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RESEARCH

Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies

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Abstract

Objective To assess the effect of 25-hydroxyvitamin D (25-OHD) levels on pregnancy outcomes and birth variables.

Design Systematic review and meta-analysis.

Data sources Medline (1966 to August 2012), PubMed (2008 to August 2012), Embase (1980 to August 2012), CINAHL (1981 to August 2012), the Cochrane database of systematic reviews, and the Cochrane database of registered clinical trials.

Study selection Studies reporting on the association between serum 25-OHD levels during pregnancy and the outcomes of interest (pre-eclampsia, gestational diabetes, bacterial vaginosis, caesarean section, small for gestational age infants, birth weight, birth length, and head circumference).

Data extraction Two authors independently extracted data from original research articles, including key indicators of study quality. We pooled the most adjusted odds ratios and weighted mean differences. Associations were tested in subgroups representing different patient characteristics and study quality.

Results 3357 studies were identified and reviewed for eligibility. 31 eligible studies were included in the final analysis. Insufficient serum levels of 25-OHD were associated with gestational diabetes (pooled odds ratio 1.49, 95% confidence interval 1.18 to 1.89), pre-eclampsia (1.79, 1.25 to 2.58), and small for gestational age infants (1.85, 1.52 to 2.26). Pregnant women with low serum 25-OHD levels had an increased risk of bacterial vaginosis and low birthweight infants but not delivery by caesarean section.

Conclusion Vitamin D insufficiency is associated with an increased risk of gestational diabetes, pre-eclampsia, and small for gestational age infants. Pregnant women with low 25-OHD levels had an increased risk of bacterial vaginosis and lower birth weight infants, but not delivery by caesarean section.

Introduction

Vitamin D insufficiency has been associated with several adverse health outcomes, including pregnancy outcomes, and is increasingly recognised as a public health concern. Observational data suggest a link between low 25-hydroxyvitamin D (25-OHD) levels—the best measure of vitamin D status in humans—and an increased risk of adverse pregnancy outcomes such as gestational diabetes, pre-eclampsia, infections, caesarean section, and fetal growth restriction.[1] Despite these findings, the knowledge and understanding of the clinical importance and implications of these associations are limited. A systematic review of the association between 25-OHD levels in the first trimester and subsequent adverse pregnancy outcomes concluded that there was no clear definition of vitamin D deficiency in pregnancy.[2] Although this review identified several studies showing inverse associations between maternal 25-OHD levels in the first trimester and the risk of adverse pregnancy outcomes, the authors did not perform a meta-analysis of the data and quantification of the association, citing inconsistent reporting of results across studies.[2]

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Forest plots

The literature on vitamin D insufficiency in pregnancy is growing rapidly, with several studies examining a variety of populations and outcomes. This literature requires to be reviewed comprehensively to characterise the associations of vitamin D insufficiency with these disparate outcomes. A recent systematic review found a significant inverse relation between serum 25-OHD levels and the incidence of gestational diabetes.[3] Since the publication of this review, further studies have been published on this topic, with other clinically important outcomes that have not yet been effectively summarised.

Consequently, translating the current level of knowledge into clinical recommendations has been challenging. A consensus on "target" 25-OHD levels in pregnancy is lacking, and the role of vitamin D supplementation in the prenatal period and during pregnancy and lactation is unclear. Furthermore, there is little evidence on "optimal" supplement dosing. Vitamin D supplementation has the potential to be a simple intervention with significant benefits. Before proceeding with large randomised controlled trials to deal with the efficacy of supplementation, understanding the breadth and quality of observational studies that underpin this evidence is critical.

We reviewed existing evidence on the effect of 25-OHD levels on pregnancy outcomes (pre-eclampsia, gestational diabetes, bacterial vaginosis, and caesarean section) and birth variables (small for gestational age, birth weight, birth length, and head circumference).

Methods

In accordance with a protocol developed a priori, we identified all relevant articles regardless of language by searching Medline (1966 to August 2012), PubMed (2008 to August 2012), Embase (1980 to August 2012), CINAHL (1981 to August 2012), the Cochrane database of systematic reviews, and the Cochrane database of registered clinical trials. We used search strategies recommended for systematic reviews of observational studies.[4] We also scanned the bibliographies of identified articles. To allow a systematic review of all studies assessing the association between serum 25-OHD levels and pregnancy outcomes or birth variables our initial search was not limited to observational studies.

We searched the electronic databases using three comprehensive search themes. To identify terms related to the exposure of interest, we did a Boolean search using the term "or" to explode (search by subject heading) and map (search by keyword) the MeSH headings: "vitamin D" OR "calciferol" OR "ergocalciferol" OR "cholecalciferol" OR "25-OHD". To identify relevant pregnancy outcomes, we carried out a second Boolean search using the term "or" to explode (search by subject heading) and map (search by keyword) the MeSH headings: "pregnancy" OR "pregnancy complications" OR "pregnancy outcome" OR "caesarean section". Finally, to identify relevant neonatal outcomes, we carried out a third Boolean search using the term "or" to explode (search by subject heading) and map (search by keyword) the MeSH headings: "foetal development" OR "birth weight" OR "small for gestational age". To address pregnancy outcomes we combined the exposures of interest with pregnancy outcomes using the Boolean operator "and" and to address birth variables we combined the exposures of interest with neonatal outcomes using the Boolean operator "and". We then combined the two sets of searches using the Boolean operator "or", limited to human studies. This search excluded other design types using the Boolean operator "not": case reports, comments, editorials, letters, or reviews or systematic or synthesis or quantitative or meta-analysis.

Selection criteria

Two reviewers (FA, TN) screened abstracts and titles to identify articles for further review. Articles were considered for inclusion if they reported on original data from an original study, included an outcome of interest, and utilised blood samples during pregnancy that assessed serum 25-OHD levels. We excluded articles if they used non-blood measures of 25-OHD (amniotic fluid or placenta), assessed other metabolites of vitamin D (for example, 1,25-dihydroxyvitamin D), reported on biological mechanisms of vitamin D metabolites, or were of non-human studies.

