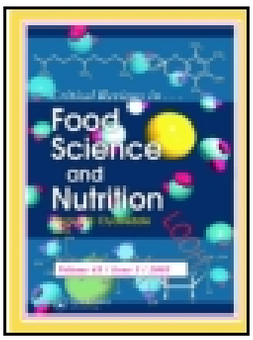

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The effects of Early Life Polyunsaturated Fatty Acids and Ruminant Trans Fatty Acids on Allergic Diseases: A systematic review and meta-analysis

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Abstract

Early life nutritional exposures could modify the gene expression and susceptibility of allergic diseases (AD). This **s**ystematic review aimed to evaluate whether early life (the first 1,000 days) natural exposure to polyunsaturated fatty acids (PUFA) and ruminant trans fatty acids (R-TFA) could affect the AD risk. We searched PubMed, EMBASE, PsycINFO, Scopus, the Cochrane

Library, and ClinicalTrials.gov from inception through September 10, 2017 for observational studies. The quality of studies was examined by the Newcastle-Ottawa Scale, and the best evidence synthesis (BES) was applied. We included 26 observational studies, and 8 of them showed high quality. BES showed a moderate evidence for the protective effect of vaccenic acid (VA) on eczema, while insufficient or no evidence was found in other associations. Meta-analysis showed that higher n-6/n-3 ratio and linoleic acid were associated with higher risk of eczema (pooled odds ratio [OR] =1.06, 95% confidence intervals [CI]: 1.00-1.13; 1.08, 95% CI: 1.02-1.15). However, VA was inversely associated with eczema pooled OR =0.43, 95% CI: 0.26-0.72). Early life natural exposure to VA showed evident benefit on decreasing the risk of eczema, while PUFA and other R-TFA showed limited effects on AD. More robust studies especially for R-TFA are required.

Keywords:

polyunsaturated fatty acids, ruminant trans fatty acids, allergic diseases, eczema, meta-analysis, humans.

Introduction

The prevalence of Allergic Diseases (AD) is rising dramatically worldwide especially in more industrialized countries during the past two decades, representing a substantial disease burden of individuals and health service cost (Gupta et al., 2004; Wu et al., 2011). Although AD is caused by the complex interaction between the genetic predisposition and the environmental factors (Hide et al., 1996), it is generally accepted that the current allergy epidemic is attributable to the environmental exposures in early life (Arshad et al., 2005). Early life is restricted to the first 1,000 days during fetus and infancy from conception to age two years (Arabena et al., 2014). The hygiene hypothesis suggested that the lack of microbial exposure resulted from the very hygienic conditions in early life might show an impact on the imbalance of the immune system, primarily the imbalance of T helper cell type 1 and type 2 (Th1/Th2) (Kuo et al., 2013). As the prenatal and infancy period are the critical window for children's development, early prevention of AD is becoming progressively important.

Previous studies stated that natural nutritional exposures in early life play a crucial role in modifying the gene expression and susceptibility of AD (Prescott et al., 2016). It was suggested that polyunsaturated fatty acids (PUFA) exposed in early life could affect the development of the immune system by regulating numerous metabolic processes and the gene expressions of

important proteins, such as enzymes and cytokines (Enke et al., 2008). Studies suggested that n-3 PUFA may decrease the risk for the future development of AD, while n-6 PUFA showed opposite associations (Rosenlund et al., 2016; Rucci et al., 2016; Yu et al., 2015). However, prior systematic reviews and meta-analyses showed mixed results on the effect of PUFA (Anandan et al.,2009; Best et al.,2016; Foiles et al.,2016). These findings were limited by the length of the exposure period (e.g., pregnancy), exclusively focusing on a specific category of PUFA (e.g., n-3 PUFA) or a certain AD (e.g., only asthma), or including only a small number of studies.

Although the relationships between PUFA and AD have been studied more thoroughly, the potentially beneficial effect of trans fatty acids (TFA) was seldom discussed.

A recent study evaluated the impact of TFA on human health and indicated that different type of TFA have different health effect (Kuhnt et al., 2016). TFA include unsaturated and polyunsaturated fatty acids that contain at least one double bond in the trans configuration. Dietary TFA mainly come from two sources—industrial production (industrial TFA) and natural sources (ruminant TFA, which is abundant in human milk and cow's milk) (Albuquerque et al., 2011). An ecological study investigated the relationship between intake of TFA and the prevalence of AD in ten European countries, which demonstrated that ruminant TFA (R-TFA) might play a desirable role on AD when compared with industrial TFA (Weiland et al., 1999). Evidences from animal studies also indicated that R-TFA, including conjugated linoleic acid (CLA) and vaccenic acid (VA), have favorable effects on immune and inflammatory responses

(Bocking et al., 2014; Jaudszus et al., 2008). Meanwhile, several the population studies demonstrated that natural exposure to R-TFA in early life has desirable effects on the human AD (Chisaguano et al., 2014; Rucci et al., 2016). Based on the current evidence, the health hazard of TFA should be separately assessed, especially for R-TFA. However, we still lack the evidence based on the systematic review and meta-analysis to confirm whether R-TFA has an effect on the human AD.

Therefore, we conducted a systematic review and meta-analysis of observational studies aimed to systematically assess whether early life natural exposure to PUFA and R-TFA showed sufficient effects on the development of AD. We were especially interested in evaluating the following: (1) the potential differential effects of the n-3 PUFA and n-6 PUFA on AD and (2) the association between R-TFA and the AD risk.

Methods

Search Strategy and Selection Criteria

We conducted a systematic literature search of EMBASE, PubMed, PsycINFO, Scopus, and the Cochrane Library for relevant literatures from inception through September 12, 2017. Reference lists of key publications and narrative review were hand-searched. In order to check for a possible publication bias, we undertook a systematic search of the gray literature by using the identical inclusion criteria in the ClinicalTrials.gov to identify unpublished studies.

We identified observational studies performed in humans estimating the relationship between early life PUFA or R-TFA and the development of AD in children. Exposure period was restricted to the first 1,000 days during fetus and infancy from conception to age 2 years. Studies needed to provide endpoints of AD, and risk estimates [odds risk (OR), relative risk (RR) or hazard ratio (HR)] for PUFA or R-TFA as the exposure. We included English language articles only, while scanned titles /abstracts of non-English language articles to evaluate agreement with the results published in English. Studies were excluded if they didn't report the profile of PUFA or R-TFA, or if they targeted participants with the medical condition.

Data Extraction and Quality Assessment

Pairs of investigators independently performed title scans and abstract reviews, and they reviewed the full-text studies to evaluate their eligibility for inclusion. A predesigned standardized form was used for data extraction and discrepancies were resolved by repeat discussion until consensus was reached. In order to ensure the completeness and accuracy of extracted data, the second reviewer verified the first reviewer's data abstraction for all included articles. Investigators extracted information on the basic study characteristics, exposure components, exposure assessments, assessments and main findings.

