

***In utero* exposure to 25(OH) D and risk of childhood asthma, wheeze and respiratory tract infections: a meta-analysis of birth cohort studies**

Haixia Feng, MS,<sup>a,\*</sup> Pengcheng Xun, MD, PhD,<sup>b,\*</sup> Katharine Pike, PhD,<sup>c</sup> Andrew K Wills, PhD,<sup>d</sup> Bo L Chawes, MD, PhD,<sup>e</sup> Hans Bisgaard, MD, DMSc,<sup>e</sup> Wei Cai, MD, PhD,<sup>f,g,h</sup> Yanping Wan, MD,<sup>a,†</sup> and Ka He, MD, ScD<sup>b,†</sup>

<sup>a</sup>Department of Clinical Nutrition, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China;

<sup>b</sup>Department of Epidemiology and Biostatistics, School of Public Health-Bloomington, Indiana University, Bloomington, IN, USA;

<sup>c</sup>Respiratory, Critical Care and Anaesthesia, UCL Institute of Child Health, London, UK;

<sup>d</sup>School of Clinical Sciences, University of Bristol, Bristol, UK;

<sup>e</sup>COPSAC; Copenhagen Prospective Studies on Asthma in Childhood, Herlev and Gentofte Hospital, University of Copenhagen, Copenhagen, Denmark;

<sup>f</sup>Department of Clinical Nutrition, Xin Hua Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China;

<sup>g</sup>Shanghai Key Laboratory of Pediatric Gastroenterology and Nutrition, Shanghai, China; Shanghai Institute of Pediatric Research, Shanghai, China;

<sup>h</sup>Department of Pediatric Surgery, Xin Hua Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai, China.

\*These authors contribute equally to this work.

**†Corresponding authors:**

Professor Yanping Wan; Address: Department of Clinical Nutrition, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China; Phone: +86-13916315626; Fax: +86-21-68383328; Email: wanyp204@163.com.

Professor Ka He; Address: Department of Epidemiology and Biostatistics, School of Public Health-Bloomington, Indiana University, 1025 E. Seventh Street, C042, Bloomington, Indiana 47405, USA; Phone: +01-812-856-2260; Fax: +01-812-855-7345; E-mail: [kahe@indiana.edu](mailto:kahe@indiana.edu).

**Funding:** This study was supported by Abbott Fund Institute of Nutrition Sciences (AFINS-2013-06), the key Laboratory of Pediatric Gastroenterology and Nutrition, Shanghai (11DZ2260-500), and the National Natural Science Foundation of China (81573140).

## **Abstract**

**Background:** Studies of the associations between *in utero* 25-hydroxyvitamin D [25(OH) D] exposure and childhood asthma risk, wheeze and respiratory tract infections are inconsistent and inconclusive.

**Objectives:** To assess the associations between 25(OH) D levels in cord blood or maternal venous blood and risk of offspring's asthma, wheeze and respiratory tract infections.

**Methods:** Data were derived from PubMed, EMBASE, Google Scholar, references from relevant articles, and *de novo* results from published studies until December, 2015. Random-effects meta-analysis was conducted among 16 birth cohort studies.

**Results:** Comparing the highest to the lowest category of 25 (OH) D levels, the pooled ORs were 0.84 (95% CI, 0.70 to 1.01;  $P = 0.064$ ) for asthma, 0.77 (0.58 to 1.03;  $P = 0.083$ ) for wheeze, and 0.85 (0.66 to 1.09;  $P = 0.187$ ) for respiratory tract infections. The observed inverse association for wheeze was more pronounced and became statistically significant in the studies that measured 25 (OH) D levels in cord blood (0.43, 0.29 to 0.62;  $P < 0.001$ ).

**Conclusions:** Accumulated evidence generated from this meta-analysis suggests that increased *in utero* exposure to 25 (OH) D is inversely associated with the risk of asthma and wheeze during childhood. These findings are in keeping with the results of two recently published randomized clinical trials of vitamin D supplementation during pregnancy.

## Key Messages

- Two recent randomized clinical trials suggest a non-significant protective effect of prenatal vitamin D supplementation on the risk of persistent wheeze/asthma in early childhood. However, the trials may be underpowered.
- After meta-analyzing data from 16 birth cohorts, we show that increased *in utero* exposure to 25 (OH) D is inversely related to risk of offspring's asthma and wheeze, but not respiratory tract infections.

## **Capsule Summary**

- Combining data from 16 birth cohort studies, this meta-analysis suggests that *in utero* exposure to 25(OH) D is inversely associated with risk of offspring's wheeze and asthma during childhood, supporting findings from two recent RCTs.

**Keywords:** 25 (OH) D, asthma, wheeze, respiratory tract infections, meta-analysis,  
*birth cohort studies*

<i>Abbreviations used:</i>	
25(OH) D:	25-hydroxyvitamin D
CI:	Confidence intervals
CLIA:	Chemiluminescence immunoassay
ECL:	Electrochemiluminescence
EIA:	Enzyme immunoassay
HPLC:	High-performance liquid chromatography
HPLC-MS:	High-performance liquid chromatography-tandem mass spectrometry
HR:	Hazards ratio
LC-MS:	Liquid chromatography-tandem mass spectrometry
NOS:	Newcastle-Ottawa quality assessment scale
OR:	Odds ratio
PRISMA:	Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines
RIA:	Radioimmunoassay
RR:	Relative risk
RTI:	Respiratory tract infection

## INTRODUCTION

Asthma, characterized by variable airway obstruction, wheeze, bronchial hyper-responsiveness and airway inflammation,<sup>1</sup> is very common worldwide and usually starts in childhood.<sup>2</sup> While allergic constitution, sensitization to allergens<sup>3-5</sup> and/or familial history of allergic disease<sup>6</sup> are recognized risk factors for asthma, studies also suggest that maternal vitamin D status during pregnancy may be associated with the risk of asthma in childhood.<sup>7,8</sup> To date, some birth cohort studies have shown that vitamin D status may play an important role in the development of fetal lungs.<sup>9-11</sup> Also, epidemiologic studies have suggested that maternal vitamin D intake<sup>12, 13</sup> as well as vitamin D levels in blood<sup>14-16</sup> were inversely associated with respiratory tract infections and other wheezing illnesses, presumably because of its multiple immune effects including induction of antibacterial responses and modulation of T-lymphocytes to suppress inflammation.<sup>17,18</sup> Data from randomized clinical trials are sparse, but two recently-published trials reported a non-significant more than 20% reduced risk of persistent wheeze/asthma at age 0-3 years from prenatal vitamin D supplementation<sup>19,20</sup> In addition to the relatively short follow-up period, the investigators acknowledged that the studies might be underpowered.