On the initial screen for eligibility of articles the observed agreement between reviewers was 97%, corresponding to substantial agreement (κ=0.79). An article was retained if either reviewer believed that it should be retained. To determine which papers were to be included two reviewers (FA, TN) independently screened the full text of identified articles against inclusion and exclusion criteria. Articles were excluded when serum 25-OHD levels were sampled during or after delivery, the outcomes of interest were not reported, or a control or comparison group was not identified. There was no disagreement between the reviewers on articles for inclusion.

Data extraction

We developed a data extraction form to collect key indicators of study quality using meta-analysis of observational studies in epidemiology standards.[5] The key indicators were study design, use of a comparison or control group, definition of 25-OHD cut-off levels, gestational age at serum sampling, quantification method, location or latitude of population, and description of important baseline confounders (ethnicity or race, skin colour, clothing, body mass index, use of vitamin D supplementation, exposure to ultraviolet B, use of sunscreen, and season).

Articles were categorised on the basis of the outcomes of interest: gestational diabetes, pre-eclampsia, bacterial vaginosis, caesarean section, small for gestational age infants, birth weight, birth length, and head circumference. We examined the definitions of gestational diabetes, pre-eclampsia, bacterial vaginosis, and small for gestational age as reported in the articles. Articles reporting on multiple outcomes were included. For studies that measured 25-OHD levels multiple times during pregnancy, we included the earliest measurement in the analysis. Two reviewers (FA, TN) independently extracted information from each article and compared findings; any discrepancies were resolved by consensus. Attempts were made to contact authors of studies with unclear data.

Statistical analysis

The associations between 25-OHD levels and pregnancy outcomes and birth variables were reported in various ways, including proportions, odds ratios (95% confidence intervals), means (standard deviations), and medians (interquartile ranges). We converted medians and interquartile ranges to means and standard deviations using previously outlined methods.[6] Studies varied in their definition of cut-offs for 25-OHD levels (deficiency, insufficiency, and sufficiency). In studies that reported outcomes as proportions in two cut-off categories (deficiency and insufficiency), we combined the numbers to create a category of insufficiency for pregnancy outcomes, defined as a serum concentration less than 75 nmol/L. For birth variables, we defined insufficiency as a serum concentration less than 37.5 nmol/L. To ensure inclusion of all available data, cut-offs were not specified a priori; instead we used the cut-offs

given within the included studies. If studies reported 25-OHD levels in ng/mL we converted the values to nmol/L.

Given the variability in measurement, we used the adjusted odds ratio and weighted mean difference as the common measures of association across studies. As studies control for potential confounding in different ways and to different degrees, we used the most adjusted reported odds ratio when more than one was reported. In those studies that did not report an adjusted odds ratio, we calculated the odds ratio using proportions. We converted relative risks to odds ratios using the formula: $RR\times(1-P)/(1-(P\times RR))$ in which P is the incidence of the outcome of interest in the non-exposed group.[7] For studies that reported mean levels of 25-OHD, we used the weighted mean difference to compare the levels of 25-OHD between women who did and did not develop the outcome of interest.

We used the "metan" command in Stata to pool the odds ratios and weighted mean differences across studies. Forest plots were used to visually assess pooled estimates and corresponding 95% confidence intervals. To assess for heterogeneity, we calculated the Q statistic (significance level of $P<0.1$) and the I^2 statistic. To account for potential heterogeneity between studies we used random effect models to obtain pooled effect estimates across studies.

Stratified meta-analyses were done on factors considered to be clinically important, and on those related to study quality or potential heterogeneity. These variables included adjustment for critical confounders, country of origin, 25-OHD cut-off levels, gestational age at sampling, study design, and methods used to quantify 25-OHD. When the number of studies reporting on a specific outcome was small, we did not carry out meta-analysis and stratification. Finally, we carried out metaregression to assess the predictive effect of the variables on heterogeneity. Publication bias was also assessed using Begg's test and visual inspection of funnel plots. All analyses were done with Stata 11.0.

Results

The literature search identified 3357 articles pertaining to the relevant exposure, outcomes, and study designs of interest (figure⇓). After the initial screening of abstracts and titles, 51 papers remained for full text review. Hand searching of the bibliographies of these articles identified two additional articles. After full text review, 22 articles were excluded, leaving 31 articles for final inclusion. Ten studies on gestational diabetes, nine on pre-eclampsia, three on bacterial vaginosis, two on caesarean section, and 10 on birth variables were included in the systematic review. One study reported on more than one outcome of interest.

Study characteristics

Table 1⇓ shows the characteristics of the 31 included studies.[8-38] The studies were published between 1980 and 2012 and the number of participants per study ranged from 95 to 1100. Studies reporting on gestational diabetes included 687 cases and 3425 controls, whereas studies reporting on pre-eclampsia included 350 cases and 2841 controls.

Women were diagnosed as having gestational diabetes if they had abnormal oral glucose tolerance test results two or three hours after receiving 75 or 100 g of oral glucose between 24 and 28 weeks of gestation. Pre-eclampsia was defined by new onset hypertension after 20 weeks of gestation (systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg) and proteinuria 0.3 g or more per day or 2 or more + on dipstick testing. Bacterial vaginosis was diagnosed based on a score of 7-10 from a Gram stained vaginal smear, interpreted using Nugent et al's method.[39] Small for gestational age was defined as a birth weight less than the 10th centile in all of the eligible studies,[11 30 33 35 36] except one (less than fifth centile).[38]

Quality assessment

Fifteen studies were case-control studies,[8 13-23 25 30 38] 11 cohort studies,[9-11 24 26 29 31 33-35 37] and five other designs.[12 27 28 32 36] All the studies used a comparison group. Some articles reported on confounding factors, such as age, body mass index, use of vitamin D supplementation, gestational age at sampling, season, and race. Several studies used multivariable logistic regression to adjust for these confounding factors, whereas others only assessed the correlation. In addition, studies differed on the number of confounding factors adjusted for, as well as the reporting of effect measures (table 2⇓).