We applied the Newcastle-Ottawa quality-assessment scale (NOS) to evaluate the quality of our eligible studies (Wells et al., 2010). A maximum of 9 points was assigned to each study: representativeness of study selection (4 points); comparability (2 points); and assessment of

either the outcome or exposure (3 points). Total scores of NOS were divided into two parts and regarded scores of 0-6 and 7-9 as low and high quality, respectively.

Levels of scientific evidence

To reach conclusions about the evidence for the relationships between fatty acids (PUFA and R-TFA) and AD, the best evidence synthesis (BES) was conducted (Singh et al., 2008). We performed the BES with the identified studies and took the number of studies, the consistency of findings, and the methodological quality of identified studies into consideration. The results from the most fully adjusted model of each study were included in the evidence synthesis. We performed the BES for AD overall as well as each different type of AD. The strengths of evidence were classified into four categories, including strong, moderate, insufficient, and no evidence (Ekris et al., 2016).

Data synthesis and Meta-Analysis

We performed 2 types of data synthesis: (1) comparison of effects of early life PUFA and R-TFA on AD for all the included papers and (2) performance of meta-analyses for AD. The primary association measures were odds ratios (OR) between extreme profiles of fatty acids (high vs. low) for most of the study. Risk ratios (RR) and hazard ratios (HR) also showed in only a few studies. Meta-analysis was considered only for included studies with comparable OR and corresponding 95% confidence intervals (95% CI) of the most adjusted model and the longest follow-up for each outcome. Pre-specified meta-analyses were conducted to determine the

effects of various fatty acids (FA) on specific AD. Potential heterogeneity among included studies was estimated using the I^2 statistic. Random-effects model was applied to our meta-analysis due to the significant heterogeneity. We used the specified sensitivity analysis to assess the impact of each individual study on the overall pool estimate by omitting one study at a time. Egger's linear regression and Begg's rank correlation were conducted to evaluate the potential publication bias. The funnel plot was also executed when we pooled 10 or more studies. The above analyses were undertaken with STATA 12.0 (Stata Crop., College Station, TX, USA).

Results

Literature Flow and Study Characteristics

We identified 4,274 potentially relevant citations, and 119 full citations were retrieved. Overall, 26 unique citations were deemed eligible for systematic review, and 19 provided sufficient data for meta-analysis (Chisaguano et al., 2014; Dirix et al., 2011; Jonsson et al., 2016; Lowe et al., 2008; Lumia et al., 2014; Lumia et al., 2011; Miyake et al., 2013; Miyake et al., 2009; Montes et al., 2013; Morales et al., 2012; Newson et al., 2004; Notenboom et al., 2011; Nwaru et al., 2012; Nwaru et al., 2011; Nwaru et al., 2010; Pike et al., 2012; Rosenlund et al., 2016; Rucci et al., 2016; Saito et al., 2010; Soto-Ramirez et al., 2012; Standl et al., 2014; Thijs et al., 2011; van Elten et al., 2015; Waidyatillake et al., 2017; Wijga et al., 2006; Yu et al., 2015) (**Figure 1**).

The characteristics of 26 studies (including 18, 416 participants) were showed in **Table 1**. Among these studies, 25 were cohort study and one was nested case-control study. Nineteen

were performed in Europe, four in Asia, two in Australia, and one in the United States. The sample size ranged from 65 to 4,976, with a median sample size of 708. One study included only boys, while the others included both sexes. Mother's mean age ranged from 27.5 to 38.7 years. Twenty-two reported the OR as effect estimate, three reported RR, and two reported HR. The quality scores ranged from three to nine, with eight studies (30.8%) rated as high quality **(Supplement Table 1)**.

Exposures and clinical outcomes

Eleven targeted exposures were identified in 26 studies (some studies had more than one exposures). FA exposure was assessed mainly from maternal dietary, blood sample or breast milk. Exposure period was restricted to early life. Dietary PUFA and R-TFA were measured by food-frequency questionnaire (FFQ) or diet history questionnaire (DHQ). The profile of FA in the blood sample and breast milk were examined by using gas chromatography **(Table 1)**.

There were five targeted clinical outcomes in 26 studies, including eczema, wheeze, asthma, allergic rhinitis, and sensitization. Nineteen of 26 studies (73%) reported eczema as the endpoint, 42.3% reported wheeze, 38.4% reported asthma, 23.07% reported allergic rhinitis, and 34.7% reported sensitization. Outcome measurements were determined by using the validated International Study of Asthma and Allergic in Children (ISAAC) questionnaire, non-validated health questionnaire, or doctors' diagnosis. **(Table 1)**.

The effects of PUFA and R-TFA on AD

The majority of 26 studies (25 or 96%) evaluated the effect of PUFA on AD and seven of them (26.9 %) evaluated the effect of R-TFA. Most of the studies (84.6 %) provided multiple results regarding the association between FA and AD.

As for PUFA, there were 60 results evaluating the effects of different n-3 PUFA on AD, and 33 (55%) of them reported desirable effects with 7 (13%) were of statistical significance. Only 4 studies (6%) showed statistically significant effect of n-6 PUFA. For R-TFA, the majority studies showed a desirable effect of CLA (77%) or VA (100%) on AD, but few were statistically significant. However, both two studies (100%) reported significant favorable effect of VA on eczema **(Table 2)**.

Meta-Analysis

Effectiveness of PUFA and R-TFA on eczema

As shown in **Table 3**, meta-analysis showed no significant association of early life n-3 PUFA and n-6 PUFA with eczema (pooled OR=0.92, 95% CI: 0.78-1.08; and 1.00, 95% CI: 0.90-1.11). However, significant increase in the risk of eczema was observed with higher n-6/n-3 ratio (pooled OR: 1.06, 95% CI: 1.00-1.13) **(Figure 1)**. Furthermore, the positive association between LA and eczema was observed (pooled OR: 1.08, 95% CI: 1.02-1.15) **(Figure 2)**. Regarding R-TFA, two separate studies (800 participants) were combined in the meta-analysis, showing a significant reduction in the incidence of eczema with higher VA (pooled OR: 0.42, 95% CI: 0.25-0.72) **(Figure 3)**. Overall, the BES presented a moderate evidence for the protective effect

of VA on eczema, while there was insufficient or no evidence for the relationship of other R-TFA and PUFA with eczema (**Table 4**).

Effectiveness of PUFA and R-TFA on other AD

The pooled data of meta-analysis revealed non-significant effect of PUFA on asthma, wheeze, allergic rhinitis, and sensitization (**Table 3**). Meanwhile, the BES showed insufficient or no evidence for the association between different PUFA and other AD (**Table 4**). The results of R-TFA on other AD could not be combined in meta-analysis because of insufficient data.

