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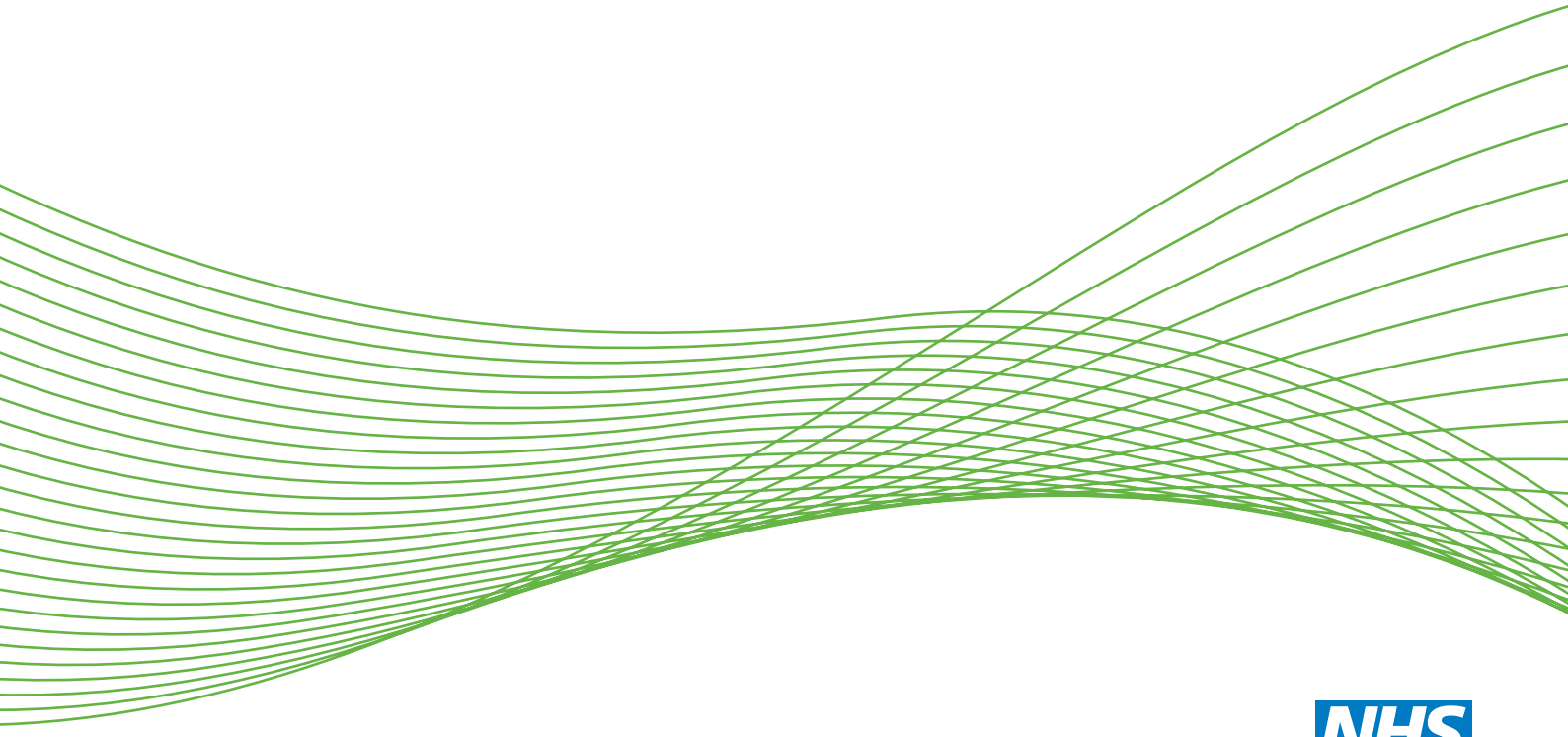

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Abstract

Vitamin D supplementation in pregnancy: a systematic review

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Background: It is unclear whether or not the current evidence base allows definite conclusions to be made regarding the optimal maternal circulating concentration of 25-hydroxyvitamin D [25(OH)D] during pregnancy, and how this might best be achieved.

Objectives: To answer the following questions: (1) What are the clinical criteria for vitamin D deficiency in pregnant women? (2) What adverse maternal and neonatal health outcomes are associated with low maternal circulating 25(OH)D? (3) Does maternal supplementation with vitamin D in pregnancy lead to an improvement in these outcomes (including assessment of compliance and effectiveness)? (4) What is the optimal type (D_2 or D_3), dose, regimen and route for vitamin D supplementation in pregnancy? (5) Is supplementation with vitamin D in pregnancy likely to be cost-effective?

Methods: We performed a systematic review and where possible combined study results using meta-analysis to estimate the combined effect size. Major electronic databases [including Database of Abstracts of Reviews of Effects (DARE), Centre for Reviews and Dissemination (CRD), Cochrane Database of Systematic Reviews (CDSR) and the Health Technology Assessment (HTA) database] were searched from inception up to June 2012 covering both published and grey literature. Bibliographies of selected papers were hand-searched for additional references. Relevant authors were contacted for any unpublished findings and additional data if necessary. Abstracts were reviewed by two reviewers.

Inclusion and exclusion criteria: Subjects: pregnant women or pregnant women and their offspring. Exposure: either assessment of vitamin D status [dietary intake, sunlight exposure, circulating 25(OH)D concentration] or supplementation of participants with vitamin D or food containing vitamin D (e.g. oily fish). Outcomes: offspring – birthweight, birth length, head circumference, bone mass, anthropometry and body composition, risk of asthma and atopy, small for gestational dates, preterm birth, type 1 diabetes

mellitus, low birthweight, serum calcium concentration, blood pressure and rickets; mother – pre-eclampsia, gestational diabetes mellitus, risk of caesarean section and bacterial vaginosis.

Results: Seventy-six studies were included. There was considerable heterogeneity between the studies and for most outcomes there was conflicting evidence. The evidence base was insufficient to reliably answer question 1 in relation to biochemical or disease outcomes. For questions 2 and 3, modest positive relationships were identified between maternal 25(OH)D and (1) offspring birthweight in meta-analysis of three observational studies using log-transformed 25(OH)D concentrations after adjustment for potential confounding factors [pooled regression coefficient 5.63 g/10% change maternal 25(OH)D, 95% confidence interval (CI) 1.11 to 10.16 g], but not in those four studies using natural units, or across intervention studies; (2) offspring cord blood or postnatal calcium concentrations in a meta-analysis of six intervention studies (all found to be at high risk of bias; mean difference 0.05 mmol/l, 95% CI 0.02 to 0.05 mmol/l); and (3) offspring bone mass in observational studies judged to be of good quality, but which did not permit meta-analysis. The evidence base was insufficient to reliably answer questions 4 and 5.

Limitations: Study methodology varied widely in terms of study design, population used, vitamin D status assessment, exposure measured and outcome definition.

Conclusions: The evidence base is currently insufficient to support definite clinical recommendations regarding vitamin D supplementation in pregnancy. Although there is modest evidence to support a relationship between maternal 25(OH)D status and offspring birthweight, bone mass and serum calcium concentrations, these findings were limited by their observational nature (birthweight, bone mass) or risk of bias and low quality (calcium concentrations). High-quality randomised trials are now required.