Association between gestational diabetes and 25-OHD insufficiency

All 10 studies reporting on gestational diabetes presented their findings as proportions[8-17] and five also presented their findings as means and medians.[9 12 13 16 17] Two meta-analyses were conducted. In the first analysis, studies reporting odds ratios (calculated and most adjusted) were pooled to quantify the association between 25-OHD insufficiency and gestational diabetes. The second analysis pooled weighted mean differences to determine if there were significant differences between the mean 25-OHD levels among women who did and did not develop gestational diabetes.

In the first analysis, gestational diabetes was found to be associated with insufficient 25-OHD levels compared with the comparison group, with a pooled odds ratio based on a random effects model of 1.49 (95% confidence interval 1.18 to 1.88). There was no evidence of heterogeneity across studies ($P=0.58$; $I^2=0.0\%$). (See supplementary file.) To examine the robustness of the risk estimate, several stratified analyses were done based on adjustment for critical confounders, country of origin (developed and developing countries), 25-OHD cut-off concentration (<50 and <75 nmol/L), gestational age at sampling among the studies (<16 weeks and >16 weeks), study design (case-control and other design), and 25-OHD quantification methods (high performance liquid chromatography and mass spectrometry assay, or radioimmunoassay) (table 3⇓). Stratification did not significantly alter the pooled estimate of association in each stratum of interest, with one exception. In the three studies that adjusted for critical confounders the pooled odds ratio increased to 1.98 (95% confidence interval 1.23 to 3.23). Metaregression analyses did not show adjustment for critical confounders, country of origin, 25-OHD cut-off level, gestational age at sampling, study design, and 25-OHD quantification method to be predictive of heterogeneity.

The second analysis showed that pregnant women with gestational diabetes had significantly lower 25-OHD levels than the comparison group (pooled weighted mean difference −7.36 nmol/L, 95% confidence interval −10.16 to −4.56 nmol/L). Furthermore, the weighted mean difference did not change significantly when stratifed by country of origin and study design.

Association between pre-eclampsia and 25-OHD insufficiency

Of the nine studies that reported on pre-eclampsia,[11 18-25] seven presented their findings as proportions[11 18-20 22-24] and five as

means.[19 21-23 25] The overall meta-analysis using the most adjusted odds ratio showed a significant association between pre-eclampsia and 25-OHD insufficiency compared with the comparison group, with a pooled odds ratio based on a random effects model of 1.79 (95% confidence interval 1.25 to 2.58). (See supplementary file.)

There was no evidence of heterogeneity across studies (P=0.81; I^2=0.0%). Stratified analyses were done based on adjustment for critical confounders, 25-OHD cut-off concentrations (<50 and <75 nmol/L), gestational age at sampling among the studies (<16 weeks and >16 weeks), study design (case-control and other design), and 25-OHD quantification methods (high performance liquid chromatography and mass spectrometry assay, or radioimmunoassay). In the stratified analyses, the pooled estimate of the association varied significantly across strata. Adjustment of critical confounders led to a more conservative, and in fact non-significant, pooled estimate of the association between pre-eclampsia and 25-OHD concentration (odds ratio 1.51, 95% confidence interval 0.89 to 2.57). Conversely, when there was no adjustment for confounding, the pooled odds ratio increased to 2.09 (95% confidence interval 1.26 to 3.46). Similarly, when case-control studies were pooled, the estimate of association increased (2.05, 1.33 to 3.14). Together these suggest that the pooled estimate of association varies with indicators of study quality. The estimate of association also varied by gestational age at sampling, definition of insufficiency, and method for quantification (table 3). Owing to a small number of studies in each strata, metaregression analyses did not show the following to be predictive of heterogeneity: adjustment for critical confounders, 25-OHD concentration cut-offs, gestational age at sampling, study design, and 25-OHD quantification methods.

When the analysis was conducted on the studies reporting means and standard deviations,[19 21-23 25] pregnant women with pre-eclampsia had significantly lower concentrations of 25-OHD than the comparison group (pooled weighted mean difference −14.53 nmol/L, 95% confidence interval −22.57 to −6.49 nmol/L). All studies reporting on means and standard deviations were of case-control design.

Association between bacterial vaginosis or caesarean section and 25-OHD insufficiency

Three studies[26-28] reported an increased risk of bacterial vaginosis in pregnant women with low 25-OHD levels (table 3). One of the studies[28] reported a significant association between 25-OHD concentrations less than 37.5 nmol/L and risk of bacterial vaginosis (adjusted odds ratio 4.4; P=0.02). Another of the studies[27] also found that 25-OHD deficiency was associated with bacterial vaginosis in pregnant women (adjusted odds ratio 2.87, 95% confidence interval 1.13 to 7.28). Similarly, the remaining study[26] found that women with bacterial vaginosis had lower unadjusted 25-OHD levels than women with normal vaginal flora (geometric mean difference −10.6; P<0.001). A meta-analysis of these three studies could not be conducted owing to differential statistical reporting (adjusted odds ratio and geometric mean).

One group of researchers[11] found no increased risk of caesarean section in pregnant women with 25-OHD insufficiency, whereas others[29] showed an increased risk of primary caesarean section among women with 25-OHD concentrations <37.5 nmol/L compared with those with concentrations >80 nmol/L (table 3).