Sensitivity Analysis and Publication Bias

In the sensitivity analyses, none of the exposures omitted in turn seemed to substantially influence the AD (data not shown). Funnel plots showed no evidence of publication bias for AD (funnel plots not shown). Results of Begg's and Egger's tests were consistent with the funnel plots, except for the evidence of eczema [alpha-linolenic acid (ALA): Begg P =0.013, Egger P =0.001; arachidonic acid (AA): Begg P =0.216, Egger P =0.002]. The trim and fill analysis was performed to correct the publication bias for eczema. Results showed that no trimming was required and the data were unchanged.

Discussion

To our knowledge, this is the most comprehensive study that has systematically and quantitatively assessed the association between early life natural exposure to PUFA and R-TFA and risk of AD. We found that early life exposures to higher n-6/n-3 ratio and LA were

significantly associated with increased risk of incident eczema, while the BES showed insufficient evidence. Moderate evidence was found for an inverse relationship between early life VA and the future development of eczema. No significant associations were observed between AD and other PUFA or R-TFA in early life.

In general, our results concerning the effect of n-3 and n-6 PUFA on AD were consistent with the previous meta-analysis, which concluded that early life n-3 and n-6 PUFA is unlikely to play a crucial role in children's AD (Anandan et al., 2009). However, we found a positive association between early life exposure to higher n-6/n-3 ratio and the development of eczema. It was first proposed by Black and Sharpe that the increase of AD was associated with changes in dietary fat intake, notably the increase of n-6 PUFA and the decrease of n-3 PUFA (Black et al., 1997). Early life exposure to higher n-6 /n-3 ratio had both short and long-term effect on the risks of AD in children (Thijs et al., 2011; van Elten et al., 2015) , and the mechanism of n-6/n-3 ratio on eczema is biologically plausible. The functional disturbance of T cell, especially the status of Th2 predominance played an important role in the development of AD (Chu et al., 2007). Pro-inflammatory n-6 PUFA series, such as AA, can strengthen inflammatory processes by its derivatives prostaglandin E2 (PGE2) and leukotriene B4 (L-B4) (Enke et al., 2008). Subsequently, PGE2 will lead to a reduction in the Th1/Th2 cytokine ratio, promoting the development of AD (Gold et al., 1994). Conversely, n-3 PUFA exert anti-inflammatory effects via inhibiting AA metabolism to decrease the production of PGE2 and L-B4 (Calder et al.,

2006). Moreover, n-3 PUFA can decrease the secretion of inflammatory cytokines, such as interleukin-1β and interleukin-6 (Zhao et al., 2005). Taking together, higher n-6/n-3 ratio will disturb the balance of the Th1/Th2 cytokine ratio in the immune system, subsequently affecting the development of AD. Similarly, early life exposure to higher linoleic acid (LA), a member of the n-6 PUFA, was positively associated with the risk of eczema. LA is a precursor of PGE2 which inhibit the formation of interferon-γ to develop AD (Black et al., 1997). Nevertheless, the pooled estimates of higher n-6/n-3 ratio and LA were relatively small on AD (pooled OR=1.06 and 1.08, respectively). In the BES, insufficient or no evidence were also found for the above relationships. Therefore, more robust studies should conduct to confirm these associations.

In our study, we were the first to find systematic evidence that higher VA exposure in early life has prominent protective effect on eczema. It could be explained partly by the anti-inflammatory properties of VA. Studies suggested that VA attenuate allergic dermatitis and suppress airway inflammation in animal models (Kanwar et al., 2008; Sun et al., 2011). In the present study, the magnitude of the association between VA and eczema was strong (pooled OR=0.43) and the BES confirmed a moderate evidence for this association. However, it should be noted that only two related cohort studies were included, which were both conducted in Europe. Most of the European countries, such as Netherlands and Spain, had a high consumption of dairy products (Hjartåker et al., 2002), which are generally considered to be a major source of VA (Albuquerque et al., 2011). A large European cross-sectional study investigated the

association between farm-produced dairy products and AD, which indicated that high consumption of dairy products may offer protection against AD, such as asthma (Waser et al., 2007). Additionally, a later study further found that whole milk intake during pregnancy appeared protective for the development of childhood asthma and allergic rhinitis, while low-fat yogurt was directly associated with increased risk of AD (Maslova et al., 2012). The favorable effect of whole dairy products on AD might in part due to the content of VA (Hjartåker et al., 2002). It is noteworthy that the above research populations had a high consumption of dairy products, i.e. their concentration of VA is comparatively high. However, in the case of low consumption of dairy products, the protective effect of early life VA on AD remains unclear. To better understand the contribution of VA during early life to the future development of eczema, more research should be conducted in different regions and populations with different genetic background, especially in those with low consumption of dairy products.

Our study comprehensively evaluated the association of PUFA and R-TFA with AD. However, no significant effects were observed on AD except for eczema. Two attentive explanations should be taken into consideration. Firstly, according to the atopic march (Hahn et al., 2005), the initial events of AD are eczema and food allergy, which showed the greatest incidence before three years old. However, most of the allergic respiratory diseases, such as wheeze, asthma and allergic rhinitis, appeared at the age of five. Due to the limited length of follow-up in our systematic review, we were unable to determine the potential effect of early life

PUFA and R-TFA on later-onset AD. Secondly, as various environmental and social components come into play in later life, the natural exposures to FA in early life might be ineffective in the long-term (Bamai et al., 2014; Wood et al., 2015). To date, the evidence regarding the effect of early life PUFA supplement on the risk of AD has been controversial (Anandan et al., 2009; Best et al., 2016; Gunaratne et al., 2015). Meanwhile, no relevant randomized controlled trials have estimated the effect of early life R-TFA on AD. Future studies should extend the length of follow-up to determine whether early life natural exposure to PUFA and R-TFA have sufficient impact on AD, or apply interventions to evaluate the potential effect of early life PUFA and R-TFA supplements on the late-onset AD.

Our findings have potential implications for the prevention of AD. New data from ISACC revealed that the prevalence of eczema has been rise dramatically both in developing and developed countries (Odhiambo et al., 2009), which places a large burden on infants, their families, and society (Carroll et al., 2005). Eczema in early childhood may trigger the other AD. Infants with eczema had a 3-fold increase in developing asthma and a nearly 3-fold increase in developing rhinitis at follow-up compared with those without eczema (von Kobyletzki et al., 2012). In addition, infant eczema is accompanied by sleep disturbance and mental health problems, affecting the future development of physiology and psychology (Schmitt et al., 2011). Our study found that early life natural exposure to PUFA and R-TFA were associated with the

risk of eczema in future, which show the importance of appropriate early nutritional prevention strategies for AD.

