

Prenatal Intake of Vitamins and Allergic Outcomes in the Offspring: A Systematic Review and Meta-Analysis



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What is already known about this topic? Few observational studies suggest that vitamin deficiency is associated with developing higher prevalence of allergic diseases in children; however, we need robust evidence from randomized controlled trials to determine if this is the case.

What does this article add to our knowledge? This systematic review indicates that prenatal intake of vitamin D may protect against the development of recurrent childhood wheeze. Because early childhood wheeze is not necessarily the same as asthma, longer-term follow-ups of these trials are required to establish the efficacy of vitamin D in the prevention of actual asthma in later childhood.

How does this study impact current management guidelines? Consumption of higher doses of vitamin D during pregnancy needs to be considered in pregnancy management policies. However, the effective dose could vary depending on the baseline level of vitamin D in different regions.

BACKGROUND: Allergic diseases have seen a rise worldwide, with children suffering the highest burden. Thus, early prevention of allergic diseases is a public health priority.

OBJECTIVE: To synthesize the evidence from randomized controlled trials (RCTs) assessing the effect of vitamin interventions during pregnancy on developing allergic diseases in offspring.

METHODS: We searched CENTRAL, MEDLINE, SCOPUS, World Health Organization's International Clinical Trials Registration, E-theses, and Web of Science. Study quality was evaluated using Cochrane's risk of bias tool. Included RCTs had a minimum of 1-month follow-up postgestation.

RESULTS: A total of 5 RCTs met the inclusion criteria, including 2456 children who used vitamins C + E (1 study), vitamin C (1 study), and vitamin D (3 studies) compared with placebo/control. Two studies were judged to have a high risk of bias for performance bias or a high rate of loss to follow-up. All were rated as low risk of bias for blinding of outcome

assessment. We did not perform meta-analysis with vitamin C or vitamin C + E studies due to high heterogeneity between the 2 included studies. However, we did conduct a meta-analysis with trials on vitamin D (including 1493 children) and the results showed an association between the prenatal intake of vitamin D and the risk of developing recurrent wheeze in offspring (relative risk (RR), 0.812; 95% CI, 0.67-0.98).

CONCLUSIONS: The current evidence suggests that prenatal supplementation of vitamin D might have a beneficial effect on recurrent wheezing in children. Longer-term follow-up of these studies is needed to ascertain whether this observed effect is sustained. There is lack of evidence on the effect of other vitamins for the prevention of respiratory and/or allergic outcomes. © 2016 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2017;5:771-8)

Key words: Vitamins; Allergic outcomes; Asthma; Wheeze; Wheezing; Respiratory outcomes; Eczema; Offspring; Clinical trial; Intervention; Efficacy; Effectiveness; Systematic review; Meta-analysis

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In the last 2 decades, allergic diseases have seen a rise worldwide, with children suffering the highest burden of the condition.¹ Food allergies, eczema, and asthma are the most common allergic disorders in children.^{1,2} Because of the increasing burden of allergic diseases, they are a key focus for public health.

The Developmental Origins of Health and Disease theory proposes that development is not dictated by a hard-wired genetic program; instead, the organism responds to the surrounding environment and the risk of many diseases is set during this time.³ It has become increasingly evident that there is an important role for environmental factors in the onset of complex conditions such as allergic diseases and that the role of fixed

Abbreviations used

HR- Hazard ratio

RCT- Randomized controlled trial

RR- Relative risk

genetic variation is far less than previously believed.⁴ Therefore, new approaches toward disease prevention with an emphasis on early interventions, that is, prepregnancy and/or during pregnancy, need to be widely investigated. Current evidence suggests that the role of maternal diet during pregnancy in subsequent disease development is a priority area for future studies,⁵ because many of the immune modulatory processes may start *in utero*.

The role of environmental and lifestyle factors in developing allergies has been examined in a number of epidemiological studies. A systematic review has investigated the association of nutrient deficiencies with the risk of development of asthma and allergic diseases in children.⁶ This review included 62 observational studies and indicated that vitamins A, D, and E; zinc; fruits and vegetables; and a Mediterranean diet during pregnancy may prevent asthma and wheeze. However, this review was based on observational studies, which carry a high risk of bias, and there is a need for secondary research based on summary of more robust interventional studies.

The purpose of this systematic review was to summarize the existing evidence from randomized controlled trials (RCTs) for the association between intake of vitamin supplements during pregnancy and the risk of developing allergic disorders in the offspring.

METHODS**Criteria for considering studies for this review**

Types of studies. Only RCTs (including cluster RCTs and quasi-RCTs) with a minimum follow-up of 1 month postnatally were included. The review considered studies that documented clinical outcome data and used any types of vitamins. No language restriction was applied.

Types of participants. Pregnant women and their offspring, regardless of their location, were considered as the target group for this systematic review. High-risk populations were not excluded.

Types of interventions. Studies that used any vitamin supplementation during pregnancy, irrespective of dose, formulation or mode of delivery, and composition, for example, oil and tablet, were included.

Trials were also included if the intervention(s) had been extended after pregnancy either during breast-feeding or with the infants or both.

Outcomes of interest. Trials were included if they had reported clinical outcomes of allergy in the offspring, either as a primary end point or as a secondary end point. Allergic outcomes were defined as asthma, wheeze, rhinitis, eczema, food allergy, and positive skin prick test result (to any allergen) and elevated specific IgE level. Outcomes included were those that had used a validated method as opposed to parental reports.

Search strategy for identification of studies. A comprehensive search strategy, including all the relevant synonyms for the main concepts, was developed covering the main

bibliographic databases (see this article's Online Repository at www.jaci-inpractice.org). Trials were identified through systematic searches within 3 main electronic databases, as advised by the Cochrane collaboration⁷:

- a. Cochrane Library (current issue) including the following:
 - Cochrane Database of Systematic Reviews (CDSR)
 - CENTRAL (trials)
 - Database of Reviews of Effectiveness
- b. MEDLINE (EBSCOhost)
- c. SCOPUS

When searching MEDLINE, the subject-specific terms were combined with the Cochrane Highly Sensitive Search Strategy for identifying randomized trials in MEDLINE: sensitivity-maximising version.⁷ We adapted the preliminary search strategy for MEDLINE (EBSCOhost) for use in the other databases when relevant. The last search for literature was conducted in January 2016.

The clinical trials registry and World Health Organization platform were searched for ongoing and recently completed trials. Conference proceedings were identified through the Institute for Scientific Information Web of Science, and the British Library E-Theses Online Service was searched for retrieving theses. No language or publication status restrictions were imposed. References of included studies were crosschecked for additional studies.

