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Article

Omega-3 Fatty Acids and Allergic Disease

Effect of Omega-3 Fatty Acids Supplementation during Childhood in Preventing Allergic Disease: A Systematic Review and Meta-Analysis

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Abstract

Background: Early omega-3 fatty acids exposure can influence early immune development and potentially prevent allergic disease.

Objectives: To review the effects of omega-3 fatty acids during childhood on allergic disease outcomes.

Methods: We conducted searches of the PubMed, EMBASE and Cochrane Central Register of Controlled Trials and international trial registers (ClinicalTrials.gov and ISRCTN Registry) to September 30, 2018. We included randomized controlled trials (RCTs) and prospective cohort studies regarding the effect of omega-3 fatty acids during childhood on allergic disease outcomes. A total of 8 publications from 2 prospective cohort studies and 6 reports representing 5 unique RCTs were included.

Results: The results of meta-analysis showed that omega-3 fatty acids during childhood did not appear to significantly alter the risk of any atopy (≤ 3 years old: RR 0.70, 95% CI 0.47 to 1.04, $p=0.08$; > 3 years old: RR 0.98, 95% CI 0.82 to 1.16, $p=0.77$), wheeze (≤ 3 years old: RR 0.82, 95% CI 0.54 to 1.26, $p=0.375$; > 3 years old: RR 1.03, 95% CI 0.53 to 2.00, $p=0.929$) and eczema (≤ 3 years old: RR 0.86, 95% CI 0.68 to 1.08, $p=0.20$; > 3 years old: RR 0.90, 95% CI 0.60 to 1.35, $p=0.60$).

Conclusions: There is limited evidence to support omega-3 fatty acids during childhood could reduce the risk of allergic disease.

Key words: omega-3 fatty acids; allergic disease; asthma; wheeze; eczema; meta-analysis

Introduction

Worldwide prevalence of allergic disease has risen dramatically in recent decades, which places a burden on families, society and healthcare system[1, 2]. Although the etiology of allergic disease has not been fully elucidated, environmental factors including diet seem to be of great importance. Considerable research showed nutrition exposure including omega-3 fatty acids in early life could improve early immune development and reduce inflammatory responses[3-5]. Supplementation of EPA and DHA was reported to increase the composition of omega-3 fatty acids in immune cell membranes[6, 7] decreasing the production of inflammatory eicosanoids (prostaglandin E₂, leukotriene B₄ and etc.), cytokines (interleukin-1 β , IFN- γ and etc.) and expression of adhesion molecules[8-11]. Early omega-3 fatty acids exposure is shown to influence early immune development and potentially prevent allergic disease[12, 13].

A number of observational studies found that children with allergic disease consumed breast milk with lower EPA and DHA in infancy than those healthy children[14-16]. Some studies observed a protective effect of omega-3 fatty acids or oily fish supplementation during childhood on allergic outcomes in children[17-19]. Until recently, the evidence of the effects of childhood omega-3 fatty acids supplementation on allergic outcomes in later life is lacking as previous research provided inconsistent answers to this question. One previous meta-analysis suggested that omega-3 fatty acids supplementation in infants could not reduce the risk of asthma in later life[20]. One descriptive review summarizing the effect of omega-3 fatty acids supplementation during childhood on the prevention of allergic disease suggested omega-3 fatty acids could play a positive role in decreasing the risk of developing some manifestations of allergic disease in the short term[21]. However, the data were not pooled in that review, which provided weak evidence. In all, there is not yet a quantitative analysis assessing the effect of omega-3 fatty acids supplementation during childhood on the risk of various kinds of allergic disease and sensitive status of children.

Considering factors described above and that more studies have been published lately[22-29], we have incorporated studies till now and performed a systematic review and meta-analysis to identify the effect of omega-3 fatty acids during childhood on the risk of allergic disease. This review should provide more evidence on whether it is beneficial for preventing allergic disease by supplementing omega-3 fatty acids during childhood.

Methods

The protocol was registered with the PROSPERO International Prospective Register of Systematic Reviews (CRD42018112023).

Search Strategy

A comprehensive search for publications was conducted using the following databases from inception to September 30, 2018: PubMed (1966 to September, 2018), EMBASE (1974 to September,

2018), Cochrane Central Register of Controlled Trials (CENTRAL, through September, 2018), and international trial registers (ClinicalTrials.gov and ISRCTN Registry) from their inception to September, 2018 without any restriction. Detailed search strategy is shown in **Table S1**. We also reviewed the reference lists of included studies and pertinent reviews for supplementing the search.