The present systematic review and meta-analysis has several strengths. Firstly, a systematic approach to identify the relevant publications and a sequence of screening procedures were performed. Secondly, the prospective nature of the included studies avoided the influence of selection and recall bias. Thirdly, we systematically reviewed and assessed the summarized association between AD with different types of individual PUFA and R-TFA, including n-3 PUFA, n-6 PUFA, DHA, LA, CLA, and VA. These data showed a most comprehensive sign of the association between natural exposure to PUFA and risk of AD based on the previous evidence. In addition, this study is the first to quantify the effect of early life exposure to R-TFA on AD in children.

Potential limitation of our study should be considered. Firstly, we cannot exclude the possibility of residual confounders because of the observational nature of the included studies. However, most of the studies had adjusted for the major risk factors of AD. Secondly, language bias could occur because we only included studies published in English. Our eligible studies, however, covered a wide range of non-English countries, such as countries across Europe and Asia. We also searched Chinese language studies and did not identify qualified studies. Thirdly, different methods of assessments (dietary and biomarkers) were applied in the included studies, and the units were heterogeneous across different studies. Nevertheless, we used odds risks for the

higher versus lower category of fatty acids exposure, which could, to some degree, reduce the bias caused by different exposure assessment methods or units. Fourthly, Subgroup analysis by exposure time window were limited due to the few studies evaluating the effect of infancy PUFA or R-TFA on AD. Finally, the impact of heterogeneity on the results of meta-analysis were uncertain. Given the limitations proposed earlier, future studies should focus on using standardized study design and measures to decrease heterogeneity and increase the evidence of the findings.

In conclusion, our study reported the systematic, quantitative analysis of the effect of early life PUFA and R-TFA on AD. We found moderate evidence for an inverse association between early life VA and the future development of eczema, and non-convincing evidence for negative association of n-6/n-3 ratio or LA with eczema. No significant associations were observed between other PUFA or R-TFA and other AD. Future studies involving early life PUFA and R-TFA are recommended to assess other AD such as wheeze, asthma, and allergic rhinitis in order to fully evaluate their potential health benefits.

Conflicts of interest

The authors declare that they have no competing interest.

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Table 1. Observational studies of PUFA and R-TFA status during early life and allergic disease in the offspring.

Study	Design	Population	Participants	Age (years)	Exposure assessment	FA type	Outcome assessment	Main findings
Waidyatillake 2017	Prospective cohort study	The MACS cohort in Australia,1990 1994	N=312	18	Colostrum breast milk GC	N-3 PUFA N-6 PUFA N-6/n-3 ratio	ISAAC	Higher levels of n-3 in colostrum were associated with increased asthma at 18 years (OR: 4.43, 95%CI: 1.46, 13.39) and eczema at 18 years (OR: 9.89, 95%CI: 1.44, 68.49).
Jonsson 2016	Prospective cohort	The FARMFLORA birth cohort in Sweden, 2005-2008	N=65	3	Maternal serum GC	EPA	Doctor diagnose	Serum proportions of EPA can lower allergy in children (OR: 0.47, 95%CI: 0.27, 0.83).
Rucci 2016	Prospective cohort study	The Generation R Study in Netherlands, 2000	N=4,976	6	Maternal plasma GC	N-3 PUFA, ALA, EPA, DHA N-6 PUFA, LA, AA N-6/n-3 ratio	ISAAC	Maternal n-6 PUFA levels were associated with a decreased risk of childhood asthma (OR: 0.71; 95% CI: 0.52, 0.96) and with an increased risk of childhood eczema

								(OR: 1.21; 95% CI: 1.07, 1.37).
Rosenlund 2016	Prospective cohort study	The ALADDIN cohort study in Sweden, 2004-2007	N=225	2	Breast milk GC	N-3 PUFA, ALA, DHA N-6 PUFA, LA, AA N-6/n-3 ratio CLA,VA	IgE test	An inverse association was observed between n-3 PUFA in breast milk and sensitization in the child up to 24 months of age (RR: 0. 49, 95% CI: 0.23-1.05).
Yu 2015	Prospective cohort study	The GUSTO birth cohort in Singapore, 2009-2010	N=960	1.5	Maternal plasma GC	N-3 PUFA, ALA, EPA, DHA N-6 PUFA, LA, AA	ISAAC	Maternal total n-3 PUFA, n-6 PUFA status and the n-6/n-3 PUFA ratio were not significantly associated with offspring rhinitis, eczema, wheezing, a positive SPT in offspring.
Standl 2015	Prospective cohort	The LISAplus birth cohort study in German, 1997-1999	N=436	2/6/10	Cord blood GC	N-3 LC-PUFAs N-6 LC-PUFAs N-6/n-3 ratio	SAQ	No significant association between n-3 LC-PUFA, n-6 LC-PUFA, or the n-6/n-3 ratio in serum with eczema, asthma,

								allergic rhinitis, or sensitization.

(Continued)

Table 1. *(Continued)*

Study	Design	Population	Participants	Age (years)	Exposure assessment	FA type	Outcome assessment	Main findings
Van 2015	Prospective cohort	The PIAMA birth cohort in Netherlands 1996-1997	N=274	3/8/11/14	Breast milk GC	N-3 PUFAs, ALA, EPA, DHA N-6 PUFAs, LA, AA N-6/n-3 ratio	ISAAC	Maternal breast milk with higher levels of n-3 PUFA were less likely to have asthma in allergic mothers (OR: 0.50; 95% CI: 0.31-0.79) ; maternal higher levels of n-6 FAs were more likely to have asthma in non-allergic mother (OR: 1.86, 95% CI:1.14-3.03)
Lumia 2014	Nested case-control study	The DIPP study in Finland 1996-2004	N=410	5	Maternal plasma GC	N-3 PUFAs, ALA, DHA N-6 PUFAs,	Doctor diagnose	EPA, CLA, DHA, total n-6 PUFA were

						LA, AA N-6/n-3 ratio CLA,VA		associated with a decreased risk of asthma (OR: 0.77, 0.73, 0.74, 0.75; 95%CI: 0.63-0.93, 0.60-0.89, 0.61-0.91, 0.62-0.90); LA and total n-6 PUFA were associated with an increased risk of atopic asthma (OR: 1.43, 1.18; 95% CI: 1.08-1.89, 1.00-1.39).
Chisaguano 2014	Prospective cohort	The INMA-Sabadell cohort study in Spain 2004-2006	N=274	1	Maternal plasma GC	CLA,VA	QNR	VA was found to be linked to a lower risk of atopic eczema (OR = 0.373; 95%CI: 0.171-0.811);
Montes 2013	Prospective cohort	The network INMA-Sabadell cohort study in Spain 2004-2006	N=211	1.1	Maternal plasma GC	N-3 PUFAs, ALA, EPA, DHA N-6 PUFAs, LA,	Doctor diagnose	Regarding cord blood samples, DHA and total n-3 PUFAs showed a negative