A systematic review of 3 cohort studies qualitatively summarized the evidence suggesting a potential link between serum levels of vitamin D and the diagnosis of asthma in childhood.<sup>21</sup> Another systematic review of 23 published articles including 9 cross-sectional, 2 case-control, and 12 cohort studies in both children and adults suggested that higher serum levels of 25-hydroxyvitamin D [25(OH) D] were associated with lower risk of asthma exacerbations, but provided little evidence on whether lower *in utero* exposure to 25(OH) D has a programming effect on development of asthma in childhood.<sup>22</sup> Notably, neither of these reviews considered

wheeze and respiratory tract infections as outcomes.

Since placental transfer of 25(OH) D is the major source of vitamin D in the developing fetus, we conducted this meta-analysis to quantitatively summarize the up-to-date literature to examine the overall associations between 25 (OH) D levels of cord blood or maternal venous blood and risk of childhood asthma, wheeze, and respiratory tract infections.

## **METHODS**

### **Search strategy and selection criteria**

Two investigators (HF and PX) scrutinized publications independently based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.<sup>23</sup> The complete search process is outlined in **Fig 1** and the completed PRISMA checklist is available in **E Table 1** at the Online Repository. First, PubMed [1966-] and EMBASE [1974-] were searched from inception to December 31, 2015 using the terms “vitamin D” and “lung disease or asthma or respiratory tract diseases” and “epidemiological studies” and “cohort / prospective or follow-up or longitudinal studies”. Second, Google Scholar was used to search for any other studies that were not identified from the aforementioned literature review. Third, the references from the retrieved articles were manually searched for possible additional studies. Conference abstracts and unpublished studies were not included. Finally, to get additional information, we requested *de novo* data specifically for this meta-analysis from some primary studies.<sup>24-26</sup>

The following inclusion criteria were used in this meta-analysis: 1) English article; 2) prospective birth cohort design; 3) cord blood or maternal venous blood 25 (OH) D levels as



exposure; 4) offspring's asthma, wheeze or respiratory tract infections, which were diagnosed by physicians or meeting the international guidelines, or the parents reported as outcomes; and 5) available data on the relevant odds ratio (OR) or relative risk (RR) or hazards ratio (HR) and the corresponding 95% confidence intervals (CIs).

### **Quality assessment and data extraction**

Two investigators (HF and PX) independently evaluated the quality of each primary study based on the criteria derived from the Newcastle-Ottawa quality assessment scale (NOS) with a maximum score of 9 points.<sup>27</sup> Any discrepancy was adjudicated by a third reviewer (KH). The NOS for cohort studies was divided into three groups: selection of cohort (4 points), comparability of cohort (2 points) and assessment of outcome (3 points). The quality of study was considered high or moderate if the sum score was  $\geq 8$  points or between 5-7 points, respectively. We limited our meta-analysis to those studies that were rated as high or moderate quality. One study was excluded from the main analysis due to low quality.<sup>28</sup> **E Table 2** at the Online Repository shows the details of the study quality assessment.

Data were extracted from all the eligible studies by two independent researchers (HF and PX) based on a standardized form, and any controversy was resolved through consensus by discussion with a third researcher (KH). From each included study, we extracted the following information: name of the first author, year of publication, the country of origin, study name, proportion of boys, duration of follow-up, numbers of participants/events, exposure assessment and categorization, outcome definition and assessment, adjusted covariates in the final model, and the OR / RR / HR estimates with corresponding 95% CIs for all categories and/or continuous

exposure of interest.

To examine dose-response relationship, linear associations were standardized to per 10 nmol/L increment in blood 25 (OH) D levels. If the linear association was not reported in the primary study, we estimated it using Greenland and Longnecker's generalized least square method if the participants and the events in each category were available, or using variance-weighted least-squares estimation if this information was unavailable.<sup>29,30</sup> If the highest exposure category was open-ended (e.g., >89 nmol/L), its upper limit was determined by assuming its range was the same interval as that of the adjacent category. Twelve out of 16 included studies reported ORs (95% CIs). In addition, we obtained *de novo* data for ORs (95% CIs) from two studies,<sup>25,26</sup> and converted RRs (95% CIs) to ORs (95% CIs) in other two studies<sup>31,32</sup> by using the formula:  $OR = [(1 - P_0) * RR / (1 - RR * P_0)]$ , where  $P_0$  indicated the incidence of the outcome of interest in the non-exposed/reference group.<sup>33</sup>

### **Statistical analysis**

The ORs and corresponding 95% CIs extracted from individual studies were transformed into natural logarithms to normalize their distribution, stabilize the variances, and facilitate the calculation of its standard errors. The pooled ORs and corresponding 95% CIs for each outcome of interest were computed using a random-effects model by weighting the inverse of variance. To get the pooled association for risk of asthma and wheeze, we pooled the two outcomes within each study first using a random-effects model if the study reported both of the two outcomes. The heterogeneity among studies was tested by Cochran's  $Q$  test with a significance level of 0.10 and quantified by the  $I^2$  statistic. An  $I^2$  value of 0-25%, 26-50%, 51-75%, or >75% represents no,

low, moderate, or high heterogeneity, respectively. Publication bias was determined by Egger's regression asymmetry test also with a significance level of 0.1 The Duval and Tweedie nonparametric "trim and fill" method was used to adjust for publication bias if applicable.<sup>34</sup>

Stratified analyses were performed to determine modification effects by a few pre-specified factors, including blood source (cord blood vs. maternal venous blood), outcome diagnosis (clinician diagnosis vs. parental report), and season variation consideration (yes vs. no). A random-effects meta-regression model was used to obtain the *P* value for interaction between each of these factors and the exposure.<sup>35</sup> Sensitivity analyses were conducted to evaluate the influence of replacing a random-effects model with a fixed-effects model and the influence of a single study on the associations by omitting one primary study each time in the meta-analysis.

