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[Page 1] Title: The role of fish intake on asthma in children: A meta-analysis of observational studies.

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Supplement: Quality assessment tool (1)

Supplement: Meta-analysis-Forest plots (6) (Fig(s): S1-S6)

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Supplement: Table S1

[Page 2] *Pediatr Allergy Immunol.*

The role of fish intake on asthma in children: A meta-analysis of observational studies

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ABSTRACT

BACKGROUND

The evidence is mixed on the use of long chain omega 3 fatty acids in the prevention and management of childhood asthma.

METHODS

We conducted a systematic search and meta-analysis investigating the role of fish intake, the main dietary source of long chain omega-3 fatty acids, on asthma in children.

RESULTS

A total of 1,119 publications were identified. Twenty-three studies on fish intake in association with childhood asthma were included in the final review. In 15/23 studies early introduction of fish (6-9 months) and regular consumption (at least once a week) improved asthma symptoms and reduced risk in children 0-14 years as compared to no fish consumption; 6/23 showed no effect and 2/23 studies suggest adverse effects. Meta-analysis revealed an overall 'beneficial effect' for 'all fish' intake on 'current asthma' [OR: 0.75; 95%CI: 0.60-0.95] and 'current wheeze' [OR: 0.62; 95%CI: 0.48-0.80] in children up to 4.5 years old. An overall protective effect of 'fatty fish' intake as compared to 'no fish' intake in children 8-14 years old was also observed [OR: 0.35; 95%CI: 0.18-0.67].

CONCLUSION

This meta-analysis suggests that introduction of fish early in life (6-9 months) and regular consumption of all fish (at least once a week) reduces asthma and wheeze in children up to 4.5 years old, while fatty fish intake may be beneficial in older children. Future well-designed clinical trials are recommended to confirm the promising findings documented in this literature analysis.

Keywords: asthma, children, fish, nutrition, oily fish, omega 3 fatty acids

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INTRODUCTION

Despite advances in pharmacotherapy, globally, the asthma epidemic in children is a major public health problem as it continues to rise. Asthma is associated with morbidity, substantial health care costs and decreased quality of life (1, 2). There is no cure for asthma, symptoms can only be controlled by medication which on a long-term basis may cause side-effects. Therefore, identifying potential non-pharmacological interventions which improve management of asthma is of great public health significance.

The modern diet is low in fruits, vegetables, fish and high in refined grains, red meat and fast food (3) which may have contributed to the high prevalence of asthma in Westernized countries (1). As a result today's diet is deficient in anti-inflammatory nutrients (from fruit/vegetables) and long chain omega 3 fatty acids (LC n-3 PUFA) (obtained from fish) and high in omega 6 fatty acids mainly from processed fats and oils such as margarine and vegetable oils (3, 4). It is believed that the change in anti-inflammatory status, fatty acid composition, increase in omega 6 : omega 3 fatty acid ratio is associated with an increase in oxidative stress and airway inflammation promoting the development and symptoms of asthma (5, 6).

[Page 4] There has been considerable interest in the potential therapeutic and protective properties of omega-3 fatty acids (EPA/DHA) in the pathogenesis of asthma due to anti-inflammatory and immune-modulating properties (4, 7). To date, the evidence for the beneficial effects of omega 3 fatty acid intake in asthma is controversial and intervention trials investigating the impact of fish or fish oil supplementation in children are lacking (8). Most of the epidemiological evidence which gave rise to the hypothesis that marine omega 3 fatty acids may have a protective effect is based on studies documenting that regular consumption of fish has a prophylactic effect. (9-11). In addition, many systematic reviews have investigated the impact of omega 3 fatty acid intake during pregnancy on allergy outcome in offspring suggesting a beneficial effect (12-14). Little is known about fish intake on asthma during childhood (post-infancy) and this warrants further investigation.

The purpose of this study is to conduct an up-to-date systematic search, qualitatively synthesize the available evidence and perform a meta-analysis to determine the role of fish (lean and fatty) on childhood asthma. The impact of fish intake during pregnancy is beyond the scope of this review.

METHODS

This review was conducted according to PRISMA guidelines (15) (Supplement 4).

Literature Search strategy

We conducted a comprehensive literature search of publications up to July 2017 using the following bibliographic databases: Cochrane Central Register of Controlled Trials, MEDLINE, PUBMED, CINAHL (EBSCO), SCOPUS and EMBASE. No restrictions were applied on language or publication dates. Supplementary studies were sought from conference [Page 5] proceedings as well as clinical trials registries (International, European, Australia and New Zealand) in order to identify published/unpublished trials Other potential relevant citations were identified by hand search of the reference lists of relevant articles, reviews, systematic reviews and meta-analysis. Databases were searched for relevant publications, by a two-step search strategy using the following search terms:

Search 1: [“fish OR fatty fish OR oily fish OR omega 3 fatty acids OR n-3 long chain polyunsaturated fatty acids AND “childhood asthma”]

Search 2: [“fish OR fatty fish OR oily fish OR omega 3 fatty acids OR n-3 long chain polyunsaturated fatty acids AND “children” AND “asthma”].

The final search was a combination of both searches using the term “OR”. The full search strategy is provided in (Supplement 1).