						AA		correlation with the prevalence of the disease (OR: 0.50, 0.49; 95%CI: 0.30-0.84, 0.30-0.79).
Miyake 2013	Prospective cohort study	The KOMCHS cohort study in Japan, 2007-2008.	N=1354	1.9/2.4	Maternal diet FFQ	N-3 PUFA, ALA, DHA N-6 PUFA, LA, AA N-6/n-3 ratio	ISAAC	Maternal EPA levels were associated with a de-creased risk of infantile wheeze (OR: 0.70; 95% CI: 0.49-0.99);

(Continued)

Study	Design	Population	Participants	Age (years)	Exposure assessment	FA type	Outcome assessment	Main findings
Soto-Ramirez 2012	Prospective cohort study	The obstetric clinics And prenatal classes in American, 2008-2010	N=115	1.9/2.4	Breast milk GC	N-3 PUFA N-6 PUFA	ISAAC	Total n-6 PUFA were associated with an increased risk of asthma in infants (RR: 2.91; 95% CI: 1.37-6.18).
Pike 2012	Prospective cohort study	The Southampton Women's Survey in England, 1998-2002	N=865	3	Maternal plasma GC	N-3 PUFA, ALA, DHA N-6 PUFA, LA, AA N-6/n-3 ratio	ISAAC	EPA, DHA, and total n-3 PUFA were associated with reduced risk of wheezing (RR: 0.57, 0.67, 0.69; 95% CI: 0.37-0.89, 0.49-0.93, 0.51-0.95).
Nwaru 2012	Prospective cohort study	The DIPP study in Finland,1996-1997	N=2441	5	Maternal diet FFQ	N-3 PUFA, ALA, EPA, DHA N-6 PUFA, LA, AA N-6/n-3 ratio CLA	ISAAC	ALA was associated with a decreased risk of allergic rhinitis (HR: 0.74; 95 % CI: 0.56-0 99); Higher ratio of n-6/n-3 PUFA were associated with an increased risk of allergic rhinitis (HR: 1.35; 95 % CI: 1.05-1.73).
Morales 2012	Prospective cohort study	The INMA Project in Spain, 2004-2006	N=580	0.5/1.1	Maternal plasma GC	N-3 PUFA, ALA, DHA N-6 PUFA, LA, AA	Parent report	No association was found between FAs and eczema and wheezing.
Notenboom 2011	Prospective cohort study	The KOALA Birth Cohort Study in Netherlands, 2000	N=1275	6-7	Breast milk Fast-GC	N-3 PUFA N-6 PUFA N-6/n-3 ratio	ISAAC	N-3 PUFA was associated with a lower risk of eczema (OR: 0.6; 95% CI: 0.42-0.87).
Nwaru 2011	Prospective cohort study	The DIPP study in Finland, 1996-1997	N=652	1.9/2.4	Maternal plasma Fast-GC	N-3 PUFA N-6 PUFA	IgE test	N-3 PUFA as well as n-6 PUFA were associated with lower risk of wheat allergen (OR: 0.38, 0.32; 95% CI: 0.18-0.82, 0.12-0.84).

Table 1. *(Continued)*

Table 1. *(Continued)*

Study	Design	Population	Participants	Age (years)	Exposure assessment	FA type	Outcome assess-ment	Main findings
Thijs 2011	Prospective cohort study	The KOALA Birth Cohort Study in Netherlands, 2000-2003	N=312	2	Breast milk GC	N-3 PUFA CLA, VA	ISAAC	N-3 PUFA and CLA were associated with lower risk of eczema (OR: 0.62, 0.48; 95% CI: 0.30-1.29, 0.24-0.96) at 1 year.
Dirix 2011	Prospective cohort study	The MEFAB cohort study in Nethlands,1990-1994	NR	6.5	Maternal plasma NR	AA	NR	A major impact of early-life exposure to AA on atopic risk at 7 years of age seems rather unlikely.
Lumia 2011	Prospective cohort study	The DIPP Nutrition and allergy study in Finland, 1996-1997	N=2679	1	Maternal plasma GC	N-3 PUFA, ALA, EPA,DHA N-6 PUFA, LA, AA N-6/n-3 ratio CLA	ISAAC	CLA were associated with a decreased risk of asthma (HR: 0.64; 95% CI: 0.41-1.00).
Saito 2010	Prospective cohort study	The OMCHS cohort study in Japan, 2002-2003	N=771	0.3	Maternal diet FFQ	N-3 PUFA, ALA, EPA,DHA N-6 PUFA, LA, AA N-6/n-3 ratio	SAQ	No association was found between FAs and eczema.
Nwaru 2010	Prospective cohort study	The DIPP study in Finland, 1998-2000	N=936	5	Maternal diet FFQ	N-3 PUFA N-6 PUFA	Ig-E test	No association was found between FAs and allergic sensitization.
Miyake 2009	Prospective cohort study	The OMCHS in Japan, 2001-2003	N=763	1.3-2	Maternal diet DHQ	N-3 PUFA, ALA, EPA, DHA N-6 PUFA, LA, AA N-6/n-3 ratio	ISAAC	ALA and DHA was associated with a reduced risk of wheeze (OR:0.52, 0.37; 95% CI: 0.28-0.97,0.15-0.91); n-6 PUFA and LA were related to an in creased risk of eczema (OR: 2.25, 2.11; 95% CI: 1.13-4.54, 1.06-4.26).

(Continued)

Table 1. *(Continued)*

Study	Design	Population	Participants	Age (years)	Exposure assessment	FA type	Outcome assessment	Main findings
Lowe 2008	Prospective cohort study	The MACS in Australia, 1990-1994	N=224	2/6/7	Colostrum EBM GC	N-3 PUFA, ALA, EPA, DHA N-6 PUFA, LA, AA N-6/n-3 ratio	Parent report	N-3 PUFA in breast milk was associated with increased risk of eczema (OR: 1.60; 95% CI: 03-2.50); Higher levels of n-6 PUFA in colostrum were positively associated with rhinitis (OR: 1.59; 95% CI: 1.12-2.25).
Wijga 2006	Prospective cohort study	The PIAMA study in Netherlands, 1996-1997	N=265	1	Breast milk GC	N-3 PUFA, ALA, EPA, DHA N-6 PUFA, LA, AA N-6/n-3 ratio Σtrans	Doctor diagnose	In mothers with allergy, breast milk n-3 PUFA was inversely associated with asthma (OR: 0.50, 95% CI: 0.16-1.00); In mothers without allergy, ALA was positively associated with sensitization (OR: 2.43; 95% CI: 1.01-5.88).
Newson 2004	Prospective cohort study	The ALSPAC in the UK, 1991-1992	N=265	2/6/7	Maternal blood GC	N-3 PUFA, ALA, EPA, DHA N-6 PUFA, LA, AA	Parent report	Fetal exposure to n-6 and n-3 PUFA is an important determinant of early childhood wheezing and AD.