Association between birth variables and 25-OHD insufficiency

Of the 10 studies that reported on birth variables,[11 30-38] six reported on small for gestational age infants,[11 30 33 35 36 38] four on birth weight,[31 34 35 37] and two on birth length and head circumference.[34 37]

The overall meta-analysis using the most adjusted odds ratio showed a significant association between small for gestational age infants and 25-OHD insufficiency compared with the comparison group (random effects model, pooled odds ratio 1.85, 95% confidence interval 1.52 to 2.26). (See supplementary file.) There was no evidence of heterogeneity across studies (P=0.37; I^2=7.8%). A sensitivity analysis excluding a study that was conducted on women who were positive for HIV antibodies[36] still found a significant effect of 25-OHD insufficiency on small for gestational age infants (table 3). Stratified analyses were done based on adjustment for critical confounders, 25-OHD concentration cut-offs (<37.5 and <80 nmol/L), gestational age at sampling among the studies (<16 and >16 weeks), and study design (case-control and other). The association between small for gestational age infants and 25-OHD insufficiency remained significant at all levels of stratification. Stratified analysis based on 25-OHD quantification method was not conducted because only one study[38] used the high performance liquid chromatography assay method. Metaregression analyses did not show adjustment for critical confounders, 25-OHD level cut-offs, gestational age at sampling, and study design to be predictive of heterogeneity. A small randomised controlled trial of supplementation of vitamin D during pregnancy among Asian women showed almost twice as many small for gestational age infants in the control group (29% *v* 15%).[32] This study was not included in the meta-analysis because participants received additional vitamin D supplementation during pregnancy.

Of the four studies[31 34 35 37] that reported on birth weight, infants of mothers with 25-OHD concentrations less than 37.5 nmol/L during pregnancy had lower birth weight (random weighted mean difference −130.92 g, 95% confidence interval −186.69 to −75.14 g). However, birth length and head circumference did not differ significantly (table 3).

Publication bias

Visual inspection of Begg's funnel plot of included studies on gestational diabetes, pre-eclampsia, and birth variables revealed asymmetry, raising the possibility of publication bias. The Begg's test was not, however, significant (P=0.79 for gestational diabetes, P=0.65 for pre-eclampsia, P=0.57 for small for gestational age infants, and P=0.50 for birth weight). The observed asymmetry was possible due to the small number of studies included in the meta-analysis.

Discussion

In this systematic review and meta-analysis we found an association between 25-OHD insufficiency and adverse pregnancy outcomes and birth variables. These findings are of concern, particularly given recent evidence suggesting that 25-OHD deficiency or insufficiency is common during pregnancy, especially among high risk groups, including vegetarians, women with limited sun exposure (for example, those who live in cold climates or in northern latitudes, wear sun screen, or wear protective clothing), and those from ethnic minority groups with darker skin.[40-43] Vitamin D supplementation may be a simple way to reduce the risk of these adverse outcomes. A recent systematic review showed that evaluation

of the effect of supplementation during pregnancy on maternal, perinatal, or infant health outcomes is based on limited evidence.[44] However, despite this limitation the researchers were able to show that daily vitamin D supplementation (800-1000 IU/day) had a protective effect on low birth weight.[44] These findings, in combination with our results, suggest that low levels of 25-OHD may be a modifiable risk factor in pregnancy, and healthcare providers should at least be encouraging pregnant women to follow current guidelines on recommended daily allowances for vitamin D. While this would seem to be a simple directive, there is active debate on what is considered the appropriate intake of vitamin D in pregnancy, as the recommended intake by bodies that advice on best practices in pregnancy care varies from 600 to 2000 IU/day.

The effect of vitamin D has been described in several organ systems within the human body.[45] Gene array studies in many cells and tissues show that 1,25-dihydroxycholecalciferol ($1,25(OH)_2D$) regulates several genes throughout the body, or as much as 5% of the human genome.[46 47] How $1,25(OH)_2D$ functions in these tissues and the physiological consequences are not clearly known however.[46 47] Several mechanisms may explain the observed association between 25-OHD level and risk of gestational diabetes. Gestational diabetes is a result of pregnancy induced insulin resistance and impaired compensatory insulin secretion.[17 48] Evidence suggests that vitamin D improves insulin sensitivity by enhancing insulin responsiveness to glucose transport.[17 48] In addition, vitamin D may play a role in early placental development through gene regulation and expression, which may affect the development of pre-eclampsia. Although 25-OHD deficiency may affect fetal growth through its effect on fetal bone development,[46] the biological basis for the association between 25-OHD deficiency and birth weight is unclear. Currently, there is a lack of defined pathways for the relation between biomolecular mechanisms and pregnancy complications and fetal outcomes.

Although it is biologically plausible that low 25-OHD levels could be responsible for the adverse pregnancy and neonatal outcomes examined in this review, owing to the observational nature of the data reviewed we cannot infer causality from these findings. However, when we consider this review and the body of literature with the Bradford Hill[49] criteria in mind, certain criteria indicate that these associations are possibly not spurious and may be shown to be causal in randomised controlled trials. Our review adds to the biological plausibility argument and shows a consistency in association and an appropriate temporal relation between exposure and outcome. The association between 25-OHD levels and pregnancy related outcomes has been consistently observed in diverse patient populations, and similar results were found after conducting several stratified and sensitivity analyses. We also documented that low 25-OHD levels preceded the outcomes. We cannot be certain, however, that low 25-OHD levels predated pregnancy. Furthermore, 25-OHD levels at different stages of pregnancy may be associated with different clinical outcomes and we were unable to identify such "critical windows" for 25-OHD insufficiency and specific pregnancy outcomes in this review. One study[44] also suggests that experimental evidence shows that low birth weight might be reduced with vitamin D supplementation.

