A Dose–Response Meta-Analysis Between Maternal Fish Oil Supplement And Risk of Asthma/Wheeze In Offspring

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Abstract

Background: Prenatal exposure to omega-3 polyunsaturated fatty acids (n-3 PUFA) present in oily fish may prevent asthma or wheeze in childhood.

Objective: By limiting this systematic review to fish oil intervention that commenced in the gestational period, we aim to find more clear evidences about the relationship between supplement with fish oil during pregnancy and the risk of asthma/wheeze in offspring, and to improve the life satisfaction of children who suffered asthma.

Methods: A comprehensive literature search was conducted in the following database: PubMed, Medline, Web of Science, the Cochrane library, and Embase up to February 2021. Two reviewers independently selected studies, extracted data of the characteristics, and assessed risk of bias. Eight randomized controlled trials totaling 3,037 mother-infant pairs were analyzed in the end. “Allergic asthma” and “asthma and/or wheeze” were assessed in our meta-analysis. Subgroup analysis and sensitivity analysis were conducted. Dose–response data was examined using the robust-error meta-regression method.

Results: This meta-analysis showed that n-3 PUFA during pregnancy did not significantly reduce the risk of asthma/wheeze (RR 0.93; 95% CI 0.82 to 1.04, p=0.21) and allergic asthma (RR 0.66, 95% CI 0.24 to 1.86, p=0.44). Subgroup analyses revealed that the risk of childhood asthma/wheeze was significantly decreased: (1) in Europe (RR 0.69; 95% CI 0.53 to 0.89), (2) when the dose was ≥ 1200 mg/d (RR 0.69; 95% CI 0.55 to 0.88), (3) when supplementation started after gestational age 22 (RR 0.65; 95% CI 0.50 to 0.85), (4) when supplementation was from pregnancy to lactation (RR 0.69; 95% CI 0.51 to 0.95). Furthermore, the linear dose–response analysis showed that when maternal supplementation of n-3 PUFA increased by 100mg/d, the risk of asthma/wheeze was reduced by 2%.

Conclusions: Although perinatal replenishment of n-3 PUFA did not prevent allergic disease in offspring, under some conditions, it could reduce the incidence of asthma/wheeze and allergic asthma in children, and the higher the dose, the better the protective effect it has. Additional research is needed to confirm the hypothesis of a link between n-3 PUFA intake and prevention of childhood asthma/wheeze.

Background

As the most common allergic disease in childhood, the prevalence of asthma has increased rapidly in the past 20–30 years [1–4]. Many asthma children’s life satisfaction had been affected, not only physical activity was limited, but also emotional and mental health had been impaired[5–9]. These effects create huge burdens on families, society, and medical care systems.

The modern diet life style, especially the balance of n-3 polyunsaturated fatty acids (n-3 PUFA), is widely accepted as a reason for an increased risk of allergic disease, although genetic, epigenetic, and environmental factors likely also contribute [10–11]. In many countries, high consumption of vegetable oils and meat results in an increased intake of n-6 PUFA and arachidonic acid (AA, 20:4, n-6), respectively. Diets high in n-6 PUFA lead to a high concentrations of AA in tissues. Arachidonic acid gives rise to prostaglandin and leukotrienes, both of which are highly active mediators of inflammation and allergic reactions [12]. Conversely, n-3 PUFA has multiple anti-inflammatory actions, such as reduce leucocyte chemotaxis, adhesion molecule expression and leucocyte-endothelium interaction, decrease production of inflammatory cytokines and the reactivity of T-cell, and increase production of eicosanoids with lower biological potency and inflammation resolving resolvins from eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [13].

Observational studies have shown that maternal fish intake during pregnancy is associated with decreased asthma/wheeze in children [14–19]. However, the results of randomized controlled trials (RCTs) are inconsistent; some trials showed a beneficial effect [20, 21], whereas others have not demonstrated benefit [22–25].