Study eligibility criteria

Inclusion criteria

Inclusion criteria was based on Participants, Intervention, Comparator and Outcomes (PICO) (16)**.**

Type of participants

This systematic review considered publications that included children younger than 18 years old.

Type of intervention

Publications reporting the effect of fish as the primary exposure measurement

Outcome measures

Primary outcomes of interest were all asthma symptoms (wheeze, dyspnoea, shortness of breath) and prevalence or incidence of asthma.

[Page 5] A publication was considered when exposures were measured and the presence of asthma was based on the manifestation of symptoms, pulmonary tests or doctor-diagnosis

(17). In addition, studies examining the effect of omega 3 fatty acid intake on atopy in children were considered relevant when results on fish intake and asthma outcome in children could be separated.

Type of studies:

We considered experimental and epidemiological study designs including randomized controlled trials (RCTs), non-randomized controlled trials, before and after intervention studies, cohort, case-control, and cross-sectional studies.

Exclusion criteria

Exclusion criteria were based on PICO characteristics and reasons for exclusion are summarized in (Fig. 1). Publications not included were: reviews, systematic reviews, editorials, comments, letters, case-studies, animal studies, and those with no abstract, full-text or English translation available. Interventions or risk factors other than fish consumption such as: formula supplementation, dietary patterns, food groups, individual nutrients, fish oil, obesity, dust mites, as well as studies focusing on maternal diet or asthma genotypes were considered to be irrelevant.

Selection of studies

MP and CI initially screened the titles, abstracts or descriptors of all publications retrieved by the search and duplicates removed.

When there was insufficient information in the abstract to warrant exclusion of the article, the full-text of the article was retrieved. Full-text papers were then independently appraised by both reviewers for inclusion and details extracted. Discrepancies were resolved by discussion [Page 6] and consensus that led to agreement. The search was supplemented by cross-checking reference and bibliographies of relevant publications, systematic reviews and reviews.

[Page 7]