Study registration: This study is registered as PROSPERO CRD42011001426.

Funding: The National Institute for Health Research Health Technology Assessment programme.

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List of abbreviations

25(OH)D	25-hydroxyvitamin D
ABCD	Amsterdam Born Children and their Development
ABCVitamin D	Vitamin D Supplementation During Pregnancy for Prevention of Asthma in Childhood trial
aBMD	areal bone mineral density
ALP	alkaline phosphatase
ALSPAC	Avon Longitudinal Study of Parents and Children
AMED	Allied and Complementary Medicine Database
BA	bone area
BIOSIS	Bioscience Information Service
BMC	bone mineral content
BMD	bone mineral density
BMI	body mass index
CD4	cluster differentiation 4
CDSR	Cochrane Database of Systematic Reviews
CENTRAL	Cochrane Central Register of Controlled Trials
CI	confidence interval
CRD	Centre for Reviews and Dissemination
CSA	cross-sectional area
DARE	Database of Abstracts of Reviews of Effects
DBP	vitamin D-binding protein
DEQAS	Vitamin D External Quality Assessment Scheme
DEXA	dual-energy X-ray absorptiometry
FEV_1	forced expiratory volume in 1 second
FFQ	Food Frequency Questionnaire
FVC	forced vital capacity
HbA_{1c}	glycated haemoglobin
HIV	human immunodeficiency virus
HLA	human leucocyte antigen
HMIC	Health Management Information Consortium
HPLC	high-performance liquid chromatography
HTA	Health Technology Assessment
IgE	immunoglobulin E
ISRCTN	International Standard Randomised Controlled Trial Number
LMP	last menstrual period
MAVIDOS	Maternal Vitamin D Osteoporosis Study
MoM	multiple of the median
NHANES	National Health and Nutrition Examination Survey
NICE	National Institute for Health and Care Excellence
OR	odds ratio
pQCT	peripheral quantitative computed tomography
PTH	parathyroid hormone
RCT	randomised controlled trial
REM	random-effects model
RIA	radioimmunoassay
SACN	Scientific Advisory Committee on Nutrition
SD	standard deviation
SGA	small for gestational age
SPA	single photon absorptiometry

SWS	Southampton Women's Survey	VDAART	Vitamin D Antenatal Asthma Reduction Trial
UKCRN	United Kingdom Clinical Research Network	VDR	vitamin D receptor
UVB	ultraviolet B	Zetoc	The British Library's Electronic Table of Contents

Scientific summary

Background

Low levels of serum 25-hydroxyvitamin D [25(OH)D] have been observed in many populations, including pregnant women. Studies have demonstrated associations between low levels of serum 25(OH)D during pregnancy and maternal/offspring health outcomes. However, many of these studies are observational in nature and it is unclear whether or not the current evidence base allows definite conclusions to be made regarding the optimal maternal circulating concentration of 25(OH)D during pregnancy, and how this might best be achieved. The aim of this work was to provide a systematic review of the current evidence base linking maternal 25(OH)D status to both maternal and offspring health outcomes, in order to answer the specific questions below.

Objectives

What are the clinical criteria for vitamin D deficiency in pregnant women?

What adverse maternal and neonatal health outcomes are associated with low maternal circulating 25(OH)D?

Does maternal supplementation with vitamin D in pregnancy lead to an improvement in these outcomes (including assessment of compliance and effectiveness)?

What is the optimal type (D_2 or D_3), dose, regimen and route for vitamin D supplementation in pregnancy?

Is supplementation with vitamin D in pregnancy likely to be cost-effective?

Methods

Data sources

Completed studies (systematic reviews): Database of Abstracts of Reviews of Effects (DARE), Centre for Reviews and Dissemination (CRD), Cochrane Database of Systematic Reviews (CDSR), Health Technology Assessment (HTA) database. Completed studies (other study types): Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE, Bioscience Information Service (BIOSIS), Google Scholar, Allied and Complementary Medicine Database (AMED). Ongoing studies: National Research Register archive, United Kingdom Clinical Research Network (UKCRN) Portfolio, Current Controlled Trials, ClinicalTrials.gov. Grey literature: Conference Proceedings Citation Index-Science (1990–present), The British Library's Electronic Table of Contents (Zetoc) conference search, Scientific Advisory Committee on Nutrition (SACN) website, Department of Health website, The King's Fund Library database, Trip database, HTA website, Health Management Information Consortium (HMIC) database. Bibliographies of selected papers were hand-searched for additional studies. We contacted first authors and experts in several fields including metabolic bone disease, obstetrics, infant nutrition, child development and allergy for any unpublished findings. Citations were independently reviewed by two reviewers according to CRD guidelines.

Inclusion and exclusion criteria

Studies were selected if they fulfilled criteria based on the sample studied, the independent variable of interest (exposure), the outcomes and the study design.

Sample studied
Pregnant women or pregnant women and their offspring.

Exposure
Either assessment of vitamin D status [dietary intake, sunlight exposure, circulating 25(OH)D concentration] or supplementation of participants with vitamin D or food containing vitamin D (e.g. oily fish).

Outcomes
Primary: maternal osteomalacia, neonatal hypocalcaemia, rickets and reduced bone mass.

Secondary: maternal quality of life, neonatal body composition, and later offspring health outcomes (including asthma, diabetes mellitus and immune disease).

Study design
Observational studies (case–control, cohort, cross-sectional), intervention studies.

Studies were excluded if they were not written in English, were non-human studies, did not measure maternal vitamin D status in or immediately after pregnancy or supplement participants with vitamin D in pregnancy, or where an outcome of interest was not measured. Systematic reviews were not included in the formal review, but were used as a potential source of additional references via hand-searching.

Data extraction
Data extraction was carried out by two reviewers. Disagreements were resolved in the same way as for screening of abstracts according to CRD guidelines. Separate forms were used to mark or correct errors or disagreements, and a database was kept for potential future methodological work. Data were abstracted onto an electronic form. This contained the following items: general information (e.g. date of data extraction, reviewer ID); study characteristics (e.g. study design, inclusion/exclusion criteria); study population characteristics; method of assessment of vitamin D status; baseline data (e.g. age, sex, ethnicity, measures of vitamin D status/supplementation); quality criteria; outcomes (what they were and how they were ascertained); confounding factors; analysis (statistical techniques, sample size based on power calculation, adjustment for confounding, losses to follow-up); and results [direction of relationship, size of effect, and measure of precision of effect estimate such as 95% confidence interval (CI) or standard error].

Assessment of validity and quality
Quality assessment of studies occurred first during data extraction and second in the analysis of review findings. The quality of included studies was assessed by the two reviewers, using a checklist of questions. The questions used, although based initially on CRD guidelines, were refined through piloting and agreement with the advisory group. Aspects of quality assessed included appropriateness of study design, ascertainment of exposure and outcome, and consideration of the effects of important confounding factors. Quality assessment also incorporated specific issues related to vitamin D. Quality data were used in narrative description of quality, and to produce composite validity scores with which to assign a quality level to each study such that studies could be stratified during synthesis of evidence.

