

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

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22 **Abstract**

23 **Background:** Allergic diseases have seen a rise worldwide with children
24 suffering the highest burden. Thus early prevention of allergic diseases is a
25 public health priority.

26 **Objective:** To synthesise the evidence from randomised controlled trials
27 (RCTs) assessing the efficacy of vitamin interventions during pregnancy on
28 developing allergic diseases in offspring.

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

33 **Results:** A total of five RCTs met the inclusion criteria, including 2456
34 children that used vitamins C+E (one study), vitamin C (one study) and
35 vitamin D (three studies) compared with placebo/control. Two studies were
36 judged to have a high risk of bias for performance bias or high rate of loss to
37 follow-up. All were rated as low risk of bias for blinding of outcome
38 assessment. We did not perform meta-analysis with vitamin C or C+E studies
39 due to high heterogeneity between the two included studies. However we did
40 conduct a meta-analysis with trials on vitamin D (including 1493 children) and
41 the results showed an association between prenatal intake of vitamin D and
42 the risk of developing recurrent wheeze in offspring (RR=0.812, 95 %
43 CI=0.67-0.98).

44 **Conclusion:** The current evidence suggests that prenatal supplementation of
45 vitamin D, might have a beneficial effect on recurrent wheezing in children.
46 Longer-term follow-up of these studies are needed to ascertain whether this
47 observed effect is a sustained. There is lack of evidence on the effect of other
48 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.

49

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

53

54 **List of abbreviations:**

55 **WHO:** World Health Organisation

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

58 **RCT:** Randomised Clinical Trial

59 **SPT:** Skin Prick Test

60 **sIgE:** specific Immunoglobulin E

61 **DARE:** Database of Reviews of Effectiveness

62 **RR:** Relative Risk or Risk Ratio

63 **CI:** Confidence Interval

64 **ISI:** Institute for Scientific Information

65 **Introduction**

66 In the last two decades allergic diseases have seen a rise worldwide with
67 children suffering the highest burden of the condition¹. Food allergies, eczema
68 and asthma are the most common allergic disorders in children¹⁻². Due to the
69 increasing burden of allergic diseases they are a key focus for public health.

70

71 The Developmental Origins of Health and Diseases theory proposes that
72 development is not dictated by a hard-wired genetic programme, instead the
73 organism responds to the surrounding environment and the risk of many
74 diseases is set during this time³. It has become increasingly evident that there
75 is an important role for environmental factors in the onset of complex
76 conditions such as allergic diseases and that the role of fixed genetic variation
77 is far less than previously believed⁴. Therefore, new approaches towards
78 disease prevention with an emphasis on early interventions i.e. pre-pregnancy
79 and/or during pregnancy need to be widely investigated. Current evidence
80 suggests that the role of maternal diet during pregnancy on subsequent
81 disease development is a priority area for future studies⁵, as many of the
82 immune modulatory processes may start in-utero.

83

84 The role of environmental and life-style factors on developing allergies has
85 been examined in a number of epidemiological studies. A systematic review
86 has investigated the association of nutrient deficiencies on the risk of
87 development of asthma and allergic diseases in children⁶. This review
88 included 62 observational studies and indicated that vitamins A, D, and E;
89 zinc; fruits and vegetables; and a Mediterranean diet during pregnancy may
90 prevent asthma and wheeze. However, this review was based on
91 observational studies which carry a high risk of bias and there is a need for
92 secondary research based on summary of more robust interventional studies.

93

94 The purpose of this systematic review is to summarise the existing
95 randomised controlled trials evidence of the association between vitamin
96 supplements during pregnancy and the risk of developing allergic disorders in
97 the offspring.

98 **Methods**

99 **Criteria for considering studies for this review**

100 **Types of studies**

101 Only randomised controlled trials (RCT) (including cluster randomised
102 controlled trials and quasi-randomised controlled trials) with a minimum
103 follow-up of one month postnatally were included. The review considered
104 studies which documented clinical outcome data and used any types of
105 vitamins. No language restriction was applied.