Data collection and analysis

Selection of studies. The main reviewer (M.V.) screened all the search results against the eligibility criteria and all those that were clearly irrelevant were excluded from further consideration. Thereafter, a tailored eligibility form was used by M.V. to appraise the retrieved studies, abstract, and full text for relevance against the full inclusion criteria. Where there was uncertainty about the inclusion of a particular study, other members of the review team (H.M. and T.D.) were consulted and a consensus was reached about the study eligibility. All the included studies were discussed and approved by the review team.

Data extraction. M.V. extracted the data using a tailored data extraction form (Table E1, available in this article's Online Repository at www.jaci-inpractice.org). Detailed information on study characteristics was recorded. Throughout the data extraction process, any disagreements about the interventions and outcomes were discussed and resolved within the review team. There was no blinding to the name of authors, institutions, journals, or the outcomes of the trials during the process. Ten percent of all the extracted data was randomly selected and double checked by a second reviewer (H.M.) for accuracy against the trial reports.

Assessment of risk of bias in included studies. The risk of bias tool described in the Cochrane Handbook for Systematic Reviews for Interventions was used to appraise the studies.⁸ The tool includes 7 domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessments, incomplete outcome data, selective outcome reporting, and other bias.

Measurement of treatment effect

Dichotomous data were analyzed as relative risk (RR) with 95% CI and continuous data as mean difference or standardized mean difference, with 95% CI.

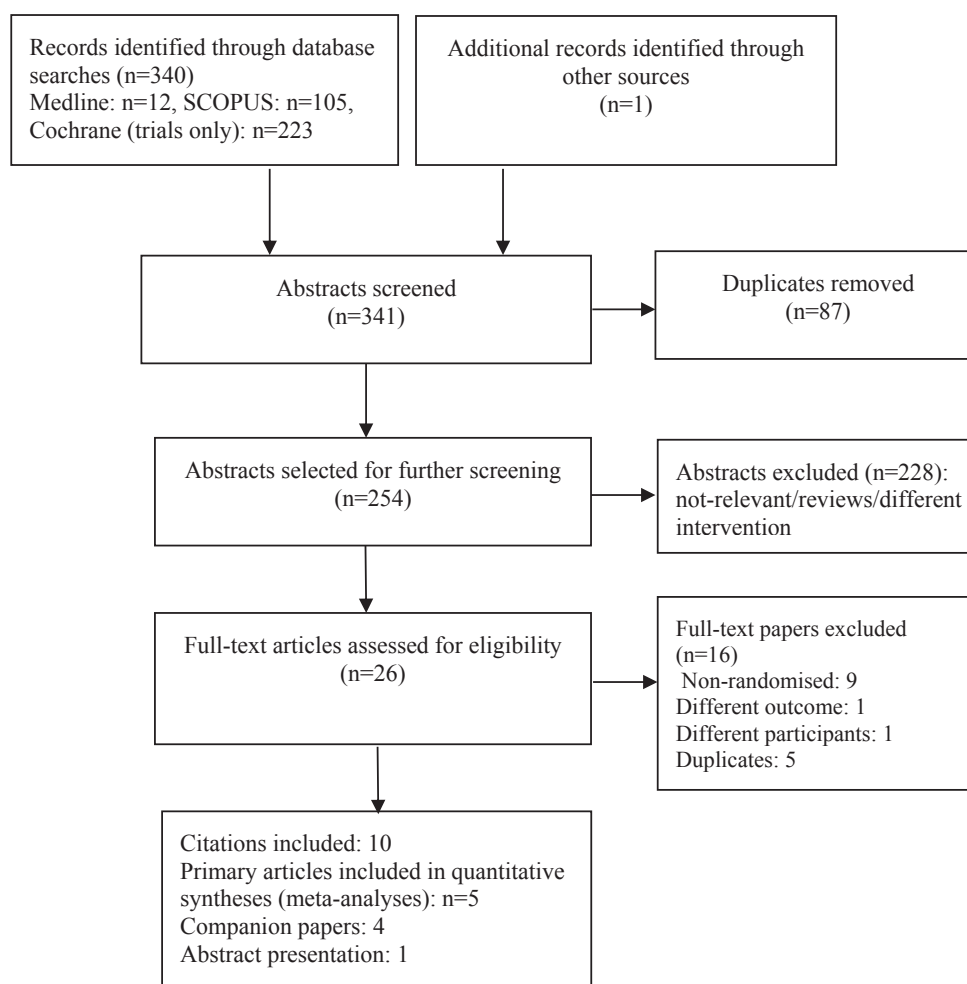


FIGURE 1. Study flow diagram, following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria.

Unit of analysis issues. In trials with more than 1 intervention arm, multiple pairwise comparisons of intervention groups versus comparator were avoided. Therefore, data from different intervention arms were pooled for an overall comparison with the control group or the placebo arm. The weight assigned to the control group was considered as the total number of participants in the comparator group versus the total number of participants in the combined intervention arms.⁹

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

Assessment of heterogeneity. We used visual inspection of Forest plots and also the chi-square test to measure statistical heterogeneity between effect sizes of included studies ($P < .05$).¹⁰ I^2 statistics were used to quantify the amount of possible variability in effect estimates that is due to heterogeneity rather than chance ($I^2 > 30\%$, moderate heterogeneity; $I^2 \geq 75\%$, considerable heterogeneity).

Assessment of reporting biases. Every effort was made to identify unpublished studies through searching abstracts and ongoing trials databases. Publication bias was assessed using funnel plots.¹¹

The asymmetry was assessed visually in the plots and no formal statistical tests were conducted. The funnel plot was helpful to explore possible small study biases for some of the primary outcomes (see this article's Online Repository at www.jaci-inpractice.org).

Data synthesis. We used Eppi Reviewer version 4.4.3.0. for conducting meta-analyses using random-effects model. Dichotomous data were entered as events and the number of participants. Data were pooled using the random-effects model where heterogeneity was reported as 75% or less.⁷ We also reported RR as a statistical choice in conducting the meta-analyses because it is easy to interpret.¹²

Subgroup analysis and investigation of heterogeneity. We performed subgroup analyses on the basis of type of vitamin and type of control group (ie, placebo vs no treatment).

Sensitivity analysis. We did not conduct any sensitivity analysis because of the small number of studies that contributed to meta-analyses.

