Prenatal intake of vitamins and allergic outcomes in the offspring: a systematic review and meta-analysis

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Abstract

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 synthesise the evidence from randomised controlled trials (RCTs) assessing the efficacy of vitamin interventions during pregnancy on developing allergic diseases in offspring.

Methods: We searched CENTRAL, MEDLINE, SCOPUS, WHO's Int. Clin. Trials Reg., E-theses and Web of Science. Study quality was evaluated using the Cochrane's risk of bias tool. Included RCTs had a minimum of 1-month follow-up post gestation.

Results: A total of five RCTs met the inclusion criteria, including 2456 children that used vitamins C+E (one study), vitamin C (one study) and vitamin D (three studies) compared with placebo/control. Two studies were judged to have a high risk of bias for performance bias or 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 C+E studies due to high heterogeneity between the two included studies. However we did conduct a meta-analysis with trials on vitamin D (including 1493 children) and the results showed an association between prenatal intake of vitamin D and the risk of developing recurrent wheeze in offspring (RR=0.812, 95 % CI=0.67-0.98).

Conclusion: 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 are needed to ascertain whether this observed effect is a sustained. There is lack of evidence on the effect of other vitamins for prevention of respiratory and/or allergic outcomes.

- **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 randomised 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 development of recurrent childhood wheeze. As 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 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.

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

List of abbreviations:

WHO: World Health Organisation

WHO's Int. Clin. Trials. Reg.: World Health Organisation International Clinical Trials Registration

RCT: Randomised Clinical Trial

SPT: Skin Prick Test

sIgE: specific Immunoglobulin E

DARE: Database of Reviews of Effectiveness

RR: Relative Risk or Risk Ratio

CI: Confidence Interval

ISI: Institute for Scientific Information

Introduction

In the last two 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]. Due to 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 programme, 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 genetic variation is far less than previously believed[4]. Therefore, new approaches towards disease prevention with an emphasis on early interventions i.e. pre-pregnancy and/or during pregnancy need to be widely investigated. Current evidence suggests that the role of maternal diet during pregnancy on subsequent disease development is a priority area for future studies[5], as many of the immune modulatory processes may start in-utero.

The role of environmental and life-style factors on developing allergies has been examined in a number of epidemiological studies. A systematic review has investigated the association of nutrient deficiencies on 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 summarise the existing evidence form randomised controlled trials for the association between 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 randomised controlled trials (RCT) (including cluster randomised controlled trials and quasi-randomised controlled trials) with a minimum follow-up of one month postnatally were included. The review considered studies which 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 e.g. oil, tablet.

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 or secondary endpoint. Allergic outcomes were defined as: asthma, wheeze, rhinitis, eczema, food allergy and positive skin prick test (to any allergen) and elevated specific IgE. Outcomes included were those, which had utilised 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 (online repository). Trials were identified through systematic searches within three main electronic databases, as advised by the Cochrane collaboration[7]:

a. Cochrane Library (current issue) including:
    - Cochrane Database of Systematic Reviews (CDSR)
    - CENTRAL (trials)
    - DARE

b. MEDLINE (EBSCOhost)
c. SCOPUS

When searching MEDLINE, the subject-specific terms were combined with the Cochrane Highly Sensitive Search Strategy for identifying randomised 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 WHO platform were searched for ongoing and recently completed trials. Conference proceedings were identified through the ISI Web of Science and, for retrieving theses the British Library E-Theses Online Service was searched. 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 (MV) screened all the search results against the eligibility criteria and all those which were clearly irrelevant were excluded from further consideration. Thereafter, a tailored eligibility form was used by MV to appraise the retrieved studies, abstract and full text for relevance against the full inclusion criteria. Where there was uncertainty about inclusion of a particular study, other members of the review team (HM & TD) 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

MV extracted the data using a tailored data extraction form (online repository). Detailed information on study characteristics were 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 (HM) 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 seven 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 was analysed as risk ratios or relative risk (RR) with 95% CI and continuous data as mean difference or standardised mean difference, with 95% CI.

Unit of analysis issues

In trials with more than one 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 or 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^2 test to measure statistical heterogeneity between effect sizes of included studies ($P<0.05$)[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 ≥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 (online repository).

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 random-effects model where heterogeneity was reported as ≤75%[7]. We also reported relative risk as a statistical choice in conducting the meta-analyses, as it is easy to interpret[12].

Subgroup analysis and investigation of heterogeneity

We performed sub-group analyses based on the type of vitamin and type of the control group (i.e. placebo versus 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 (Figure1). We included 5 RCTs comparing at least one vitamin with a control that met the inclusion criteria for this systematic review.