106 **Types of participants**

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

110 **Types of interventions**

111 Studies that used any vitamin supplementation during pregnancy, irrespective
112 of dose, formulation or mode of delivery and composition e.g. oil, tablet.

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

115 **Outcomes of interest**

116 Trials were included if they had reported clinical outcomes of allergy in the
117 offspring, either as a primary or secondary endpoint. Allergic outcomes were
118 defined as: asthma, wheeze, rhinitis, eczema, food allergy and positive skin
119 prick test (to any allergen) and elevated specific IgE. Outcomes included were
120 those, which had utilised a validated method as opposed to parental reports.

121 **Search strategy for identification of studies**

122 A comprehensive search strategy, including all the relevant synonyms for the
123 main concepts, was developed covering the main bibliographic databases
124 (online repository). Trials were identified through systematic searches within
125 three main electronic databases, as advised by the Cochrane collaboration⁷:

126 a. Cochrane Library (current issue) including:

- 127 • Cochrane Database of Systematic Reviews (CDSR)
- 128 • CENTRAL (trials)
- 129 • DARE

130 b. MEDLINE (EBSCOhost)

131 c. SCOPUS

132 When searching MEDLINE, the subject-specific terms were combined with the
133 Cochrane Highly Sensitive Search Strategy for identifying randomised trials in
134 MEDLINE: sensitivity-maximising version⁷. We adapted the preliminary
135 search strategy for MEDLINE (EBSCOhost) for use in the other databases
136 when relevant. The last search for literature was conducted in January 2016.

137 The clinical trials registry and WHO platform were searched for ongoing and
138 recently completed trials. Conference proceedings were identified through the
139 ISI Web of Science and, for retrieving theses the British Library E-Theses
140 Online Service was searched. No language or publication status restrictions
141 were imposed. References of included studies were crosschecked for
142 additional studies.

143 **Data collection and analysis**

144 **Selection of studies**

145 The main reviewer (MV) screened all the search results against the eligibility
146 criteria and all those which were clearly irrelevant were excluded from further
147 consideration. Thereafter, a tailored eligibility form was used by MV to
148 appraise the retrieved studies, abstract and full text for relevance against the
149 full inclusion criteria. Where there was uncertainty about inclusion of a
150 particular study, other members of the review team (HM & TD) were consulted
151 and a consensus was reached about the study eligibility. All the included
152 studies were discussed and approved by the review team.

153 **Data extraction**

154 MV extracted the data using a tailored data extraction form (online repository).
155 Detailed information on study characteristics were recorded. Throughout the
156 data extraction process, any disagreements about the interventions and
157 outcomes were discussed and resolved within the review team. There was no
158 blinding to the name of authors, institutions, journals or the outcomes of the
159 trials during the process. Ten percent of all the extracted data was randomly
160 selected and double checked by a second reviewer (HM) for accuracy against
161 the trial reports.

162 **Assessment of risk of bias in included studies**

163 The risk of bias tool described in the Cochrane Handbook for Systematic
164 Reviews for Interventions was used to appraise the studies⁸. The tool includes
165 seven domains: random sequence generation, allocation concealment,
166 blinding of participants and personnel, blinding of outcome assessments,
167 incomplete outcome data, selective outcome reporting and other bias.

168 **Measurement of treatment effect**

169 Dichotomous data was analysed as risk ratios or relative risk (RR) with 95%
170 CI and continuous data as mean difference or standardised mean difference,
171 with 95% CI.

172 **Unit of analysis issues**

173 In trials with more than one intervention arm, multiple pairwise comparisons of
174 intervention groups versus comparator were avoided. Therefore, data from
175 different intervention arms were pooled for an overall comparison with the
176 control or placebo arm. The weight assigned to the control group was
177 considered as the total number of participants in the comparator group versus
178 the total number of participants in the combined intervention arms⁹.