RESULTS

The results of the search strategy yielded 341 studies, of which 26 were selected for full-text assessment (Figure 1). We included

TABLE I. Characteristics of included trials and study population for vitamins and prevention of respiratory and/or allergic outcomes in offspring

Primary article	Companion articles*	Country, enrollment period	No. of participants [†]	Age at last F-U	Sample: high risk of atopy	Intake of intervention from/until	Duration of intervention (mo) [‡]	Vitamin product	Placebo	Total daily dose	Outcomes reported
Greenough et al, ¹³ 2010	Poston et al, ¹⁸ 2006	UK 2003-2005	2404 mothers	2 y	No	From the second trimester of pregnancy to delivery	6-6.5	Vitamins C & E	Microcrystalline cellulose with addition of tartaric & citric acid + sunflower seed oil	1000 mg vitamin C & 400 IU RRR α -tocopherol, daily	- Wheeze - Eczema - Asthma - Cough - Breathing difficulty
Goldring et al, ¹⁴ 2013	Yu et al, ¹⁹ 2009	UK 2007-not mentioned	180 mothers	3 y	No	27 wk to delivery	3 mo + 1 wk	Vitamin D (cholecalciferol) or vitamin D (ergocalciferol)	No treatment	Single oral dose of 200,000 IU (bolus) or 800 IU daily	- Wheeze - Eczema - Food allergy - Rhinitis - Atopy - URTI - LRTI - Inhaled bronchodilator or steroid
McEvoy et al, ¹⁵ 2014	McEvoy 2013 (conference abstract)	USA 2007-2011	179 mothers	1 y	No	22 wk to delivery	4-4.5	Crushed vitamin C	Ground cornstarch	500 mg, daily	- Wheeze - Breathing difficulty
Chawes et al, ¹⁶ 2016	Bisgaard et al, ²⁰ 2013	Denmark 2008-2010	623	3 y	No	24 wk to 1 wk after delivery	3.5-4 + 1 wk	Vitamin D ₃ (cholecalciferol)	Tablets containing no active substance	2400 IU, once a day	- Persistent wheeze - Asthma - URTI - LRTI - Episodes of lung symptoms - SPT - Specific IgE
Litonjua et al, ¹⁷ 2016	Litonjua et al, ²¹ 2014	USA 2009-2011	880	3 y	Yes	Between 10 and 18 wk to delivery	5-7.5	Vitamin D & placebo	Not mentioned	4000 IU, daily	- Wheeze or asthma - Eczema with rash - LRTI - Total IgE (mean) - Sensitization (aeroallergens) - Specific IgE

LRTI, Lower respiratory tract infection; SPT, skin prick test; URTI, upper respiratory tract infection.

The outcomes of interest for this systematic review are in bold.

*Published data and conference abstracts. No unique data were extracted from conference abstracts and were not included in the reference list.

[†]Number at randomization, where recruitment occurred prenatally.

[‡]Total duration in pregnancy, including after birth, if applicable.

5 RCTs comparing at least 1 vitamin with a control that met the inclusion criteria for this systematic review.

These included trials (including a total of 2456 children) were represented by 5 original articles¹³⁻¹⁷ and 4 grouped as their companion articles.¹⁸⁻²¹ Table I presents the characteristics of the included trials, their companion articles, and the study population. The trials were conducted in the United Kingdom, Denmark, and the United States. The types of vitamin supplementations included were vitamins C + E,¹³ vitamin D,^{14,16,17} and crushed vitamin C.¹⁵ The duration of intervention and follow-up in the included studies varied from 3.5-4 to 7.5 months and 12 to 36 months, respectively. In trials that used vitamin C and vitamins C + E, a higher blood concentration of vitamins was observed in those assigned antioxidants.^{13,15} In trials that used vitamin D, level of maternal 25-hydroxyvitamin D measured either at third trimester or after delivery was significantly higher in the treatment group versus the comparison group.^{14,16,17} The most frequently reported outcomes were wheeze and eczema. As expected with systematic reviews, there were differences between the included trials in terms of the type of the population, the supplementation used, and the comparators. We have therefore described the results of individual studies narratively and conducted meta-analysis only when there was no evidence of statistical heterogeneity. The definition and diagnosis method of the outcomes in each study are presented in Table E2, available in this article's Online Repository at www.jaci-inpractice.org.

Vitamin C studies

Greenough et al's (2010) study. The study was conducted in the United Kingdom between August 2003 and June 2007.¹³ The studied sample was pregnant women at risk of developing preeclampsia. Women were supplemented with daily vitamin C (1000 mg) tablets and vitamin E (400 IU) gelatin capsules, from 16 to 22 gestation weeks until delivery. Women in the control group received identical tablets of microcrystalline cellulose with addition of tartaric acid and citric acid along with gelatin capsules of sunflower seed oil. Compliance with the intervention was measured by counts of returned pills. Primarily, this study was designed to prevent the risk of fetal growth restriction and premature delivery in the women¹⁸ and the extended follow-up at 2 years has assessed the effect of the vitamin intervention on respiratory outcomes in children.

The list of the reported outcomes in the study is presented in Table I. The outcomes of "asthma" and "eczema" are reported at age 1 year and "recurrent wheeze" at age 2 years. No statistically significant association was observed between the intervention and control groups for prevention of recurrent wheeze (10 of 386 vs 11 of 366; odds ratio [OR], 0.83; 95% CI, 0.26-2.59; $P = .66$) and asthma (23 of 386 vs 23 of 366; OR, 0.94; 95% CI, 0.42-2.11; $P = .85$). In addition, the results did not show a significant association between prenatal intake of vitamins C + E and prevention of eczema (98 of 386 vs 86 of 366; OR, 1.10; 95% CI, 0.70-1.74; $P = .58$).

McEvoy et al's (2014) study. The study was conducted in the United States between March 2007 and January 2011.¹⁵ The studied sample was smoking pregnant women. Women were supplemented with daily crushed vitamin C (500 mg) gel capsules, from 22nd gestation week until delivery. Women in the control group received ground cornstarch in gel capsules.

Adherence was measured by dividing the number of capsules taken by the total number prescribed in a given period.

The study reported the efficiency of consumption of vitamin C during pregnancy on pulmonary function tests and wheezing in children at age 1 year. The list of the reported outcomes in the study is presented in Table I. The results of the unadjusted analysis showed no significant statistical association between the intervention and control groups for outcome measure defined as "recurrent wheeze" (9 of 76 vs 17 of 83; RR, 0.56; 95% CI, 0.27-1.18; $P = .13$). A significant difference was observed for the outcome of "at least 1 episode of wheezing" between the intervention and control groups (15 of 76 vs 31 of 83; RR, 0.56; 95% CI, 0.33-0.95; $P = .03$).

Given the fact that there is high heterogeneity between the studies that supplemented pregnant women prenatally with vitamin C, we did not perform meta-analysis for these trials.

Vitamin D studies

Goldring et al's (2013) study. The study was conducted in the United Kingdom between April and November 2007.¹⁴ This study recruited pregnant women with multiple ethnicities. The study introduced 2 intervention arms, as women were randomized either to receive a daily dose of ergocalciferol (800 IU) or a single oral dose of cholecalciferol (200,000 IU, bolus), from 27 gestation weeks until delivery. The comparator in this study was defined as "no treatment." Adherence was measured by telephone calls during pregnancy.