These included trials (including total of 2456 children) were represented by five original papers[13-17] and four grouped as their companion papers[18-21]. Table 1 shows the characteristics of the included trials, their companion papers and study population. The trials were conducted in United Kingdom, Denmark and United States. The types of vitamin supplementations included were as 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 vitamins C and 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 and was significantly higher in the treatment versus 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 type of the population, supplementation used and the comparators. We have therefore described the results of individual studies narratively and only conducted meta-analysis when there was no evidence of statistical heterogeneity. The definition and diagnosis method of the outcomes in each study are presented in online repository.

Vitamin C studies

Greenough et al. (2010)[13] study

The study was conducted in the U.K between August 2003 to June 2007. The studied sample were pregnant women at risk of developing pre-eclampsia. Women were supplemented with daily vitamins C (1,000mg) tablets and E (400IU) gelatin capsules, from 16-22 gestation weeks until delivery. Women in the control group received identical tablets of microcrystalline cellulose with

addition of tartaric acid and citric acid along 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 women[18] and the extended follow-up at 2 years has assessed the efficiency of the vitamin intervention on respiratory outcomes in children.

The list of the reported outcomes in the study is shown in Table 1. The outcomes of "asthma" and "eczema" are reported at 1-year age and "recurrent wheeze" at 2 years. No statistically significant association was observed between the intervention and control group for prevention of recurrent wheeze (10/386 vs. 11/366, OR=0.83, 95% CI=0.26-2.59, p=0.66) and asthma (23/386 vs. 23/366, OR=0.94, 95% CI=0.42-2.11, p=0.85). Additionally the results did not show a significant association between prenatal intake of vitamin C+E and prevention of eczema (98/386 vs. 86/366, OR=1.10, 95% CI=0.70-1.74, p=0.58).

McEvoy et al. (2014)[15] study

The study was conducted in U.S.A between March 2007 and January 2011. The studied sample were smoking pregnant women. Women were supplemented with daily crushed vitamin C (500mg) gel capsules, from 22nd gestation weeks 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 1-year age. The list of the reported outcomes in the study is shown in Table 1. The results of the unadjusted analysis showed no significant statistical association between the intervention and control groups for outcome measure defined as "recurrent wheeze" (9/76 vs. 17/83, RR=0.56, 95% CI=0.27-1.18, p=0.13). A significant difference was observed for the outcome of "at least 1 episode of wheezing" between the intervention and control groups (15/76 vs. 31/83, RR=0.56, 95% CI=0.33-0.95, p=0.03).

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. (2013)[14] study

The study was conducted in the U.K between April and November 2007. This study recruited pregnant women with multiple ethnicities. The study introduced two intervention arms, as women were randomised either to receive a daily dose of ergocalciferol (800IU) or a single oral dose of cholecalciferol (200,000IU, 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 up 3 years of age 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 statistical significant association between prenatal intake of daily vitamin D and control group (8/56 vs. 7/50, RR=1.02, 95% CI=0.40-2.61, p=0.97). Furthermore, no significant association was observed for the outcome measure of "wheeze with positive asthma predictive index" (6/56 vs. 7/50, RR=0.77, 95% CI=0.28-2.13, p=0.61) between the study arms. The outcomes of "eczema in the last year" (11/55 vs. 7/49, RR=1.40, 95% CI=0.59-3.33, p=0.44) and "food allergy diagnosis" (8/55 vs. 3/49, RR=2.38, 95% CI=0.67-8.46, p=0.16) did not show a significant statistical association for the prenatal consumption of daily vitamin D in comparison to control.

Chawes et al. (2016)[16] study

The study was conducted in Denmark between 2008 to 2010. The studied sample were unselected pregnant women. Women were supplemented with daily vitamin D_3 (2,400IU) tablets, from 24 gestation weeks to one week after delivery. Women in the control arm received tablets containing no active substance. In addition, women assigned to both intervention and control arms