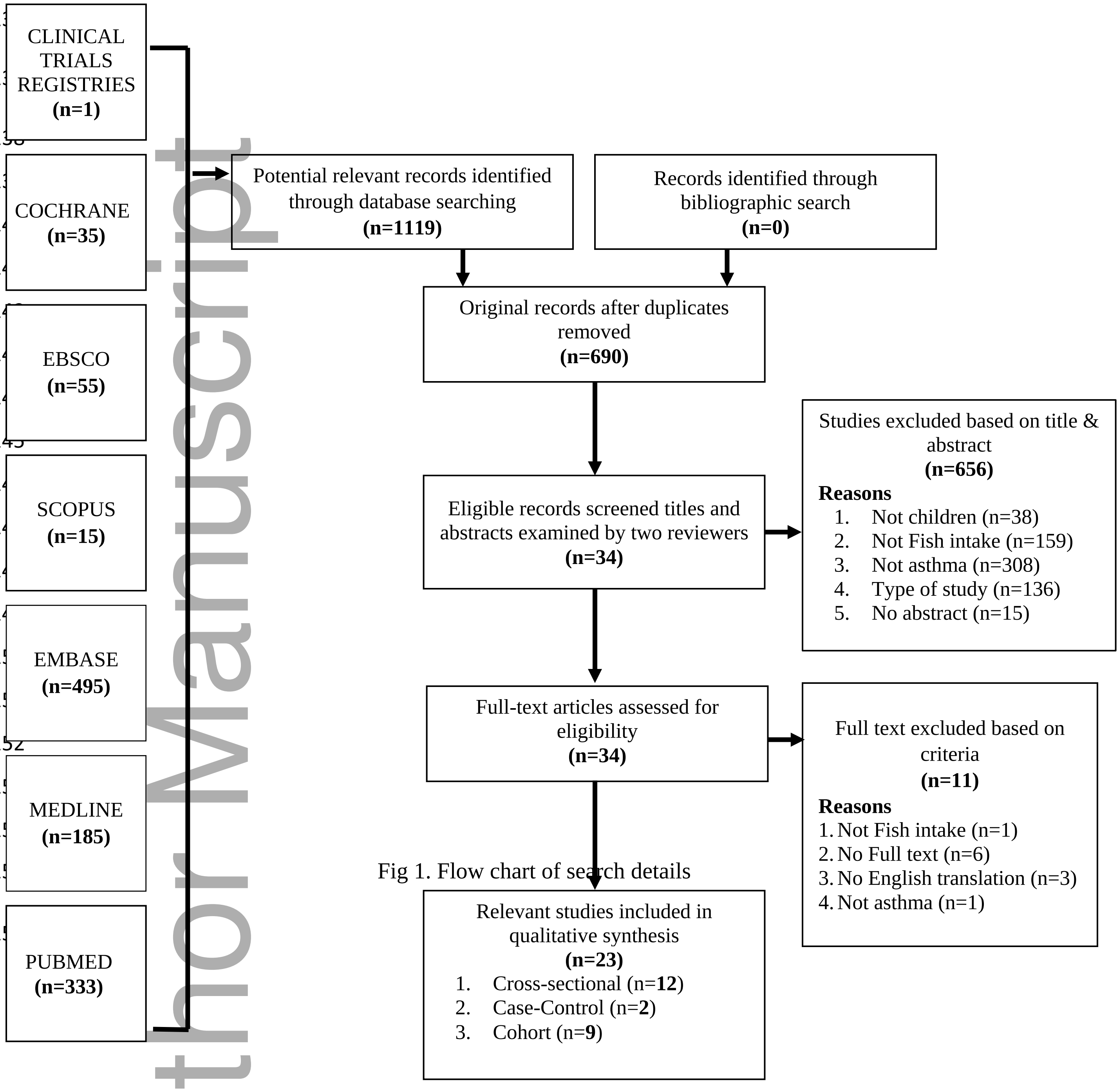


Fig 1. Flow chart of search details

[Page 8] *Quality assessment and risk of bias*

SKS and MP independently rated all publications included in the meta-analysis using a validated quality assessment tool according to Zaza et al (18) (Supplement 2). The scientific soundness was rated using twenty four validity questions with possible responses of 'yes' or 'no'. A value of one was assigned to 'yes' and zero to 'no'. The final score was presented as the percentage of total (maximum) probable scores for each study. Any discrepancies were addressed with mutual agreement between the authors or with a third reviewer (BE). Finally, the scores from both evaluators were averaged and presented as the final score. The observed median study quality score was used as the dividing point between low and high quality (19). We set the benchmark of 70% and above as a high quality study while below 70% as low quality.

Data Extraction

Data extracted included authors, year of publication, study name, study design, geographic location, sample size, age range of target population, follow-up, dietary exposure, outcome measures, dietary and respiratory assessment tools, confounders, main findings (risk estimates along with 95% CI and p-values).

Data Synthesis and statistical analysis

Study outcomes were synthesized and categorized into two groups based on the effect on asthma symptoms (improvement or no improvement). Quantitative data were pooled in statistical meta-analysis software using STATA software version 14.1 (Stata Corp, Texas). For the purpose of the meta-analyses, publications assessing 'all' types of fish and 'fatty fish' as the exposures and regular fish intake, at least once a week were deemed appropriate. Studies were included in the meta- analysis when effect size was expressed as odds ratio (OR) and their 95% confidence intervals (95%CI) were reported or could be calculated. Eligible publications [Page 8] were categorized into two groups based on the common outcome of interest "current asthma" or "current wheeze" as defined by ISAAC (20).

For the purpose of the meta-analysis and due to heterogeneity among study designs, sub-group analyses were conducted based on study design, age range, follow-up and type of fish consumed. Cut-offs for age was defined based on children's age range in publications.

Two assessments were conducted for each outcome. One was a combination of all study designs with 'all fish or 'fatty fish' exposure' and the other according to study design and similar age group. Heterogeneity was assessed using the standard Chi-square test and I^2 statistic (21). Considerable heterogeneity was considered to be an I^2 value > 75% (21) and statistical significance at the 5% level (22). We applied the random-effects model to estimate the pooled ORs and 95% CIs for 'current asthma and 'current wheeze'.

RESULTS

Electronic search:

The literature search identified a total of 1,119 potential publications of which 690 remained, after removal of duplicates (Fig. 1). No additional citations were found by cross-checking of reference lists. Only one ongoing RCT was identified from international clinical trial registries. Two reviewers (MP, CI) independently screened the 690 eligible papers by scanning titles and abstracts. A total of 656 were excluded based on publication type (136), no abstract (15) or on PICO criteria as they did not examine the effect of fish intake (160) in association with asthma symptoms (308) in children (37), leaving 34 full-text articles to be assessed for eligibility. Of the remaining 34 potential studies, 11 full-texts were deemed to be inappropriate (1 not intervention, 1 not asthma, 6 no full-text available and 3 no English translation), leaving a total of 23 relevant studies. Specifically, 12 cross-sectional (10, 23-33), 2 case-control (34, 35) and 9 cohort studies (11, 36-43) examining the effect of fish consumption on asthma symptoms in children.

 Study Characteristics

The database search identified 23 studies conducted from 1992 to July 2017 examining the effect of fish consumption on asthma in children. Two were undertaken in Australia (33, 34), Japan (28, 32), Spain (10, 25) and Finland (35, 41), three in Norway (38, 40, 44) and the Netherlands (31, 37, 43), four in Sweden (11, 36, 39, 45), one in China (30), Italy (26), France (23), Central Europe (29) and the ISAAC study which involved 20 countries globally (24). Collectively, 163,744 children and adolescents up to 15 years old were investigated with sample sizes ranging from 138 to 50,004. A variety of assessment methods were used to assess dietary intake in children. Twenty studies evaluated fish intake using a number of different questionnaires (10, 11, 23, 24, 26, 27, 29-34, 36-43) ranging from one question to questionnaires consisting of limited food groups/items and detailed Food Frequency questionnaires (FFQ) comprising of a wide-variety of foods that capture the entire dietary pattern. While two studies used a 3-day food record (25, 35) and one, a diet history questionnaire (28).