This study followed-up children to age 3 years and this systematic review only reports the results for the intervention arm of daily vitamin D. The results of unadjusted analysis for "recurrent wheezing" showed no statistically significant association between prenatal intake of daily vitamin D and the control group (8 of 56 vs 7 of 50; RR, 1.02; 95% CI, 0.40-2.61; $P = .97$). Furthermore, no significant association was observed for the outcome measure of "wheeze with positive asthma predictive index" (6 of 56 vs 7 of 50; RR, 0.77; 95% CI, 0.28-2.13; $P = 0.61$) between the study arms. The outcomes of "eczema in the last year" (11 of 55 vs 7 of 49; RR, 1.40; 95% CI, 0.59-3.33; $P = .44$) and "food allergy diagnosis" (8 of 55 vs 3 of 49; RR, 2.38; 95% CI, 0.67-8.46; $P = .16$) did not show a significant statistical association for the prenatal consumption of daily vitamin D in comparison to control.

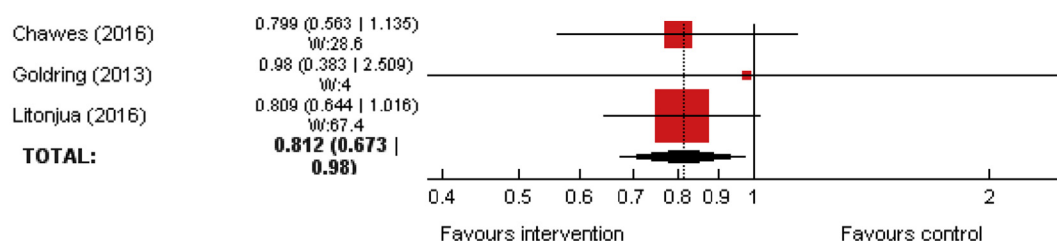
Chawes et al's (2016) study. The study was conducted in Denmark between 2008 and 2010.¹⁶ The studied sample was unselected pregnant women. Women were supplemented with daily vitamin D₃ (2400 IU) tablets, from 24 gestation weeks to 1 week after delivery. Women in the control arm received tablets containing no active substance. In addition, women assigned to both intervention and control arms received an extra 400-IU dose of vitamin D₃ as part of their routine care. Compliance to the intervention was measured by counts of returned pills.

The study reported the cumulative incidence of allergic outcomes by age 3 years. The results of unadjusted analysis indicated that the risk of developing recurrent wheeze did not show a significant difference between the intervention and control groups (47 of 295 vs 57 of 286; hazard ratio [HR], 0.76; 95% CI, 0.52-1.12; $P = .16$). Asthma was reported at age 3 years only and no significant difference was observed between the intervention and control groups (32 of 278 vs 47 of 271; OR, 0.82; 95% CI, 0.50-1.36; $P = .45$). Furthermore, there was no

Measure: Binary: risk ratio

Heterogeneity: $Q = 0.163$; $df = 2$; $p = 0.922$; $I^2 = 0\%$; $\tau^2 = 0$

Random effects model: 0.812 (0.673, 0.98)



Outcome	Vitamin D	Placebo	Participants	Study sample
Wheeze-Daily Vitamin D vs. Placebo or no treatment	n/N	n/N		
Goldring2013	7/50	8/56		Unselected
Chawes 2016	47/295	57/286		Unselected
Litonjua 2016	98/405	120/401		Atopic
Subtotal	750	743	1493	

FIGURE 2. Forest plot for daily vitamin D intake vs placebo or no treatment as the control for prevention of recurrent wheeze in offspring.

significant statistical difference between the study arms for eczema as an outcome (68 of 295 vs 72 of 286; HR, 0.90; 95% CI, 0.65-1.26; $P = .55$). Children in the intervention arm reported statistically significant “lower episodes of troublesome lung symptoms” compared with the control group (5.9 vs 7.2; incidence rate ratio (IRR), 0.83; 95% CI, 0.71-0.97; $P = .02$). The cumulative results for skin prick test and specific IgE outcomes were not statistically different between the intervention and control groups (24 of 294 vs 19 of 283; OR, 1.24; 95% CI, 0.66-2.31; $P = .51$ and 34 of 289 vs 22 of 278; OR, 1.55; 95% CI, 0.89-2.73; $P = .13$, respectively).

Litonjua et al’s (2016) study. The study was conducted in the United States between 2009 and 2011.¹⁷ The study sample was women with a history of atopy. Women were supplemented with daily vitamin D₃ (4000 IU) tablets, between 10 and 18 gestation weeks until delivery. The nature of the placebo capsules was not reported. Women in both study arms also received a multivitamin with 400 IU of vitamin D. Adherence to the intervention was measured by electronic medication container caps.

The study reported the cumulative incidence of allergic outcomes by age 3 years. The outcomes of “asthma or recurrent wheeze” were reported together and the results showed no significant statistical difference between the intervention and control groups (98 of 405 vs 120 of 401; HR, 0.8; 95% CI, 0.6-1.0; $P = .051$). There was also no significant statistical difference in the risk of developing “eczema with rash” in the study arms (83 of 405 vs 89 of 401; HR, 0.9; 95% CI, 0.7-1.2; $P = .56$). The result for positive specific IgE test results at 3 years showed a significant statistical difference between the intervention and

control groups (43 of 405 vs 50 of 401; mean difference (MD), -1.7; 95% CI, -3.4 to 0.0; $P = .02$).

Meta-analyses of vitamin D studies

We conducted a meta-analysis for the outcome measure of “recurrent wheeze” for trials that used vitamin D prenatally in pregnant women. Figure 2 shows the Forest plot for this outcome. Three trials contributed to the meta-analysis including a total of 1493 children. No statistical heterogeneity was observed between the included trials ($\chi^2 = 0.16$; $P = .92$; $I^2 = 0\%$) (Figure 2). The results of the present meta-analysis showed an association between the maternal intake of daily vitamin D during pregnancy and a lower risk of developing recurrent wheeze in offspring (RR, 0.812; 95% CI, 0.673-0.98). We also conducted the meta-analysis including only the 2 recent vitamin D trials^{16,17} and it yielded similar results (Forest plot not shown).

Risk of bias in included trials

The risk of bias figures and authors’ judgments are presented in Table E3, available in this article’s Online Repository at www.jaci-inpractice.org. Only 1 trial was deemed to have a low risk of bias across all domains.¹⁷ Of the 5 trials, most had adequate random sequence generation ($n = 3$), allocation concealment ($n = 3$), and performance bias ($n = 3$). All trials were rated as having a low risk of bias for blinding of outcome assessment and selective outcome reporting. Completeness of outcome data was rated as having high risk of bias for 1 trial¹³ because the study had a high loss to follow-up and the authors acknowledged the fact that the study was an unplanned extended follow-up of the original trial for measuring allergic outcomes in children. The original trial was primarily designed to assess the effect of

vitamins C and E supplementation on developing preeclampsia in women at increased risk.