Our review, summarising existing data, shows an increasingly compelling case for a causal relation between low 25-OHD levels and adverse maternal and neonatal outcomes. However our review also highlights the knowledge gaps in the related literature. We were unable to show a dose-response relation between low 25-OHD levels and outcomes. This may be due to a lack of data at the extremes of the 25-OHD cut-off levels. This needs to be dealt with in future studies. The quality of individual studies was not always optimal as a result of inconsistent reporting on confounding factors. For example, maternal nutrition was not systematically measured across studies, although it affects birth weight; we stratified our meta-analysis based on whether studies were done in developed and developing countries as a proxy of maternal nutritional status. All studies (with one exception)[36] were from developed countries and none reported on maternal dietary data. Future endeavours to study this association need to consider, collect, and report consistently on important factors such as nutrition, lifestyle, family history of metabolic complications of pregnancy, maternal weight, sun exposure, skin pigmentation, and exercise so that current knowledge can be refined. This review also suggests that the method used to quantify 25-OHD levels may be an important factor when evaluating the risk of vitamin D deficiency. While both measurements still suggested a risk associated with low 25-OHD levels, a combined high performance liquid chromatography and mass spectrometry assay was associated with more modest estimates of risk that did not reach statistical significance compared with radioimmunoassay methods. This was possibly due to low statistical power, and therefore more work is needed to determine if the risk associated with low 25-OHD levels reported here persists when using a combined high performance liquid chromatography and mass spectrometry assay as the quantification method.

We also acknowledge the limitations of this review and of meta-analytical methods more broadly. Owing to a lack of or limited adjustment for confounding factors in some studies, we used the most adjusted odds ratio in meta-analysis. In some studies an odds ratio was not reported but we were able to calculate an unadjusted odds ratio based on event rates reported in the exposed and unexposed groups. Unadjusted odds ratios must be interpreted with caution as confounding can result in spurious associations. Secondly, many of the studies included were of a case-control design, which could overestimate the effect size of the association and makes the temporal relation between exposure and outcome less clear. Thirdly, the studies varied in their definition of cut-offs for 25-OHD insufficiency; in those that reported separate proportions of deficiency and insufficiency, we combined the numbers to categorise concentrations less than 75 nmol/L as the insufficiency cut-off for pregnancy outcomes and concentrations less than 37.5 nmol/L for birth variables, based on the availability of data. This review did not examine the benefit or risk of having a 25-OHD level above or below a certain cut-off, but shows that within a population of pregnant women, lower levels of 25-OHD increases the risk of adverse outcomes. Further work on what defines a "normal" 25-OHD level in pregnancy is required. Finally, there is a suggestion of publication bias in this meta-analysis, which may in part be explained by the small number of studies available for each outcome of interest.

Limitations aside, this review remains the most comprehensive study of 25-OHD insufficiency in pregnancy to date, including data on over 22 000 women. The diversity of location and latitudes, seasonality, ethnicity, body mass index of participants, and dietary vitamin D intake (supplementation and fortification) reported in these studies also allows for increased generalisability of these results to other populations.

Our findings of a significant association between 25-OHD insufficiency and adverse pregnancy outcomes and birth variables are of concern. Although we recognise the methodological limitations of the studies included in this review, our study does serve as a comprehensive review of this literature.

Despite small trials of vitamin D supplementation in pregnancy showing a reduction in the risk of having small for gestational age infants, there remains a need for large, well designed randomised controlled trials to determine whether strategies to optimise maternal 25-OHD levels are effective in improving pregnancy and neonatal outcomes.

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Data sharing: The statistical code and datasets are available from the corresponding author at Doreen.Rabi@albertahealthservices.ca.

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What is already known on this topic

Evidence is emerging that lower levels of 25-hydroxyvitamin D (25-OHD) are associated with adverse health outcomes, including pregnancy outcomes

What this study adds

Vitamin D insufficiency is associated with an increased risk of gestational diabetes, pre-eclampsia, and small for gestational age infants

Pregnant women with low 25-OHD levels had an increased risk of bacterial vaginosis and lower birth weight infants, but not delivery by caesarean section