To obtain better clinical evidence, many systematic reviews have been performed. There are two systematic and meta-analyses [26, 27] of observational studies assessing maternal fish oil intake in relation to risk of some allergic diseases, while the strength evidence of which was not better than results from randomized controlled trials. And there are three systematic and meta-analyses of RCTs [28–30]. Two of them evaluated maternal omega-3 PUFA intake in relation to risk of allergic disease in offspring [28, 29], but the analysis on asthma/wheeze was not adequate. Another systematic review conducted by Lin et al. [30] found a protective effect of prenatal fish oil replenishment on wheeze/asthma in children, but the timeliness included in this meta-analysis is a little weak.
As described in this report, we undertook an updated systematic review to further measure a possible relationship between supplementation with omega-3 PUFA during pregnancy and the risk of asthma/wheeze in offspring, so as to improve the quality of life for children with asthma/wheeze.

**Methods**

This systematic review of RCTs was performed in conformity with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [31] that reported maternal fish oil intake during pregnancy and asthma/wheeze in children.

**2.1. Search Strategy**

A structured and comprehensive literature search was executed using the following databases from origin to 31 March 2021: PubMed, Medline, Web of Science, Embase, and the Cochrane library. We searched for relevant publications using the specific search criteria for each database based on PubMed search criteria as follows: The search terms in our search strategy were (((((((fatty acid) OR (omega 3 fatty acid)) OR (n-3 PUFA)) OR (n-3 fatty acid)) OR (n-3 polyunsaturated fatty acid)) OR (docosahexaenoic acid)) OR (fish oil)) OR (fish)) AND (((((pregnant) OR (perinatal)) OR (prenatal)) OR (antenatal)) OR (maternal)) OR (gestational))) AND (((((child) OR (offspring)) OR (infant)) OR (adolescent)) OR (youth))) AND (((((asthma) OR (wheeze)) OR (respiratory)) OR (Immunoglobulin E-mediated hypersensitivity)) OR (atopic)) OR (allergic)).

**2.2. Study Selection**

The meta-analysis included studies that met the criteria below: (1) Design: randomized controlled trial; (2) Participants: pregnant women and their children; (3) Fish oil group: supplement of capsules rich in n-3 PUFA or salmon commenced in the gestational stage; (4) Control group: supplement with placebo (e.g., olive oil, soya bean oil, vegetable oil); (5) Outcomes: prevalence of asthma/wheeze, and allergic asthma.

Two authors independently assessed articles for inclusion. Any discrepancies were resolved by discussion and, if necessary, by third-party arbitration.

**2.3. Outcome measures**

For this current review, the primary outcome was the prevalence of asthma/wheeze, defined as either a clinical diagnosis, a parental report of asthma symptoms, at least three instances of wheezing in the previous two years, or parental report of physician diagnosis of asthma.

The secondary outcome was prevalence of allergic asthma, defined as asthma with the presence of IgE antibodies or a positive skin-prick test.

**2.4. Data Extraction and Bias Assessment**

Data were extracted using a standardized table. Extracted data included first author with year of publication, study location, study design, participants, intervention, placebo, length of follow-up, and incidence of asthma/wheeze and allergic asthma.

The quality of studies was assessed using Cochrane Collaboration Risk of Bias Instrument [32]. A couple of authors investigators extracted the data from the selected studies and assessed quality of the articles, independently. If there were any differences or discrepancies, they would be settled through a third party discussion.

**2.5. Data Synthesis and Analysis**

Relative risk (RR) with 95% confidence interval (CI) was employed to assess the effects of n-3 PUFA supplementation during pregnancy on the incidence of allergic disease in offspring. Hazard ratio (HR) and incidence rate ratio (IRR) were directly regarded as RR. Odds ratio about the “allergic asthma” reported by Hansen et al. was considered as RR due to the lack of details [21]. When RRs were not reported in the article, we calculate the crude RRs according to the events/total of included studies.