DISCUSSION

This is the first systematic review of RCTs that investigated the association of prenatal intake of vitamins with the risk of developing allergic/respiratory diseases in the offspring. We identified 5 RCTs with a total of 2456 children. The studies were of unselected pregnant women,¹⁶ women with a history of atopy,¹⁷ pregnant women at risk of developing preeclampsia,¹³ different ethnic/race groups,¹⁴ and smoking pregnant women.¹⁵ Two studies were judged to have a high risk of bias due to their performance bias^{13,14} or a high rate of loss to follow-up.¹³ All trials were rated as having a low risk of bias for blinding of outcome assessment. It was not possible to conduct meta-analyses for vitamin C studies due to observed differences between the included trials. Maternal vitamin D consumption during pregnancy was associated with a lower risk of developing recurrent wheeze in offspring, when compared with placebo/control. However, we were not able to investigate the effect of vitamin D on other allergic outcomes because outcomes were reported differently in the included trials. In all trials, supplementation with vitamins significantly increased the concentration of vitamins in the intervention group compared with the control group by the end of the intervention.

Observational studies typically report a beneficial effect of higher intake of vitamin D as well as antioxidants during pregnancy on allergic outcomes.^{22,23} The results from this systematic review proposed a protective effect of prenatal intake of vitamin D during pregnancy for prevention of recurrent wheeze in offspring. However, we could not address the effect of prenatal intake of vitamin C or D on other allergic outcomes owing to the observed heterogeneity between the trials.

It is possible that the follow-up periods of the studies for this review have been too short to detect other allergic outcomes, that is, asthma. For example, wheezing is known as a primary symptom of asthma in early childhood²⁴ and about 40% of childhood wheeze will persist later in life and will eventually develop into asthma by age 6 years,^{25,26} indicating that majority of cases of wheeze during infancy are in fact acute respiratory infection. Therefore, extended follow-up of these trials could help to provide a clearer answer as to whether the vitamin D intervention is beneficial for asthma prevention.

There were also some limitations in the studies' design. For example, the trials were statistically underpowered to detect an effect for their primary and/or secondary outcome measures. Significant differences were observed for only some of the secondary outcomes as "at least 1 episode of wheezing,"¹⁴ "episodes of troublesome lung symptoms,"¹⁶ and "positive specific IgE"¹⁷ and trials failed to show a beneficial effect for primary allergic outcomes such as wheeze and asthma in children. Also, the trials used different doses of vitamins during pregnancy. The dose of vitamin D varied between 800 and 4000 IU and doses of vitamins C and/or E varied between 500 and 1000 mg. It is possible to hypothesize that lower doses of vitamins may have failed to reach the desirable level of 25-hydroxyvitamin D or antioxidants in pregnant women to have an influential effect on the fetal immune programming and lung function.²⁷⁻²⁹ However, this is refuted by studies that have reported similar effect size using higher doses of vitamin D.^{16,17} A previous RCT by addressing

the safety and efficacy of vitamin D supplementation during pregnancy showed that a 4000-IU dose of vitamin D is a safe approach and was necessary to optimize the circulating concentration of 25-hydroxyvitamin D levels to 80 nmol/L or more.³⁰ There is limited evidence on the safety of intake of vitamins C and E at any stage of pregnancy; however, the Institute of Medicine's Food and Nutrition Board has set an upper limit of 2000 mg and 1000 mg per day for the ingestion of vitamins C and E, respectively, during pregnancy in the United States.³¹

Furthermore, in all trials, the intervention was started in the second trimester in pregnancy. However, the development of the lungs begins in the first trimester in pregnancy and vitamin D plays an immunomodulatory role in the development of lung and immune system.³² Therefore, the interventions might have commenced too late in pregnancy or some used too low doses of vitamin D to have a beneficial impact on lung development. Finally, the studies recruited different types of population, which limits the generalizability of the studies. Baseline levels of vitamin D vary in different geographical areas³³ and this issue has not been addressed in the conducted trials. Well-designed trials are necessary to address all these possible confounders among different populations.³⁴ Further larger scale research should administer vitamin D earlier in pregnancy or prepregnancy and use appropriate doses of vitamin D to achieve a desirable level of vitamin D in maternal and fetal blood. Furthermore, studies assessing the efficiency of nutrients are required to consider the defined guidelines in their clinical design, enabling to test the associated hypothesis in a valid manner.³⁵

To date, no other systematic review has evaluated the effect of prenatal vitamins on the prevention of allergic and/or respiratory outcomes in children. The result from the current evidence is promising that prenatal intake of vitamin D could protect childhood wheeze. The role of maternal consumption of vitamins during pregnancy in the risk of developing other allergic outcomes and sensitization needs to be investigated in larger well-designed trials. Furthermore, it will be important for future research to examine the impact of the timing of the intervention and the optimum dose of vitamins. We were unable to perform any meta-analyses on the timing or dose of intervention and study populations due to the small number of trials that could contribute to meta-analyses.

The current evidence suggests that prenatal intake of daily vitamin D might protect against recurrent childhood wheeze; however, there is currently lack of evidence that prenatal intake of vitamins can prevent any other allergic/respiratory outcomes.

REFERENCES

1. Pawankar RG, Canonica W, Holgate ST, Lockey RF, Blaiss MS, editors. White book on allergy: update 2013. Milwaukee, WI: U.K. World Allergy Organization; 2013.
2. Asher J, Pearce N. Global burden of asthma among children. *Int J Tuberc Lung Dis* 2014;18:1269-78.
3. Bernal AJ, Jirtle RL. Epigenomic disruption: the effects of early developmental exposures. *Birth Defects Res Part A Clin Mol Teratol* 2010;88:938-44.
4. Barouki R, Gluckman PD, Grandjean P, Hanson M, Heindel JJ. Developmental origins of non-communicable disease: implications for research and public health. *Environ Health* 2012;11:42.
5. Sausenthaler S, Heunrich J, Koletzko S, GINIplus and LISA plus Study Group. Early diet and the risk of allergy: what can we learn from the prospective birth cohort studies GINIplus and LISAplus? *Am J Clin Nutr* 2011;94:2012S-7S.
6. Nurmatov U, Devereux G, Sheikh A. Nutrients and foods for the primary prevention of asthma and allergy: systematic review and meta-analysis. *J Allergy Clin Immunol* 2011;127:724-33.