In all studies, consumption of 'all types' of fish (lean and fatty) were investigated, while in six studies the effect of fatty fish intake on asthma symptoms was measured (28, 34, 36-38, 42). Asthma was diagnosed using a questionnaire (10, 23-32, 34-39, 41, 43), parent-report (44), doctor-diagnosed (11, 37, 40) and in one study by bronchial hyperresponsiveness (BHR) (33). The characteristics of included studies are shown in (Supplement, Table S1). Overview of the literature search will be presented based on study design and age group.

Cohort Studies: 0-12 years of age.

The nine cohort studies identified, investigated the effect of early fish intake on asthma in 33, 673 children from birth until 12 years of age (11, 36-43). Seven studies revealed that early introduction and regular consumption of 'all fish' were associated with a reduction in asthma symptoms up to 12 years of age (11, 36-41) and two showed no effect (42, 43).

[Page 10] Two studies reported that early introduction of 'all fish' (at 6-9 months of age) and regular consumption ≥ 1/week) of 'all fish' (37) and 'fatty fish' (38) reduced asthma incidence (OR_c: 0.72; 95%CI: 0.55-0.93) (38), prevalence of wheeze (OR: 0.64; 95% CI: 0.43-0.94) (37) and use of medication (OR: 0.75; 95%CI: 0.58-0.96) (38) in children up to 4 years of age (37) (38). Conversely, Kiefte-de-Jong et al, noted that no fish during the first 12 months of life and introduction of 'all fish' between 0-6 months was associated with an increase in prevalence of wheezing [(OR: 1.57; 95%CI: 1.07-2.31), (OR:1.53; 95%CI: 1.07-2.19)] respectively at 48 months as compared to a reduction in the prevalence of wheezing with fish introduction at age 6-12 months (OR: 0.64; 95% CI: 0.43-0.94).

Beneficial effects of 'all fish' intake were also documented in children during the first 5 years of life. Fish consumption before 9 months had a protective effect for recurrent wheezing (OR: 0.6; 95% CI 0.4-0.8) (39), multiple trigger wheeze (OR: 0.6; 95%CI:0.3-0.99) (39), episodic viral wheeze (OR:0.6; 95%CI:0.4-0.99) (39) , asthma risk (OR: 0.73; 95%CI: 0.55-0.97) (11), (OR: 0.84; 95% CI=0.57-1.22) (40), all asthma ($p<0.001$) (41) and atopic asthma ($p<0.05$) (41) up to 5 years as fish intake increased from two to three times per month (OR adj= 0.82 95% CI: 0.54-1.29) to once a week (OR adj= 0.66, 95% CI: 0.43-1.01) and at least once a week (OR adj= 0.55; 95% CI 0.34-0.87; p trend=0.003) (11) a dose response was observed.

A Swedish cohort which involved 3285 children with follow-up till 12 years of age, regular 'all fish' intake (≥ 2-3 times/month) at age of 1 year was associated with reduced risk of prevalence (OR_{adj}: 0.71; 95%CI: 0.57-0.87) and incidence (OR_{adj}: 0.80; 95%CI: 0.65-0.98) of asthma up to age 12 years, after adjusting for confounders of parental history of allergic disease, sex, maternal smoking during pregnancy (36). However, no significant association was observed between fish intake at age 8 years and incidence of asthma at 12 years (p trend= 0.303) (36). This finding is in agreement with two other cohorts that documented no relationship among early, late or long-term intake of any kind of fish on asthma in children at ages 2 (42) (p=0.16) and 8 years (43). Although, Willers et al, did report that early fish intake was inversely associated to BHR in children at age 8 years old (43).

[Page 11] *Case-control studies: 5-15 years of age*

A beneficial effect of fish intake on asthma in children was observed in 2 case-control studies (34, 35). In a nested-cohort study which was a sub-group of the Finnish DIPP study, 182 children with asthma and 728 controls participated and were observed at 3, 6, 12 months and thereby annually up to 6 years (35). Overall, early introduction of ‘all fish’ and fish-products (6-8 months) was associated with a reduction in ‘all asthma’ risk (OR_{adj}: 0.87; 95%CI: 0.77-0.98) in children at 6 years adjusting for confounders. Another case-control study investigating the impact of fish intake on asthma symptoms in 584 children aged 8-11 years also reported a protective effect (34). After adjusting for confounders, children who ate fresh, ‘fatty fish’ at least once a week had a significantly reduced risk of current asthma (OR_{adj}: 0.26; 95%CI: 0.09-0.72) as compared to ‘any fresh’ fish (OR_{adj}: 0.52; 95% CI: 0.24-1.15) and ‘non-fatty’ fish (OR_{adj}: 0.68; 95% CI: 0.3-1.54) consumers. No reduction in asthma risk was observed with canned fish consumption.