Tables

Table 1| Characteristics of included studies

Trials by outcome	Location/latitude	Participants	Ethnicity	Gestational age at time of sampling (weeks)	25-OHD concentration cut-off (nmol/L)	25-OHD quantification method‡	Study design
Gestational diabetes:							
Baker et al, 2012[8]	USA/35˚N	Gestational diabetes: 60, control: 120	52% white, 33% black, 10% Hispanic, 5% other	12-13	<50 (reference >75)	HPLC and MS	Nested case-control
Clifton-Bligh et al, 2008[9]	Australia/33˚S	Gestational diabetes: 81, control: 226	54.7% European, 28% South East Asian, 6.8% Asian, 6.2% Middle Eastern	28.7	<50 (reference >50)	HPLC and MS	Prospective cohort
Farrant et al, 2009[10]	India/12˚N	Gestational diabetes: 39, control: 520	Indian	30	<50 (reference >50	RIA	Prospective cohort
Fernandez-Alonso et al, 2011[11]	Spain/36˚N	Gestational diabetes: 36, control: 430	Spanish	11-14, 36-39	<75 (reference >75)	ECL	Prospective cohort
Maghbooli et al, 2008[12]	Iran/32˚N	Gestational diabetes: 52, control: 527	Iranian	24	>35 (reference >35)	RIA	Cross sectional
Makgoba et al, 2011[13]	UK/51˚N	Gestational diabetes: 90, control: 158	68.4% white, 19.6% African, 7.6% Asian	28	<50 (reference >50)	HPLC and MS	Case-control
Parlea et al, 2012[14]	Canada/43˚N	Gestational diabetes: 118, control: 219	60% white, 34% Asian, 5% Black, 1% other	15-18	<73.5 (reference >73.5)	ECL	Nested case-control
Savvidou et al, 2011[15]	UK/51˚N	Gestational diabetes: 100, control: 1000	58% white, 33% African, 9.5% Asian	12.4	<75 (reference >75)	HPLC and MS	Case-control
Soheilykhah et al, 2010[16]	Iran/32˚N	Gestational diabetes: 54, control: 111	Iranian	22.03	<50 (reference >75)	ELISA	Case-control
Zhang et al, 2008[17]	USA/47˚N	Gestational diabetes: 57, control: 114	70.2% white, 3.5% African, 26.3% other	16	<50 (reference >75)	EIA	Nested case-control
Pre-eclampsia:							
Azar et al, 2011[18]	Norway/62˚N, USA/47˚N, Australia/33˚N	Gestational diabetes, pre-eclampsia: 23, no gestational diabetes, no pre-eclampsia: 20	White	12, 21, 32, 37	<75 (reference >75)	HPLC and MS	Case-control
Baker et al, 2010[19]	USA/35˚N	Pre-eclampsia: 43 severe, severe pre-eclampsia: 43, control: 198	29% white, 40% African, 26% Hispanic, 5% other	17	<75 (reference >75)	HPLC and MS	Nested case-control
Bodnar et al, 2007[20]	USA/40˚N	Pre-eclampsia: 49, control: 216	68.5% white 31.5% African	10.4	<75 (reference >75)	ELISA	Nested case-control
Fernandez-Alonso et al, 2011[11]	Spain/36˚N	Pre-eclampsia: 7, control: 459	Spanish	11-14, 36-39	<75 (reference >75)	ECL	Prospective cohort
Kolusari et al, 2008[21]	Turkey/39˚N	Pre-eclampsia: 47, control: 48	Turkish	34	None	HPLC	Case-control
Powe et al, 2010[22]	USA/42˚N	Pre-eclampsia: 39, control: 131	53.8% white	11.6	<37 (reference >37)	HPLC and MS	Nested case-control
Robinson et al, 2010[23]	USA/32˚N	Pre-eclampsia: 50, control: 100	48% African	29	>80 (reference >80)	RIA	Case-control
Wei et al, 2012[24]	Canada/49˚N	Pre-eclampsia: 32, control: 665	89% white	12-18*	<50 (reference >50)	ECL	Prospective cohort
Yu et al, 2012[25]	UK/51˚N	Pre-eclampsia: 60, control: 1000	50% white, 38% African, 12% Asian	11-13	None	HPLC and MS	Case-control

Table 1 (continued)

Trials by outcome	Location/latitude	Participants	Ethnicity	Gestational age at time of sampling (weeks)	25-OHD concentration cut-off (nmol/L)	25-OHD quantification method‡	Study design
Bacterial vaginosis:							
Bodnar et al, 2009[26]	USA/40˚N	Cases: 192 with bacterial vaginosis, control: 277 without bacterial vaginosis	44.6% white, 55.4% African	9.5	<20, 20-<37.5, 37.5-<50, 50-<80, >80	RIA	Prospective cohort
Hensel et al, 2011[27]	Across USA	Pregnant: 440. 29% of group with bacterial vaginosis. Non-pregnant: 3523	White, African, Mexican	During pregnancy	<75	Variable, different laboratories	Cross sectional
McGuire Davis et al, 2010[28]	USA/39˚N	80 adolescents (≤18 years)	African	18 to 29	<37.5 (reference >50)	RIA	Cross sectional
Fernandez-Alonso et al, 2011[11]	Spain/36˚N	Caesarean section: 105, control: 361	Spanish	11-14, 36-39	<75 (reference >75)	ECL	Prospective cohort
Scholl et al, 2012[29]	USA/35˚N	Cases: 290, controls: 863	35% black, 51% Hispanic, 14% white	14	<37.5 (reference 37.5-80)	HPLC	Cohort
Bodnar et al, 2010[30]	USA/40˚N	Small for gestational age: 111, controls: 301	273 white, 139 black	<22	<37.5 (reference 37.5-75)	ELISA	Nested case-control
Bowyer et al, 2009[31]	Australia/33°S	971	59% dark maternal skin	23-32	<25 (reference 26-50)	ECL	Prospective cohort
Brooke et al, 1980[32]	UK/51˚N	Treatment (calciferol 1000 IU/day): 59, control: 67	Asian immigrants	28-32	NA	HPLC	Double blinded randomised controlled trial
Burris et al, 2012[33]	USA/42˚N	Small for gestational age: 7†, control: 22	72.3% black, 27.6% white	26-28	<25 (reference50-75)	ECL and RIA	Prospective cohort
Ertl et al, 2012[38]	UK/51˚N	Small for gestational age: 150, control: 1000	50% white, 50% African	11-13	<50 (reference 50-75)	HPLC and MS	Case-control
Fernandez-Alonso et al, 2011[11]	Spain/36˚N	Small for gestational age: 46, control: 406	Spanish	11-14, 36-39	<50 (reference >75)	ECL	Prospective cohort
Gale et al, 2008[34]	UK/51˚N	466	white	32	<30 (reference >75)	RIA	Retrospective cohort
Leffelaar et al, 2010[35]	Netherlands/51°N	Vitamin D deficient 861, insufficient 797, adequate 2072	68.9% white, 31.2% non-white	<18	<29.9 (reference >50)	EIA	Prospective cohort
Mehta et al, 2009[36]	Tanzania/6°S	1078 pregnant women infected with HIV	African	12-27	<80 (reference >80)	ECL	Secondary analysis of randomised controlled trial
Morley et al, 2006[37]	Australia/38°S	374	93% Australian born	28-32	<28 (reference >28)	RIA	Prospective cohort

25-OHD=25-hydroxyvitamin D; ELISA=enzyme linked immunosorbent assay; RIA=radioimmunoassay; HPLC=high performance liquid chromatography; MS=mass spectrometry; ECL=electrochemiluminescence assay; EIA=enzymatic immunoassay; NA=not available.

*Serum concentration data from 12-18 weeks of pregnancy were utilised.