7. Lefebvre C, Manheimer E, Glanville J. Chapter 6: Searching for studies. In: Higgins JPT, Green S, editors. *Cochrane handbook for systematic reviews of interventions* version 5.1.0 (updated March 2011). The Cochrane Collaboration. Available at: www.cochrane-handbook.org; 2011. Accessed February 27, 2016.
8. Higgins JPT, Altman DG, Sterne JAC. Chapter 8: Assessing risk of bias in included studies. In: Higgins JPT, Green S, editors. *Cochrane handbook for systematic reviews of interventions* version 5.1.0 (updated March 2011). The Cochrane Collaboration. Available at: www.cochrane-handbook.org; 2011. Accessed February 27, 2016.
9. Higgins JPT, Deeks JJ, Altman DG. Chapter 16: Special topics in statistics. In: Higgins JPT, Green S, editors. *Cochrane handbook for systematic reviews of interventions* version 5.1.0 (updated March 2011). The Cochrane Collaboration. Available at: www.cochrane-handbook.org; 2011. Accessed February 27, 2016.
10. Deeks JJ, Altman DG, Bradburn MJ. Statistical methods for examining heterogeneity and combining results from several studies in meta-analysis. In: Egger M, Davey Smith G, Altman DG, editors. *Systematic reviews in health care: meta-analysis in context* (2nd ed). London, UK: BMJ Publication Group; 2001.
11. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ (Clinical research ed.)* 1997;315:629-34.
12. Deeks JJ, Higgins JPT, Altman DG. Chapter 9: Analysing data and undertaking meta-analyses. In: Higgins JPT, Green S, editors. *Cochrane handbook for systematic reviews of interventions* version 5.1.0 (updated March 2011). The Cochrane Collaboration. Available at: www.cochrane-handbook.org; 2011. Accessed February 27, 2016.
13. Greenough A, Shaheen SO, Shennan A, Seed PT, Poston L. Respiratory outcomes in early childhood following antenatal vitamin C and E supplementation. *Thorax* 2010;65:998.e1003.
14. Goldring ST, Griffiths CJ, Martineau AR, Robinson S, Yu C, Poulton S, et al. Prenatal vitamin D supplementation and child respiratory health: a randomised controlled trial. *PLoS One* 2013;8:e66627.
15. McEvoy CT, Schilling D, Clay N, Jackson K, Go MD, Spitalo P, et al. Vitamin C supplementation for pregnant smoking women and pulmonary function in their newborn infants: a randomized clinical trial. *JAMA* 2014;311:2074-82.
16. Chawes BL, Bønnelykke K, Stokholm J, Vissing NH, Bjarnadottir E, Schoos AM, et al. Effect of vitamin D3 supplementation during pregnancy on risk of persistent wheeze in the offspring: a randomized clinical trial. *JAMA* 2016;315:353-61.
17. Litonjua AA, Carey VJ, Laranjo N, Harshfield BJ, McElrath TF, O'Connor GT, et al. Effect of prenatal supplementation with vitamin D on asthma or recurrent wheezing in offspring by age 3 years. The VDAART randomized clinical trial. *JAMA* 2016;315:362-70.
18. Poston L, Briley AL, Seed PT, Kelly JF, Shenan AH, Vitamins in Pre-eclampsia (VIP) Trial Consortium. Vitamin C and vitamin E in pregnant women at risk for pre-eclampsia (VIP trial): randomised placebo-controlled trial. *Lancet* 2006;367:1145-54.
19. Yu CKH, Sykes L, Sethi M, Teoh TG, Robinson S. Vitamin D deficiency and supplementation during pregnancy. *Clin Endocrinol* 2009;70:685-90.
20. Bisgaard H, Vissing NH, Carson CG, Bischoff AL, Følsgaard NV, Kreiner-Møller E, et al. Deep phenotyping of the unselected COPSAC2010 birth cohort study. *Clin Exp Allergy* 2013;43:1384-94.
21. Litonjua AA, Lange NE, Carey VJ, Brown S, Laranjo N, Harshfield BJ, et al. The Vitamin D Antenatal Asthma Reduction Trial (VDAART): rationale, design, and methods of a randomized, controlled trial of vitamin D supplementation in pregnancy for the primary prevention of asthma and allergies in children. *Contemp Clin Trials* 2014;38:37-50.
22. Palmer DJ, Sullivan TR, Skeaff CM, Smithers LG, Makrides M, DOMInO Allergy Follow-up Team. Higher cord blood 25-hydroxyvitamin D concentrations reduce the risk of early childhood eczema in children with a family history of allergic disease. *World Allergy Organ J* 2015;8:28-34.
23. Allan KM, Prabhu N, Craig LCA, McNeill G, Kirby B, McLay J, et al. Maternal vitamin D and E intakes during pregnancy are associated with asthma in children. *Eur Respir J* 2015;45:1027-36.
24. Stevens CA, Turner D, Kuehni CE, Couriel JM, Silverman M. The economic impact of preschool asthma and wheeze. *Eur Respir J* 2003;21:1000-6.
25. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. The Group Health Medical Associates. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995;332:133-8.
26. Akinbami LJ, Moorman JE, Garbe PL, Sondik EJ. Status of childhood asthma in the United States, 1980-2007. *Pediatrics* 2009;123:S131-45.
27. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266-81.
28. Litonjua AA, Weiss ST. Is vitamin D deficiency to blame for the asthma epidemic? *J Allergy Clin Immunol* 2007;120:1031-5.
29. Yurt M, Liu J, Sakurai R, et al. Vitamin D supplementation blocks pulmonary structural and functional changes in a rat model of perinatal vitamin D deficiency. *Am J Physiol Lung Cell Mol Physiol* 2014;307:L859-67.
30. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res* 2011;26:2341-57.
31. Institute of Medicine. Dietary reference intakes for vitamin C, vitamin E, selenium, and carotenoids. Washington, DC: National Academy Press; 2000.
32. Henderson AJ, Warr JO. Fetal origins of asthma. *Semin Fetal Neonatal Med* 2012;17:82-91.
33. Kimlin MG, Olds WJ, Moore MR. Location and vitamin D synthesis: is the hypothesis validated by geophysical data? *J Photochem Photobiol* 2007;86:234-9.
34. Karras SN, Anagnostis P, Naughton D, Annweiler C, Petroczi A, Goulis DG. Vitamin D during pregnancy: why observational studies suggest deficiency and interventional studies show no improvement in clinical outcomes? A narrative review. *J Endocrinol Invest* 2015;38:1265-75.
35. Heaney RP. Guidelines for optimising design and analysis of clinical studies of nutrient effects. *Nutr Rev* 2014;72:48-54.

RESEARCH QUESTION USING PATIENT INTERVENTION COMPARISON OUTCOME STUDY STRUCTURE AND SEARCH STRATEGY

The following 4 important concepts were identified on the basis of the research question:

- Population: Pregnancy
- Intervention: Vitamins and/or dietary supplements
- Outcome(s): Allergic outcomes
- Study: Randomized controlled trial

Various keywords and synonyms were identified using the Patient Intervention Comparison Outcome Study approach. A full list of the used keywords for each concept is presented below.

Within groups of terms, the terms were combined using OR and the groups of terms themselves were then combined in the following manner: #1 AND #2 AND #3 AND #4.