309 received an extra 400IU dose of vitamin D3, as part of their routine care.
310 Compliance to the intervention was measured by counts of returned pills.
311
312 The study reported cumulative incidence of the allergic outcomes by 3 years
313 of age. The results of unadjusted analysis indicated that the risk of developing
314 recurrent wheeze did not show a significant difference between the
315 intervention and control group (47/295 vs. 57/286, HR=0.76, 95% CI=0.52-
316 1.12, p=0.16). Asthma was reported at 3 years of age only and no significant
317 difference was observed between the intervention and control groups (32/278
318 vs. 47/271, OR=0.82, 95% CI=0.50-1.36, p=0.45). Furthermore there was not
319 a significant statistical difference between the study arms for eczema as an
320 outcome (68/295 vs. 72/286, HR=0.90, 95% CI=0.65-1.26, p=0.55). Children
321 in the intervention arm reported statistically significant "lower episodes of
322 troublesome lung symptoms" compared to the control group (5.9 vs. 7.2,
323 IRR=0.83, 95% CI=0.71-0.97, p=0.02). The cumulative results for SPT and
324 sIgE outcomes were not statistically different between the intervention and
325 control group (24/294 vs. 19/283, OR=1.24, 95% CI=0.66-2.31, p=0.51) and
326 (34/289 vs. 22/278, OR=1.55, 95% CI=0.89-2.73, p=0.13) respectively.
327
328 **Litonjua et al. (2016)[17] study**
329 The study was conducted in U.S.A between 2009 to 2011. The study sample
330 were women with a history of atopy. Women were supplemented with daily
331 vitamin D_3 (4,000IU) tablets, between 10-18 gestation weeks until delivery.
332 The nature of the placebo capsules was not reported. Women in both study
333 arms also received a multivitamin with 400IU of vitamin D. Adherence to the
334 intervention was measured by electronic medication container caps.
335
336 The study reported cumulative incidence of the allergic outcomes by 3 years
337 of age. The outcomes of "asthma or recurrent wheeze" were reported together
338 and the results showed no significant statistical difference between the
339 intervention and control groups (98/405 vs. 120/401, HR=0.8, 95% CI=0.6-
340 1.0, p=0.051). There was also no significant statistical difference in the risk of
341 developing "eczema with rash" in the study arms (83/405 vs. 89/401, HR=0.9,
342 95% CI=0.7-1.2, p=0.56). The result for positive sIgE tests at 3 years showed

a significant statistical difference between the intervention and control group (43/405 vs. 50/401, MD=-1.7, 95% CI=-3.4-0.0, p=0.02).

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 1,493 children. No statistical heterogeneity was observed between the included trials (Chi^2=0.16, p=0.92, I^2=0%) (Figure 2). The results of the present meta-analysis showed an association between 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 two 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 online repository. Only one trial was deemed to have 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 one trial[13] since 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 efficacy of vitamins C and E supplementation on developing pre-eclampsia in women at increased risk.

Discussion

This is the first systematic review of randomised controlled trials that investigated the association of prenatal intake of vitamins on the risk of developing allergic/respiratory diseases in the offspring. We identified five 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 pre-eclampsia[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 high rate of loss to follow-up[13]. All trials were rated having 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 to placebo/control. However we were not able to investigate the efficiency of vitamin D on other allergic outcomes since 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 to 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 i.e. asthma. For example, wheezing is known as a primary symptom of asthma in early childhood[24] and about 40% of childhood wheeze will persist later in life and will eventually develop into asthma by 6 years of age[25-26], indicating majority 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 only observed for some of the secondary outcomes as "at least 1 episode of wheezing"[14], "episodes of troublesome lung symptoms"[16] and "positive sIgE"[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 800-4000IU and doses of vitamin C and/or E, varied between 500-1000mg. It is possible to hypothesis 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 which 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 4000IU vitamin D is a safe approach and was necessary to optimise the circulating concentration of 25-hydroxyvitamin D levels to ≥ 80nmol/L[30]. There is limited evidence on the safety of vitamins C and E intake at any stage of pregnancy; however the Institute of Medicine's Food and Nutrition Board have set an upper limit of 2000mg and 1000mg per day for vitamins C and E ingestion respectively during pregnancy in the United States[31].

Further, in all trials the intervention was started in the 2nd 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 dose of vitamin D to have a beneficial impact on lung development. Finally, the studies

recruited different types of population, which limits the generalisability of the studies. Baseline levels of vitamin D vary in different geographical areas[33] 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 pre-pregnancy and employs 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 efficacy 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 on the risk of developing other allergic outcomes and sensitisation needs to be investigated in larger well-designed trials. Further 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.