All analyses were performed by using STATA statistical software (Version 13.0; STATA Corporation LP, College Station, Texas, USA). A two-sided *P* value  $\leq 0.05$  was considered statistically significant if not otherwise specified.

## **RESULTS**

### **Literature search**

**Fig 1** shows the flow of the study selection process. A total of 130 articles in PubMed and 356 in EMBASE were identified initially. Of them, 8 in PubMed (5 reviews and 3 case reports) and 102 in EMBASE (28 reviews and 74 case reports) were excluded. Also, 111 in PubMed and 249 in EMBASE were excluded after reviewing the title and abstract. Of the 11 studies in PubMed and 5 in EMBASE, 3 studies were further excluded after full-text review because of substantial

overlap with other studies. In addition, 4 studies were found via Google Scholar. Thus, by systematically searching the literature, 17 studies<sup>10,11,24-26,31-33,36-44</sup> were identified. After quality assessment, one study was excluded.<sup>33</sup> Finally, 16 birth cohort studies were included in this meta-analysis.

### **Study characteristics**

Characteristics of the 16 included studies are summarized in **E Table 3** at the Online Repository. These primary studies were conducted in Asia,<sup>38,40,43</sup> Europe,<sup>10,24-26,31,32,36,41,44</sup> North America,<sup>42</sup> and Australia.<sup>11,37,39</sup> Of the 16 studies, 10 studies focused on asthma, including 8 871 participants (1 494 incident cases) with an average 5.9-year follow-up; 10 studies reported results on wheeze, including 9 072 individuals (2 277 incident cases) with an average 5.5-year follow-up; and 10 studies presented findings on respiratory tract infections, including 8 359 individuals (2 562 incident cases) with an average 1.4-year follow-up. The blood 25(OH) D levels were measured with various methods including chemiluminescence immunoassay (CLIA), electrochemiluminescence (ECL), enzyme immunoassay (EIA), high-performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC-MS), high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS), and radioimmunoassay (RIA). The blood samples were either cord blood or maternal venous blood during pregnancy in different stages. Seven studies were evaluated as high quality,<sup>24-26,36,37,40,44</sup> while the other 9 studies as moderate quality.<sup>10,11,29,30,38,39,41-43</sup>

### **Primary analyses**

#### **Asthma**

According to the available data from 8 studies,<sup>10,11,24-26, 37,38,41</sup> a borderline significant inverse association between *in utero* exposure to vitamin D and risk of asthma in offspring was found. The pooled OR for the offspring's asthma was 0.84 (95%CI, 0.70 to 1.01;  $P = 0.064$ ) comparing the highest to the lowest category of 25 (OH) D levels (**Fig 2**). No evidence of heterogeneity ( $I^2 = 10.4\%$ ,  $P = 0.349$ ) and publication bias (Egger's test:  $P = 0.308$ ) was found.

No statistically significant linear association was observed based on available data derived from 8 studies.<sup>10,24-26,36,37,41,42</sup> The pooled OR for the offspring's asthma was 0.99 (95%CI, 0.97 to 1.02;  $P = 0.691$ ) with a 10 nmol/L increment in 25 (OH) D levels. Neither heterogeneity ( $I^2 = 0.0\%$ ,  $P = 0.520$ ) nor publication bias (Egger's test:  $P = 0.570$ ) was evidenced.

## **Wheeze**

Similar to asthma, a borderline significant inverse association was observed between *in utero* exposure to vitamin D and risk of wheeze in offspring when combining data from 8 eligible studies.<sup>11, 24-26,31,37,41,44</sup> The pooled OR for wheeze was 0.77 (95%CI, 0.58 to 1.03;  $P = 0.083$ ) comparing the highest to the lowest category of 25 (OH) D levels (**Fig 3**). A moderate heterogeneity exists across included studies ( $I^2 = 62.1\%$ ,  $P = 0.010$ ). No publication bias was evident (Egger's test:  $P = 0.122$ ).

No statistically significant linear association was determined using available data from 9 studies.<sup>24-26,31,36,37,39,41,44</sup> The pooled OR for offspring's wheeze was 0.98 (95%CI, 0.96 to 1.004;  $P = 0.114$ ) with every 10 nmol/L increment in 25 (OH) D levels. No heterogeneity ( $I^2 = 5.3\%$ ,  $P = 0.391$ ) nor publication bias (Egger's test:  $P = 0.286$ ) exists.

## Respiratory tract infections

Ten studies examined cord blood or maternal venous blood 25 (OH) D levels in relation to offspring's respiratory tract infections.<sup>11,24,25,31,32,37,40,41,43,44</sup> The combined results did not reveal a significant inverse association. The pooled OR was 0.85 (95%CI, 0.66 to 1.09;  $P = 0.187$ ) as compared the highest to the lowest category of 25 (OH) D levels (**Fig 4**). No evidence on publication bias (Egger's test:  $P = 0.486$ ), but a moderate heterogeneity existed across included studies ( $I^2 = 66.1\%$ ,  $P = 0.003$ ).

A non-significant linear association was found. The pooled OR was 0.97 (95%CI, 0.94 to 1.01;  $P = 0.156$ ) with a 10 nmol/L increment in 25 (OH) D levels. Neither heterogeneity ( $I^2 = 0.0\%$ ,  $P = 0.495$ ) nor publication bias (Egger's test:  $P = 0.798$ ) existed.