179 **Handling missing data**

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

184 **Assessment of heterogeneity**

185 We used visual inspection of forest plots and also, the Chi² test to measure
186 statistical heterogeneity between effect sizes of included studies (P<0.05)¹⁰. I²
187 statistics were used to quantify the amount of possible variability in effect
188 estimates that is due to heterogeneity rather than chance (I²>30% moderate
189 heterogeneity, I²≥75% considerable heterogeneity).

190 **Assessment of reporting biases**

191 Every effort was made to identify unpublished studies through searching
192 abstracts and ongoing trials databases. Publication bias was assessed using
193 funnel plots¹¹. The asymmetry was assessed visually in the plots and no
194 formal statistical tests were conducted. The funnel plot was helpful to explore

195 possible small study biases for some of the primary outcomes (online
196 repository).

197 **Data synthesis**

198 We used Eppi Reviewer version 4.4.3.0. for conducting meta-analyses using
199 random-effects model. Dichotomous data were entered as events and the
200 number of participants. Data were pooled using random-effects model where
201 heterogeneity was reported as $\leq 75\%$ ⁷. We also reported relative risk as a
202 statistical choice in conducting the meta-analyses, as it is easy to interpret¹².
203 Studies were grouped under one umbrella as “any vitamins” for performing
204 meta-analyses.

205 **Subgroup analysis and investigation of heterogeneity**

206 We performed sub-group analyses based on the type of vitamin and type of
207 the control group (i.e. placebo versus no treatment).

208 **Sensitivity analysis**

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

211 **Results**

212 The results of the search strategy yielded 341 studies, of which 26 were
213 selected for full-text assessment (Figure1). We included 5 RCTs comparing at
214 least one vitamin with a control that met the inclusion criteria for this
215 systematic review.

216

217 These included trials (including total of 2456 children) were represented by
218 five original papers¹³⁻¹⁷ and four grouped as their companion papers¹⁸⁻²¹.
219 Table 1 shows the characteristics of the included trials, their companion
220 papers and study population. The trials were conducted in United Kingdom,
221 Denmark and United States. The types of vitamin supplementations included
222 were as vitamins C+E¹³, vitamin D^{14,16-17} and crushed vitamin C¹⁵. The
223 duration of intervention and follow-up in the included studies varied from 3.5-4
224 to 7.5 months and 12 to 36 months respectively. In trials that used vitamins C
225 and C+E, a higher blood concentration of vitamins was observed in those
226 assigned antioxidants^{13&15}. In trials that used vitamin D, level of maternal 25-
227 hydroxyvitamin D measured either at third trimester or after delivery and was
228 significantly higher in the treatment versus comparison group^{14, 16&17}. The
229 most frequently reported outcomes were wheeze and eczema. As expected
230 with systematic reviews there were differences between the included trials in
231 terms of type of the population, supplementation used and the comparators.
232 We have therefore described the results of individual studies narratively and
233 only conducted meta-analysis when there was no evidence of statistical
234 heterogeneity. The definition and diagnosis method of the outcomes in each
235 study are presented in online repository.

236

237 **Vitamin C studies**

238 **Greenough et al. (2010)¹³ study**

239 The study was conducted in the U.K between August 2003 to June 2007. The
240 studied sample were pregnant women at risk of developing pre-eclampsia.
241 Women were supplemented with daily vitamins C (1,000mg) tablets and E
242 (400IU) gelatin capsules, from 16-22 gestation weeks until delivery. Women in
243 the control group received identical tablets of microcrystalline cellulose with

244 addition of tartaric acid and citric acid along gelatin capsules of sunflower
245 seed oil. Compliance with the intervention was measured by counts of
246 returned pills. Primarily this study was designed to prevent the risk of fetal
247 growth restriction and premature delivery in the women¹⁸ and the extended
248 follow-up at 2 years has assessed the efficiency of the vitamin intervention on
249 respiratory outcomes in children.