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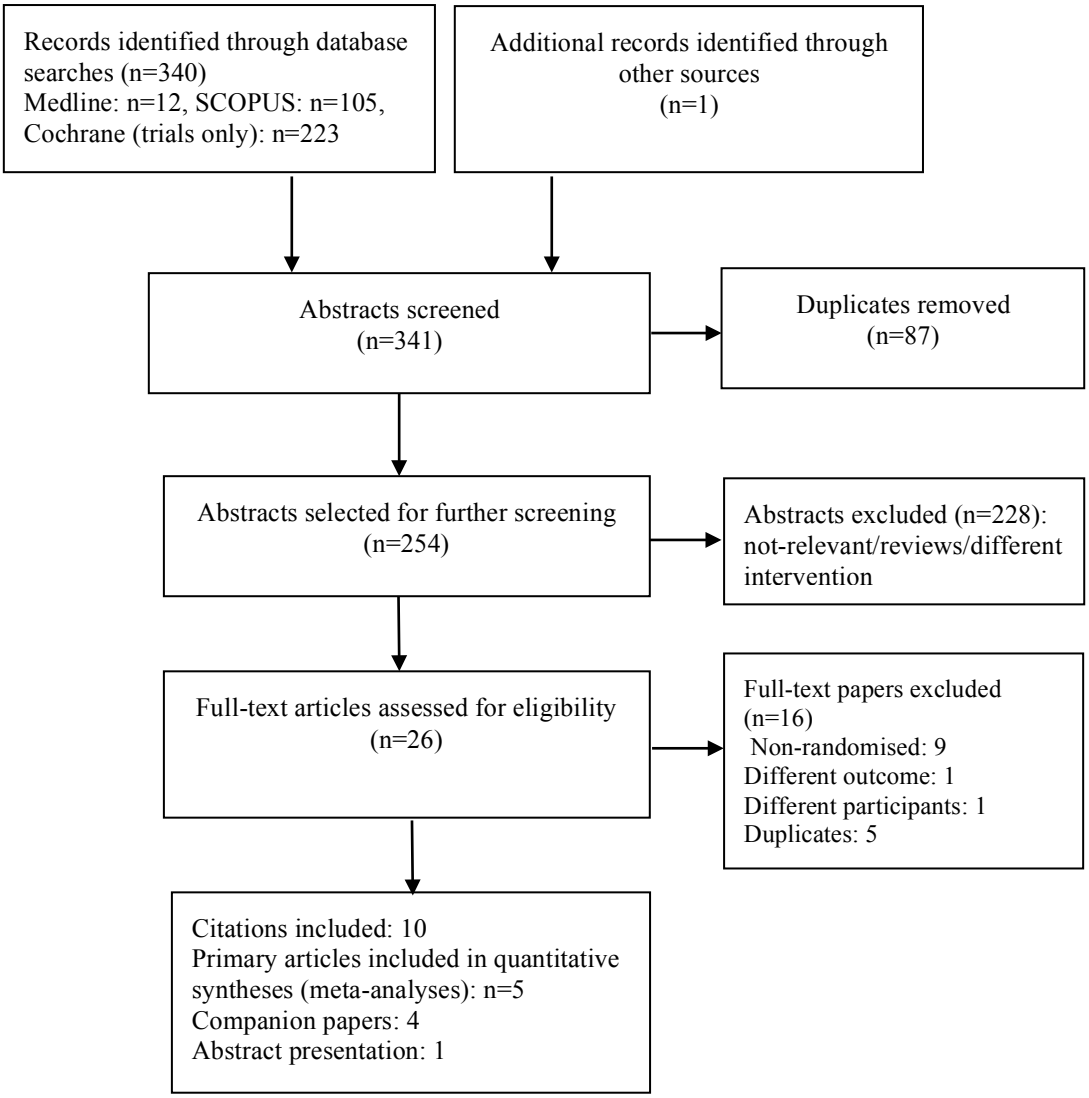
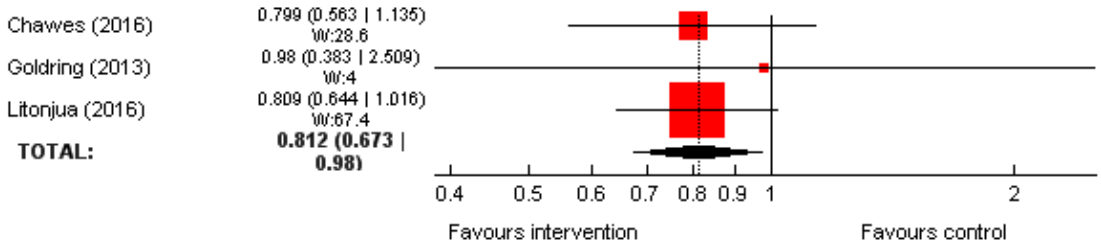


Figure 1: Study flow diagram, following PRISMA criteria

Measure: Binary: risk ratio
Heterogeneity: Q = 0.163; df = 2; p = 0.922; I-squared = 0%; tau-squared = 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