Study Selection

The inclusion criteria were as follows: 1). Design: RCTs or prospective cohort studies, 2). Population: children whose allergic outcomes were measured under 18 years old, 3). Intervention: supplementation of omega-3 fatty acids during childhood (0 to 18 years old), 4). Comparison: placebo without supplementation of omega-3 fatty acids, 5). Outcomes: primary outcomes: the incidence of any atopy: any of atopic disease (asthma/wheeze, eczema, atopic rhinitis and food allergy with/without IgE sensitivity) or sensitization in childhood, secondary outcomes: the incidence of specific atopic disease (asthma/wheeze, eczema, atopic rhinitis and food allergy with/without IgE sensitivity) or sensitization in childhood. Outcomes were assessed as short term (up to 3 years of age) and long term (beyond 3 years of age). The presence of allergic disease is defined as a clinician diagnosis, parent report of symptoms of allergic disease, or parent report of a physician's diagnosis. Sensitization is defined as a positive skin-prick test (SPT) or IgE serology indicating sensitive status with/without clinically relevant symptoms. Studies that met all inclusion criteria were included in this review. All of the identified studies were reviewed independently by two investigators.

Data Extraction

Study characteristics were extracted independently by two reviewers using a self-designed data extraction sheet. Extracted data included: name of the first author, year of publication, study design, area, number of participants, intervention, placebo, starting time and duration of intervention, length of follow-up and main outcomes. Extracted data of outcomes were presented (risk ratio (RR) with 95% confidence interval (CI)) in an individual table. We contacted each first author or corresponding author of included studies asking for additional information if needed. A discussion was held upon the differences about the interventions and outcomes until agreement was reached between the reviewers before data analysis.

Assessment of Quality

Newcastle-Ottawa Scale[30] was employed to judge the methodological quality of cohort studies. This scale uses a star system to evaluate the quality of included studies on the basis of three broad perspectives: the selection of study cohort, the comparability of groups, and the ascertainment of either the exposure or outcome. After judging these three perspectives, a maximum of nine stars would be assigned to each study. A study with six or more stars was thought to be of high quality. We used the Cochrane Collaboration risk of bias tool to assess the risk of bias in RCTs[31]. Seven sections including generation of allocation sequence, allocation concealment, blinding of participants and researchers, blinding of outcome assessors, completeness of outcome data, selective outcome reporting, other risk of bias, were evaluated. Each item was marked by low, high or unclear risk of

bias. Every disagreement was settled by two data extractors, all team members would discuss if necessary.

Statistical Analysis

Included studies varied markedly in terms of the methods, intervention and reported outcome measures. We therefore reported study results for all cohort studies and some RCTs descriptively if meta-analysis could not be carried out. Meta-analysis was conducted only for RCTs with comparable timing of outcome assessment, and the effect of omega-3 fatty acids was analyzed at two points in the child's life short term (up to 3 years old) and long term (beyond 3 years old). Risk ratio (RR) with 95% confidence interval (CI) was used to assess the effects of omega-3 fatty acids on the incidence of childhood allergic disease. We used data of outcomes with the longest follow-up in two terms (less or beyond 3 years old) when multiple reports originated from the same population. Adjusted odds ratio (OR) of eczema in one study was directly regarded as RR. A random-effects model was used due to the variability among included studies. We used I^2 statistic to assess the statistical heterogeneity within studies. A percentage less than 50% ($I^2 \leq 50$) indicates a low heterogeneity[32]. Prespecified subgroup analyses were performed to determine whether the observed associations were modified by timing of supplementation, allergic risk of infants (whether first degree relatives had allergic history) and study location. To assess the association of different characteristics observed in subgroup analyses and the incidence of outcomes, univariable meta-regression analyses were conducted. The factors for the stratification of subgroups were regarded as co-variables in regression models, the restricted maximum likelihood method was used for fitting the univariable meta-regression.

Two methods of sensitivity analysis were used to determine the stability of summary risk estimates, namely repeating meta-analysis with a fixed-effects model and removing studies rated as 'high' risk of selection, performance or attrition bias. We did not test publication bias with the formal statistical tests, because they have limited power when there are less than 10 studies[33]. Data synthesis and analysis were performed with Stata 12.0. A p value less than 0.05 was considered statistically significant unless otherwise specified.

Quality of Evidence Assessment

Quality assessment of the outcomes was conducted based on the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) System. The GRADE approach specifies 4 levels of quality: high, moderate, low, or very low. The highest quality rating is for randomized trial evidence. Reviewers can downgrade randomized trial evidence to moderate, low, or even very low quality evidence, depending on the presence of the 5 factors, including limitations in the design and implementation, indirectness of evidence, unexplained heterogeneity or inconsistency of results, imprecision of results and high probability of publication bias.

Results

Literature Search

A total of 1406 records were obtained resulting from electronic searching. 446 duplicate records were removed and 960 records remained. 907 publications were removed after reading titles and abstracts by two independent reviewers, resulting in 53 publications which needed to be read in full-text. 45 full publications were excluded for different reasons, detailed information is shown in **Figure.1**. Consequently, 6 publications[24-29] from 5 unique RCTs were included in quantitative synthesis and 2 prospective cohort studies[22, 23] were included in descriptive analysis in this review. One ongoing study was searched in the international trial registers but there is no data available (NCT01473823). No study was included in manual retrieval by reviewing relevant studies and reviews.