†Serum concentration data from <25 nmol/L were utilised.

Table 2| Reported measures, method of adjustment, and confounding factors

Source	Reported measures	Method of adjustment	Confounding factors
Gestational diabetes:			
Baker et al, 2012[8]	Adjusted odds ratio	Logistic regression model	Age, insurance status, body mass index, gestational age at serum collection, season
Clifton-Bligh et al, 2008[9]	Mean (SD) odds ratio	No adjustment	None
Farrant et al, 2009[10]	Geometric mean (interquartile range), proportions	Multiple linear and logistic regression model	Age, fat mass or body mass index, diabetes status
Fernández-Alonso et al, 2011[11]	Proportions	None	None
Maghbooli et al, 2008[12]	Mean (SD) proportions	Assessment of correlation or no adjustment	None
Makgoba et al, 2011[13]	Mean (SD) proportions	Assessment of correlation/no adjustment	None
Parlea et al, 2012[14]	Adjusted odds ratio	Logistic regression model	Age and weight
Savvidou et al, 2011[15]	Median (interquartile range), proportions	Multiple logistic regression model	Age, body mass index, smoking status, method of conception, season, race
Soheilykhah et al, 2010[16]	Proportions, median (interquartile range)	Assessment of correlation/no adjustment	None
Zhang et al, 2008[17]	Adjusted odds ratio, mean (SD)	Logistic regression model	Age, prepregnancy body mass index, family history of type 2 diabetes, race
Pre-eclampsia:			
Azar et al, 2011[18]	Proportions	Adjusted method not specified	Body mass index
Baker et al, 2010[19]	Adjusted odds ratio	Multiple logistic regression model	Age, body mass index, gestational age at serum sampling, season, parity
Bodnar et al, 2007[20]	Adjusted odds ratio, adjusted geometric mean (95% CI), proportions	Multiple regression models	Prepregnancy body mass index, gestational age at serum sampling, education, season, race
Fernández-Alonso et al, 2011[11]	Proportions	None	None
Kolusari et al, 2008[21]	Mean (SD)	None	NA
Powe et al, 2010[22]	Adjusted odds ratio, mean (SD)	Multiple logistic regression model	Body mass index, season, race
Robinson et al, 2010[23]	Adjusted odds ratio for continuous level, median (interquartile range), proportions	Multiple linear regression model	Age, prepregnancy body mass index, gestational age at serum sampling, race
Wei et al, 2012[24]	Adjusted odds ratio	Multiple logistic regression model	Age, smoking status, body mass index, season
Yu et al, 2012[25]	Median (interquartile range)	None	NA
Bacterial vaginosis:			
Bodnar et al, 2009[26]	Prevalence ratio	Multivariable Poisson regression model	Presence of sexually transmitted disease, race
Hensel et al, 2011[27]	Adjusted odds ratio	Multiple logistic regression model	Age, body mass index, education, race, poverty index, marital status, number of lifetime partners, unprotected sex, current contraceptives use
McGuire Davis et al, 2010[28]	Odds ratio	Multiple logistic regression model	Season
Fernández-Alonso et al, 2011[11]	Proportions	None	None
Scholl et al, 2012[29]	Adjusted odds ratio	Multiple logistic regression model	Age, parity, ethnicity, smoking status, gestational age at serum sampling, season, body mass index
Birth variables			
Bodnar et al, 2010[30]	Adjusted odds ratio	Multiple logistic regression model	Age, gestational age at serum sampling, marital status, season, body mass index, smoking during pregnancy, socioeconomic status, periconceptional multivitamin use, preconceptional physical activity
Bowyer et al, 2009[31]	Adjusted mean difference	Linear regression model	Gestational age at serum sampling, age, maternal birth place
Brooke et al, 1980[32]	Mean (SE)	None	None
Burris et al, 2012[33]	Adjusted odds ratio	Logistic regression model	Season, age, prepregnancy body mass index, race
Ertl et al, 2012[38]	Proportions	None	None
Fernández-Alonso et al, 2011[11]	Proportions	None	None
Gale et al, 2008[34]	Mean (SD)	None	None

Table 2 (continued)

Source	Reported measures	Method of adjustment	Confounding factors
Leffelaar et al, 2010[35]	Odds ratio	Multiple logistic regression model	Infant sex, maternal height, parity, age, smoking, prepregnancy body mass index, educational level, ethnicity, vitamin D status
Mehta et al, 2009[36]	Relative risk	Multivariable analysis	Multivitamin supplementation, maternal age, CD4 cell count, and HIV disease stage at baseline
Morley et al, 2006[37]	Adjusted mean difference	Linear regression model	Infant sex, maternal height, first child, smoking, season

NA=not available.

Table 3| **Results of insufficient 25-hydroxyvitamin D (25-OHD) levels and pregnancy and neonatal outcomes**