Search strategy

1. pregnan* OR ((antenatal or ante-natal or ante natal)) OR ((prenatal or pre-natal or pre natal)) OR ((mother or maternal))
2. (MH "Pregnancy+")
3. vitamin* OR (supplement* or dietary supplement*) OR nutrient* OR mineral* OR micronutrient*
4. vitamins (MeSH terms)
5. (allerg* or atopy or dermatitis or eczema) OR (asthma or asthma prevention) OR (wheez*) OR (food hypersensitivity or food allergy or food intolerance) OR (non IgE or non-IgE or mediated allerg*) OR (hypersensitivity or sensitisation) OR rhinitis
6. (MH "Hypersensitivity+")
7. (randomised controlled trial or randomised control trial or randomised controlled study or randomised clinical trial or controlled clinical trial or controlled trial or random allocation) OR (single blind or single blind method or single blind

trial or double blind method OR double-blind method) OR (clinical trial* or quasi-experimental study or placebo-controlled trial or placebo control or intervention study or follow-up stud*)

8. (MH "Clinical Trials as Topic+") OR (MH "Controlled Clinical Trials as Topic+")
9. (#1 OR #2) AND (#3 OR #4) AND (#5 OR #6) AND (#7 OR #8)

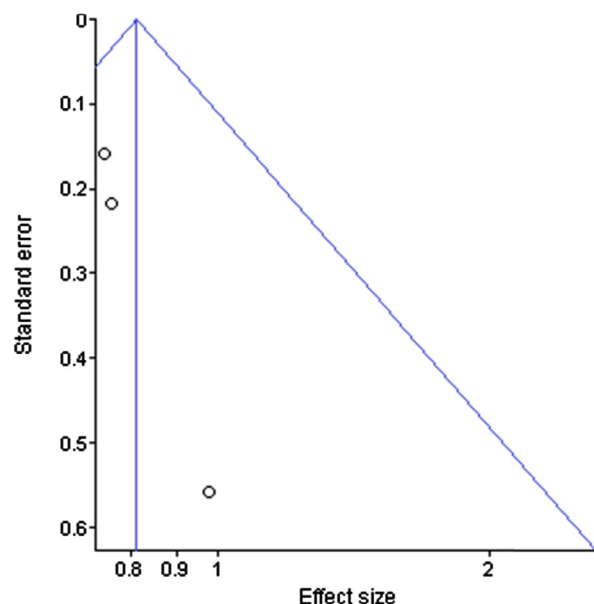


FIGURE E1. Funnel plot for vitamin D versus placebo or no treatment for prevention of recurrent wheeze in offspring.

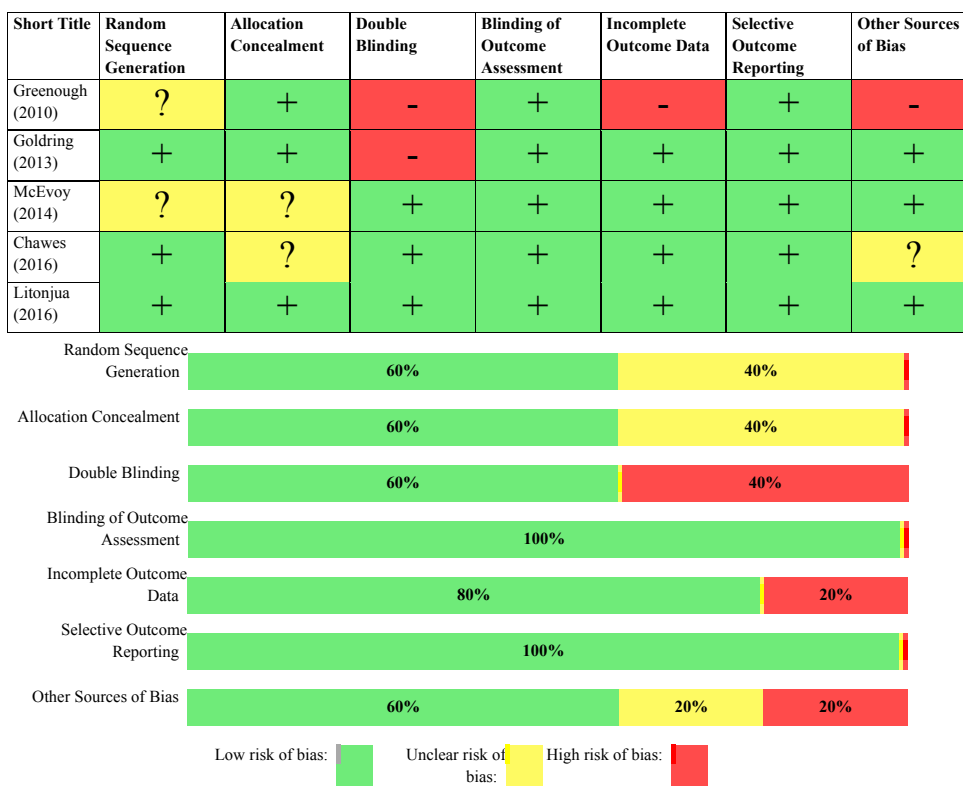
**FIGURE E2.** Summary of risk of bias assessment.

TABLE E1. Data extraction tool

1. Exclude nonrandomized
2. Study protocol
3. Linked record-Do not obtain data
4. Study details
Country
Recruitment period
Setting
Informed consent
Ethical approval
Source of funding
5. Trial type
PC-RCT
<i>Randomized controlled trial-placebo controlled</i>
RCT
<i>Randomized controlled trial</i>
Quasi experimental
No. & name of study groups/arms
ITT
<i>Intention-to-treat analysis</i>
6. Study sample
Matched baseline characteristics
Women's age
Exclusion criteria
FH of atopy
Unselected sample
<i>Subjects are taken from an unselected population</i>
No. of participants at randomization
No. at F-U
Missing participants
Reasons missing
Time points measured
<i>Earlier F-U(s) reported time points</i>
Infant's age at last F-U
7. Intervention
Vitamin/supplement type
Vitamin C
Vitamins C & E
Vitamin D (cholecalciferol)
Vitamin D (ergocalciferol)
Combined vitamin D
Daily dosage
Comparisons
Placebo
No treatment
When Int. has been applied?
Pregnancy alone
Pregnancy & after delivery
Timing in pregnancy
Duration of Int. in pregnancy
Total duration of Int.
Side effects
Compliance
8. Outcomes
<i>The defined and measured end point(s) in the study</i>
Allergic disease(s)
Wheeze

TABLE E1. (Continued)

Eczema
Asthma
SPT (any positive)
Specific IgE
Food Allergy
9. Diagnosis method
<i>Please tick all that apply</i>
F-U questionnaires, eg, ISAAC
Clinician diagnosed
SPT
<i>If the test is conducted vs any specific allergen, eg, egg-peanut-milk, please record the details and mean diameter of the wheal, eg, ≥ 3 mm</i>
Self-report
Physical examination
Specific IgE

F-U, Follow-up; Int., intervention; ISAAC, International Study of Asthma and Allergies; SPT, skin prick test.