Observational Studies

Study Characteristics and Quality Assessment

The characteristics of observational studies are summarized in Table 1. Two included studies[22, 23] were multicenter, prospective cohort studies. In one study[22], the gestation of included infants was at least 34 weeks. Infants in exposed group were supplemented with a daily dose of fish oil containing 650 mg of DHA and 350 mg of EPA of monounsaturated fatty acids. Infants not compliant with exposure factors were treated as control group. The supplementation was started before 15 months of age and continued until 2.5 years of age. The process was followed by routine and unscheduled visits or telephone calls. The other study[23] included term infants, and infant and follow-on formulas containing 17 mg of DHA/100 kcal and 34 mg of ARA/100 kcal were supplied in the exposed group at approximately 4 months of age and continued until 12 months of age. Children fed with formulas without added DHA/ARA were treated as control group. The process was followed by routine visits and unscheduled visit or telephone call. For quality assessment, the quality of both of the included observational studies was assessed by using the Newcastle-Ottawa Scale[30]. One[22] was awarded 8 stars and the other[23] scored 9. Overall, the two studies were both of high quality. (**Table 2**)

Outcomes of Observational Studies

Clausen et al's study[22] showed omega-3 fatty acids intake in children at early stage may decrease food sensitization and food allergy in young children by more than half until age 15 months. Regular fish oil could even decrease the severity of food allergy. Lapillonne et al's study[23] showed there was no statistically significant difference in the incidence of eczema between two groups($p=0.083$).

Randomized Controlled Trials

Study Characteristics and Quality Assessment

The characteristics of included RCTs are summarized in **Table 3**. Detailed information of outcomes is shown in **Table S2**. Six included studies[24-29] were from five unique RCTs. Omega-3 fatty acids were supplemented with different forms, doses and frequencies in experimental group. The control group was not supplemented with omega-3 fatty acids. Four studies[24-27] started the intervention several days after birth. Two publications[28, 29] from the Childhood Asthma Prevention Study (CAPS) commenced the intake of omega-3 fatty acids from the age of 6 months. The follow-up of studies lasted until 9 months to 8 years. For quality assessment, risk of bias of six included RCTs was assessed according to the *Cochrane Handbook*. Three studies were assessed with high risk in attrition bias(24), reporting bias(27) and other bias(26), respectively. Others were evaluated as low risk for correct method utilization or unclear risk for inadequate information.(**Figure.2**)

Primary Outcome of RCTs

Any Atopy

Four included RCTs[24-26, 29] reported the incidence of any atopy of children. The pooled data of three RCTs[24-26] indicated the incidence of any atopy in children up to 3 years old was not reduced in experimental group compared with placebo group(RR 0.70, 95% CI 0.47 to 1.04, $p=0.08$) with significant heterogeneity ($I^2=64.8\%$). The pooled data of two RCTs[26, 29] showed there was also no difference between groups beyond 3 years old (RR 0.98, 95% CI 0.82 to 1.16, $p=0.77$) without significant heterogeneity ($I^2=0\%$). (**Figure. 3**)

Secondary Outcomes of RCTs

Asthma/wheeze

Asthma or symptoms of wheeze were assessed in six included studies[24-29]. Three studies[27-29] showed that omega-3 fatty acids intake was not associated with a reduced risk of asthma. However, we did not pool the data due to the significant heterogeneity of the definition of asthma among the studies (**Supplemental file**). The pooled data indicated there was no significant reduction on the incidence of wheeze in experimental group compared with placebo group(≤ 3 years old: RR 0.82, 95% CI 0.54 to 1.26, $p=0.375$; > 3 years old: RR 1.03, 95% CI 0.53 to 2.00, $p=0.929$) with significant heterogeneity ($I^2=66.2\%$ and $I^2=72.7\%$, respectively).(**Figure.4**)

Eczema

Five included studies[24-28] reported the incidence of eczema in children up to 3 years old, and two[26, 29] reported the outcome beyond 3 years old. The pooled data indicated the incidence of eczema was not significantly decreased in experimental group compared with placebo group (≤ 3 years old: RR 0.86, 95% CI 0.68 to 1.08, $p=0.20$; > 3 years old: RR 0.90, 95% CI 0.60 to 1.35, $p=0.60$), without significant heterogeneity ($I^2=35\%$ and $I^2=29.6\%$, respectively).(**Figure. 5**)

Atopic Rhinitis

One included RCT[29] indicated that the risk of atopic rhinitis in children beyond 3 years old was not reduced in experimental group compared with placebo group(RR 1.03, 95% CI 0.78 to 1.37, $p=0.84$).

Food Allergy

One included RCT[25] indicated that the risk of food allergy in children up to 3 years old was not reduced in experimental group compared with placebo group(RR 0.81, 95% CI 0.47 to 1.42, $p=0.47$).