Data synthesis
The aim of this part of the review was to investigate whether or not effects were consistent across studies and to explore reasons for apparent differences. We used both descriptive (qualitative) and quantitative synthesis; our capacity for the latter was determined by the evidence available. Where meta-analysis was possible, we used standard analytical procedures. Only independent studies were meta-analysed. Thus, where a study contained two treatment arms, these were not included in the same analysis. We used the Q-statistic to define statistical heterogeneity, with a $p < 0.1$ to define statistical significance. The I^2 statistic (percentage of variability in the results that is due to heterogeneity) was used to quantify the degree of heterogeneity across studies. Results were presented as forest plots, either as random-effects models

(REMs), if significant heterogeneity was detected, or as fixed-effects models if minimal heterogeneity was detected. All analysis was performed using Stata v11.0 (StataCorp LP, College Station TX, USA).

Results

Included/excluded studies: 22,961 citations were identified from the initial database search up to 3 January 2011. A subsequent additional search from 3 January 2011 to 18 June 2012 identified another 2448 citations, yielding a total of 25,409 citations. A further 66 citations were identified from other sources (e.g. grey literature and bibliographies). After duplicate citations were removed, 16,842 citations were screened. Of these, 16,669 were excluded on the basis of the content of the title and/or the abstract (if available). A further eight papers could not be found despite thorough searching; thus, 16,677 records were excluded. A total of 165 full-text articles were retrieved for detailed assessment and, of these, 76 papers were included in the review. A total of 89 papers retrieved for assessment were excluded. Around a third of these ($n = 34$) were abstracts. Twenty-one papers had no relevant maternal or offspring outcome; 11 papers had no estimate of maternal vitamin D status; 10 papers used data from other papers included in the review; eight papers were either review articles, letters, editorials or commentaries with no new results; one paper was of a non-human study; and four papers reported on an outcome not assessed in any other paper (maternal breast cancer, offspring schizophrenia, offspring multiple sclerosis and offspring influenza A). The results relating to the specific research questions are detailed below.

What are the clinical criteria for vitamin D deficiency in pregnant women? The highly heterogeneous and variable quality of the identified studies resulted in an evidence base that did not allow this question to be reliably answered, in terms of either biochemical relationships or disease outcomes. *What adverse maternal and neonatal health outcomes are associated with low maternal circulating 25(OH)D? Does maternal supplementation with vitamin D in pregnancy lead to an improvement in these outcomes (including assessment of compliance and effectiveness)?* The results relevant to these two study questions are itemised by individual health outcomes below.

Birthweight

Nineteen observational studies were identified. Composite bias scores ranged from –2 to +8, with seven of the 19 studies scored as having a low risk of bias. Six studies demonstrated a significant positive relationship between maternal vitamin D status and offspring birthweight; one study found a significant negative association. Of the remaining studies, seven suggested a non-significant positive association between the two variables and three found a non-significant negative association.

Nine intervention trials were identified. Seven of these studies were rated as having a high chance of bias on the composite score (–2 to –9); only the two most recent studies were assessed as having a low risk of bias (composite bias score of 5 and 10). Sample sizes ranged from 40 to 350 patients and interventions were highly variable. Three studies demonstrated significantly greater birthweight in offspring of supplemented mothers. The remainder showed no significant difference in infant birthweight regardless of supplementation (birthweight was non-significantly higher in the supplemented group in two of these, non-significantly lower in the supplemented group in one, and was not presented in the remaining two).

Meta-analysis of three observational studies found weak positive associations between log-transformed maternal 25(OH)D concentrations and offspring birthweight after adjustment for potential confounders [pooled regression coefficient 5.63 g/10% change in maternal 25(OH)D, 95% CI 1.11 g to 10.16 g].

Birth length

Twelve observational studies were identified. One study was assessed as having a high risk of bias (composite score –2, high risk) with the others demonstrating composite scores between +1 and +8. Two studies found a significantly positive relationship between maternal vitamin D status and offspring birth length; however, neither study directly measured maternal serum 25(OH)D concentration in

pregnancy. Of the remaining studies, four showed a non-significant positive association and four showed a non-significant inverse association. A further study observed a significant positive association between maternal vitamin D status and offspring length at 1 month.

Two intervention trials were identified. Both were assessed to have a high risk of bias (composite bias score of both –2, high risk). In one, birth length was higher in the offspring of women supplemented with vitamin D than in the offspring of unsupplemented women; the other found no significant association but a trend towards higher birth length in the supplemented group. Both studies were assessed to have a high risk of bias.

Head circumference

Eleven observational studies were identified, none of which found a significant relationship between maternal vitamin D status and offspring head circumference. Composite bias scores ranged from –2 to +8, with six studies having a low risk of bias. There was a non-significant trend towards greater head circumference with greater maternal vitamin D status in five studies, and a non-significant inverse relationship in four studies.

Two intervention studies were identified, both of which were assessed as having a high risk of bias (composite bias score –2 in both). One study demonstrated significantly greater offspring head circumference in supplemented mothers; the other found no association, but a non-significant trend towards greater head circumference in supplemented mothers.

Offspring bone mass

Eight observational studies were identified, all of which were assessed as being of medium to low risk of bias, with composite bias scores ranging from +3 to +7. Five studies demonstrated a significant positive relationship between maternal vitamin D status and offspring bone outcomes [which included whole-body, lumbar, femoral and tibial bone mineral content (BMC), and whole-body and lumbar spine bone mineral density (BMD)]. Of the remaining studies, no significant association was observed between maternal vitamin D status and offspring radial and whole-body BMC.

One intervention study was identified, which found no difference in offspring forearm BMC (measured within 5 days of birth) between supplemented and unsupplemented mothers. There was a non-significant trend towards higher forearm BMC in the supplemented group. This study was assessed to have a high risk of bias.

Offspring anthropometry and body composition

Six observational studies were identified, four of which demonstrated a significant relationship between maternal vitamin D status and offspring body composition and anthropometric variables (including skinfold thickness, lean mass and fat mass). Two studies found no significant relationship between maternal vitamin D status and the offspring anthropometric variables measured. Composite bias scores ranged from +3 to +8, indicating a medium to low risk of bias. Two intervention studies were identified; both were assessed to have a high risk of bias (composite bias score –2 for both). One demonstrated no effect of maternal vitamin D supplementation on offspring triceps skinfold thickness, whereas the other did find evidence of a positive effect.

Offspring asthma and atopy

Ten observational studies were identified. Five studies found a significantly reduced risk of offspring asthma or atopy with higher maternal vitamin D status; conversely, three studies found a significant positive association between maternal vitamin D status and offspring risk of asthma or atopy. The remaining two studies found no significant association between late-pregnancy 25(OH)D and lung function in offspring aged 6–7 years. All but one study were judged to be at moderate to high risk of bias, and no intervention studies were identified.