$I^2$ statistics were employed for quantifying the potential variability among included studies. When the heterogeneity was not obvious ($I^2 \leq 50\%$), the fixed-effect model was implemented to summarize the results. When there was substantial heterogeneity ($I^2 > 50\%$), the meta-analysis was undertaken by a random effect model [33]. Egger's test were conducted in this meta analysis to evaluate potential
publication bias. Dose–response data using the robust-error meta-regression method was implemented by Stata 15.1/SE. A funnel plot was not executed because the number of studies was less than ten.

The above analyses and forest plots contained in this review were performed by the Stata 15.1/SE. The “risk of bias graph” was created by Review Manager 5.3.

**Results**

### 3.1. Literature search

From the database search, we initially identified 3,310 publications from online-search in PubMed, Medline, Web of Science, Embase, and the Cochrane library. After deleting duplicate articles, 1,302 publications remained. Of that remainder, we removed 985 publications after reading the titles and 259 publications were removed after two independent reviewers read abstracts; this process resulted in 58 publications that required reading in full. Forty-eight full-text publications were rejected for various reasons. Finally, we selected 10 studies from 8 unique randomized controlled trials with the longest follow-up time for each outcome. Among them, 32 articles did not report the outcomes we needed, 8 articles were observational studies rather than RCTs, and 8 articles were not supplemented with n-3 long-chain PUFAs. Flow diagram named Fig. 1 elaborated the details of the study selection and progress of RCTs.

### 3.2. Participants

This review was composed of data for 3,553 women who had been pregnant, and 3,037 mother-infant pairs completed the randomized controlled trials. There were 1,603 women in the experimental groups and 1432 women in the control groups. All participants were recruited from clinics and hospitals. The selected studies were conducted worldwide, three studies in Australia [22, 34, 35], two randomized controlled trials from North America [25, 35], and five studies performed in Europe [20–21, 23–24, 37].

Participants in four randomized controlled trials [22–24, 34] had an atopic disease or a family history of allergic disease. Two trials [20, 21] included pregnant women who did not have medical problems. One trial included atopic and non-atopic mothers [36], and one trial recruited women with depression [25]. The length of follow-up was from 6 months to 24 years. Table 1 presents the characteristics and results of these randomized controlled trial studies.

Table 1. RCTs of maternal n–3 PUFA supplementation during pregnancy and allergic disease in the offspring.

### 3.3. Intervention

In six of the eight unique randomized controlled trials, the participants were divided into an experimental group and a control group [20, 22–24, 34–36], whose experimental group received salmon or fish oil, and control group received olive oil, vegetable oil, corn and/or soy oils, or nothing. In two trials [21, 25], the participants were divided into three groups. In one trial [25], an eicosapentaenoic acid-rich fish oil group, a docosahexaenoic acid-rich fish oil group, and a control group (soy oil) were constituted. The other trial included [21] one experimental group (fish oil) and two control groups (olive oil and no oil). The amount of daily n-3 PUFA supplementation was from 400 mg to 3700 mg.

In six of the trials, omega-3 polyunsaturated fatty acid supplementation commenced at gestational ages 12 to 30, and supplementation continued until delivery [21, 22, 24, 25, 34, 35]; in two trials, supplementation continued into lactation period [20, 23]. The duration of intervention was from 10 to 29 weeks, after statistical calculation.

### 3.5. Quality of randomized controlled trials

A bias risk assessment was performed employing the modified models of the Cochrane Collaboration Risk of Bias tool for intervention trials for the following six aspects, such as selection bias, performance bias, measurement bias, attrition bias, reporting bias, and other bias[32]. Individual item was assessed as low-risk, high-risk or unclear (not given).