Sensitization

Two included RCTs[25, 29] reported sensitization of patients. No significant reduction in sensitization was obtained in experimental group in children up to 3 years old(RR 1.00, 95% CI 0.70 to 1.42, $p=1.00$) and beyond 3 years old(RR 1.11, 95% CI 0.89 to 1.37, $p=0.35$). Two studies[25, 28] reported results of some specific SPT. No significant difference was found in the results of SPT+egg, peanuts, milk, house dust mites(HDM) and cat between two groups in one study[25]. Another study[28] showed there was no difference in the results of SPT+inhaled allergens (salmon, tuna, peanuts, cow's milk and egg), and ingested allergens(HDM, grasses, cockroach, cat, Alternaria; Hollister-Stier, Spokane, Wash) between two groups.

Subgroup Analysis

Only data reported at last time point of each unique RCT were pooled in the subgroup analysis. As a result, there were 5 studies included in the subgroup analysis. The detailed results are presented in **Table 4**. We stratified the outcomes by start-timing of supplementation, allergic risk of children and study location.

Start-timing of supplementation(<6 months and ≥ 6 months)

Four included studies[24-27] started the supplementation from age of under 6 months. There were no significant differences between experimental group and control group for any outcomes according to pooled data. One study[29] started the supplementation ≥ 6 months of age. It indicated that the risk of wheeze was increased among children in experimental group compared with those in control group.

Allergic risk of children

We regarded children with high allergic risk if their first degree relatives had allergic history. Two studies[25, 29] included children with high risk of allergy, while the other three studies[24, 26, 27] did not strictly restrict on this. There were no significant differences between experimental group and control group for any of the outcomes according to pooled data.

Study location

Two studies conducted in Australia[25, 29] indicated that the risk of wheeze was increased among children in experimental group compared with those in control group. There were no significant differences between experimental group and control group in studies conducted in USA[24,26] and UK[27] for any of the outcomes according to pooled data.

Meta-regression

In subgroup analyses for the incidence of any allergy, wheeze and eczema, no evidence of modification effect by start-timing of supplementation, allergic risk of children, study location was obtained according to meta-regression analysis(all $P_{\text{interaction}} > 0.05$, **Table 4**).

Sensitivity Analysis

Sensitivity analyses were conducted for the all outcomes by repeating meta-analysis with a fixed-effects model and removing studies with high risk of bias. The risk of any atopy for children up to 3 years old was significantly changed from (RR 0.70, 95% CI 0.47 to 1.04) to (RR 0.78, 95% CI 0.63 to 0.96) when transferring random to fixed effects model. Changing the pooling model for other outcomes and removing one study[24] with high risk of bias on incomplete outcomes did not change the results. (**Table 5**)

Quality of Evidence

We conducted Quality Assessment of the outcomes based on the GRADE System. The evidence of the outcomes was from different types of studies, so the evaluation was conducted separately according to the type of the study. Overall, the evidence was of moderate quality regarding the incidence of eczema, and the evidence was of low or even very low quality regarding other outcomes. The main defect of the quality of this meta-analysis existed in inconsistency, such as significant heterogeneity among studies. The main defects in the quality of descriptive outcomes were the limited number of studies without large sample size and the inconsistent results among the studies. Therefore, we chose to downgrade the quality of most outcomes in the relevant items. Detailed information is shown in **Table S3&S4**.

Discussion

To the best of our knowledge, this is the first quantitative analysis of evidence from RCTs and prospective cohort studies to date assessing the effects of omega-3 fatty acids in childhood on the risk of allergic disease. Only one observational study[22] indicated provision of omega-3 fatty acids in children at early stage may decrease food allergy and even the severity of food allergy in young children. The result was inconsistent with one included RCT[25], which may due to the characteristics of included infants, different types of supplementation, the presence of confounding factors, the start and follow-up between two studies. Our meta-analysis showed no beneficial effect of omega-3 fatty acids was found for preventing allergic disease. However, sensitivity analysis indicated the result of any atopy was not stable. The insignificant protective effect of omega-3 fatty acids intake on any atopy was changed to significant when transferring random to fixed effects model to pool data. The marginal results suggested omega-3 fatty acids not be widely used in clinical practice instantly for preventing allergy in childhood, whereas more studies should be carried out to add new evidence in this topic.

Previous review[21] indicated that fish oil intake in children may be associated with immunologic benefits and may decrease the risk of some manifestations of allergic disease, but evidence was almost from observational studies and there was no any pooled data of the results. The previous meta-analysis[20] showed no benefit of omega-3 fatty acids intake during childhood was found in primary prevention of asthma. However, three included studies originated from the same cohort with different follow-up period and the outcome 'wheeze' in some included studies was directly regarded as 'asthma' in that meta-analysis. It overlooked the fact that wheeze can not be equated to asthma simply. Synthesizing the current evidence in this review, the hypothesis that there is a protective association between omega-3 fatty acids intake during childhood and allergic disease cannot be confirmed or rejected unequivocally due to the current limited evidence.