Offspring born small for gestational age

Seven observational studies were identified. All achieved a composite bias score of between +1 and +7, indicating a low to medium risk of bias. One study found a significantly increased risk of infants being small for gestational age (SGA) if maternal 25(OH)D was < 30 nmol/l. A second study found a U–shaped relationship between SGA and maternal 25(OH)D concentration in white women only, with the lowest risk between 60 and 80 nmol/l. No relationship was seen in black women. A third study of pregnant women with early-onset pre-eclampsia found significantly lower serum 25(OH)D in those women with SGA infants compared with the control groups. The four remaining studies found no significant relationship; two of these found a non-significant trend towards greater SGA risk in women with lower vitamin D status. Data were not given for the other two studies.

Two intervention trials were identified, one judged at low risk of bias and the other at high risk of bias, and neither of which found a significant difference in SGA risk in women supplemented with vitamin D compared with unsupplemented mothers. There was, however, a non-significant trend towards higher SGA risk in the unsupplemented group in both studies.

Offspring preterm birth

Seven observational studies were identified, ranging from low to high risk of bias. One study found that the risk of threatened premature delivery was significantly increased in mothers with lower 25(OH)D. Six studies found no significant relationship. No intervention trials were identified.

Offspring type 1 diabetes mellitus

Three observational studies were identified, judged to be at medium or low risk of bias. One study found a significantly increased risk of type 1 diabetes mellitus in the offspring of mothers with lower concentration of 25(OH)D in late pregnancy. The remaining studies found no significant relationship. No intervention studies were identified.

Offspring low birthweight

Three observational studies were identified, with composite bias scores ranging from –2 to +3, indicating a medium to high risk of bias. One study found a significantly reduced risk of low-birthweight offspring with adequate, compared with inadequate, maternal vitamin D and calcium intake. The remaining studies found no significant association. No intervention studies were identified.

Offspring serum calcium concentration

One observational study, at low risk of bias, was identified which found no significant association between maternal 25(OH)D at delivery and offspring cord calcium.

Six intervention trials were identified, all judged to be at high risk of bias (composite scores –9 to –1). Offspring serum calcium was significantly higher in the supplemented group in five of these studies. The remaining study found a non-significant trend towards higher cord blood calcium in the supplemented group. Meta-analysis of the intervention studies demonstrated a weak positive association (mean difference in serum calcium concentration in offspring of supplemented vs. unsupplemented mothers: 0.05 mmol/l, 95% CI 0.02 mmol/l to 0.05 mmol/l). Factors which might increase risk of symptomatic hypocalcaemia, such as ethnicity and breast (compared with formula) feeding, were not adequately addressed.

Offspring blood pressure

Two observational studies were identified, judged to be at medium risk of bias, and neither of which found a significant relationship between maternal 25(OH)D concentration and offspring blood pressure. No intervention trials were identified.

Pre-eclampsia

Eleven observational studies were identified, judged to be at low to medium risk of bias. Five studies found a significant inverse relationship between maternal vitamin D status and risk of pre-eclampsia; the remaining six studies found no significant relationship. Meta-analysis was possible for four studies, suggesting an inverse relationship between 25(OH)D and pre-eclampsia risk, but did not achieve statistical significance. One intervention trial was identified; no difference in risk of pre-eclampsia was seen in mothers supplemented with vitamin D compared with unsupplemented women.

Gestational diabetes mellitus

Eight observational studies were identified, judged to be at low to medium risk of bias. Three studies found a significant inverse relationship between risk of gestational diabetes mellitus and maternal vitamin D status. No intervention studies were identified.

Caesarean section

Six observational studies were identified, judged to be at low to medium risk of bias. Two studies found an inverse relationship between risk of caesarean section and maternal vitamin D status. The remaining four studies found no significant relationship, although a non-significant inverse trend was observed in two studies (the remaining two studies did not provide adequate data to assess trend). No intervention trials were identified.

Maternal bacterial vaginosis

Three observational studies were found, judged to be at low to medium risk of bias, and all of which found that lower maternal 25(OH)D was significantly associated with an increased risk of bacterial vaginosis in pregnancy. No intervention trials were identified.

What is the optimal type (D_2 or D_3), dose, regimen and route for vitamin D supplementation in pregnancy?

The marked variation in dose, route, study population, methods of exposure and outcome evaluation, and lack of comparative investigations, meant that the evidence base was insufficient to reliably answer this question.

Is supplementation with vitamin D in pregnancy likely to be cost-effective?

No studies including health economic evaluations in relation to specific disease outcomes were identified.

Conclusions

There was some evidence to support a positive relationship between maternal vitamin D status and offspring birthweight (meta-analysis of observational studies), neonatal calcium concentrations [meta-analysis of randomised controlled trials (RCTs)] and offspring bone mass (observational studies). Recurring themes in each disease area included marked heterogeneity between studies in terms of design, definition of exposure and outcome, dose, timing, route, statistical analysis, treatment of potential confounding factors. In no single disease area did the evidence base unequivocally support the use of vitamin D supplementation during pregnancy.

Implications for health care

The fundamental conclusion is that the current evidence base does not allow the study questions to be definitively answered. It is therefore not possible to make rigorously evidence-based recommendations regarding maternal vitamin D supplementation during pregnancy.

Recommendations for research

This systematic review has identified important gaps in the evidence, and further high-quality research is clearly needed. In many areas, well-designed large prospective cohort studies are most appropriate as the next step. In others, the evidence base is sufficient to suggest RCTs. Without such a rigorous approach, there is a risk that public health policy will be made on the basis of optimistic evaluations of conflicting and heterogeneous studies. Although modest doses of vitamin D during pregnancy are likely to be relatively safe, at least in the short term, there is a dearth of long-term data to inform the potential long-term effects of maternal vitamin D supplementation on offspring health. As with most interventions, it is probably optimistic to expect that there will be no risk of adverse events.

Study registration

This study is registered as PROSPERO CRD42011001426.

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Chapter 1 Background

Epidemiology of vitamin D serum concentrations

There are very few data on vitamin D levels in pregnant women across a population representative of the UK as a whole; the available studies, however, suggest that low serum 25-hydroxyvitamin D [25(OH)D] concentrations are common in this group. In one cohort in Southampton, composed of white Caucasians, 31% had concentrations of circulating 25(OH)D < 50 nmol/l and 18% had concentrations < 25 nmol/l.[1] A recent US study of a population representative of the national demographic distribution revealed that 80% of black pregnant women had levels < 50 nmol/l; the figures for Hispanic and white pregnant women were 45% and 13% respectively.[2] In Asian cohorts in the northern hemisphere the burden is even higher,[3–7] possibly reaching ≥ 90%. A study of non-pregnant South Asian women in the north of England, many of whom were of child-bearing age, demonstrated that 94% had circulating levels of 25(OH)D ≤ 37.5 nmol/l and 26% had levels ≤ 12.5 nmol/l;[8] a survey of the UK (non-pregnant) population revealed low levels of 25(OH)D in 50%.[9] As the main source of vitamin D is synthesis in the skin under the influence of ultraviolet B (UVB) radiation from sunlight exposure, ethnicity (dark skin), covering and northerly latitudes (as in UK) are all major risk factors for low concentrations.[10] The vitamin D axis is thought to be highly influential in the acquisition of bone mineral, and significant changes in women's vitamin D and calcium homeostasis occur during pregnancy in order to provide the fetus with adequate calcium to mineralise its rapidly growing skeleton. Evidence that maternal vitamin D status influences neonatal calcium homeostasis has come from studies of Asian immigrants, among whom reduced serum 25(OH)D concentrations are accompanied by increased parathyroid hormone (PTH) levels. Maternal vitamin D deficiency in pregnancy has been associated with neonatal hypocalcaemia[11] and other adverse birth outcomes, such as craniotabes and widened growth plates, suggestive of rachitic (rickets-like) change.[12] Indeed, a recent study demonstrated rachitic-like widening of the fetal distal femoral metaphysis relative to its length, scanned by ultrasound at 19 and 34 weeks, in fetuses of mothers with low levels of circulating 25(OH)D. This implies a relatively early effect,[13] and consistent findings have come from a further cohort.[14] Infants of mothers with low vitamin D intake may have lower calcium levels at day 4 post delivery.[15] Anecdotally, infant rickets is becoming more common in dark-skinned communities in the UK, probably due to low infant intake of vitamin D from the mother, secondary to maternal deficiency, initially via the placenta in utero and then via breast milk postnatally.[16–19] However, accurate population-wide epidemiological data are lacking, and the 25(OH)D concentration, below which an individual is considered deficient, is the subject of much debate.