The generation of random sequences was assessed as low-risk in all studies. Nine studies [20–23, 25, 34–37] showed ample allocation concealment, and one study [24] did not describe the allocation concealment; thus, it was not possible for the experimental subjects and researchers to predict the results of the study. The pregnant women and medical staff were blinded in seven studies and unblinded in three studies. In the unblinded studies, the pregnant women in the control group did not receive any supplement [21, 24, 37]. Outcome assessors, investigators, and research workers were blinded in all studies. The data were clearly explained in five studies,
| First author, country, year | Setting and participants | Intervention and timing | outcomes | Follow-up (age; completed/enrolled, n; rate, %) | Results |
|-----------------------------|--------------------------|-------------------------|----------|------------------------------------------------|---------|
| Dunstan et al. Australia, 2003 | n=98 pregnant, atopic women, were recruited between January 1999 and September 2001 in Western Australia. | Fish oil group: fish oil capsules 4x1g/d; 3700mg n-3 LCPUFA (56.0% DHA, and 27.7% EPA) | Asthma; recurrent wheeze. | 1 year; 83/98; 84.7% | No difference was seen in the incidence of recurrent wheeze (10/40 vs. 12/43). No difference was seen in the incidence of asthma (2/40 vs. 6/43). |
| Olsen et al. Denmark, 2008 | n=533 Danish pregnant women with singleton pregnancies through antenatal care clinics in 1990. | Fish oil capsules, 4x1g/d (32% EPA, 23% DHA). Control 1: olive oil capsules. Control 2: no oil capsules. | Asthma of any types; allergic asthma | 16 years; 528/533; 99.1% | Asthma (any type) was significantly reduced in the fish oil group compared with the olive oil group (8/263 vs. 11/136; P=0.03). There was a significant effect on allergic asthma (OR 0.27; 95% CI, 0.08-0.91; P=5.03). |
| Furuhjelm et al., Sweden, 2011 | N=145 pregnant women, at risk of having an allergic infant, were recruited through antenatal care clinics in 2003–2005, in Sweden. | The -3 group: nine capsules (35% EPA, 1.6 g/day and 25% DHA, 1.1 g/day). The placebo group: Nine soya bean oil capsules. | Any asthma; IgE-associated asthma. | 2 years, 143/145; 98.6% | No difference in the prevalence of any asthma and IgE-associated asthma between the intervention groups (7/54 vs. 8/65; 2/54 vs. 4/64, respectively; NS). |
| Authors                | Population | Intervention                   | Outcome | Results                                                                 |
|------------------------|------------|--------------------------------|---------|-------------------------------------------------------------------------|
| Noakes et al.,         | N=123      | The salmon group: 2 portions   | 6 months| No significant differences in the incidence of wheeze were observed      |
| United Kingdom, 2012   | pregnant women in the area of Princess Anne Hospital (Southampton, United Kingdom) | per week of farmed salmon contained 1.14g EPA, 2.32g DHA. | 86/123;69.9% | between the groups (11/46 vs. 7/37).                                    |
|                        |            | control group: continue their habitual diet. | From 20 weeks GA until delivery. |                                                                         |
| Palmer et al.,         | N=706      | The n-3LCPUFA group: 3×500 mg capsules/d, 800mgDHA, 100mgEPA. | 3 year  | No significant differences were seen in the incidence of IgE-associated asthma (6/368 vs. 14/338). |
| Australia, 2013        | infants, at high hereditary risk of developing allergic Disease, whose mothers were participating in the DOMInO trial, were recruited from 20th March 2006 and to 8th May 2008, in South Australia. | control group: 3×500mg vegetable oil capsules. | 638/706;90.4% |                                                                         |
|                        |            | From 21 weeks GA to birth. |                                             |                                                                         |
| María Consuelo et al., | N=1,094    | DHA group: 2 capsules/d, contained 400mg DHA/d. | 18 months | No statistically significant protective effect of DHA treatment in the offspring of maternal atopic, compared with the placebo group was observed on the incidence of wheeze (IRR,0.88; 95% CI, 0.64 to 1.21; p=0.42), and the same to the offspring whose mothers are non-atopic (IRR,1.03; 95% CI, 0.83 to 1.27; p=0.80). |
| Mexico, 2014           | pregnant women were recruited between February 2005 and February 2007, in Mexico. | Placebo group: 2 capsules/d, contained a mixture of corn and soy oil. | 869/1094;79.4% |                                                                         |
| Study | Participants | Intervention | Outcome | Results |
|-------|--------------|--------------|---------|---------|
| Berman et al., United States, 2016 | N=118 women, both with and without history of allergic disease, were recruited in the United States. | Group 1: EPA-rich fish oil (1060 mg EPA plus 274 mg DHA). Group 2: DHA-rich fish oil (900 mg DHA plus 180 mg EPA). The placebo: soy oil. | Asthma/wheezing 36months | 84/118; 71.2% No significant differences were seen in the incidence of asthma/wheeze in the offspring between the fish oil group and the placebo group (14/57 vs. 7/27). |
| Best et al., Australia, 2016 | N=706 children, born to mothers who participated in the (DOMInO) RCT, with a family history of allergic disease, were recruited in South Australia. | The n-3 LCPUFA group: 500 mg fish oil capsules (800mg DHA/d, and 100mgEPA/d). Control:500mg vegetable oil capsules. | Wheeze symptoms with sensitization; parent-reported asthma ever 6years; | 603/706; 85.4% There was no difference between the n-3 LC-PUFA and control groups in the percentage of children with parent-reported asthma (79/367 vs. 73/336). No significant differences were seen in the incidence of wheeze symptoms with sensitization in the offspring between the groups (60/367 vs. 45/336). |
| Bisgaard et al., Denmark, 2016 | N=736 pregnant women recruited into the Copenhagen Prospective Studies on | Fish oil capsules, 4x1g/d; 2.4 g/d n-3 LCPUFA (55% EPA and 37% DHA); the control group: olive oil. | Persistent wheeze or asthma. From birth to date of submission (5–7 yr; mean age, 6.0 yr); not reported. | The risk of persistent wheeze or asthma in the treatment group was 19.0% vs. 29.2% in the control group (HR, 0.65; 95% CI, 0.47 to 0.91; P = 0.011). |
Asthma in Childhood 2010 (COPSAC2010) pregnancy cohort, between November 2008 and November 2010. From 24 weeks GA until 1 week after delivery. 647/695; 93.1% (5 years)