Strength of this review should be noted. This review qualitatively and quantitatively assessed evidence from prospective cohort studies and RCTs with rigorous and transparent methods. Considering the high heterogeneity in the assessment of outcomes, we grouped included studies according to different duration of follow-up. Data from children with similar age could be pooled. To dismiss the heterogeneity and explore more precise outcomes, subgroup analysis was carried out regarding factors that may cause the heterogeneity.

This review also has limitations which need to be mentioned. The number of studies was not large, sample size of some included studies was small. It's difficult to pool the data of asthma because of obvious difference in definition of asthma. The risk of publication bias cannot be excluded because only published studies were included in meta-analysis and we did not obtain any reply about the extra unpublished data. Sensitivity analysis showed that a small number of results were not stable. Furthermore, the quality of outcomes is low.

According to the hypothesis of atopic march, symptoms of allergy occur sequentially in childhood[35]. The follow-up of current studies should continue to school age even to 18 years old based on well controlled confounding factors, especially for asthma. Some results of allergic disease cannot be pooled in this meta-analysis, so further studies are warranted. The optimal doses, tolerability of omega-3 fatty acids supplementation, and the effect of long term should be focused on in the future. Although the subgroup analysis showed allergic risk of children may not significantly change the outcomes, further studies still need pay more attention to children with or without high risk of allergy.

Conclusion

The pooled data showed that there was no statistical reduction in allergic disease outcomes (the incidence of any atopy, asthma/wheeze, eczema, atopic rhinitis, food allergy, sensitization) with omega-3 fatty acids supplementation during childhood. Thus, omega-3 fatty acids should not be recommended for routine clinical application during childhood for preventing allergic disease. However, the results should be interpreted with caution due to the limited number of studies and the heterogeneity among studies.

Author contributions

Yin Zhang is responsible for data search, inclusion and exclusion of studies, data extraction, data analysis and writing the manuscript. Jilei Lin is responsible for data search, inclusion and exclusion of studies, data extraction and data analysis. Rui Zhou is responsible for data search, inclusion and exclusion of studies, assessment of methodological quality and checking the data extraction. Xuexiang Zheng is responsible for data search, assessment of methodological quality and checking the data extraction. Jihong Dai is responsible for conception of this systematic review, supervision, interpretation of results, and checking the first and the final versions of the manuscript.

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Declaration of Interest statement

The authors declare no conflict of interest

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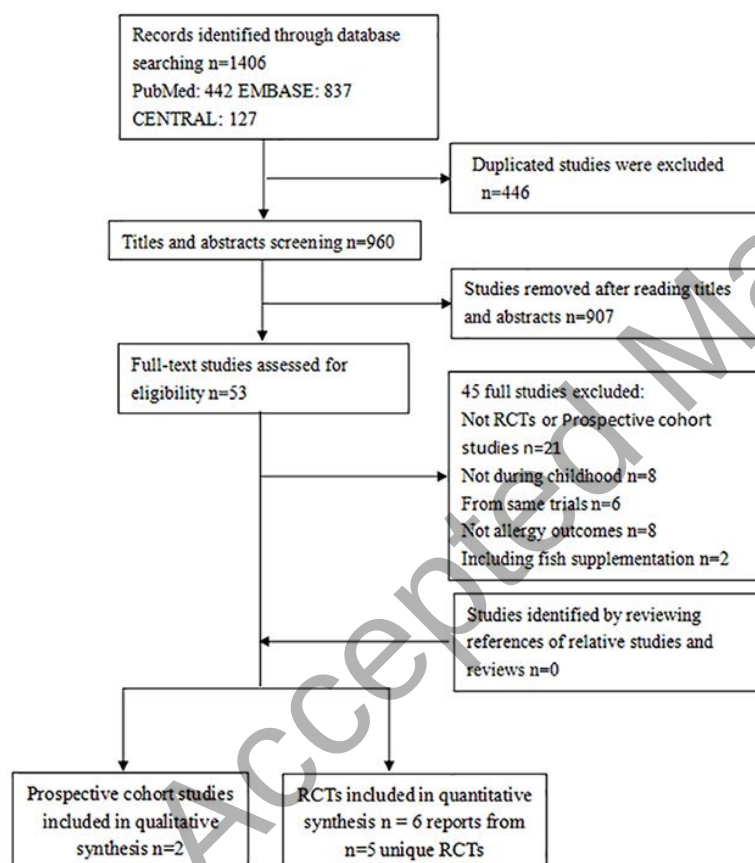


Figure 1: The flow diagram of identifying relevant studies.