Intervention studies

There have been several, mainly small, intervention studies examining this issue (*Table 1*). Thus, in one study, 506 women were supplemented with vitamin D at 12 weeks' gestation with 400 IU per day and compared with 633 women supplemented with placebo.[20] Levels of 25(OH)D were higher in maternal, umbilical cord, and infant serum (days 3 and 6) in the supplemented group. This was not a randomised trial, but supplemented women from one clinic compared with placebo in another clinic. Another study compared 59 Asian women, supplemented with 1000 IU vitamin D in the last trimester of pregnancy,[3] with 67 controls. Calcium levels were higher in the supplemented mothers, and there was a lower incidence of symptomatic neonatal hypocalcaemia and growth retardation among babies of supplemented mothers. Again, in an Asian population,[4] 25 mothers were randomised to 1200 IU vitamin D per day, 20 mothers to 600,000 IU twice (seventh and eighth months) and 75 mothers to placebo. In this study there was no difference in calcium and alkaline phosphatase (ALP) levels between mothers taking 1200 IU per day and those taking placebo. However, those taking 600,000 IU twice had higher maternal and cord calcium and lower ALP than those taking placebo. In a second study,[5] the same group compared maternal and cord

TABLE 1 Trials of vitamin D supplements in pregnancy

Trial	*n*	Location	Intervention	Outcome	Direction of effect
Cockburn *et al.* (1980)[20]	1139	Scotland	400 IU/day or placebo	Maternal 25(OH)D	↑
				Cord 25(OH)D	↑
				Infant 25(OH)D	↑
Brooke *et al.* (1980)[3]	126	UK (Asian population)	1000 IU/day or placebo	Maternal calcium	↑
				Cord calcium	→
				Neonatal calcium	↑
				Maternal weight	↑
Marya *et al.* (1981)[4]	120	Asian (India)	600,000 IU (twice), 1200 IU/day or placebo	Maternal calcium	↑
				Cord calcium	↑
				Maternal ALP	↓
				Cord ALP	↓
Marya *et al.* (1988)[5]	200	Asia (India)	600,000 IU (twice) or placebo	Maternal calcium/ALP	↑
				Cord calcium/ALP	↑
				Maternal ALP	↓
				Cord ALP	↓
Delvin *et al.* (1986)[6]	34	France	1000 IU/day or no vitamin D	Cord 25(OH)D	↑
				Neonatal 25(OH)D	↑
Mallet *et al.* (1986)[7]	68	France	200,000 IU (once), 1000 IU/day or no vitamin D	Maternal 25(OH)D with both regimes	↑

↑, elevation; →, no change; ↓, decrease; ALP, alkaline phosphatase.

calcium and ALP in supplemented 100 Asian-Indian women with 600,000 IU twice (again at the seventh and eighth months) and 100 controls and again found higher maternal and cord calcium and lower ALP in the supplemented group. There have been two studies in French populations. In the first study, 15 women randomised to receive 1000 IU vitamin D per day from the third trimester were compared with 15 controls.[6] Day 4 neonatal calcium and 25(OH)D levels were higher in the supplemented group. In the second study, 21 French women received 1000 IU per day in the last trimester and 27 received 200,000 IU once during the seventh month; 29 unsupplemented women served as a control goup.[7] In this study, neonatal calcium at days 2 and 6 was similar in all groups, but maternal serum 25(OH)D was greater in both intervention groups than in the controls. In one study, measuring bone mineral content (BMC) at birth,[21] there was no difference in radial BMC in offspring of 19 Asian mothers who had taken 1000 IU vitamin D per day and in the offspring of 45 controls. However, this lack of observed effect is likely to reflect both the small numbers of subjects and the poor sensitivity of single photon absorptiometry (SPA) in measuring the tiny amount of bone mineral in the baby's distal radius.

Safety of vitamin D supplementation in pregnancy

None of the studies listed in *Table 1* suggested that vitamin D supplementation during pregnancy carries a significant risk. Human beings have evolved to cope with as much as 25,000 IU vitamin D formation daily in the skin. Although in rat studies the equivalent of 15,000,000 IU per day resulted in extraskeletal calcifications, there is no evidence that doses < 800,000 IU per day have any adverse effect.

Two studies[22,23] have examined the children of hypoparathyroid women given 100,000 IU vitamin D daily for the duration of pregnancy and found no morphological or physiological adverse consequences. These children were followed for up to 16 years. Recent work has demonstrated a moderate increase in atopy in children of mothers in the highest quarter of serum vitamin D in pregnancy, where levels were > 30 ng/ml.[24] However, in this study the numbers were small, with only six cases of atopy (asthma, eczema) by 9 years in the top quarter of maternal vitamin D, four each in the middle quarter and two in the bottom. These numbers, even in the highest quarter, were actually lower than the figure for the general population. Additionally, in the Southampton Women's Survey (SWS), there was no association between maternal 25(OH)D status and atopic or non-atopic eczema at 9 months of age.[25] This finding needs to be further examined in larger studies, but suggests, for safety, that the optimal intervention would be to supplement those mothers found to be deficient in vitamin D, rather than all pregnant mothers.