| Study | Participants | Intervention | Outcomes | Duration | Results |
|-------|--------------|--------------|----------|----------|---------|
| Hansen et al., Denmark, 2017 | n=533 Danish pregnant women with singleton pregnancies through antenatal care clinics in 1990. | Fish oil capsules, 4×1g/d (32% EPA, 23% DHA). Control 1: olive oil capsules. Control 2: no oil capsules. | Asthma medication used; asthma discharge diagnosis; allergic asthma. | From 30 weeks GA to delivery. | 24 years; 522/533; 98.0% Asthma medication prescribed was significantly reduced in the fish oil group compared with the olive oil group (31/262 vs. 28/134; P=0.02). There was a significant effect on allergic asthma (OR 0.27; 95% CI, 0.08-0.91; P=0.03). Asthma discharge diagnosis was significantly reduced between groups (8/262 vs. 13/134; P=0.01). |

GA, gestational age; HDM, house dust mite; LC-PUFA, long chain PUFA; NR, not reported; RCT, randomized controlled trial; NS, no significance, SPT, skin-prick test.

while not in the others [20, 22, 24–25, 35]. Five studies [20–21, 34–37] showed a low risk of reporting bias, and four studies had high risk of reporting bias [22–23, 25, 35]. In nine studies, there was not enough information to evaluate whether there were other risks of bias or whether current problems introduced bias, and only one study was rated as a low risk of other bias because of no obvious biases in the report [23]. Figure 2 shows the risk assessment of bias for all the studies.