Cross-sectional studies: 0-15 years

The literature search retrieved 12 cross-sectional studies undertaken in 128,577 children aged 0-15 years (10, 23-26, 28-33, 45). Five studies observed that regular ‘all fish’ intake (>1/week) was associated with a reduction in lifetime prevalence of asthma (OR_{adj}: 0.92; 95%CI: 0.78-1.08) (24), current wheeze [(OR_{adj}: 0.85; 95%CI:0.74-0.97)(24); (OR: 0.44; 95%CI: 0.21-0.93)] (31), current wheeze with atopy positive (OR_{adj}: 0.51; 95%CI: 0.32-0.81) (24), doctor-diagnosed asthma (OR: 0.54; 95%CI: 0.35-0.84) (45) , night-time breathlessness (OR: 0.36; 95%CI:0.17-0.78) (45), current asthma (OR: 0.51; 95%CI: 0.31-0.84) (45), (OR: 0.34; 95%CI: 0.13-0.85) (31), past-year wheeze (OR adj: 0.75; 95%CI: 0.53-0.93) (23), past-year wheeze and atopy negative (OR: 0.61; 95%CI: 0.43-0.87) (23), atopic asthma with BHR (OR:0.12; 95%CI: 0.02-0.66) (31), atopic wheeze with BHR (OR: 0.15; 95%CI: 0.03-0.63) (31) and BHR (OR:0.35; 95%CI: 0.1-0.9) (33). While one study (29) reported that, irregular fish intake [Page 11] (<1/month) was associated with an increase in ‘persistent cough’ (OR: 1.18; 95%CI:1.04-1.34), ‘wheeze ever’ (OR: 1.14; 95%CI:1.03-1.25) and ‘current wheeze’ (OR: 1.21; 95%CI: 1.06-1.39) A recent study undertaken by Xu et al, including 13, 877 children, 0-14 years old, observed that fish and shrimp intake triggered asthma episodes by

14% ($p<0.05$) (30), although estimated effect size was lacking. Adverse effects of 'all fish' intake were also reported in a Japanese study of 1,673 asthmatic children and 22,109 controls aged 6-15 years (32). After adjustment for confounders (age, gender, parental asthma), a statistically significant higher prevalence of asthma was observed in children consuming fish 1-2 times/week (OR_{adj}: 1.117; 95%CI: 1.005-1.241) and more than or equal to 3-4 times/week (OR_{adj}: 1.319; 95%CI: 0.896-1.943) than among those who consumed no fish (OR_{adj}: 1.039; 95%CI: 0.785-1.376).

Quality assessment

Twenty one out of 23 studies were rated as 'high quality'. The average quality score was 82.1% with the highest score 100% and the lowest score 64% (Table 1). Most of the cohort studies included in the meta-analysis were of high quality, 8 out of 9 studies scored above 90%. The studies that were rated lower quality were due to their failure to control for potential confounders and address possible biases. Using t-test, we checked if there were any differences in the quality score for the selected studies between two reviewers and found no major difference in average score (p-value=0.33).

[Page 13] Table 1. Summary table of Quality Assessment of relevant studies in this systematic review (Zaza, 2000).

Study	Reviewers	Study design	Population Density	Population Description	Time points	Entire sampling or Probability sampling	Eligibility Criteria	Exposure Measures		Outcome Measures		Statistical Test Suitable	Adjustment For Confounders	Bias Discussed	Bias not covered	Overall Average Score (%)
								Validity	Reliability	Validity	Reliability					
Peat et al, 1992	SKS/MP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	✗	67.5%
Hodge et al, 1996	SKS/MP	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✗	✗	70.5%
Takemura et al, 2002	SKS/MP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	83.0%
Farchi et al, 2003	SKS/MP	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✗	73.0%
Nafstad et al, 2003	SKS/MP	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	91.0%
Kim et al, 2005	SKS/MP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	79.5%
Kull et al, 2006	SKS/MP	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	95.0%
Tabak et al, 2006	SKS/MP	✓	✗	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✗	75.0%
Chatzi et al, 2007	SKS/MP	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	82.0%
Antova et al, 2003	SKS/MP	✓	✓	✓	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✗	75.0%
Nagel et al, 2010	SKS/MP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	83.0%
Oien et al, 2010	SKS/MP	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	95.0%
Rodriguez et al, 2010	SKS/MP	✓	✗	✓	✗	✗	✓	✓	✓	✓	✓	✓	✓	✗	✗	73.0%
Goksor et al, 2011	SKS/MP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	91.0%
Willers et al, 2011	SKS/MP	✓	✗	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✗	93.0%

Key: ✓: criteria satisfied ; ✕ : criteria not satisfied

[Page 14] Table 1. Summary table of Quality Assessment of relevant studies in this systematic review (Zaza, 2000).

Study	Reviewers	Study design	Population Density	Population Description	Time points	Entire sampling or Probability sampling	Eligibility Criteria	Exposure Measures		Outcome Measures		Statistical Test Suitable	Adjustment For Confounders	Bias Discussed	Bias not covered	Overall Average Score (%)
								Validity	Reliability	Validity	Reliability					
Kunitsugu et al, 2012	SKS/MP	✓	✕	✓	✓	✕	✓	✓	✓	✓	✓	✓	✓	✓	✕	73.0%
Kiefte de Jong et al, 2012	SKS/MP	✓	✕	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✕	95.0%
Dotterund et al, 2013	SKS/MP	✓	✓	✓	✓	✓	✕	✓	✓	✓	✓	✓	✓	✕	✕	80.5%
Magnusson et al, 2013	SKS/MP	✓	✕	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✕	✕	91.0%
Nwaru et al, 2013	SKS/MP	✓	✕	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	97.5%
Lumia et al, 2015	SKS/MP	✓	✕	✓	✓	✕	✓	✓	✓	✓	✓	✓	✓	✕	✕	78.0%
Saadeh et al, 2015	SKS/MP	✓	✕	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✕	82.0%

Xu et al, 2016	SKS/MP	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✕	✓	✕	64.0%

Key: ✓: criteria satisfied ; ✕: criteria not satisfied

[Page 16] ***Meta-analysis***

A total of nineteen studies were used in the meta-analysis (10, 11, 23-29, 31, 32, 34-37, 39, 40, 42, 43).