## Stratified analysis

We examined potential effect modifications of a few pre-specified factors (**Table 1**). The association with risk of wheeze was modified by the source of 25 (OH) D ( $P$  for interaction = 0.007). A statistically significant inverse association was documented when combining data from studies with 25(OH) D measured in cord blood. The pooled OR was 0.43 (95%CI, 0.29 to 0.62;  $P < 0.001$ ) comparing the highest to the lowest category of cord blood 25(OH) D levels. The risk of offspring's wheeze was lowered by 5% with every 10 nmol/L increment in cord blood 25 (OH) D levels (0.95, 95%CI, 0.91 to 0.99;  $P = 0.009$ ). By contrast, no significant association was found when combining studies with 25(OH) D measured in maternal venous blood. Other factors including outcome diagnosis and season variation consideration did not appreciably modify the

associations of interest. Of note, one primary study<sup>11</sup> reported sex difference. Hence, we requested *de novo* data on sex difference and received data from 3 primary studies.<sup>24-26</sup> After combining data from these 4 available studies, no significant sex difference was found (data not shown).

### **Sensitivity analysis**

When replacing a random-effects model with a fixed-effects model, the results were generally consistent, except that the association between *in utero* exposure to 25 (OH) D (highest vs. lowest) and risk of respiratory tract infection became statistically significant (0.93; 95% CI, 0.91 to 0.95;  $P < 0.001$ ) (**E Table 4** at the Online Repository). Since the most common cause of recurrent wheezing is asthma attacks, we combined these two endpoints with available data from 10 studies.<sup>10,11,24-26,31,37,38,41,44</sup> The pooled OR was 0.80 (95% CI, 0.66 to 0.98;  $P = 0.028$ ) comparing the highest to the lowest category of 25 (OH) D levels.

A couple of studies substantially affect the pooled results (**E Table 5** at the Online Repository). The overall association between 25 (OH) D and asthma was strengthened and became statistically significant when the study by Camargo and colleagues<sup>37</sup> (Categorical analysis: 0.82; 95% CI, 0.67 to 0.999;  $P = 0.049$ ) or the study by Wills and colleagues<sup>26</sup> (Categorical analysis: 0.78; 95% CI, 0.64 to 0.96;  $P = 0.017$ ) was omitted. Similarly, the overall association between 25 (OH) D and wheeze was strengthened and became statistically significant by omitting the study by de Jongh and colleagues<sup>31</sup> (Categorical association: 0.72; 95% CI, 0.53 to 0.99;  $P = 0.041$ ) or by omitting the study by Wills and colleagues<sup>26</sup> (Linear association: 0.97; 95% CI, 0.95 to 0.9996;  $P = 0.047$ ).

## **DISCUSSION**

In this meta-analysis, we found that increased *in utero* exposure to 25(OH) D was inversely associated with risk of offspring's wheeze and asthma in childhood, which is in adherence with the findings from two recent randomized clinical trials of vitamin D supplementation during pregnancy. The evidence was strengthened when 25 (OH) D levels were measured in cord blood. The observed associations were not appreciably modified by sex, outcome diagnosis methods, and season variation consideration in primary studies.

### **Strengths and limitations**

One unique strength of this meta-analysis is that we obtained *de novo* data from a few primary studies,<sup>24-26</sup> which provides more accurate and/or additional information specifically for this meta-analysis. Another main strength of this study is that *in utero* exposure to vitamin D is measured by objective biomarkers (i.e., cord blood or maternal venous blood) that account for vitamin D from diet and sun exposure. However, we realize that serum 25 (OH) D may not be the best biomarker, alternative vitamin D biomarkers (e.g., tissues and intracellular fluid) are definitely needed in future studies depending on the scale of the study and the budget and ethical considerations.

Despite the above merits, the results should be interpreted with caution because of some limitations. First, moderate heterogeneity existed in some pooled analyses. The variations of several factors may generate the heterogeneity. For example, different study population, study location, sample size, duration of follow-up, source of blood samples for measuring vitamin D



status, outcome diagnosis, season variation consideration, and adjustment for different covariates in primary studies. In the analyses, a random-effects model was used in concordance with the heterogeneity. In addition, stratified analyses were conducted to explore any potential effect modifier. Second, asthma and respiratory tract infections in some primary studies and wheeze in all the studies were reported by parents except the COPSAC study where wheeze was diagnosed at acute visits to the research clinic and by a daily diary of respiratory symptoms. Although the signs of these health conditions may be clearly visible, the likelihood of misclassification could not be completely ruled out, e. g., parents might report wheeze as respiratory tract infection. Nevertheless, the potential misclassification should not substantially affect our results since the great majority of the parental reports were based on clinician's diagnosis. Third, maternal 25(OH) D levels in some studies were measured in venous blood during pregnancy at different time points. This inconsistency of the measurement time window might introduce errors in the exposure assessment, but this would presumably lead to a bias towards the null. However, most of the studies (12 out of 16) measured 25 (OD) D in the later stage of pregnancy (i.e., 2 studies at 34-week gestation) or at birth (10 studies). Thus, the different measurement timing in a few studies should not substantially bias our findings. Fourth, most of the primary studies measured 25 (OH) D levels only once, which might not adequately reflect the long-term exposure and may attenuate any possible associations of interest because of random variation. Our findings are more likely to suggest a beneficial effect of higher levels of vitamin D in late pregnancy. Further longitudinal studies with repeat measurements in 25 (OH) D levels at different stages of pregnancy are warranted. Fifth, the baseline vitamin D levels varied across studies, but we were not able to adjust for this variation in the meta-analysis due to lack of original data. Sixth, we were not able to pool the data comparing individuals with vitamin D levels  $\geq 75$  nmol/L vs.

everyone else due to data insufficiency. Finally, although the pooled analyses were based on fully adjusted models in the individual studies, our results might be biased by the inherent limitations in the primary studies such as residual confounding or confounding from unknown or unmeasured factors.