Abbreviations: AA, arachidonic acid; AD, allergic diseases; ALA, a-linolenic acid; ALADDIN, assessment of lifestyle and allergic disease during infancy; ALSPAC, Avon Longitudinal Study of Parents and Children; BF, breastfeeding; BMI, Body Mass Index; BW, birth weight; CI, confidence interval; DIPP, type 1 diabetes prediction and prevention; DHA, docosahexaenoic acid; DHQ, diet history questionnaire; EBM, expressed breast milk; EPA, eicosapentaenoic acid; FAs, fatty acids; FFQ, food-frequency questionnaire; GA, gestational age; GUSTO, growing up in singapore towards healthy outcomes; HDM, house dust mite; INMA, the infancy medio ambiente; ISAAC, international study on asthma and allergy in childhood; KOMCHS, the kyushu okinawa maternal and child health study; LA, linoleic acid; LISAplus, life-style related factors on the immune system and the development of allergies in childhood plus the influence of traffic emissions and genetics; MACS, melbourne atopy cohort study; MEFABNR, Maastricht Essential Fatty Acid Birth; NR, not reported; OMCHS, osaka maternal and child health study; OR odds ratio; PIAMA, prevention and incidence of asthma and mite allergy; PUFA, polyunsaturated fatty acids; QNR, questionnaire; SAQ, self-administered questionnaires; SPT, skin-prick test; UK, United Kingdom; wt%, weigh of total fat/fatty acids.

Table 2. Percentage (%) of observational studies where early life fatty acids status showing favorable effects for allergic disease in children by type of fatty acids.

Fatty acids	Type of allergic outcomes: number of results						% with statistically significant and favorable effect ($P<0.05$)						% with favorable effect ($P<0.05$ not necessary)					
							Eczema	Asthma	Wheeze	Allergic rhinitis	Sensitization	Total	Eczema	Asthma	Wheeze	Allergic rhinitis	Sensitization	Total
PUFA	E	A	W	R	S	T												
n-3 PUFA	16	9	8	7	7	60	6	0	12	14	14	13	56	56	37	43	43	55
n-6 PUFA	15	7	8	6	5	55	6	14	0	0	0	6	54	58	63	33	80	58
n-6/n-3 ratio	8	5	3	5	1	25	0	0	0	0	0	0	37	40	33	20		32
n-3 PUFA																		
n-3 LC PUFA	9	7	2	3	8	34	0	14	0	33	11	13	67	57	100	67	75	71
ALA	17	9	7	3	7	51	6	0	0	0	0	4	71	22	63	67	58	57
EPA	17	9	8	4	6	52	0	11	13	0	17	10	53	56	75	25	67	58
DHA	17	9	8	3	7	51	12	22	13	0	0	12	59	78	63	33	58	61
n-6 PUFA																		
LA	17	9	8	4	7	51	0	0	0	0	14	2	29	56	50	50	25	37
AA	18	9	9	5	8	61	0	0	0	0	0	0	66	44	44	20	40	51
R-TFA																		
CLA	2	2	2	1	3	13	0	50	0	0	0	15	100	100	50		67	77
VA	2	1	1	0	3	9	100			.	0	22	100			.	100	100

Abbreviations: A, asthma; ALA, alpha-linolenic acid; AA, arachidonic acid; CI, confidence interval; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; E, eczema; EPA, eicosapentaenoic acid; LA, linoleic acid; n-3 PUFA, polyunsaturated fatty acids; n-3 LC PUFA, n-3 long chain polyunsaturated fatty acids; n-6 PUFA, polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; R, allergic rhinitis; R-TFA, ruminant trans fatty acids; S, sensitization; T, total; VA, vaccenic acid; W, wheeze; **.**, indicates no results.

Table 3. Meta-analyses of early life fatty acids status and allergic diseases.

Fatty acids	Eczema			Asthma			Wheeze			Allergic rhinitis			Sensitization		
	N * α	OR (95%CI)	I^2(%)	*N*	OR (95%CI)	I^2(%)	*N*	OR (95%CI)	I^2(%)	*N*	OR (95%CI)	I^2(%)	*N*	OR (95%CI)	I^2(%)
PUFA															
n-3 PUFA	10	0.92 (0.78, 1.08)	52.80	4	0.89 (0.75, 1.05)	0.00	4	1.06 (0.63, 1.80)	53.60	3	1.29 (0.78, 2.14)	61.90	2	1.04 (0.86 1.27)	9.10
n-6 PUFA	10	1.00 (0.90, 1.11)	49.70	4	0.97 (0.70, 1.35)	68.80	4	0.99 (0.87, 1.11)	30.20	3	1.03 (0.96, 1.10)	0.00	2	1.08 (0.94, 1.23)	9.40
n-6/n-3 ratio	**4**	**1.06 (1.00, 1.13)**	**55.20**	3	1.07 (0.86, 1.32)	43.70	2	0.99 (0.88, 1.10)	30.00	2	1.08 (0.94, 1.23)	9.40	1	.	.
n-3 PUFA															
n-3 LC PUFA	5	1.03 (0.76, 1.40)	48.80	4	0.89 (0.75, 1.06)	0.00	2	0.88 (0.60, 1.29)	19.90	3	0.92 (0.55, 1.52)	68.10	3	1.00 (0.69, 1.45)	0.00
ALA	9	0.98 (0.93, 1.04)	0.00	3	1.04 (0.87, 1.25)	0.00	4	1.02 (0.93, 1.12)	0.00	1	.	.	.1	.	.
EPA	9	0.97 (0.91, 1.05)	15.80	3	0.99 (0.71, 1.39)	73.20	4	0.95 (0.87, 1.04)	0.00	1	.	.	.1	.	.
DHA	9	0.97 (0.88, 1.08)	44.70	3	0.90 (0.73, 1.11)	0.00	4	1.00 (0.92,1.10)	15.90	2	1.09 (0.73,	55.30	1	.	.

											1.63)				
n-6 PUFA															
LA	**9**	**1.08 (1.01, 1.15)**	**0.00**	3	0.93 (0.66, 1.32)	65.70	4	1.14 (0.93, 1.40)	15.60	1	.	.	1	.	.
AA	10	0.94 (0.86, 1.02)	28.70	4	1.03 (0.84, 1.25)	0.00	5	1.01 (0.91, 1.13)	0.00	2	1.06 (0.51, 1.56)	0.00	2	0.90 (0.51, 1.56)	38.80
R-TFA															
CLA	2	0.67 (0.34, 1.32)	44.20	1	.	.	1	.	.	1	.	.	1	.	.
VA	**2**	**0.42 (0.25, 0.72)**	**0.00**	1	.	.	1	.	.	.	.	.	1	.	.