Current Asthma

Assessment of cohort studies alone (11, 40, 42) revealed a statistical significant effect (21) of ‘all fish’ intake on ‘current asthma’ in children 0-4 years (I^2 =11.5%; p=0.32; (OR: 0.75; 95% CI: 0.60-0.95). Overall, a 25% reduction in ‘current asthma’ was observed with ‘all fish’ intake in children up to 4 years old (Fig 2).

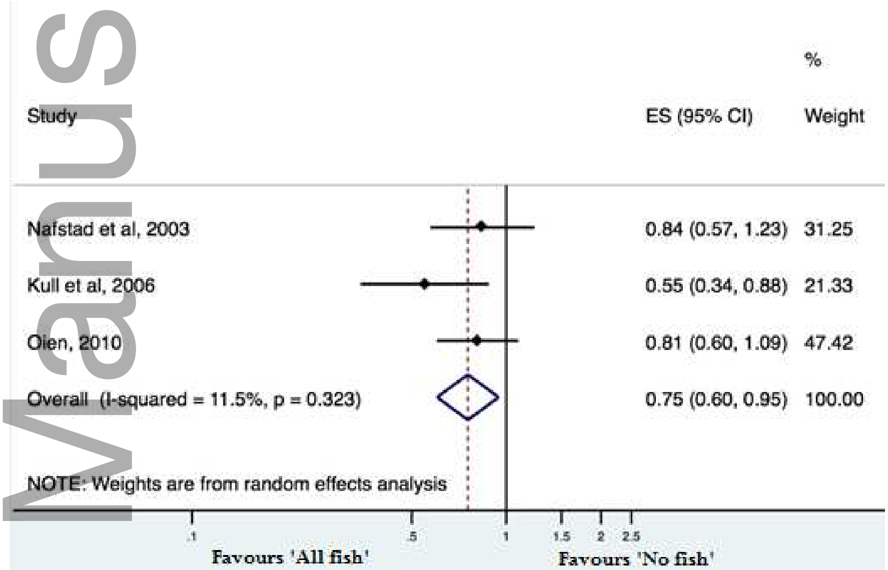


Fig 2. Forest plot of cohort studies children (0-4 years old) for ‘All fish’ intake versus ‘No fish’ and outcome ‘current asthma’.

Current Wheeze

A pooled effect for current wheeze and ‘All fish’ intake was found in cohort studies regarding children up to 4.5 years [I^2 = 0%, p=0.809; (OR: 0.62; 95%CI: 0.48-0.80)] [Page 16] (Fig 3). A 38% reduction in ‘current wheeze’ was observed with ‘All fish’ intake in children up to 4.5 years old.

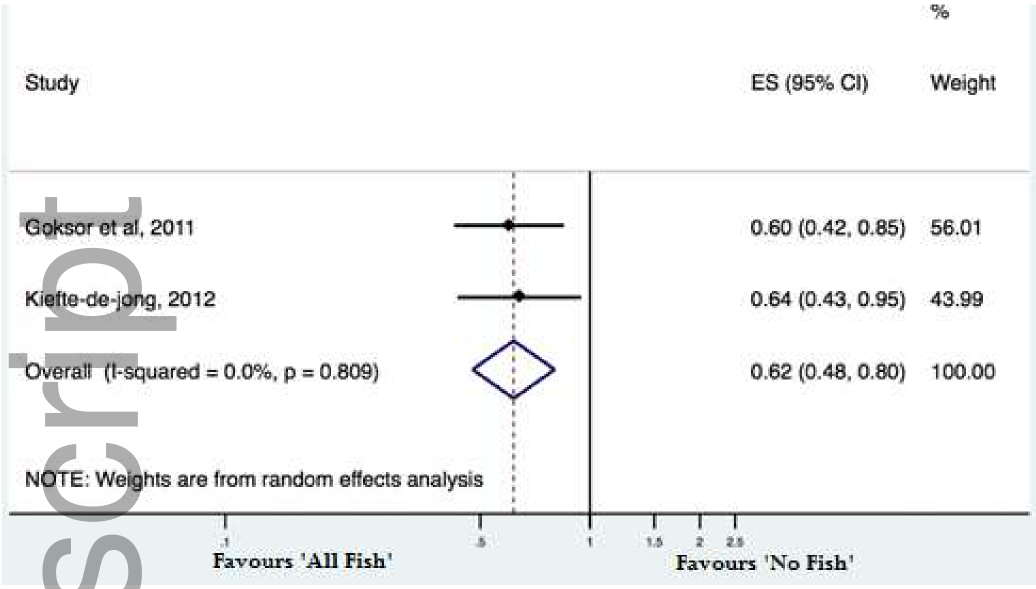
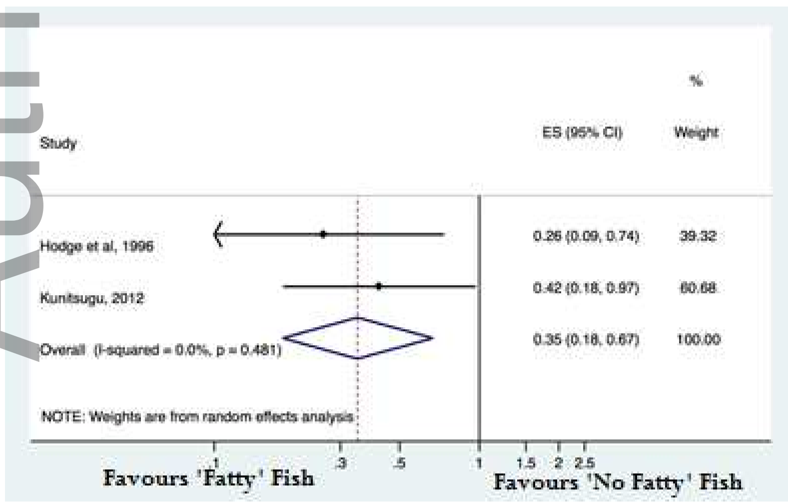