	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Selective reporting (reporting bias)	Other bias
Birch 2010	?	?	+	+	-	+	?
Dvaz 2012	+	+	+	?	+	+	+
Foiles 2016	?	?	+	?	+	?	-
Lucas 1999	+	+	+	?	+	-	+
Peat 2004	+	+	?	+	?	+	+
Toelle 2010	+	+	?	+	?	+	+

Figure 2: Summary of risk of bias assessment for included RCTs

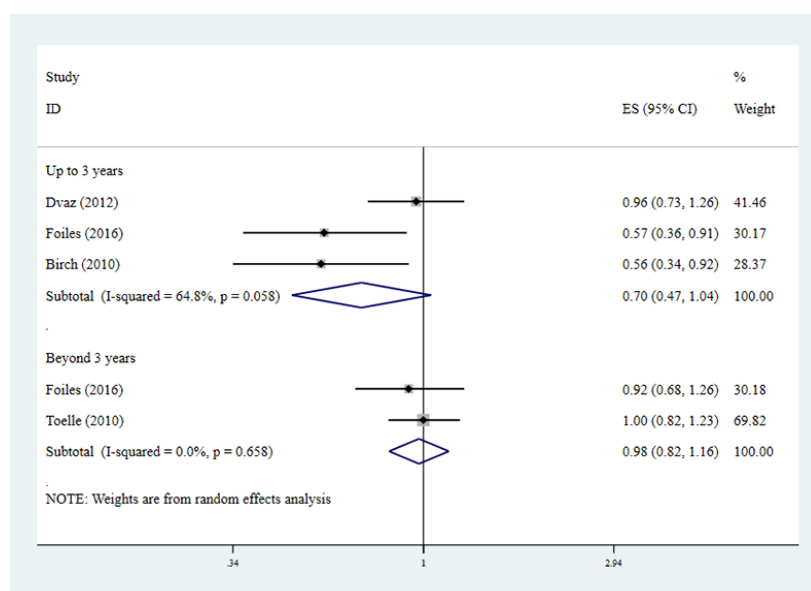


Figure 3: Relative risks and 95% confidence intervals of any atopy (≤ 3 years old and > 3 years old).

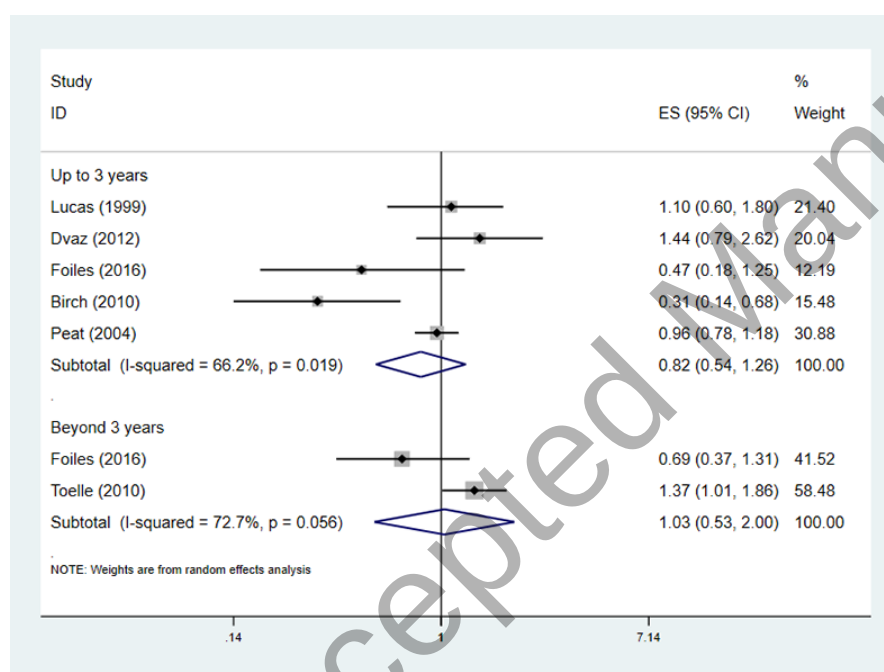


Figure 4: Relative risks and 95% confidence intervals of wheeze (≤ 3 years old and > 3 years old).