3.6. Meta-analysis results of the trials

Asthma/wheeze

In the clinical practice, it is difficult to explicitly diagnose "asthma" in children because of the strict diagnostic criteria of asthma compared with wheeze [38, 39]. Therefore, we chose the prevalence of asthma/wheeze as the main outcome to evaluate the effect of fish oil intake during pregnancy. Four reports were from two randomized controlled trials; thus, we assessed eight studies with longer follow-up for the incidence of asthma/wheeze.

Six trials did not reveal any significant differences for asthma/wheeze between the fish oil group and the placebo groups [22–25, 34–35]. Two trials showed significant protective effects of the intervention during pregnancy [20, 21]. The pooled data of the eight trials [20–25, 34–35] showed that consumption of n-3 PUFA during pregnancy did not have a significant protective effect against asthma/wheeze compared with placebo (RR 0.93; 95% CI 0.82 to 1.04; p = 0.21) (Fig. 3-A).

Allergic asthma

In three randomized controlled trials, investigators reported the effect of n-3 PUFA intake on the incidence of allergic asthma [21–22, 34], and only one trial showed a significant association between maternal fish oil intake and childhood allergic asthma [21].
pooled data of three trials [21–22, 34] indicated that the prevalence of allergic asthma in children was not reduced in experimental group compared with the placebo controlled group (RR 0.66, 95% CI 0.24 to 1.86, \( p = 0.44 \); Fig. 3-B).

**Subgroup analysis**

We analyzed the outcomes by stratified study location, family history of allergic disease, gestational stage when supplementation commenced, duration of supplementation, and age of offspring.

The risk of our main outcome endpoint in offspring, asthma/wheeze, was notably decreased under the following conditions: (1) in Europe, (2) with \( \geq 1200 \) mg/d, (3) when gestational age supplementation started after 22 weeks, (4) and when supplementation continued into lactation. Table 2 shows the detailed results of asthma/wheeze subgroup analysis. We found that prenatal n-3 PUFA supplementation could decrease the incidence of allergic asthma in preschool children (aged 6 years or less). Table 3 presents the detailed information about the age subgroup of allergic asthma.

**Table 2**

Subgroup analyses of n-3 PUFAs supplementation during pregnancy on the incidence of asthma / wheeze.

| Asthma / wheeze                        | Subgroup classification | Number of studies | RR (95% CI)      | P  | \( I^2 \) (%) |
|----------------------------------------|-------------------------|-------------------|------------------|----|---------------|
| Australia                              | 2                       | 1.07 (0.85, 1.34) | 0.57             |    | 0             |
| Study location                          |                          |                   |                  |    |               |
| Europe                                 | 4                       | 0.69 (0.53, 0.89) | \(< 0.01\)       | 17.4|               |
| North America                          | 2                       | 0.97 (0.82, 1.16) | 0.78             |    | 0             |
| Dose                                    |                          |                   |                  |    |               |
| \( \geq 1200 \) mg/d                   | 5                       | 0.69 (0.55, 0.88) | \(< 0.01\)       | 0  |               |
| \(< 1200 \) mg/d                       | 3                       | 1.02 (0.89, 1.18) | 0.74             |    | 0             |
| Family history of allergic disease      |                          |                   |                  |    |               |
| Yes                                    | 5                       | 1.01 (0.85, 1.21) | 0.89             |    | 0             |
| No                                     | 4                       | 0.86 (0.73, 1.01) | 0.07             | 64 |               |
| Gestational stage when supplementation commenced |                  |                   |                  |    |               |
| \( \leq 22 \) week                     | 5                       | 1.01 (0.89, 1.16) | 0.84             |    | 0             |
| \( > 22 \) week                        | 3                       | 0.65 (0.50, 0.85) | \(< 0.01\)       | 0  |               |
| Duration of supplementation             |                          |                   |                  |    |               |
| Pregnancy                              | 6                       | 0.97 (0.85, 1.11) | 0.69             | 20.8|               |
| Pregnancy and lactation                 | 2                       | 0.69 (0.51, 0.95) | 0.02             |    | 0             |
| Age of offspring                        |                          |                   |                  |    |               |
| \(< 5 \) years                         | 5                       | 0.98 (0.83, 1.16) | 0.83             |    | 0             |
| 5–18 years                             | 3                       | 0.88 (0.73, 1.06) | 0.20             | 78.9|               |
| \( > 18 \) years                       | 1                       | 0.54 (0.32, 0.91) | 0.02             |    |               |