### **Comparison to previous studies**

In light of the considerable complexity of the pathogenesis of these respiratory outcomes, it is not surprising to see the inconsistency in the available data on vitamin D status and the offspring's asthma, wheeze and respiratory tract infections. One meta-analysis nested in a systematic review based only on 3 birth cohort studies suggested a possible association between low serum vitamin D levels and risk of asthma in children.<sup>21</sup> Another review, which summarized data from 9 cross-sectional, 2 case-control, and 12 cohort studies in both children and adults, suggested that higher serum levels of 25 (OH) D were related to lower risk of asthma exacerbations. However, the study did not provide information on the association of *in utero* exposure to 25 (OH) D with the development of asthma in childhood.<sup>22</sup> Notably, neither of these two reviews considered wheeze and respiratory tract infections as outcomes. In addition, one recent meta-analysis combined data from 4 cohort studies found that maternal dietary intake of vitamin D was associated with lower odds of offspring's wheeze during childhood.<sup>45</sup> However, dietary intake only accounts for 10% of circulating 25 (OH) D. Findings from the present meta-analysis are generally consistent with that of the previous reviews and provide important additional information on *in utero* exposure to 25 (OH) D and risk of the offspring's wheeze and respiratory tract infections in childhood. Furthermore, the findings are in line with two recently published randomized clinical trials of vitamin D supplementation during pregnancy showing a

non-significant more than 20 % reduced risk of persistent wheeze/asthma at age 0-3 years.<sup>19,20</sup>

Of note, a recent randomized clinical trial found that the maternal or cord blood vitamin D levels should be at least 75 nmol/L in order to result in reversal or protection of the autoimmune effects.<sup>20</sup>, but the optimal level during pregnancy for lung and immune maturation is still unknown. In our meta-analysis, the distribution of 25 (OH) D levels varied across the primary studies, but overall approximately 28% participants had 25 (OH) D levels  $\geq$  75 nmol/L (**E Table 6** at the Online Repository). Thus, the pooled results are biologically in concordance with findings from the randomized clinical trials.

### **Possible explanations**

Vitamin D is an immune-regulator, involved in both cellular immunity and humeral immunity.<sup>46</sup>

There are several explanations for the potential beneficial effect of vitamin D on the development of asthma and wheeze, including: 1) 25 (OH) D can modify T-cell differentiation and dendritic cell activation,<sup>17</sup> which plays an important role in the inflammatory response; 2) 25 (OH) D can induce a shift in the profile of cytokine secretion during an immune response to an anti-inflammatory phenotype, with regulatory T lymphocytes predominating in the cell population;<sup>47</sup> and 3) In macrophages, vitamin D can promote antimicrobial responses through the induction of antibacterial proteins,<sup>18</sup> and stimulation of autophagy and autophagosome activity.

One study demonstrated a very high correlation between maternal and umbilical cord blood vitamin D levels (Spearman's correlation = 0.91),<sup>48</sup> which supports a common belief that 25 (OH) D measured in maternal venous blood should be parallel to the measures in cord blood.

However, we found that the association was more pronounced in the studies that 25 (OH) D was

measured in cord blood. Notably, the heterogeneity of the time window for measuring 25 (OH) D levels in maternal venous blood during pregnancy may attenuate any possible associations.

Studies suggest that early lung development differs between boys and girls,<sup>49,50</sup> which may explain the higher prevalence of asthma in boys than that of girls before puberty.<sup>51</sup> Although the present meta-analysis did not observe sex difference, a potential effect modification of sex cannot be completely ruled out because the available data were derived from limited number of studies.<sup>11,24-26</sup>

Because of seasonal variations in ultraviolet radiation exposure, seasonal variation in vitamin D status is recognized.<sup>52</sup> One study found that vitamin D deficiency was marginally associated with an increased risk of lower respiratory tract infections in children born in fall, but not in children born in other three seasons.<sup>32</sup> Some, but not all primary studies considered vitamin D seasonal variation in the analysis. This inconsistency may affect the pooled results and/or contribute to the heterogeneity. Nevertheless, seasonal variation consideration did not materially modify the associations of interest in this meta-analysis.

## **CONCLUSION**

In conclusion, this meta-analysis of birth cohort studies accumulates evidence supporting that increased *in utero* exposure to 25(OH) D is inversely associated with the risk of asthma and wheeze during childhood. These findings are in keeping with the results of two recently published randomized clinical trials of vitamin D supplementation during pregnancy showing a more than 20% reduced risk of persistent wheeze/asthma at age 3 years.

## **ACKNOWLEDGEMENTS**

Author contribution: H. Feng, P. Xun, Y. Wan, and K. He contributed to concept and design of the study; H. Feng and P. Xun developed the search strategy, performed the literature review, data extraction, and study quality assessment; P. Xun designed and conducted the data analysis and interpretation; P. Xun contacted the authors of primary studies for additional information; K. Pike, A. Wills, B. Chawes, and H. Bisgaard provided *de novo* data specifically for this meta-analysis; P. Xun and H. Feng contributed to tables and figures. H. Feng, P. Xun, Y. Wan, and K. He drafted the manuscript; P. Xun and K. He had full access to all the data in this meta-analysis; and all the authors contributed to the manuscript revision, reviewed and approved the final manuscript. H. Feng and P. Xun contributed equally.

Disclosure of potential conflict of interest: H. Bisgaard reports personal fees from Chiesi, outside the submitted work; other authors have no relevant interests to declare.

## FIGURE LEGENDS

**Fig 1.** Flowchart of study selection

**Fig 2.** Multivariable-adjusted ORs and 95% CIs (horizontal lines) of asthma. The summary estimates (diamond data markers) were obtained by using a random-effects model. The dots indicate the adjusted ORs comparing the highest to lowest category of, or per 10 nmol/L increment in 25(OH) D levels. The size of the shaded square is proportional to the weight of each study. CI: confidence interval; OR: odds ratio.

**Fig 3.** Multivariable-adjusted ORs and 95% CIs (horizontal lines) of wheeze. The summary estimates (diamond data markers) were obtained by using a random-effects model. The dots indicate the adjusted ORs comparing the highest to lowest category of, or per 10 nmol/L increment in 25(OH) D levels. The size of the shaded square is proportional to the weight of each study. CI: confidence interval; OR: odds ratio.