250

251 The list of the reported outcomes in the study is shown in Table 1. The
252 outcomes of “asthma” and “eczema” are reported at 1-year age and “recurrent
253 wheeze” at 2 years. No statistically significant association was observed
254 between the intervention and control group for prevention of recurrent wheeze
255 (10/386 vs. 11/366, OR=0.83, 95% CI=0.26-2.59, p=0.66) and asthma
256 (23/386 vs. 23/366, OR=0.94, 95% CI=0.42-2.11, p=0.85). Additionally the
257 results did not show a significant association between prenatal intake of
258 vitamin C+E and prevention of eczema (98/386 vs. 86/366, OR=1.10, 95%
259 CI=0.70-1.74, p=0.58).

260

261 **McEvoy et al. (2014)¹⁵ study**

262 The study was conducted in U.S.A between March 2007 and January 2011.
263 The studied sample were smoking pregnant women. Women were
264 supplemented with daily crushed vitamin C (500mg) gel capsules, from 22nd
265 gestation weeks until delivery. Women in the control group received ground
266 cornstarch in gel capsules. Adherence was measured by dividing the number
267 of capsules taken by the total number prescribed in a given period.

268

269 The study reported the efficiency of consumption of vitamin C during
270 pregnancy on pulmonary function tests and wheezing in children at 1-year
271 age. The list of the reported outcomes in the study is shown in Table 1. The
272 results of the unadjusted analysis showed no significant statistical association
273 between the intervention and control groups for outcome measure defined as
274 “recurrent wheeze” (9/76 vs. 17/83, RR=0.56, 95% CI=0.27-1.18, p=0.13). A
275 significant difference was observed for the outcome of “at least 1 episode of
276 wheezing” between the intervention and control groups (15/76 vs. 31/83,
277 RR=0.56, 95% CI=0.33-0.95, p=0.03).

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

281

282 **Vitamin D studies**

283 **Goldring et al. (2013)¹⁴ study**

284 The study was conducted in the U.K between April and November 2007. This
285 study recruited pregnant women with multiple ethnicities. The study
286 introduced two intervention arms, as women were randomised either to
287 receive a daily dose of ergocalciferol (800IU) or a single oral dose of
288 cholecalciferol (200,000IU, bolus), from 27 gestation weeks until delivery. The
289 comparator in this study was defined as “no treatment”. Adherence was
290 measured by telephone calls during pregnancy.

291

292 This study followed up children to up 3 years of age and this systematic
293 review only reports the results for the intervention arm of daily vitamin D. The
294 results of unadjusted analysis for “recurrent wheezing” showed no statistical
295 significant association between prenatal intake of daily vitamin D and control
296 group (8/56 vs. 7/50, RR=1.02, 95% CI=0.40-2.61, p=0.97). Furthermore, no
297 significant association was observed for the outcome measure of “wheeze
298 with positive asthma predictive index” (6/56 vs. 7/50, RR=0.77, 95% CI=0.28-
299 2.13, p=0.61) between the study arms. The outcomes of “eczema in the last
300 year” (11/55 vs. 7/49, RR=1.40, 95% CI=0.59-3.33, p=0.44) and “food allergy
301 diagnosis” (8/55 vs. 3/49, RR=2.38, 95% CI=0.67-8.46, p=0.16) did not show
302 a significant statistical association for the prenatal consumption of daily
303 vitamin D in comparison to control.

304

305 **Chawes et al. (2016)¹⁶ study**

306 The study was conducted in Denmark between 2008 to 2010. The studied
307 sample were unselected pregnant women. Women were supplemented with
308 daily vitamin D₃ (2,400IU) tablets, from 24 gestation weeks to one week after
309 delivery. Women in the control arm received tablets containing no active
310 substance. In addition, women assigned to both intervention and control arms

311 received an extra 400IU dose of vitamin D3, as part of their routine care.
312 Compliance to the intervention was measured by counts of returned pills.