Maternal vitamin D status, offspring wheezing and diabetes mellitus

In contrast to the findings above, another epidemiological study suggested an inverse relationship between maternal dietary intake of vitamin D in pregnancy and later wheezing in the offspring.[26] However, a study of vitamin D supplementation in infants again suggested a positive relationship such that greater infant supplementation was associated with increased later wheezing.[27] Hypponen *et al.*[28] found, in an adult population cohort, that circulating immunoglobulin E (IgE) levels (a marker of atopic tendency) were positively related to concentrations of 25(OH)D, but this was only apparent at very high concentrations (> 125 nmol/l). Animal studies have implicated 1,25(OH)D as a modulator of immune balance between a tendency to autoimmunity and atopy, but these studies have again suggested influences in both directions.[29] Thus, the data are inconsistent, and clearly any studies using dietary intake of vitamin D, rather than blood levels, as the marker of vitamin D status have the potential for confounding by UVB exposure and other lifestyle, anthropometric and health factors. It is possible that the relationships between vitamin D and atopy differ depending on timing (e.g. in pregnancy or postnatal life), with 25(OH)D or 1,25(OH)D, or are U-shaped such that both low and very high levels are detrimental. Finally, a birth cohort study from Finland demonstrated a reduced risk of type 1 diabetes mellitus in children who had been supplemented with vitamin D as infants.[30]

Longer-term importance of maternal vitamin D repletion for offspring bone size and density

Recent work has suggested that maternal vitamin D deficiency during pregnancy may not solely influence the offspring's skeleton through overt rachitic change. Evidence is accruing that less profound maternal 25(OH)D insufficiency may lead to suboptimal bone size and density in the offspring postnatally, a situation likely to lead to an increased risk of osteoporotic fracture in the offspring in later life. Evidence that the risk of osteoporosis might be modified by environmental influences in early life comes from two groups of studies: (1) those evaluating bone mineral and fracture risk in cohorts of adults for whom birth and/or childhood records are available; and (2) those studies relating the nutrition, body build and lifestyle of pregnant women to the bone mass of their offspring.[31] Cohort studies in adults from the UK, USA, Australia and Scandinavia have shown that those who were heavier at birth or in infancy have a greater bone mass[32–35] and a reduced risk of fracture[36] in later life. These associations remain after adjustment for potential confounding factors, such as physical activity, dietary calcium intake, smoking and alcohol consumption. In a cohort of twins, intrapair differences in birthweight were associated with BMC in middle age, even among monozygous pairs.[37] Mother–offspring cohort studies based in Southampton have shown that maternal smoking, poor fat stores and excessive exercise in late pregnancy all have a detrimental effect on bone mineral accrual by the fetus, leading to reduced bone mass at birth.[38]

However, the strongest risk factor for poor bone mineral accrual documented in these mother–offspring cohort studies has been maternal vitamin D insufficiency. There was already some indication of the potential role played by maternal vitamin D status in pregnancy from a retrospective cohort study[39] showing that premature babies who were supplemented with vitamin D had an increased whole-body bone mass at 12 years of age, but these recent findings provided the first direct evidence for the importance of maternal vitamin D status during pregnancy on the child's skeletal growth. In a Southampton mother–offspring cohort, data on anthropometry, lifestyle and diet were collected from women during pregnancy and venous 25(OH)D was measured by radioimmunoassay (RIA) in late pregnancy.[1] Whole-body, hip and lumbar spine bone area (BA), BMC and bone mineral density (BMD) were measured in the healthy, term offspring at age 9 years. Thirty-one per cent of the mothers had reduced (insufficient or deficient) circulating concentrations of 25(OH)D in late pregnancy. There was a positive association between maternal 25(OH)D concentration in late pregnancy and whole-body BMC ($r = 0.21$, $p = 0.0088$) and bone density ($r = 0.21$, $p = 0.0063$) in the offspring at 9 years old, with a suggestion of a threshold effect at 40 nmol/l. Both the estimated exposure to UVB radiation during late pregnancy and use of vitamin D supplements predicted maternal 25(OH)D concentration ($p < 0.001$ and $p = 0.01$) and childhood bone mass ($p = 0.03$). Reduced concentration of umbilical venous calcium also predicted lower childhood bone mass ($p = 0.03$), suggesting a possible role for placental calcium transport in this process.

Similar findings linking reduced maternal 25(OH)D concentration with lower offspring bone mass have come from the SWS.[40] In this ongoing prospective cohort study of women aged 20–34 years, characterised before and during pregnancy, maternal 25(OH)D status was measured by RIA in late pregnancy and 556 healthy term-born neonates underwent whole-body dual-energy X-ray absorptiometry (DEXA) within 20 days of birth. Bone mass was lower in the offspring of mothers who were insufficient or deficient (< 40 nmol/l) in vitamin D in late pregnancy than in the offspring of mothers who were replete. Thus, the mean whole-body BA of the female offspring of deficient mothers was 112 cm^2 compared with 120 cm^2 in the offspring of replete mothers ($p = 0.045$). The mean whole-body BMC of offspring of deficient compared with replete mothers was 59 g versus 64 g ($p = 0.046$). There were weaker associations in the boys and there was no association with maternal ALP. Additionally, maternal UVB exposure during pregnancy was positively associated with whole-body BMC in offspring aged 9 years in the Avon Longitudinal Study of Parents and Children (ALSPAC).[41]

Summary

Maternal vitamin D deficiency is important for maternal health, and also has implications for the offspring. In frank deficiency, most common in dark-skinned/covered populations in the UK, neonatal hypocalcaemia, craniotabes and infant rickets are an increasing problem. However, evidence is accruing for the longer-term implications of milder maternal vitamin D insufficiency in the broader population (including white Caucasian women). Thus, children of mothers with low levels of circulating 25(OH)D in pregnancy have reduced bone size and density, even in the absence of definite rachitic change. This is likely to lead to reduced peak bone mass and increased risk of osteoporotic fracture in later life. Furthermore, maternal vitamin D status has been linked to allergy and asthma in the offspring. Thus, the outcomes considered for this proposal will encompass both immediate maternal and neonatal health, but also longer-term skeletal development and atopy in the child.

Considerations for appraisal of data

There are several factors which make any study of evidence surrounding vitamin D problematic. First, the main source of vitamin D is from synthesis in the skin by the action of UVB radiation, with dietary intake usually forming a minor contribution to overall levels. Second, the physiology of vitamin D in pregnancy and its role in placental calcium transfer and offspring bone development (both linear growth and mineralisation) is unclear. Third, the definition of a normal range is difficult, even in non-pregnant

populations, and techniques used to measure 25(OH)D concentrations have widely different characteristics. Fourth, dose–response and differences between use of vitamin D_2 and vitamin D_3 are unclear. Fifth, postnatal vitamin D intake by the offspring may confound any pregnancy relationships. Finally, the definition of osteomalacia used is important (clinical syndrome or histological definition from bone biopsy). A detailed appraisal of these factors is given below.

