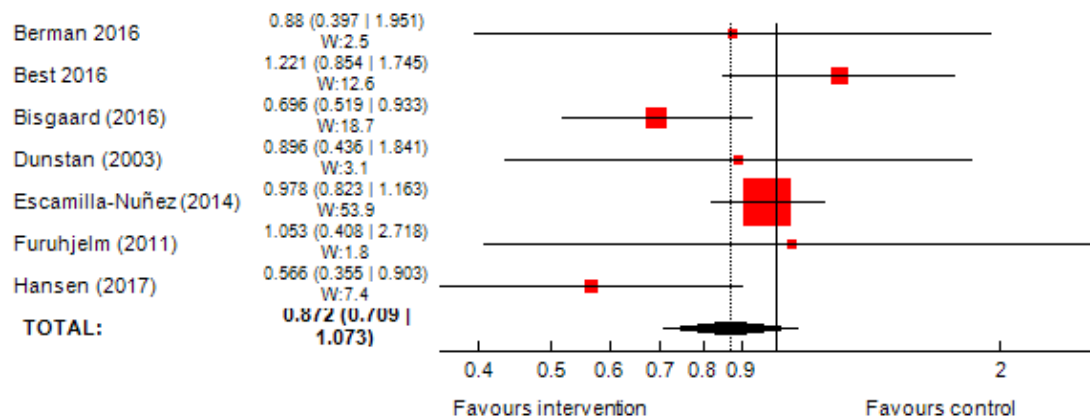Low risk of bias: [green]   Unclear risk of bias: [yellow]   High risk of bias: [red]

Figure 3. Forest plot for prenatal intake of fatty acid vs. placebo for prevention of asthma/wheeze in offspring

Heterogeneity: Q = 10.5; df = 6; p = 0.104; I-squared = 43.1%; tau-squared = 0.0292
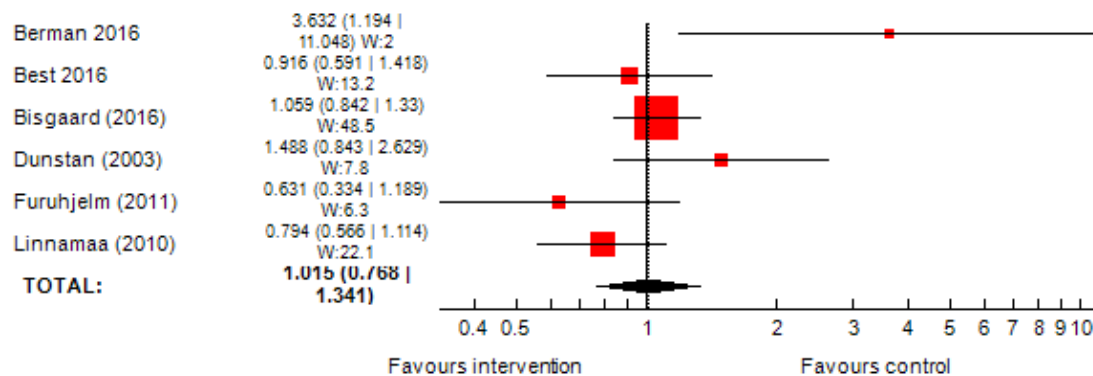
Random effects model: 0.872 (0.709, 1.07)

Outcome: Asthma/Wheeze	Fatty acid n/N	Placebo n/N	Study sample
Berman 2016	13/57	7/27	Unselected
Best 2016	60/367	45/336	Atopic
Bisgaard 2016	60/346	87/349	Unselected
Dunstan 2003	10/40	12/43	Atopic
Escamila-Nunez 2014	252/429	262/440	Unselected
Furuhjelm 2011	7/54	8/65	Atopic
Hansen 2016	31/262	28/136	Unselected
Total children: 2,951	1,555	1,396	

Figure 4. Forest plot for prenatal intake of fatty acid vs. placebo for prevention of eczema in offspring

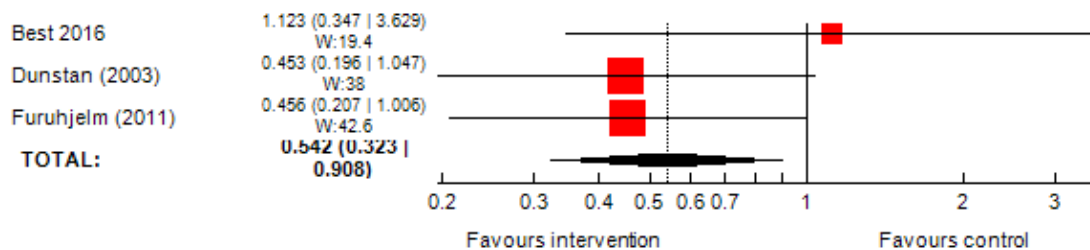Heterogeneity: Q = 11.2; df = 5; p = 0.0467; I-squared = 55.5%; tau-squared = 0.0599

Random effects model: 1.01 (0.768, 1.34)

Outcome: Eczema	Fatty acid n/N	Placebo n/N	Study sample
Berman 2016	23/57	3/27	Unselected
Best 2016	36/367	36/336	Atopic
Bisgaard 2016	114/346	98/349	Unselected
Dunstan 2003	18/40	13/43	Atopic
Furuhjelm 2011	11/54	21/65	Atopic
Linnamaa 2010	33/85	45/92	Unselected
Total children: 1,861	949	912	

Figure 5: Forest plot for prenatal intake of fatty acid vs. placebo for prevention of sensitisation to egg, measured by SPT in offspring
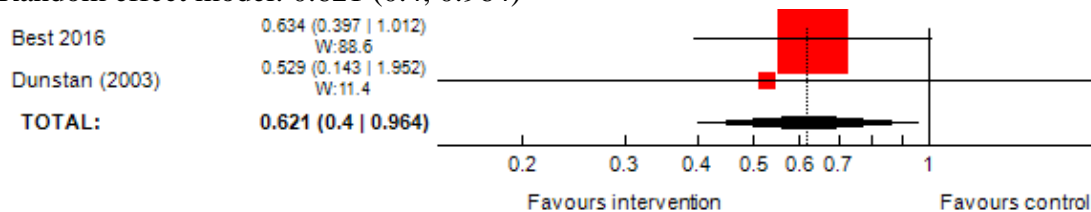
Heterogeneity: Q = 1.84; df = 2; p = 0.399; I-squared = 0%; tau-squared = 0
Random effect model: 0.542 (0.323, 0.908)

Outcome: Sensitisation to egg (SPT)	Fatty acid n/N	Placebo n/N	Study sample
Best 2016	6/248	5/232	Atopic
Dunstan 2003	9/35	14/37	Atopic
Furuhjelm 2011	7/52	18/61	Atopic
Total children: 665	335	330	

Figure 6: Forest plot for prenatal intake of fatty acid vs. placebo for prevention of sensitisation to peanut, measured by SPT in offspring

Heterogeneity: Q = 0.0658; df = 1; p = 0.798; I-squared = 0%; tau-squared = 0
Random effect model: 0.621 (0.4, 0.964)

Outcome: Sensitisation to peanut (SPT)	Fatty acid n/N	Placebo n/N	Study sample
Best 2016	27/367	39/336	Atopic
Dunstan 2003	3/35	6/37	Atopic
Total children: 775	402	373	