**Fig 4.** Multivariable-adjusted ORs and 95% CIs (horizontal lines) of respiratory tract infections. The summary estimates (diamond data markers) were obtained by using a random-effects model. The dots indicate the adjusted ORs comparing the highest to lowest category of, or per 10 nmol/L increment in 25(OH) D levels. The size of the shaded square is proportional to the weight of each study. CI: confidence interval; OR: odds ratio; RTI: respiratory tract infection.

## REFERENCES

- 1 Busse WW, Lemanske RF, Jr. Asthma. *N Engl J Med* 2001;344:350-62.
- 2 Thomas PK. Guideline based management of bronchial asthma. *J Assoc Physicians India* 2014;62:27-31.
- 3 Becerir T, Akcay A, Duksal F, Ergin A, Becerir C, Guler N. Prevalence of asthma, local risk factors and agreement between written and video questionnaires among Turkish adolescents. *Allergol Immunopathol (Madr)* 2014;42:594-602.
- 4 Peat JK, Tovey E, Toelle BG, et al. House dust mite allergens. A major risk factor for childhood asthma in Australia. *Am J Respir Crit Care Med* 1996;153:141-6.
- 5 Stevens E, Cullinan P, Colvile R. Urban air pollution and children's asthma: what do parents and health professionals think? *Pediatr Pulmonol* 2004;37:530-6.
- 6 Burke W, Fesinmeyer M, Reed K, Hampson L, Carlsten C. Family history as a predictor of asthma risk. *Am J Prev Med* 2003;24:160-9.
- 7 Duijts L. Fetal and infant origins of asthma. *Eur J Epidemiol* 2012;27:5-14.
- 8 Mebrahtu TF, Feltbower RG, Greenwood DC, Parslow RC. Birth weight and childhood wheezing disorders: a systematic review and meta-analysis. *J Epidemiol Community Health* 2015;69:500-8.
- 9 Hart PH, Lucas RM, Walsh JP, et al. Vitamin D in fetal development: findings from a birth cohort study. *Pediatrics* 2015;135:e167-73.
- 10 Magnus MC, Stene LC, Haberg SE, et al. Prospective study of maternal mid-pregnancy 25-hydroxyvitamin D level and early childhood respiratory disorders. *Paediatr Perinat Epidemiol* 2013;27:532-41.
- 11 Zosky GR, Hart PH, Whitehouse AJ, et al. Vitamin D deficiency at 16 to 20 weeks' gestation is associated with impaired lung function and asthma at 6 years of age. *Ann Am Thorac Soc* 2014;11:571-7.
- 12 Devereux G, Litonjua AA, Turner SW, et al. Maternal vitamin D intake during pregnancy and early childhood wheezing. *Am J Clin Nutr* 2007;85:853-9.

- 13 Miyake Y, Tanaka K, Okubo H, Sasaki S, Arakawa M. Maternal consumption of dairy products, calcium, and vitamin D during pregnancy and infantile allergic disorders. *Ann Allergy Asthma Immunol* 2014; 113: 82-7.
- 14 Demirel S, Guner SN, Celiksoy MH, Samcak R. Is vitamin D insufficiency to blame for recurrent wheezing? *Int Forum Allergy Rhinol* 2014;4:980-5.
- 15 Pletz MW, Terkamp C, Schumacher U, et al. Vitamin D deficiency in community-acquired pneumonia: low levels of 1,25(OH)<sub>2</sub> D are associated with disease severity. *Respir Res* 2014;15:53. 10.1186/1465-9921-15-53.
- 16 Science M, Maguire JL, Russell ML, Walter SD, Loeb M. Low serum 25-hydroxyvitamin D level and risk of upper respiratory tract infection in children and adolescents. *Clin Infect Dis* 2013;57:392-7.
- 17 Baeke F, Takiishi T, Korf H, Gysemans C, Mathieu C. Vitamin D: modulator of the immune system. *Curr Opin Pharmacol* 2010;10:482-96.
- 18 Tamblyn JA, Hewison M, Wagner CL, Bulmer JN, Kilby MD. Immunological role of vitamin D at the maternal-fetal interface. *J Endocrinol* 2015;224:R107-21.
- 19 Chawes BL, Bønnelykke K, Stokholm J, et al. Effect of vitamin D<sub>3</sub> supplementation during pregnancy on risk of persistent wheeze in the offspring: a randomized clinical trial. *JAMA* 2016;315:353-61.
- 20 Litonjua AA, Carey VJ, Laranjo N, 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.
- 21 Rajabbik MH, Lotfi T, Alkhaled L, et al. Association between low vitamin D levels and the diagnosis of asthma in children: a systematic review of cohort studies. *Allergy Asthma Clin Immunol* 2014;10:31. 10.1186/1710-1492-10-31.
- 22 Cassim R, Russell MA, Lodge CJ, Lowe AJ, Koplin JJ, Dharmage SC. The role of circulating 25



- hydroxyvitamin D in asthma: a systematic review. *Allergy* 2015;70:339-54.
- 23 Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg* 2010;8:336-41.
- 24 Chawes BL, Bonnelykke K, Jensen PF, Schoos AM, Heickendorff L, Bisgaard H. Cord blood 25(OH)-vitamin D deficiency and childhood asthma, allergy and eczema: the COPSAC2000 birth cohort study. *PLoS One* 2014;9:e99856. 10.1371/journal.pone.0099856.
- 25 Pike KC, Inskip HM, Robinson S, et al. Maternal late-pregnancy serum 25-hydroxyvitamin D in relation to childhood wheeze and atopic outcomes. *Thorax* 2012;67:950-6.
- 26 Wills AK, Shaheen SO, Granell R, Henderson AJ, Fraser WD, Lawlor DA. Maternal 25-hydroxyvitamin D and its association with childhood atopic outcomes and lung function. *Clin Exp Allergy* 2013;43:1180-8.
- 27 Wells GA, Shea B, O'Connell D, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. *Secondary The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses.* [http://www.ohri.ca/programs/clinical\\_epidemiology/oxford.asp](http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp) (Accessed Feb 2, 2016).
- 28 Gale CR, Robinson SM, Harvey NC, et al. Maternal vitamin D status during pregnancy and child outcomes. *Eur J Clin Nutr* 2008;62:68-77.
- 29 Greenland S, Longnecker MP. Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol* 1992;135:1301-9.
- 30 Orsini N, Li R, Wolk A, Khudyakov P, Spiegelman D. Meta-analysis for linear and nonlinear dose-response relations: examples, an evaluation of approximations, and software. *Am J Epidemiol* 2012;175:66-73.
- 31 de Jongh RT, Crozier SR, D'Angelo S, et al. Maternal 25-hydroxyvitamin D levels in relation to offspring respiratory symptoms and infections. *Eur Respir J* 2014;43:1181-3.
- 32 Luczynska A, Logan C, Nieters A, et al. Cord blood 25(OH)D levels and the subsequent risk of