313

314 The study reported cumulative incidence of the allergic outcomes by 3 years
315 of age. The results of unadjusted analysis indicated that the risk of developing
316 recurrent wheeze did not show a significant difference between the
317 intervention and control group (47/295 vs. 57/286, HR=0.76, 95% CI=0.52-
318 1.12, p=0.16). Asthma was reported at 3 years of age only and no significant
319 difference was observed between the intervention and control groups (32/278
320 vs. 47/271, OR=0.82, 95% CI=0.50-1.36, p=0.45). Furthermore there was not
321 a significant statistical difference between the study arms for eczema as an
322 outcome (68/295 vs. 72/286, HR=0.90, 95% CI=0.65-1.26, p=0.55). Children
323 in the intervention arm reported statistically significant “lower episodes of
324 troublesome lung symptoms” compared to the control group (5.9 vs. 7.2,
325 IRR=0.83, 95% CI=0.71-0.97, p=0.02). The cumulative results for SPT and
326 sIgE outcomes were not statistically different between the intervention and
327 control group (24/294 vs. 19/283, OR=1.24, 95% CI=0.66-2.31, p=0.51) and
328 (34/289 vs. 22/278, OR=1.55, 95% CI=0.89-2.73, p=0.13) respectively.

329

330 **Litonjua et al. (2016)¹⁷ study**

331 The study was conducted in U.S.A between 2009 to 2011. The study sample
332 were women with a history of atopy. Women were supplemented with daily
333 vitamin D₃ (4,000IU) tablets, between 10-18 gestation weeks until delivery.
334 The nature of the placebo capsules was not reported. Women in both study
335 arms also received a multivitamin with 400IU of vitamin D. Adherence to the
336 intervention was measured by electronic medication container caps.

337

338 The study reported cumulative incidence of the allergic outcomes by 3 years
339 of age. The outcomes of “asthma or recurrent wheeze” were reported together
340 and the results showed no significant statistical difference between the
341 intervention and control groups (98/405 vs. 120/401, HR=0.8, 95% CI=0.6-
342 1.0, p=0.051). There was also no significant statistical difference in the risk of
343 developing “eczema with rash” in the study arms (83/405 vs. 89/401, HR=0.9,
344 95% CI=0.7-1.2, p=0.56). The result for positive sIgE tests at 3 years showed

345 a significant statistical difference between the intervention and control group
346 (43/405 vs. 50/401, MD=-1.7, 95% CI=-3.4-0.0, p=0.02).

347

348 **Meta-analyses of vitamin D studies**

349 We conducted a meta-analysis for the outcome measure of “recurrent
350 wheeze” for trials that used vitamin D prenatally in pregnant women. Figure 2
351 shows the Forest plot for this outcome. Three trials contributed to the meta-
352 analysis including a total of 1,493 children. No statistical heterogeneity was
353 observed between the included trials ($\text{Chi}^2=0.16$, $p=0.92$, $I^2=0\%$) (Figure 2).
354 The results of the present meta-analysis showed an association between
355 maternal intake of daily vitamin D during pregnancy and a lower risk of
356 developing recurrent wheeze in offspring (RR=0.812, 95% CI=0.673-0.98).
357 We also conducted the meta-analysis including only the two recent vitamin D
358 trials^{16&17} and it yielded similar results (Forest plot not shown).

359

360 **Risk of bias in included trials**

361 The risk of bias figures and authors’ judgments are presented in online
362 repository. Only one trial was deemed to have low risk of bias across all
363 domains¹⁷. Of the 5 trials, most had adequate random sequence generation
364 (n=3), allocation concealment (n=3) and performance bias (n=3). All trials
365 were rated as having a low risk of bias for blinding of outcome assessment
366 and selective outcome reporting. Completeness of outcome data was rated as
367 having high risk of bias for one trial¹³ since the study had a high loss to follow-
368 up and the authors acknowledged the fact that the study was an unplanned
369 extended follow-up of the original trial for measuring allergic outcomes in
370 children. The original trial was primarily designed to assess the efficacy of
371 vitamins C and E supplementation on developing pre-eclampsia in women at
372 increased risk.