RR: risk ratio; CI: confidential interval. P is for statistical significance of the subgroup results. \( I^2 \) is for statistical heterogeneity within studies.
### Table 3

| Subgroup classification | Number of studies | RR (95% CI) | P   | I² (%) |
|-------------------------|-------------------|-------------|-----|--------|
| < 5 years               | 2                 | 0.43 (0.19, 0.99) | 0.047 | 0      |
| Age of offspring*       | 5–18 years        | 0.45 (0.05, 3.99) | 0.47 | 87.7   |
| > 18 years              | 1                 | 0.27 (0.08, 0.91) | 0.04 | /      |

RR: risk ratio; CI: confidence interval. P is for statistical significance of the subgroup results. I² is for statistical heterogeneity within studies.

### 3.7. Sensitivity analysis

Sensitivity analysis demonstrated an insignificant protective effect of n-3PUFA on allergic asthma, which changed to significant risk ratio 0.35 (95% CI 0.13 to 0.95) after omitting the study of Best et al [34]. Sensitivity analysis did not show significant changes in the outcome of asthma/wheeze for which the RRs ranged from 0.87 (95% CI 0.76–1.01) to 0.98 (95% CI 0.86–1.11). Appendix 1 contains a detailed record of the sensitivity analysis.

### 3.8. Dose-response analysis

In this study, RMER (robust error meta-regression) model was used for dose-response regression analysis. The p of non-linear chi square test was 0.3212, so we chose linear model for dose analysis. The results showed that there was a linear dose-response relationship between the daily fish oil supplement dose during pregnancy and the incidence of asthma/wheeze, and the higher the dose, the lower the incidence, and when perinatal n-3 PUFA supplementation increased by 100mg/d, the risk of asthma/wheeze was reduced by 2%. Details of linear regression model was in the following Fig. 4, and other detailed data and code of Stata on dose-response analysis was shown in Appendix 2.

### 3.9. Publication bias

No statistically significant publication bias was suggested by the outcome of Egger's test of asthma/wheeze (p = 0.54) and allergic asthma (p = 0.34) for each outcome in this meta-analysis.

### Discussion

The current systematic review did not show an explicit relationship between prenatal intake of n-3 PUFA and the prevalence of asthma/wheeze in all offspring, which was similar to a recent study by Vahdaninia et al. [29] and as summarized in a review by Best et al. [28]. Differently, Lin et al. reported a protective effectiveness of prenatal fish oil replenishment on wheeze/asthma in children in his meta-analysis [30], while two RCTs [23, 36] in which were not included, especially one large-sample RCT performed in Mexico [36].

The result of subgroup analysis was interesting. Studies in Europe showed a protective effect on wheeze/asthma in offspring. For children without family histories of allergic diseases (i.e., medically diagnosed allergic disease, e.g., eczema, asthma, or hay fever), maternal supplementation with fish oil in gestational period did not show any advantage for prevention of asthma/wheeze after removal of a study conducted in Mexico, a large sample trial included in our review (at the same time, the heterogeneity decreased from 64–0%; Appendix 3). We attribute those changes to differences in ethnicity and environment. And the association between the prevalence of allergic diseases and race/ethnicity has also been confirmed in other studies [40–43]. Further research is needed to explore whether fish oil replenishment during pregnancy benefits children in an ethnicity-dependent manner.

Compared with the effective dose of 2000 mg/d reported in previous reviews [30], we found that only 1200 mg DHA and/or EPA per day significantly prevented asthma/wheeze. Further studies should more thoroughly examine by detailed dose-response meta-analysis the effective dose of maternal fish oil intake.