Photosynthesis and metabolism of vitamin D

Vitamin D is a secosteroid which is synthesised in the skin by the action of sunlight. It plays a crucial role in bone metabolism and skeletal growth.[42] Around 95% is acquired via photosynthesis in the skin, with the minority from the diet.[43] There are two dietary forms: D_2, from plants, and D_3, from animals (the latter mainly found in oily fish and fortified margarines and breakfast cereals).[43] Vitamin D is synthesised from the action of sunlight (wavelengths 290–315 nm) on cutaneous 7-dehydrocholesterol, converting it to pre-vitamin D_3.[10,42] Once formed, pre-vitamin D_3 undergoes membrane-enhanced temperature-dependent isomerisation to vitamin D_3,[42] which is translocated into the circulation, where it binds to vitamin D-binding protein (DBP).[10] The main determinant of vitamin D synthesis in the skin is the level of sun exposure. The total amount of energy accrued from sunlight is dependent on duration and extent of skin exposure, but also on latitude and season. Thus, pigmented skin and covering, particularly relevant to the dark-skinned, and potentially covered, ethnic minority groups in the UK, reduce synthesis; using sun block with a factor higher than 8 almost completely prevents formation of vitamin D.[43] At latitudes of 48.5° (Paris, France), the skin is unable to form vitamin D between the months of October through to March.[42] In northern latitudes this results in a seasonal variation in levels of vitamin D, with a peak over the summer months and a trough in the winter.[10] Use of sunscreen during the summer may prevent adequate synthesis of vitamin D and subsequent storage in fat for the winter months, thus leading to deficiency; greater adiposity is also associated with reduced levels.[10] Circulating vitamin D is converted in the liver to 25(OH)D (calcidiol), which is the main circulating store. This step, which involves the cytochrome P450 system, is not tightly regulated, and thus an increase in photosynthesis of vitamin D in the skin will lead to an increase in 25(OH)D in the circulation,[10,44] bound to DBP. Excess 25(OH)D is converted to 24,25(OH)D, which is thought be relatively metabolically inactive.[10] The 25(OH)D–DBP complex enters renal tubule cells by membrane-bound megalin transport, where the enzyme 1-α-hydroxylase converts it to $1,25(OH)_2$-vitamin D (calcitriol), which is the active compound.[44] Although the kidney is the primary site for conversion of circulating 25(OH)D, many cells and tissues, such as macrophages, osteoblasts, keratinocytes, prostate, colon and breast, express the 1-α-hydroxylase enzyme.[42,45,46] As anephric patients have very low levels of $1,25(OH)_2$-vitamin D in the blood, it seems likely that these extrarenal sites function at the paracrine level, and do not play a major role in calcium homeostasis.[43]

Food sources, recommended intakes and dose response

Few foods contain significant amounts of vitamin D. The most effective sources are oily fish (e.g. salmon, mackerel) and fortified foods such as margarine and breakfast cereal. The amount of vitamin D derived from fish is modest: wild salmon contains around 400 IU per 3.5 oz (100 g).[10] There is much controversy over the recommended daily intake of vitamin D. Older guidance has suggested 200 IU per day for children and adults aged $\leq$ 50 years and 400–600 IU for older adults.[47] However, humans have evolved to synthesise much higher levels of vitamin D in the skin: 30 minutes exposure at mid-day in the summer sun at a southerly latitude in a bathing suit will release around 50,000 IU into the circulation within 24 hours in white persons.[48] Previous guidelines were not based on any rigorous assessment of the effects of levels and more recent dosing studies have shown that supplementation with 200–400 IU per day is unlikely to maintain levels of 25(OH)D over winter months, let alone replenish stores in somebody who is frankly vitamin D deficient.[49] Thus, a daily maintenance dose of around 1000 IU per day may be more appropriate in people without adequate sunshine exposure, with higher initial dosing required to reverse frank deficiency.[50]

Physiology of vitamin D in pregnancy

During pregnancy there is an increase in $1,25(OH)_2$-vitamin D, which may be largely due to an increase in DBP.[51] This rise is associated with an increase in intestinal calcium absorption (to around 80% intake), and

an absorptive hypercalciuria.[51] There does not seem to be a rise in maternal PTH or 25(OH)D during pregnancy, suggesting that the rise in 1,25$(OH)_2$-vitamin D may be due to another factor, such as PTH-related peptide, which may be secreted by the placenta.[52] Studies of maternal bone mass in pregnancy have been conflicting, but most suggest a probable decrease, with a possibly greater decrease in lactation.[53–57] The vitamin D receptor (VDR) appears to develop after birth in the infant intestine, and thus calcium absorption is a passive process immediately after birth.[58] The role of vitamin D in utero is uncertain, although 25(OH)D does cross the placenta.[59] In a mouse model, lack of VDR did not significantly affect placental calcium transport or skeletal mineralisation;[58] conversely, in the rat, 1,25$(OH)_2$-vitamin D did seem to influence placental calcium flux.[60] Additionally, chondrocytes are an extrarenal source of 1-α-hydroxylase activity [and so conversion of 25(OH)D to 1,25$(OH)_2$-vitamin D].[61] This observation therefore suggests a possible mechanism by which maternal 25(OH)D status might influence bone size in the fetus. Further evidence to support this notion comes from mouse models in which the gene for 1-α-hydroxylase (*Cyp27b1*) was either knocked out or overexpressed in chondrocytes, leading to altered growth plate morphology.[62] Few data exist in humans at the level of cell biology. Some suggestions have come from recent epidemiological work described above, in which maternal 25(OH)D concentrations positively predicted offspring bone mass at birth,[40] and at 9 years old,[1,41] with umbilical cord calcium concentrations and placental calcium transporters[63] implicated in the mechanisms.

Normal range and measurement of vitamin D

Circulating 25(OH)D is the major store of vitamin D and is the most appropriate for measurement. 1,25$(OH)_2$-vitamin D is an adaptive hormone, and therefore its level will reflect prevailing conditions such as calcium intake, and thus defining a normal level may not be meaningful.[43] The concept of what is the normal range for 25(OH)D is highly controversial at the moment. One view is that, given that humans seem to have evolved to require much higher levels of vitamin D than are observed in the UK currently, the process of measuring levels in a population and defining a lower cut-off of the distribution as deficient is likely not to be valid. Historically in the UK, serum levels have been classed as 'replete' (> 50 nmol/l), insufficient (25–50 nmol/l) or deficient (< 25 nmol/l). (Older studies often use ng/ml as the unit of measurement: 1 ng/ml = 2.5 nmol/l.) The Institute of Medicine in the USA has recently reiterated the 50 nmol/l threshold as the desirable level of circulating 25(OH)D.[64] The distinction between replete and insufficient/deficient has been made on the basis of whether or not there is a secondary rise in PTH. Other approaches to definition have been based on fractional calcium absorption and bone turnover markers. However, a recent review of the available studies relating 25(OH)D concentration to PTH concentration found, across the 70 studies, that a continuous relationship was observed in eight studies, no relationship in three and a thresholded relationship in the remaining 59.[65] Where a threshold was detected, this varied between 25 and 125 nmol/l. Studies of fractional calcium absorption are similarly heterogeneous.[66] Furthermore, in an autopsy-based study of 675 cadavers,[67] although bone mineralisation defects (osteomalacia) were not observed in any individual with 25(OH)D > 75 nmol/l, in those with levels < 25 nmol/l, a substantial proportion were found to have normal bone histology. Taken with the range of attempts to define cut-offs for deficiency, these results clearly make the point that extrapolation from 25(OH)D concentration alone to disease is difficult at the level of the individual.