373 **Discussion**

374 This is the first systematic review of randomised controlled trials that
375 investigated the association of prenatal intake of vitamins on the risk of
376 developing allergic/respiratory diseases in the offspring. We identified five
377 RCTs with a total of 2456 children. The studies were of unselected pregnant
378 women¹⁶, women with a history of atopy¹⁷, pregnant women at risk of
379 developing pre-eclampsia¹³, different ethnic/race groups¹⁴ and smoking
380 pregnant women¹⁵. Two studies were judged to have a high risk of bias due to
381 their performance bias¹³⁻¹⁴ or high rate of loss to follow-up¹³. All trials were
382 rated having low risk of bias for blinding of outcome assessment. It was not
383 possible to conduct meta-analyses for vitamin C studies due to observed
384 differences between the included trials. Maternal vitamin D consumption
385 during pregnancy was associated with a lower risk of developing recurrent
386 wheeze in offspring, when compared to placebo/control. However we were
387 not able to investigate the efficiency of vitamin D on other allergic outcomes
388 since outcomes were reported differently in the included trials. In all trials,
389 supplementation with vitamins significantly increased the concentration of
390 vitamins in the intervention group compared to the control group by the end of
391 the intervention.

392

393 Observational studies typically report a beneficial effect of higher intake of
394 vitamin D as well as antioxidants during pregnancy on allergic outcomes²²⁻²³.
395 The results from this systematic review proposed a protective effect of
396 prenatal intake of vitamin D during pregnancy for prevention of recurrent
397 wheeze in offspring. However we could not address the effect of prenatal
398 intake of vitamin C or D on other allergic outcomes owing to the observed
399 heterogeneity between the trials.

400

401 It is possible that the follow-up periods of the studies for this review have been
402 too short to detect other allergic outcomes i.e. asthma. For example,
403 wheezing is known as a primary symptom of asthma in early childhood²⁵ and
404 about 40% of childhood wheeze will persist later in life and will eventually
405 develop into asthma by 6 years of age²⁶, indicating majority of wheeze during

406 infancy are in fact acute respiratory infection. Therefore, extended follow-up of
407 these trials could help to provide a clearer answer as to whether the vitamin D
408 intervention is beneficial for asthma prevention.

409

410 There were also some limitations in the studies' design. For example, the
411 trials were statistically underpowered to detect an effect for their primary
412 and/or secondary outcome measures. Significant differences were only
413 observed for some of the secondary outcomes as "at least 1 episode of
414 wheezing"¹⁴, "episodes of troublesome lung symptoms"¹⁶ and "positive sIgE"¹⁷
415 and trials failed to show a beneficial effect for primary allergic outcomes such
416 as wheeze and asthma in children. Also, the trials used different doses of
417 vitamins during pregnancy. The dose of vitamin D varied between 800-4000IU
418 and doses of vitamin C and/or E, varied between 500-1000mg. It is possible
419 to hypothesize that lower doses of vitamins may have failed to reach the
420 desirable level of 25-hydroxyvitamin D or antioxidants in pregnant women to
421 have an influential effect on the fetal immune programming and lung
422 function²⁷⁻²⁹. However this is refuted by studies which have reported similar
423 effect size using higher doses of vitamin D^{16&17}. A previous RCT by
424 addressing the safety and efficacy of vitamin D supplementation during
425 pregnancy showed that a 4000IU vitamin D is a safe approach and was
426 necessary to optimise the circulating concentration of 25-hydroxyvitamin D
427 levels to $\geq 80\text{nmol/L}$ ³⁰. There is limited evidence on the safety of vitamins C
428 and E intake at any stage of pregnancy; however the Institute of Medicine's
429 Food and Nutrition Board have set an upper limit of 2000mg and 1000mg per
430 day for vitamins C and E ingestion respectively during pregnancy in the
431 United States³¹.