- lower respiratory tract infections in early childhood: the Ulm birth cohort. *European Journal of Epidemiology* 2014;29:585-94.
- 33 Zhang J, Yu KF. What's the relative risk? A method of correcting the odds ratio in cohort studies of common outcomes. *JAMA* 1998;280:1690-1.
- 34 Duval S, Tweedie R. Trim and fill: a simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. *Biometrics* 2000;56:455-63.
- 35 Knapp G, Hartung J. Improved tests for a random effects meta-regression with a single covariate. *Stat Med* 2003;22:2693-710.
- 36 Baiz N, Dargent-Molina P, Wark JD, Souberbielle JC, Annesi-Maesano I. Cord serum 25-hydroxyvitamin D and risk of early childhood transient wheezing and atopic dermatitis. *J Allergy Clin Immunol* 2014;133:147-53.
- 37 Camargo CA, Jr., Ingham T, Wickens K, et al. Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. *Pediatrics* 2011;127:e180-7.
- 38 Chiu CY, Huang SY, Peng YC, et al. Maternal vitamin D levels are inversely related to allergic sensitization and atopic diseases in early childhood. *Pediatr Allergy Immunol* 2015; 4:337-43. 10.1111/pai.12384.
- 39 Jones AP, D'Vaz N, Meldrum S, Palmer DJ, Zhang G, Prescott SL. 25-hydroxyvitamin D3 status is associated with developing adaptive and innate immune responses in the first 6 months of life. *Clin Exp Allergy* 2015;45:220-31.
- 40 Mohamed WA, Al-Shehri MA. Cord blood 25-hydroxyvitamin D levels and the risk of acute lower respiratory tract infection in early childhood. *J Trop Pediatr* 2013;59:29-35.
- 41 Morales E, Romieu I, Guerra S, et al. Maternal vitamin D status in pregnancy and risk of lower respiratory tract infections, wheezing, and asthma in offspring. *Epidemiology* 2012;23:64-71.
- 42 Rothers J, Wright AL, Stern DA, Halonen M, Camargo CA, Jr. Cord blood 25-hydroxyvitamin D levels are associated with aeroallergen sensitization in children from Tucson, Arizona. *J Allergy*

Clin Immunol 2011;128:1093-1099 e1-e5.

- 43 Shin YH, Yu J, Kim KW, et al. Association between cord blood 25-hydroxyvitamin D concentrations and respiratory tract infections in the first 6 months of age in a Korean population: a birth cohort study (COCOA). *Korean J Pediatr* 2013;56:439-45.
- 44 Stelmach I, Majak P, Jerzynska J, et al. Cord serum 25-hydroxyvitamin D correlates with early childhood viral-induced wheezing. *Respir Med* 2015;109:38-43.
- 45 Beckhaus AA, Garcia-Marcos L, Forno E, et al. Maternal nutrition during pregnancy and risk of asthma, wheeze, and atopic diseases during childhood: a systematic review and meta-analysis. *Allergy* 2015; 70:1588-604.
- 46 Hewison M. Vitamin D and the immune system: new perspectives on an old theme. *Endocrinol Metab Clin North Am* 2010; 39: 365-79.
- 47 Adorini L, Penna G, Giarratana N, Uskokovic M. Tolerogenic dendritic cells induced by vitamin D receptor ligands enhance regulatory T cells inhibiting allograft rejection and autoimmune diseases. *J Cell Biochem* 2003;88:227-33.
- 48 Jacquemyn Y, Ajaji M, Karepouan N. Vitamin D levels in maternal serum and umbilical cord blood in a multi-ethnic population in Antwerp, Belgium. *Facts Views Vis Obgyn* 2013;5:3-5.
- 49 Carey MA, Card JW, Voltz JW, et al. It's all about sex: gender, lung development and lung disease. *Trends Endocrinol Metab* 2007;18:308-13.
- 50 Townsend EA, Miller VM, Prakash YS. Sex differences and sex steroids in lung health and disease. *Endocr Rev* 2012;33:1-47.
- 51 McCallister JW, Mastrorarde JG. Sex differences in asthma. *J Asthma* 2008;45:853-61.
- 52 Zosky GR, Hart PH, Whitehouse AJ, Hall GL. Reply: Seasonality and total 25-hydroxyvitamin D levels as sources of potential misclassification of vitamin D deficiency. *Ann Am Thorac Soc* 2014;11:1337-8.