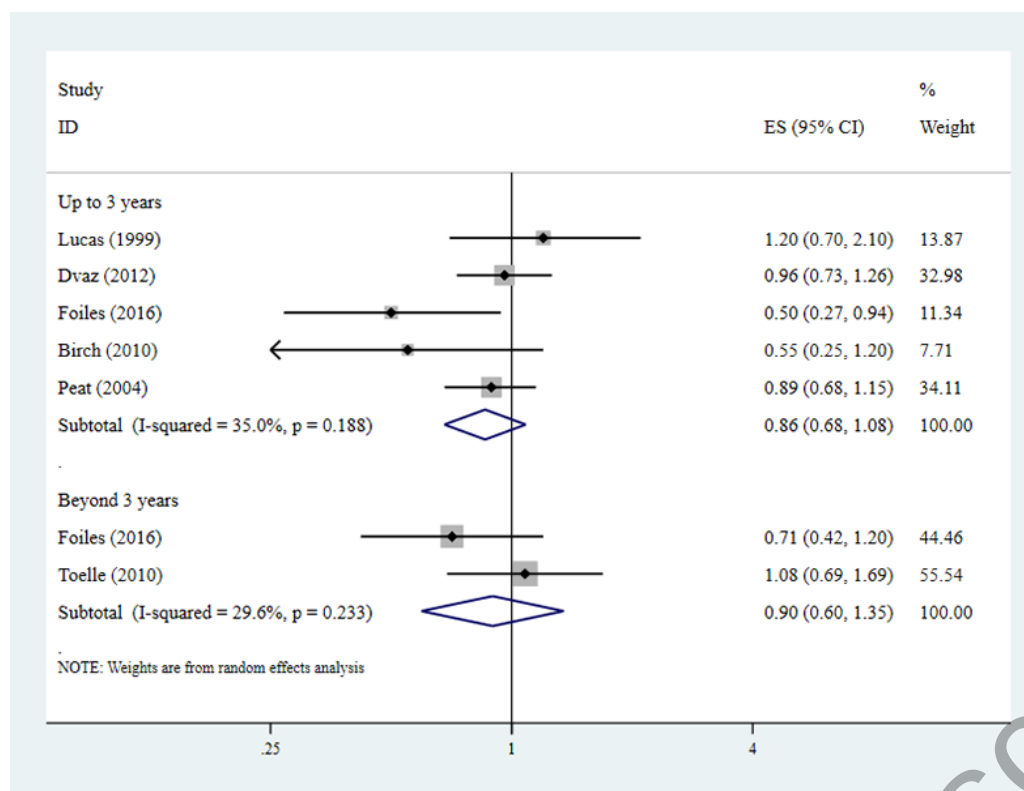


Figure 5: Relative risks and 95% confidence intervals of eczema (≤ 3 years old and > 3 years old).

Table 1: The characteristics of observational studies

	Study			Population	Exposure	Exposure			Control	Main
Author (year)	Study design	Area	Sample size	Characteristics	Int assessment	Factors	Start	Follow- up		outcom es
Clausen 2018 ²²	Multicenter, prospective, observational study	Iceland, UK, Germany, Poland, Spain, Greece, Lithuania, The Netherlands, Italy	1033/271	Infants at minimal gestational age of 34 completed weeks and an APGAR of at least seven at 5 min after birth.	Answered questionnaires over the phone at approximately 12 and 24 months	650 mg of DHA and 350 mg of EPA as well as 9.2 µg of vitamin D, 138 µg of vitamin A, 4.6 mg of vitamin E and 1.8 g of monounsaturated fatty acids	Before 15 months	From birth to 2.5 years of age	Infants not compliant with exposure factors	Food allergy, Food sensitization
Lapillonne 2014 ²³	Multicenter, prospective, observational, open-label study	France	233/92	Healthy term infants born after 37 weeks of gestation, singleton, with a birth weight, and head circumference appropriate for gestational age, exclusively fed with one of the study formulas for at least 24 hours before	Pediatricians followed participants until 12 months of age at routine visits and any unscheduled visit or telephone call	Infant and follow-on formulas containing 17 mg of DHA/100 kcal and 34 mg of ARA/100 kcal	Infant formulas until approximately 4 months of age and follow-on formulas from approximately 4 months until 12 months of age.	Through 1 year	Children fed with infant and follow-on formulas without added DHA/ARA	Eczema

Table 2: Summary of risk of bias assessment of included observational studies

Quality indicators from Newcastle-Ottawa Scale									
		Selection			Comparability		Outcome		
Study	Representativeness of the exposed cohort	Selection of the nonexposed cohort	Ascertainment of exposure	Demonstration that outcome of interest was not present at start of study	Comparability of cohorts on the basis of the design or analysis	Assessment of outcome	Was follow-up long enough for outcomes to occur?	Adequacy of follow-up of cohorts	Total score
Clausen 2018 ²²	*	*		*	**	*	*	*	8
Lapillonne 2014 ²³	*	*	*	*	**	*	*	*	9