(continued)

TABLE E2. Definition of outcomes and diagnostic criteria in individual studies

Outcomes	Diagnosis
Greenough et al's¹³ study (2010)	
Wheeze more than once/wk, in second 12 m	Asking women whether (or not) their infant had coughed and/or wheezed and the frequency of the cough and wheeze
Asthma: Chest symptoms in first 12 mo	Non-defined
Eczema, in first 12 mo	Non-defined
McEvoy et al's¹⁵ study (2014)	
Medication for wheezing, through age 1 y	By respiratory questionnaire (pediatric version) to the infant's primary caretaker asking medication for wheezing
At least 1 episode of wheezing, through age 1 y	By respiratory questionnaire (pediatric version) to the infant's primary caretaker asking for presence or absence of wheezing, maternal smoking, and exposure to second-hand smoke
Goldring et al's¹⁴ study (2013)	
Recurrent wheezing	Recurrent wheezing (≥ 2 episodes of reported wheezing since birth), defined by ISAAC criteria
Wheeze with a positive asthma predictive index	Using loose criteria
Chawes et al's¹⁶ study (2016)	
Persistent wheeze (0-3 y)	Persistent wheeze, as primary end point, was diagnosed according to a previously validated quantitative algorithm, requiring all of the following: (1) recurrent wheeze (verified diary recordings of ≥ 5 episodes of troublesome lung symptoms [cough, wheeze, and/or dyspnea] lasting ≥ 3 d within 6 mo), (2) typical symptoms of asthma (eg, exercise-induced symptoms, prolonged nocturnal cough, or persistent cough outside common cold), (3) need for intermittent bronchodilator, and (4) response to a 3-mo trial of inhaled corticosteroids and relapse upon cessation
Asthma, 3 y	Asthma, as secondary end point, was diagnosed in children fulfilling the persistent wheeze criteria at age 3 y
Eczema, 0-3 y	Eczema was diagnosed according to the criteria of Hanifin and Rajka including typical morphology and localization of skin lesions
Specific IgE, 0-3 y	Allergic sensitization was diagnosed at 6 and 18 mo by specific IgE level of 0.35 kU _A /L or higher against raw milk, pasteurized eggs, dogs, or cats
Litonjua et al's¹⁷ study (2016)	
Asthma or recurrent wheeze in the first 3 y of life	Parental report of physician's diagnosis of asthma was taken directly from the offspring questionnaires administered every 3 mo. Recurrent wheeze was defined by the occurrence of at least 1 of the following 5 conditions: (1) parental report of wheeze after child's second birthday preceded by at least 1 report of wheeze before second birthday; (2) report of child's use of asthma controller medication (defined as report of use of steroid inhalers or nebulizers, leukotriene modifiers, or steroid pills or liquids) after the second birthday, preceded by a report of wheeze before the second birthday; (3) 2 or more distinct parental reports of wheeze after the second birthday; (4) at least 1 parental report of wheeze and use of asthma controller medications at distinct visits, both subsequent to the second birthday; or (5) 2 distinct reports of use of asthma controller medications after the second birthday.
Eczema with rash, 0-3 y	Parental report of physician's diagnosis of eczema with rash in typical distribution
Positive specific IgE test results	Allergen sensitization (specific IgE to a panel of aeroallergens and food allergens)

ISAAC, International Study of Asthma and Allergies.

TABLE E3. Reviewers' judgment for risk of bias assessment in the included trials

Study author (year)	Random sequence generation	Allocation concealment	Double blinding	Blinding of outcome assessment	Incomplete outcome data	Selective outcome reporting	Other sources of bias
Greenough et al ¹³ (2010)	Unclear <i>Randomization sequence was blocked—ie, balanced—but not how randomization sequence was generated</i>	Low <i>The trial staff members were not aware of the allocation until after completion of the study</i>	High <i>There is no information whether trial staff and mothers were still blind to their allocated treatment at extended F-U</i>	Low <i>The outcome assessors were blind to the maternal allocation of treatment</i>	High <i>A high rate of loss to F-U was observed because of no response/mother declined</i>	Low <i>The predefined outcomes (as stated in the Methods) section are reported</i>	High <i>No information whether women have consumed vitamins C & E after birth</i>
Goldring et al ¹⁴ (2013)	Low <i>A computer-generated random number list in blocks of 15, stratified by 4 ethnic groups in a 1:1:1 ratio</i>	Low <i>The treatment was allocated from the hospital pharmacy</i>	High <i>Not possible to blind participants or investigators as they would know if they had no treatment, daily tablets, or a single bolus</i>	Low <i>Investigators kept blind to original treatment allocation at the extended F-U</i>	Low <i>Reasons for missing data are reported and well balanced between the groups</i>	Low <i>The predefined outcomes (as stated in the Methods section) are reported</i>	Low <i>This trial was conducted before national guidance on routinely providing advice on vitamin D intake during pregnancy was introduced in March 2008</i>
McEvoy et al ¹⁵ (2014)	Unclear <i>Randomization was stratified according to gestational age but method of randomization was not reported</i>	Unclear <i>No information on how allocation has happened</i>	Low <i>Investigators, clinicians, and patients were unaware of treatment allocation through age 1 y</i>	Low <i>Clinical research personnel were unaware of treatment assignment</i>	Low <i>Number of missing are equal in both study groups (for preclinical and clinical measurements at birth and 1 y)</i>	Low <i>The predefined outcomes (as stated in the Methods section) are reported</i>	Low <i>None identified</i>
Chawes et al ¹⁶ (2016)	Low <i>A computer-generated list of random numbers was used, supplied by an external investigator</i>	Unclear <i>No information how allocation has happened</i>	Low <i>The intervention code was unblinded when the youngest child reached age 3 y or in case of a medical emergency</i>	Low <i>The study pediatricians, who acted as general practitioners for the cohort, were blinded to the intervention</i>	Low <i>Between 6.3% and 7% of women are lost to F-U</i>	Low <i>The predefined outcomes (as stated in the Methods section) are reported</i>	Unclear <i>Women also received n-3 LCPUFA supplement and it is not stated how this could have had an effect on vitamin D₃. The statistical analyses did not show an interaction with supplementation effect</i>
Litonjua et al ¹⁷ (2016)	Low <i>Using a system that automates the random assignment of treatment groups to study ID no.</i>	Low <i>Assignment of treatment groups to study ID nos. is automatically done</i>	Low <i>Clinical center investigators and staff were blinded to the treatment code</i>	Low <i>The study protocol says clinical center investigators and staff were blind to the treatment code</i>	Low <i>Between 7% and 8% of participants are missing and reasons are similar across the study arms</i>	Low <i>The predefined outcomes (as stated in the Methods section) are reported</i>	Low <i>None identified</i>

F-U, Follow-up; LCPUFA, long-chain polyunsaturated fatty acid.

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