**TABLE 1.** Stratified analyses of the associations between *in utero* exposure to 25 (OH) D and risk of childhood asthma, wheeze and respiratory tract infections

Outcome	25(OH) D	Potential modifiers	No. of cohorts	No. of participants / events	Heterogeneity test	OR (95%CI)	P for interaction	
Asthma	Highest vs. lowest	Overall	8	8 438/1 446	$I^2 = 10.4\%$ , $P = 0.349$	0.84 (0.70 to 1.01)	--	
		Blood source	Cord blood	3	1 208/238	$I^2 = 33.3\%$ , $P = 0.223$	0.73 (0.39 to 1.37)	0.760
			Maternal venous blood	5	7 230/1 208	$I^2 = 15.2\%$ , $P = 0.318$	0.85 (0.70 to 1.04)	
		Outcome assessment	Doctor diagnosed	4	1 533/144	$I^2 = 13.3\%$ , $P = 0.326$	0.71 (0.46 to 1.09)	0.435
			Parent-report	4	6 905/1 302	$I^2 = 17.8\%$ , $P = 0.302$	0.88 (0.71 to 1.08)	
		Season Considered*	Yes	4	5 870/1 080	$I^2 = 21.1\%$ , $P = 0.284$	0.87 (0.67 to 1.11)	0.684
			No	4	2 567/366	$I^2 = 22.1\%$ , $P = 0.278$	0.78 (0.56 to 1.10)	
		↑ Per 10 nmol/L	Overall	8	8 397/1 468	$I^2 = 0.0\%$ , $P = 0.520$	0.99 (0.97 to 1.02)	--
	Blood source		Cord blood	4	1 455/260	$I^2 = 0.0\%$ , $P = 0.736$	1.04 (0.98 to 1.10)	0.137
			Maternal venous blood	4	6 942/1208	$I^2 = 0.0\%$ , $P = 0.582$	0.98 (0.96 to 1.01)	
	Outcome assessment		Doctor diagnosed	3	1 253/147	$I^2 = 15.8\%$ , $P = 0.305$	1.01 (0.93 to 1.09)	0.810
			Parent-report	5	7 144/1321	$I^2 = 0.0\%$ , $P = 0.439$	0.99 (0.97 to 1.01)	
	Season Considered*		Yes	6	6 304/1 128	$I^2 = 11.1\%$ , $P = 0.345$	1.00 (0.97 to 1.03)	0.532
			No	2	2 093/340	$I^2 = 0.0\%$ , $P = 0.757$	0.97 (0.91 to 1.04)	
Wheeze <sup>†</sup>	Highest vs. lowest		Overall	8	8 694/2 150	$I^2 = 62.1\%$ , $P = 0.010$	0.77 (0.58 to 1.03)	--
		Blood source	Cord blood	3	1 240/612	$I^2 = 0.0\%$ , $P = 0.757$	<b>0.43 (0.29 to 0.62)</b>	<b>0.007</b>
			Maternal venous blood	5	7 454/1 538	$I^2 = 0.0\%$ , $P = 0.825$	1.00 (0.83 to 1.20)	
		Season Considered*	Yes	3	4 263/920	$I^2 = 83.2\%$ , $P = 0.003$	0.60 (0.29 to 1.23)	0.427
			No	5	4 431/1 230	$I^2 = 28.8\%$ , $P = 0.230$	0.89 (0.67 to 1.18)	
	↑ Per 10 nmol/L	Overall	9	8 784/2 277	$I^2 = 5.3\%$ , $P = 0.391$	0.98(0.96 to 1.004)	--	
		Blood source	Cord blood	5	1 618/665	$I^2 = 0.0\%$ , $P = 0.438$	<b>0.95 (0.91 to 0.99)</b>	0.074
			Maternal venous blood	4	7 166/1 538	$I^2 = 0.0\%$ , $P = 0.962$	1.00 (0.97 to 1.02)	
		Season Considered*	Yes	5	4 641/973	$I^2 = 28.0\%$ , $P = 0.235$	0.97 (0.94 to 1.01)	0.512
			No	4	4 143/1 230	$I^2 = 0.0\%$ , $P = 0.485$	1.00 (0.96 to 1.03)	
RTIs	Highest vs. lowest	Overall	9	7 129/2 365	$I^2 = 66.1\%$ , $P = 0.003$	0.85 (0.66 to 1.09)	--	
		Blood source	Cord blood	6	2 753/1 250	$I^2 = 59.8\%$ , $P = 0.029$	0.74(0.53 to 1.02)	0.227
			Maternal venous blood	3	4 426/1 115	$I^2 = 81.8\%$ , $P = 0.004$	1.16 (0.58 to 2.30)	
		Outcome assessment	Doctor diagnosed	6	5 280/1 644	$I^2 = 66.4\%$ , $P = 0.011$	0.81 (0.61 to 1.07)	0.653
			Parent-report	3	1 899/721	$I^2 = 77.0\%$ , $P = 0.013$	0.95 (0.45 to 2.03)	
		Season Considered*	Yes	5	3 246/1 232	$I^2 = 73.4\%$ , $P = 0.005$	0.72 (0.41 to 1.27)	0.471
			No	4	3 933/1 133	$I^2 = 58.5\%$ , $P = 0.065$	0.91 (0.70 to 1.17)	
		↑ Per 10 nmol/L	Overall	9	8,153/2,500	$I^2 = 0.0\%$ , $P = 0.495$	0.97 (0.94 to 1.01)	--
	Blood source		Cord blood	5	2 547/1 188	$I^2 = 0.0\%$ , $P = 0.892$	0.95 (0.87 to 1.03)	0.515
		Maternal venous blood	4	5 606/1 312	$I^2 = 48.0\%$ , $P = 0.123$	0.98 (0.92 to 1.04)		
	Outcome	Doctor diagnosed	4	3 199/1 200	$I^2 = 0.0\%$ , $P = 0.878$	0.94 (0.89 to 0.99)	0.120	

assessment	Parent-report	5	4 954/1 300	$I^2 = 0.0\%$ , $P = 0.466$	1.01 (0.95 to 1.06)	
Season	Yes	6	4 426/1 429	$I^2 = 0.0\%$ , $P = 0.541$	0.99 (0.94 to 1.04)	0.811
Considered*	No	3	3 727/1 071	$I^2 = 20.3\%$ , $P = 0.285$	0.96 (0.89 to 1.04)	

25(OH) D, 25-Hydroxyvitamin D; CI, confidence interval; RTI, lower respiratory tract infection; OR, odds ratio.

\*Season of measurement of the exposure was adjusted in the model or season-standardized exposure was used.

†Wheeze, as a symptom, was parental report in all the included studies except the COPSAC study where wheeze was diagnosed at acute visits to the research clinic and by a daily diary of respiratory symptoms.