Table 3: The characteristics of RCTs								
Author	Study	Area	Sample	Participants	Timing	Experiment	Control group	outcomes
Birch 2010 ²⁴	Randomized double-blind trials	Dallas, USA	38/51	Healthy term infants who were singleton births	From age ≤5 days through 12 months	DHA/ARA formula (control + DHA as 0.32%-0.36	A similar unsupplemented formula	Any allergy, Wheeze/asthma, Eczema, Wheezing/asthma/Atopic dermatitis
Dvaz 2012 ²⁵	Double-blind randomized controlled trial	Perth, Australia	156/167	Healthy term infants born at least 36 weeks' gestation of whose mother was allergic/otherwise	From birth to 6 months of age	650 mg fish oil, 280 mg docosahexaenoic acid (DHA) and 110 mg eicosanenta	650 mg olive oil (66.6% n-9 oleic acid)	Any allergy, Wheeze, Food allergy, Skin allergic illness, Eczema, Sensitization, SPT
Foiles 2016 ²⁶	Randomized controlled	Kansas, USA	72/19	Healthy term infants who were exclusively	For the first 12 months of life	Formulas that contained	A control formula without DHA	Any allergy, Wheeze, Eczema
Lucas 1999 ²⁷	Double-blind, randomised	Nottingham and Leicester,	154/155	Healthy singletons of appropriate size	For the first 6 months	Formula with 0.30% AA and	A similar formula without the addition of	Wheeze, Asthma, Eczema
Peat 2004 ²⁸	Parallel-group randomized controlled trial	Sydney, Australia	267/259	Infants who had at least 1 parent or sibling with current asthma or frequent wheeze, multiple births, and less than 36 weeks gestation were excluded	From the age of 6 months	500 mg tuna fish oil capsules containing approximately 184 mg omega-3 fatty acid to add to	Sunola oil containing 83% monounsaturated oils, soybean-based polyunsaturated oils and margarines high in omega-6 fatty	Wheeze, Asthma, eczema, SPT
			230/220					Any allergy, Wheeze, Asthma, Eczema, Rhinitis
Toelle 2010 ²⁹								

Table 4: Subgroup analyses of omega-3 fatty acids supplementation during childhood on allergic disease outcomes

		Total	Start-timing of supplementation		Allergic risk of children		Study Location		
			<6months	≥6months	Not defined	High risk	North America	Europe	Australia
Any allergy	N	4	3	1	2	2	2	0	2
	RR (95% CI)	0.91 (0.76,1.09)	0.84 (0.65,1.1)	1.0 (0.82,1.22)	0.75 (0.46,1.21)	0.99 (0.84,1.16)	0.75 (0.46,1.21)	-	0.99 (0.84,1.16)
	I ² (%)	33.9	45	-	0	63.8	63.8		0
	P ^a	0.3	0.22	1	0.86	0.3	0.86		0.3
	P ^b		0.36		0.1		0.1		
Wheeze	N	5	4	1	3	2	2	1	2
	RR (95% CI)	0.92 (0.58,1.47)	0.80 (0.44,1.44)	1.37 (1.01,1.86)	0.65 (0.33,1.29)	1.38 (1.05,1.82)	0.48 (0.22,1.05)	0.69 (0.37,1.30)	1.38 (1.05,1.82)
	I ² (%)	72.8	71.4	-	33.2	0	58.4	-	0
	P ^a	0.73	0.45	0.04	0.22	0.02	0.07	0.1	0.02
	P ^b		0.7		0.18		0.16		
Eczema	N	5	4	1	3	2	2	1	2
	RR (95% CI)	0.94 (0.77,1.14)	0.89 (0.70,1.15)	1.08 (0.69,1.69)	0.81 (0.53,1.26)	0.99 (0.79,1.25)	0.86 (0.40,1.83)	0.71 (0.42,1.20)	0.99 (0.79,1.25)
	I ² (%)	1	15.8	-	36.1	0	60.8	-	0
	P ^a	0.5	0.38	0.74	0.36	0.94	0.69	0.2	0.94
	P ^b		0.35		0.48		0.36		

Only data reported last time point of each unique RCT were pooled in the subgroup analysis. N: number of studies; RR: risk ratio; CI: confidential interval. I^2 is for statistical heterogeneity within studies. P^a is for heterogeneity. P^b is for interaction between subgroups with meta-regression.

Table 5: Sensitivity analysis of the allergic disease outcomes

		Up to 3 years			Beyond 3 years		
		n	RR (95%CI)	I^2 (%)	n	RR (95%CI)	I^2 (%)
Any allergy	Pooling model						
	Random effects	3	0.70(0.47,1.04)	64.8	2	0.98(0.82,1.16)	0
	Fixed effects	3	0.78(0.63,0.96)	64.8	2	0.98(0.82,1.16)	0
	Removing studies with high risk	2	0.77(0.46,1.27)	72.3	2	0.98(0.82,1.16)	0
Wheeze	Pooling model						
	Random effects	5	0.82(0.54,1.26)	66.2	2	1.03(0.53,2.00)	72.7
	Fixed effects	5	0.93(0.78,1.11)	66.2	2	1.20(0.91,1.58)	72.7
	Removing studies with high risk	4	1.00(0.71,1.13)	36.0	2	1.03(0.53,2.00)	72.7
Eczema	Pooling model						
	Random effects	5	0.86(0.68,1.15)	35.0	2	0.90(0.60,1.35)	29.6
	Fixed effects	5	0.88(0.75,1.05)	35.0	2	0.91(0.64,1.27)	29.6
	Removing studies with high risk	4	0.89(0.76,1.12)	0.0	2	0.90(0.60,1.35)	29.6