Fig 3. Forest plot of cohorts comparing the effect of 'All fish' intake versus 'No fish' on 'current wheeze' in children aged 0-4.5 years.

Fatty fish versus No fatty fish

When analysing the effect of 'Fatty fish' intake in children aged 8-14 years (28, 34) for 'current asthma' in the combined sub-analysis, an overall effect was observed [I^2 =0%, p-value=0.481; (OR: 0.35; 95%CI: 0.18-0.67](Fig 4). Fatty fish intake reduced 'current asthma' in children aged 8-14 years by 65% as compared to 'no fish' intake.



[Page 18] Fig 4. Forest plot in the combined analysis comparing 'Fatty Fish' intake versus 'No Fatty Fish' in children (8-14 years) for 'current asthma'.

In contrast, no association was observed for 'all fish' intake on 'current asthma in children aged (2-15 years) when combining all study designs (11, 24, 25, 28, 31, 32, 34-36, 40, 42, 43, 45) (Supplement, Fig S1) or in sub-analysis based on study designs separately (Supplement, Fig S2). No pooled age specific effects were also found for 'current wheeze' and 'All fish' intake in children (0-13 years) irrespective of study design (Supplement, Fig(s) S3-S5). The same trend was established for 'Fatty fish' intake vs 'current asthma' in children (0-14 years) in the combined sub-analysis (Supplement, Fig S6).

DISCUSSION

The aim of this qualitative synthesis and meta- analysis was to clarify the role of fish intake on different asthma outcomes in children. Although the meta-analysis produced mixed results, three important findings were highlighted. Firstly, early introduction of ' all fish' intake during [Page 18] infancy (6-9 months of age) and regular consumption (at least once/week) of 'all fish' reduced 'current asthma' by 25% and 'current wheeze' by 38% in children up to 4.5 years old (11, 37, 39, 40, 42). While 'fatty fish' intake seems to confer an overall ' prophylactic effect' in older children 8-14 years and reduced 'current asthma' by 65% (28, 34).

Summarizing the results of the database search, in 15/23 studies a protective effect was observed between fish consumption on asthma symptoms in children (11, 23, 24, 27, 29, 31, 33-41), 6/23 no effect (10, 25, 26, 28, 42, 43) and 2/23 an adverse effect (30, 32). Two important results were highlighted in these observational studies. Early introduction during the first year of life (between 6-9 months) and regular consumption of fish (at least once a week) decreased risk, prevalence and asthma symptoms in children up to 14 years of age. This finding is in accordance with other observational studies that have found early introduction and frequent consumption of fish were associated with decreased asthma symptoms and improvement in pulmonary function in children and adolescents (9, 46-48).

Our meta-analysis of nineteen (19) publications showed an overall beneficial' effect of 'all fish' intake on 'current asthma' and 'current wheeze' in children up to 4.5 years old in five cohort studies alone (11, 37, 39, 40, 42) and of 'fatty fish' intake on 'current asthma' in 8-14 year olds(28, 34). The prophylactic effects documented in the cohort studies for young children up to 4.5 years old may be explained from research on biological mechanisms that have shown that, the development and maturation of the immune system starts early in foetal life, continues through infancy and early childhood (49); (50). Infancy is a period in which the immune cells have an increased vulnerability and susceptibility to environmental exposures such as diet. A diet rich in omega 3 fatty acids and lower omega 6: omega 3 fatty acid ratio may result in an increased incorporation of EPA and DHA into cell membranes at the expense of arachidonic acid. More EPA and DHA in the cell membrane results in decreased production of arachidonic acid-derived pro-inflammatory eicosanoids. EPA and DHA act as substrates for COX and LOX [Page 19] enzymes producing anti-inflammatory eicosanoids, protectins, resolvins and maresins that appears to exert anti-inflammatory and inflammatory resolving actions (51). Apart from anti-inflammatory properties, omega 3 fatty acids are able to modulate immune responses by promoting Th 1 cell generation thereby reducing airway inflammation, improving pulmonary function and decreasing asthma symptoms (13).