Supplementation of fish oil that began after 22 weeks gestational age and continued into early lactation significantly reduced the prevalence of asthma/wheeze in offspring. We hypothesized that n-3 PUFA might have different activities in embryo development at different stages of pregnancy because pro-inflammatory immune cell genes are expressed in late pregnancy, and the period of late pregnancy has a key regulatory function in inflammatory and immune system development [44, 45]. The immune system of a newborn is highly malleable. If the immune system does not receive appropriate signals, the neonate will incur susceptibility to allergic diseases...
Therefore, we speculate that there is a “window of opportunity” in early life during which the immune system may be acted upon by fish oil and thereby limit susceptibility to allergic diseases.

Phenotypes of asthma in children are commonly associated with allergy, and the incidence of allergic asthma will decline to some extent with advancing age [49, 50, 51]. We found supplementation of fish oil reduced the prevalence of allergic asthma in preschool children (< 5y) in subgroup analysis, but we did not find any relevant studies to explain this phenomenon. More research is needed to measure the relationship between maternal fish oil supplement and the onset of allergic asthma in childhood.

Our meta-analysis had several advantages. All included studies were recent randomized controlled trials with large sample sizes. We performed subgroup analysis and sensitivity analysis to assess potential confounding factors and evaluate stability of the outcomes. The main strength of this systematic review was that we used the most precise definition and the broadest definition of asthma – “allergic asthma” and “asthma/wheeze” as our outcome variables. However, because it was based on randomized controlled trials, our systematic review had some limitations. The primary studies had different protocols that may have affected our findings to some extent. For example, the baseline characteristics of the pregnant women were different, and the dose of intervention and the diagnosis of outcomes were also different.

Because of a lack of new and large-sized randomized controlled trials, possible confounding factors and potential bias, the hypothesis that linking maternal n–3 PUFA intake to protection against childhood asthma/wheeze or allergic asthma cannot be accepted or rejected absolutely. Ethnicity, dose, susceptibility of n-3 PUFA, and time of supplementation should be assessed in the future. Large-sample, multi-center, and randomized controlled trials need to be conducted to better comprehend the efficacy of supplementation with n-3 PUFA in pregnancy for protecting against asthma/wheeze or other relevant allergic diseases.

**Conclusion**

The available evidence showed prenatal replenishment of n-3 PUFA may reduce the incidence of asthma/wheeze or allergic asthma in offspring under certain conditions, and the dose-response analysis showed that the higher the dose, the stronger the protective effect it has. More high-quality and large sample size RCTs should be performed in the future to examine the effects of reasonable dose of prenatal n-3 PUFA intake and asthma/wheeze in offspring, especially in different races, location.

**Abbreviations**

N-3 PUFA, omega-3 polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RCTs, randomized controlled trials; PRISMA, Systematic Reviews and Meta-Analyses; RR, Relative risk; CI, confidence interval; HR, Hazard ratio; IRR, incidence rate ratio.

**Declarations**

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and materials: The datasets analyzed in this study are available from the corresponding author on reasonable request.

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**Figures**

![Flowchart of the database search process.](image)

**Figure 1**
Flow diagram depicting the study selection and progress of RCTs identified in the systematic review and meta-analysis. RCT, randomized controlled trial.

**Figure 2**

Assessment of risk of bias for included RCTs.

**Figure 3**

Effect of n-3 LC-PUFA supplementation during pregnancy compared with placebo on the incidence of asthma and/or wheeze (A) and allergic asthma (B) of children. The pooled estimate was obtained using a fixed-effects model depending on the heterogeneity test. Squares represent RRs and error bars represent 95% CIs. The diamond represents the overall effect estimate. The size of the shade square is proportional to the percent weight of each study.
Figure 4

Details of linear regression model was in the following Figure.

**Supplementary Files**

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