432

433 Further, in all trials the intervention was started in the 2nd trimester in
434 pregnancy. However the development of the lungs begins in the first trimester
435 in pregnancy and vitamin D plays an immunomodulatory role in the
436 development of lung and immune system³². Therefore the interventions might
437 have commenced too late in pregnancy or some used too low dose of vitamin
438 D to have a beneficial impact on lung development. Finally, the studies

439 recruited different types of population, which limits the generalisability of the
440 studies. Baseline levels of vitamin D vary in different geographical areas³³ and
441 this issue has not been addressed in the conducted trials. Well-designed trials
442 are necessary to address all these possible confounders among different
443 populations³⁴. Further larger scale research should administer vitamin D
444 earlier in pregnancy or pre-pregnancy and employs appropriate doses of
445 vitamin D to achieve a desirable level of vitamin D in maternal and fetal blood.
446 Furthermore, studies assessing the efficiency of nutrients are required to
447 consider the defined guidelines in their clinical design enabling to test the
448 associated hypothesis in a valid manner³⁵.

449

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

461

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

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Figure legends

Figure 1: Study flow diagram, following PRISMA criteria

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

Table 1. Characteristics of included trials and study population for Vitamins and prevention of respiratory and/or allergic outcomes in offspring

| Primary article | Companion articles+ | Country, enrolment period | No. of participants** | Age at last F-U | Sample: high risk of Atopy | Intake of intervention From/until | Duration of intervention (months)* | Vitamin product | Placebo | Total daily dose | Outcomes reported |
|------------------------------|-----------------------------------|---------------------------|-----------------------|-----------------|----------------------------|---|------------------------------------|--|---|--|---|
| Greenough 2010 ¹³ | Poston 2006 | U.K. 2003-05 | 2404 mothers | 2yrs. | No | From the 2nd trimester of pregnancy to delivery | 6-6.5 | Vitamin C & E | Microcrystalline cellulose with addition of tartaric & citric acid + sunflower seed oil | 1000mg Vit C & 400 IU RRR a-tocopherol, daily | -Wheeze -Eczema -Asthma -Cough -Breathing difficulty |
| Goldring 2013 ¹⁴ | Yu 2009 | U.K. 2007-not mentioned | 180 mothers | 3yrs. | No | 27wks to delivery | 3months + 1week | 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 2014 ¹⁵ | McEvoy 2013 (conference abstract) | U.S.A 2007-11 | 179 mothers | 1yr | No | 22wks to delivery | 4-4.5 | Crushed vitamin C | Ground cornstarch | 500 mg, daily | -Wheeze -Breathing difficulty |
| Chawes 2016 ¹⁶ | Bisgaard 2013 | Denmark 2008-2010 | 623 | 3yrs | No | 24wks to 1w after delivery | 3.5-4 + 1week | Vitamin D3 (cholecalciferol) | Tablets containing no active substance | 2400 IU, once a day | -Persistent wheeze -Asthma -URTI [#] -LRTI ^{##} -Episodes of lung symptoms |

| | | | | | | | | | | | |
|--------------------------------|------------------|------------------|-----|------|-----|------------------------------------|-------|---------------------------|------------------|-------------------|---|
| | | | | | | | | | | | -SPT -sIgE |
| Litonjua 2016 ¹⁷ | Litonjua 2014 | USA 2009-2011 | 880 | 3yrs | Yes | Between 10-18wks to delivery | 5-7.5 | Vitamin D & placebo | Not mentioned | 4000 IU, daily | -Wheeze or asthma -Eczema with rash -LRTI ^{##} -Total IgE (mean) -Sensitisation (aeroallergens) -sIgE |

[#]URTI=Upper Respiratory Tract Infection

^{##}LRTI=Lower Respiratory Tract Infection