Outcomes	No of studies	Pooled odds ratio (95% CI)	Pooled weighted mean difference (95% CI) (nmol/L)
Gestational diabetes			
Overall	10	1.49 (1.18 to 1.89)	—
Stratified analysis*:			
Adjusted for critical confounders	3	1.98 (1.23 to 3.23)	—
Unadjusted for critical confounders	7	1.37 (1.05 to 1.78)	—
Developed countries	7	1.50 (1.16 to 1.95)	—
Developing countries	3	1.45 (0.89 to 2.37)	—
25-OHD <50 nmol/L	7	1.47 (1.09 to 1.99)	—
25-OHD <75 nmol/L	3	1.52 (1.06 to 2.18)	—
Gestational age of sampling <16 weeks	5	1.55 (1.12 to 2.15)	—
Gestational age of sampling >16 weeks	5	1.44 (1.04 to 1.99)	—
Case-control study design	6	1.57 (1.19 to 2.09)	—
Other study design	4	1.34 (0.90 to 1.99)	—
HPLC-MS assay	4	1.34 (0.96 to 1.87)	—
Radioimmunoassay	6	1.65 (1.19 to 2.27)	—
Overall	5	—	−7.36 (−10.16 to −4.56)
Sensitivity analysis†	4	—	−6.75 (−9.23 to −4.26)
Stratified analysis:		—	
Developed countries	3	—	−7.12 (−10.99 to −3.25)
Developing countries	2	—	−7.89 (−9.21 to −6.58)
Case-control study design	3	—	−7.79 (−13.81 to −1.79)
Other study design	2	—	−6.53 (−9.39 to −3.66)
Pre-eclampsia			
Overall	7	1.79 (1.25 to 2.58)	—
Sensitivity analysis‡	5	1.44 (0.91 to 2.30)	—
Stratified analysis*:			
Adjusted for critical confounders	3	1.51 (0.89 to 2.57)	—
Unadjusted for critical confounders	4	2.09 (1.26 to 3.46)	—
25-OHD <50 nmol/L	2	1.27 (0.66 to 2.42)	—
25-OHD <75 nmol/L	5	2.11 (1.36 to 3.27)	—
Gestational age of sampling <16 weeks	5	1.44 (0.91 to 2.29)	—
Gestational age of sampling >16 weeks	2	2.53 (1.41 to 4.53)	—
Case-control study design	5	2.05 (1.33 to 3.14)	—
Other study design	2	1.26 (0.63 to 2.53)	—
HPLC-MS assay	3	1.91 (0.95 to 3.84)	—
Radioimmunoassay	4	2.75 (1.14 to 2.68)	—
Overall	5	—	−14.53 (−22.57 to −6.49)
Bacterial vaginosis			
Bodnar et al, 2009[20]		−10.6§ (P<0.001)	—
Hensel et al, 2011[27]		2.87¶ (1.13 to 7.28)	—
McGuire-Davies et al, 2010[28]		4.4¶ (P=0.02)	—
Caesarean section			
Fernandez-Alonso et al, 2011[11]		0.83** (0.52 to 1.29)	—
Scholl et al, 2012[29]		1.99¶ (1.20 to 3.30)	—
Small for gestational age			
Overall	6	1.85 (1.52 to 2.26)	—
5	1.98 (1.60 to 2.47)	—	
Stratified analysis*:			

Table 3 (continued)

Outcomes	No of studies	Pooled odds ratio (95% CI)	Pooled weighted mean difference (95% CI) (nmol/L)
Adjusted for critical confounders	3	2.05 (1.54 to 2.74)	—
Unadjusted for critical confounders	3	1.68 (1.28 to 2.22)	—
25-OHD <37.5 nmol/L	3	2.05 (1.54 to 2.74)	—
25-OHD <80 nmol/L	3	1.69 (1.28 to 2.22)	—
Gestational age of sampling <16 weeks	4	1.77 (1.43 to 2.19)	—
Gestational age of sampling >16 weeks	2	2.69 (1.45 to 5.01)	—
Case-control study design	2	2.16 (1.54 to 3.03)	—
Other study design	4	1.70 (1.33 to 2.18)	—
Birth weight (g)	4	—	−130.92 (−186.69 to −75.14)
Birth length (cm)	2	—	−0.194 (−0.65 to 0.26)
Head circumference (cm)	2	—	−0.048 (−0.34 to 0.24)

HPLC=high performance liquid chromatography; MS=mass spectrometry.

*P value of metaregression (P>0.05).

†Sensitivity analysis after removing one study with unclear interquartile range.[16]

‡Sensitivty analysis after removing two studies that reported on severe pre-elampsia.[19 23]

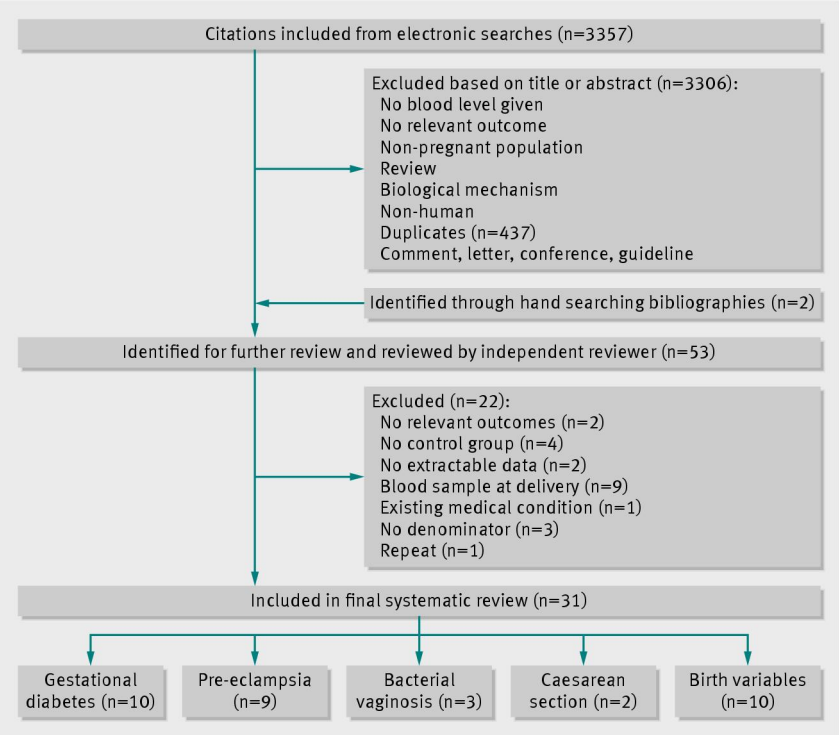
§Geometric mean difference.

¶Adjusted odds ratio (95% confidence interval).

**Odds ratio.

††Sensitivity analysis after removing one study that reported findings in population positive for HIV antibodies.[36]

Figure



Flow of studies through review