Additional protective effects on asthma symptoms were observed in two studies examining the effect of 'fatty fish' as opposed to 'all fish' in children aged 8-11 years old (34, 38). Hodge et al reported a 25% reduction in 'current asthma' when fatty fish was consumed as compared to lean or no fish in children (34). Furthermore, no reduction in asthma risk was observed with the consumption of canned and processed fish. Perhaps, processing and food additives may alter the biological activity of omega 3 fatty acids in fish oils. Dotterud et al, reported a 32% reduction in incidence of asthma with fatty fish consumption in infants at 2 years old, after adjusting for confounders (38). A beneficial effect of fatty fish intake was also noted in the studies undertaken by Kunitsugu and Oien, although results were not statistically significant, most probably explained by heterogeneity between study designs and small sample size (28, 44). Our meta-analysis confirmed an overall 'prophylactic' effect of fatty fish intake in older children (28, 34). A 65% reduction in 'current asthma' was observed in children aged 8-14 years (28, 34).

The findings of this meta-analysis highlight that the type of fish consumed whether fatty or lean may matter. Fatty fish as compared to lean fish is a rich source of omega 3 fatty acids which are able to counteract the action of omega 6 fatty acids metabolites by down-regulating pro-inflammatory and immunological pathways thereby preventing asthma development (52); (53). Another possible explanation why fish consumption reduced asthma risk in children as compared to null effects reported from fish oil supplementation during childhood (54) is that regular fish consumption may be a proxy for other healthy lifestyle habits that might promote [Page 20] the beneficial effect. In addition, fresh fish might have better bioavailability and absorption rate as opposed to fish oil. And apart from EPA/DHA, fresh fish contains other bioactive molecules such as selenium, iodine, vitamin D, potassium and B-vitamins that might interact synergistically providing these prophylactic properties (55).

On the other hand, our meta-analysis showed no statistical significant results for 14 studies (10 cross-sectional, 2 cohorts and 2 case-controls) on 'current asthma' or 'current wheeze' in children 6-15 years old (10, 23-26, 28, 29, 31, 32, 34, 35, 42, 43, 45). A possible reason for the null effects observed might be due to heterogeneity among study methodologies which included: population differences, sample size, children's age, gender, asthma definition, exposure and outcome measures, possible confounders, design of dietary questionnaires and food frequency categories, amount or type of fish consumed (fatty versus lean), quantity EPA/DHA, fish consumption patterns since nutritional guidelines may vary in each country and food preparation methods. Use of invalidated and self-administered food frequency questionnaires, 3-day diet history for the recording of dietary intake require education and expertise, are prone to information bias and response errors (43);(28). Even though data on habitual diet were collected retrospectively from parents, in young children, it is well-established that parents provide reliable information on children's diet (56). Misclassification of asthma may have occurred in young children, since the diagnosis of asthma is uncertain in children younger than 5 years and wheezing is often the result of respiratory infection (57);(58). Furthermore, spirometry which is considered to be the 'gold-standard' of pulmonary function testing in the diagnosis of asthma, cannot be performed efficiently in children younger than 5 years and diagnosis of asthma in young children is based on parents' report of symptoms (57). In addition, parental allergic disease and early onset of allergic disease in children may cause parents to delay introduction or to avoid fish in the child's diet, thus influencing asthma outcome.

[Page 21] Two cross-sectional studies documented increased risk of asthma prevalence in children consuming fish in Japan and China (30, 32). Today, the eating patterns of Japanese and Chinese children have changed to a more Westernized diet (59, 60). Processed (23, 61) and pickled foods (62) as well as a high salt intake (63, 64) have been associated with an increase in bronchial hyperresponsiveness and asthma symptoms in children. In both studies confounding factors such as overweight, socio-economic level, maternal education, type of fish consumed (lean vs fatty), poor adherence to asthma therapy were not included in regression models and may have contributed to the adverse effects.

One of the strengths of this systematic-review is the extensive literature search and large number of recent high quality publications included which increases the power of the analysis. There are very few meta-analysis in the literature that examine fish intake and asthma in children (Yang, 2013; Zhang, 2017) (14, 65) and recent reviews (Best, 2016; Miles, 2017) address omega 3 fatty acid intake during pregnancy (12, 13). Our meta-analysis focuses exclusively on children and adolescents, and adds to the existing evidence. Another strong point is the inclusion of cohort studies in the meta-analysis which are considered robust and provide strong scientific evidence because they measure events in temporal sequence thereby distinguishing causes from effects (66). Another drawback might be publication bias where studies reporting no association between fish intake and asthma may not be published by periodicals.

CONCLUSION

We conducted an up-to-date systematic search to determine the impact of fish intake in childhood asthma. This meta-analysis suggests that introduction of fish early in life (6-9 months) and regular consumption of all fish (at least once a week) reduces asthma and wheeze in children up to 4.5 years old, while fatty fish intake may be beneficial in older children. [Page 21] Future well-designed clinical trials are recommended to confirm the promising findings documented in this literature analysis.

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