Abbreviations: ALA, alpha-linolenic acid; AA, arachidonic acid; CI, confidence interval; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; n-3 LC PUFA, n-3 long chain polyunsaturated fatty acids; n-3 PUFA, polyunsaturated fatty acids; n-6 PUFA, polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; R-TFA, ruminant trans fatty acids; VA, vaccenic acid; *****, number of study; **.**, only one study; $^{\alpha}$, I^2 >50% was considered evidence of heterogeneity; Bold indicates a significant study.

Table 4. Evidence synthesis stratified by main type of fatty acids and allergic outcomes.

	Evidence synthesis stratified by main allergic outcomes					Evidence synthesis for overall allergic outcomes
Fatty acids	Eczema	Asthma	Wheeze	Allergic rhinitis	Sensitization	Overall allergic outcomes
PUFA						
n-3 PUFA	Insufficient	Insufficient	Insufficient	Insufficient	Insufficient	Insufficient
	+ - 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^- 0^- 0^- 0^+ 0^+ 0^+	**0 0^- 0^- 0^- 0^+ 0^+ 0^+** 0^- 0^-	**- 0^- 0^+ 0^+ 0^+** 0^- 0^- 0^+	**0^- 0^- 0^+** 0^+ 0^+	**- 0^- 0^- 0^+ 0^+ 0^+** 0^+	**- - - - - - + 0 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+** 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+
n-6 PUFA	Insufficient	Insufficient	Insufficient	Insufficient	Insufficient	Insufficient
	- + + 0^- 0^- 0^- 0^- 0^+ 0^+ 0^- 0^- 0^+ 0^+ 0^+ 0^+	**- + 0^- 0^- 0^- 0^+** 0^- 0^+	**+ 0^- 0^- 0^- 0^+** 0^- 0^- 0^+	+ **0 0^- 0^+** 0^- 0^+	**0^- 0^- 0^- 0^-** 0^+	**- - - - + + + + 0 0 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+** 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+
n-6/n-3 ratio	Insufficient	Insufficient	Insufficient	Insufficient	Insufficient	Insufficient
	0^+ 0^+ 0^+ 0^- 0^- 0^- 0^- 0^-	**0^- 0^+ 0^+** 0^- 0^+	**0^- 0^+ 0^+**	+ **0^- 0^+** 0^+ 0^+	**0^+**	+ **0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+** 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+
n-3 PUFA						
n-3 LC PUFA	Insufficient	Insufficient	Insufficient	Insufficient	Insufficient	Insufficient
	0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^- 0^-	**- 0 0^- 0^- 0^+ 0^+** 0^-	**0^- 0^-**	**- 0^-** 0^+	- **0^- 0^- 0^- 0^+ 0^+** 0^- 0^- 0^-	**- - - - 0 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+** 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+
ALA	Insufficient	Insufficient	Insufficient	Insufficient	Insufficient	Insufficient
	- **0 0 0^- 0^- 0^- 0^- 0^- 0^- 0^+** 0^- 0^- 0^- 0^- 0^- 0^+ 0^+	**0^+ 0^+ 0^+ 0^+ 0^+ 0^+** 0^- 0^- 0^+	**0^- 0^- 0^- 0^+** 0^- 0^- 0^+	**0^-** 0^- 0^+	**+ 0^- 0^- 0^- 0^+ 0^+** 0^-	**- - + 0 0 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+** 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+
EPA	Insufficient **0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+** 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+	Insufficient **- 0^- 0^- 0^+ 0^+ 0^+** 0^- 0^- 0^+	Insufficient **- 0^- 0^- 0^- 0^+** 0^- 0^- 0^+	Insufficient **0^+** 0^- 0^+ 0^+	Insufficient **- 0^- 0^+ 0^+** 0^- 0^-	Insufficient **- - - - - 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+** 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+

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0^+ 0^+ 0^+ 0^+ 0^+

Table 4. *(Continued)*

Fatty acids	Evidence synthesis stratified by main allergic outcomes					Evidence synthesis for overall allergic outcomes
	Eczema	Asthma	Wheeze	Allergic rhinitis	Sensitization	Overall allergic outcomes
DHA	Insufficient **- - 0 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+** 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+	Insufficient **- - 0^- 0^- 0^- 0^+ 0^+** 0^- 0^-	Insufficient **- 0^- 0^- 0^- 0^+** 0^- 0^+ 0^+	Insufficient **+ 0^+ 0^-** 0^- 0^+	Insufficient **0 0^- 0^- 0^- 0^+ 0^+** 0^-	Insufficient **- - - - - - + 0 0 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+** 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+
n-6 PUFA						
LA	Insufficient **0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+** 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+	Insufficient **0^- 0^- 0^- 0^- 0^+ 0^+** 0^- 0^+ 0^+	Insufficient **+ 0^- 0^- 0^- 0^+ 0^+ 0^+** 0^- 0^+ 0^+	Insufficient + **0^+** 0^+ 0^-	Insufficient **- +** + **0^- 0^- 0^-** 0^+	Insufficient **- + + +** + + + **0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+** 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+
AA	Insufficient + 0 **0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+** 0^- 0^- 0^- 0^+ 0^+	Insufficient **0^- 0^- 0^+ 0^+ 0^+ 0^+** 0^- 0^- 0^+	Insufficient **0^- 0^- 0^- 0^+ 0^+ 0^+** 0^- 0^+ 0^+	Insufficient **0^- 0^+** 0^- 0^+ 0^+	Insufficient **0^- 0^+ 0^+ 0^+ 0^+ 0^+** 0^- 0^+	Insufficient **+ + +** 0 **0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+** 0^- 0^- 0^- 0^- 0^- 0^- 0^- 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+ 0^+
R-TFA						
CLA	Insufficient - **0^-**	Insufficient **- 0^-**	Insufficient **0^+** 0^-	Insufficient 0^-	Insufficient 0^- 0^- 0^+	Insufficient **- - - 0^- 0^- 0^- 0^+** 0^- 0^- 0^- 0^- 0^+ 0^+
VA	Moderate **- -**	Insufficient **0^-**	Insufficient **0^-**	**..** **..**	Insufficient **0^-** 0^- 0^-	Insufficient **- - 0^- 0^- 0^- 0^-** 0^- 0^- 0^-

Abbreviations: ALA, alpha-linolenic acid; AA, arachidonic acid; CLA, conjugated linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; n-3 LC PUFA, n-3 long chain polyunsaturated fatty acids; n-3 PUFA, polyunsaturated fatty acids; n-6 PUFA, polyunsaturated fatty acids; PUFA, polyunsaturated fatty acids; R-TFA, ruminant trans fatty acids; VA, vaccenic acid; **..**, no study; +, a positive relationship; -, an inverse relationship; 0, no relationship; 0^+, no significance for a positive relationship; 0^-, no significance for an inverse relationship; Bold indicates a high quality study.

Figure 1. Flowchart of the study selection for the systematic review and meta-analysis. *Sum of excluded abstracts exceeds 223 or 93 because investigators were not required to agree on reasons for exclusion. FA, fatty acid.

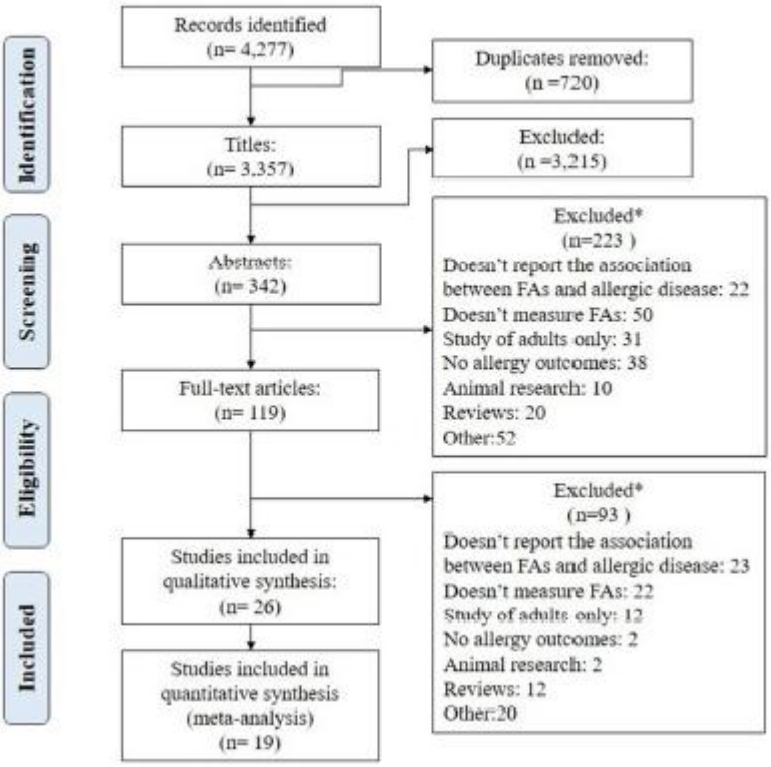

Figure 2. Forest plot of the effect of early life natural exposure to n–6/n-3 PUFA ratio on allergic diseases in children.

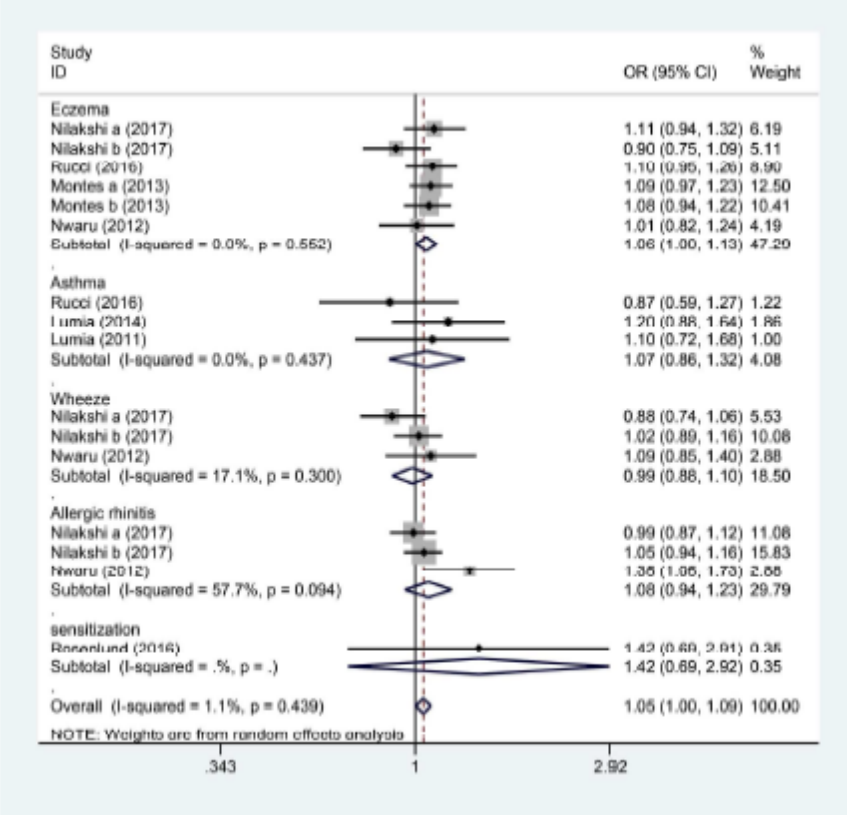

Figure 3. Forest plot of the effect of early life natural exposure to LA on allergic diseases in children.

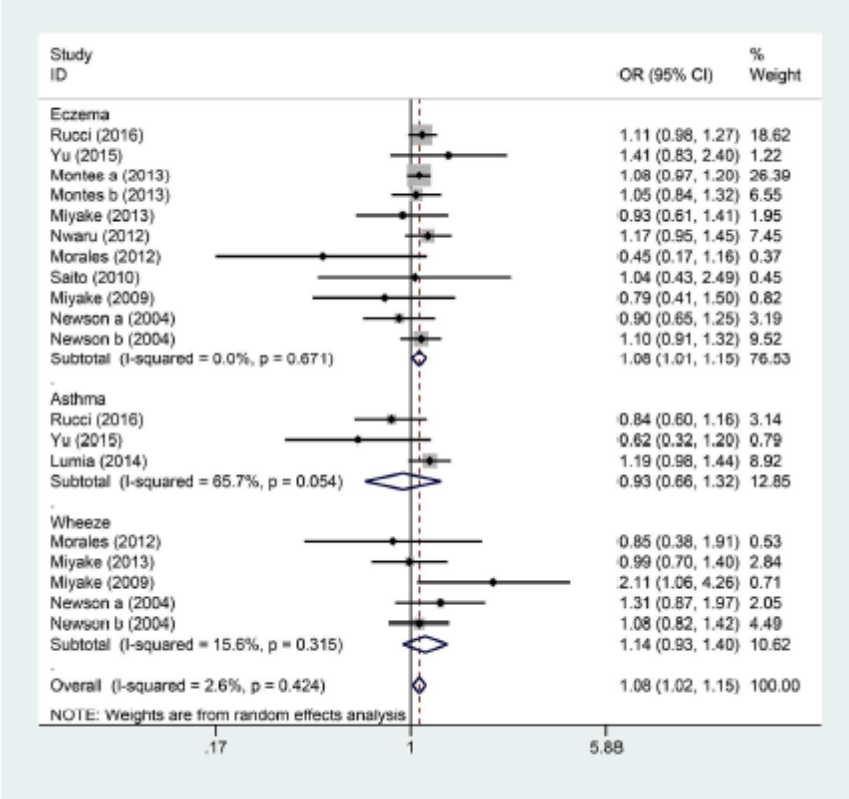

Figure 4. Forest plot of the effect of early life natural exposure to VA on eczema in children.