There are several different methods available to measure 25(OH)D. The gold standard is seen to be gas chromatography–mass spectrometry, but this technique is slow, expensive and time-consuming. Most labs use commercial kit assays, which are usually radioimmunometric assays [e.g. Immunodiagnostic Systems (IDS), DiaSorin, Nicholls], although a chemiluminescence assay also exists (e.g. LIAISON®, DiaSorin, Stillwater, MN, USA). The assays tend to be less accurate than gas chromatography–mass spectrometry and high-performance liquid chromatography (HPLC), and also discriminate less well between the D_2 and D_3 forms.[68] Comparison of the DiaSorin RIA kits with HPLC showed good correlation for D_3, but D_2 tended to be slightly underestimated.[69] A national system now exists to standardise measurement of 25(OH)D across laboratories in the UK [Vitamin D External Quality Assessment Scheme (DEQAS)],[70] and the US National Institutes of Health are leading a global programme aimed at standardisation of 25(OH)D assays across both platform and laboratory.[71]

Infant postnatal vitamin D intake

Infant feeding, supplementation and sunlight exposure are strong determinants of postnatal infant 25(OH)D levels and bone health.[72] Concentrations of 25(OH)D in breast milk depend on the mother's blood levels and so, if the mother is deficient in vitamin D during pregnancy, she is likely to continue to be deficient through lactation, yielding a double insult to the child in the absence of adequate sun exposure. Clearly, postnatal vitamin D supplementation of either the mother (during breastfeeding) or the infant directly, together with maternal or childhood sun exposure, could confound any early outcomes attributed to maternal vitamin D status in pregnancy.

Osteomalacia: definition

Osteomalacia is a bone disease caused by inadequate mineralisation of the bone protein matrix, most often, in the UK, as a result of low levels of vitamin D.[73] Inadequate calcium and phosphate are other potential causes, seen more frequently in developing countries, or as a result of genetic abnormalities leading to phosphate loss. Although osteomalacia is therefore a histological term, it is used to describe the finding of low vitamin D status in a patient with bone/muscle pain, weakness, waddling gait, skeletal fragility and appropriate biochemical abnormalities (e.g. hypocalcaemia).[73] Very few studies have examined osteomalacia in pregnancy, although, anecdotally, the incidence of the clinical syndrome is rising in dark-skinned ethnic minorities in the UK. Clearly the definition of osteomalacia used in studies considered for this review will be critical as the symptoms of osteomalacia overlap considerably with those of chronic pain syndromes such as fibromyalgia. Bone biopsy is the only way to diagnose osteomalacia histologically, but the interventional nature of this procedure means that it is unsuitable for large-scale population studies. One recent study of 675 human subjects at autopsy has demonstrated that there is no threshold in circulating 25(OH)D level below which osteomalacic changes on bone biopsy are always seen.[67]

Chapter 2 Existing evidence synthesis

Two previous systematic reviews have been performed in this area. The most recent (Mahomed and Gulmezoglu[74]), from the Cochrane group, asked the question 'What are the effects of vitamin D supplementation on pregnancy outcome?', and, although withdrawn in 2011, the actual searches and conclusions were established in 1999. The authors searched for intervention studies registered on the Cochrane Pregnancy and Childbirth Group's Trials Register (October 2001) and the Cochrane Central Register of Controlled Trials (CENTRAL) (Issue 3, 2001). Thus, more recent work and observational data, plus unpublished evidence, were not included. We believe that a further Cochrane review is under way. Two trials of vitamin D supplementation in pregnancy (Mallet *et al.*[7] and Brooke *et al.*;[3] see *Table 1*) were assessed worthy of inclusion, but the authors concluded that there was insufficient evidence on which to base any recommendations. The National Institute for Health and Care Excellence (NICE) produced guidelines for antenatal care in 2008 (CG62).[75] Again, the conclusion was that there was insufficient evidence to allow a recommendation regarding vitamin D supplementation in pregnancy, although the authors acknowledged that supplementation may be beneficial in high risk groups. Despite the lack of good evidence for population wide supplementation and the dose chosen, the Department of Health currently recommend that all pregnant women take 400 IU vitamin D daily.[76] Most recently, Aghajafari *et al.*[77] published a systematic review focused on obstetric outcomes, finding a possible beneficial effect of higher concentrations of maternal vitamin D in terms of gestational diabetes mellitus, pre-eclampsia and bacterial vaginosis, small for gestational age (SGA) infants and lower birthweight infants, but not delivery by caesarean section.

Chapter 3 Research questions

1. What are the clinical criteria for vitamin D deficiency in pregnant women?
2. What adverse maternal and neonatal health outcomes are associated with low maternal circulating 25(OH)D?
3. Does maternal supplementation with vitamin D in pregnancy lead to an improvement in these outcomes (including assessment of compliance and effectiveness)?
4. What is the optimal type (D_2 or D_3), dose, regimen and route for vitamin D supplementation in pregnancy?
5. Is supplementation with vitamin D in pregnancy likely to be cost-effective?

Chapter 4 Review methods

Design

Systematic review of evidence to address these five research questions, following the methods recommended by the Centre for Reviews and Dissemination (CRD), University of York (www.york.ac.uk/inst/crd/), with meta-analysis to generate a pooled effect size where study designs allowed.

The review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO; registration number CRD42011001426; www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42011001426).

Inclusion criteria

Studies were selected if they fulfilled criteria based on the sample studied, the independent variable of interest (exposure), the outcomes and the study design.

Sample studied

This must include pregnant women or pregnant women and their offspring.

Exposure

This must include either assessment of vitamin D status [dietary intake, sunlight exposure, circulating 25(OH)D concentration] or supplementation of participants with vitamin D or food containing vitamin D (e.g. oily fish).

Outcomes

Primary

Neonatal hypocalcaemia, rickets in the offspring, offspring bone mass and maternal osteomalacia.

Secondary

Offspring body composition (including offspring birthweight, birth length, head circumference, anthropometry, risk of being born SGA and risk of low birthweight); offspring preterm birth and later offspring health outcomes (including asthma and atopy, blood pressure and type 1 diabetes mellitus); maternal quality of life (including pre-eclampsia, gestational diabetes mellitus, risk of caesarean section and bacterial vaginosis).

Study type and setting

Studies which reported data on individuals were included. Ecological and animal studies were excluded. Examples of eligible study designs, together with associated level of resulting evidence quality (Centre for Evidence-Based Medicine)[78] are shown below:

- level 1a: systematic review (with homogeneity) of randomised controlled trials (RCTs)
- level 1b: individual RCT [with narrow confidence interval (CI)]
- level 2a: systematic review (with homogeneity) of cohort studies
- level 2b: individual cohort study
- level 3a: systematic reviews (with homogeneity) of case–control studies
- level 3b: individual case–control study.

All studies which contributed relevant information were included, regardless of the setting. However, the setting was noted as part of data abstraction and was used in narrative synthesis. Studies were not excluded on the basis of publication date.

Exclusion criteria

Studies were excluded if they were not written in English, were non-human studies, did not measure maternal vitamin D status during or immediately after pregnancy or supplement participants with vitamin D in pregnancy, or if an outcome of interest was not assessed. Systematic reviews were not included in the narrative, but used as a source of references through hand searching.

Search strategy for identification of studies

The search strategy was informed by initial scoping exercises performed by an information specialist with extensive expertise in systematic reviews of effectiveness and observational evidence. The search aimed to identify studies which describe maternal vitamin D levels/supplementation in relation to maternal and offspring outcomes which may be suitable for answering the questions posed in the review (search terms are shown in *Appendix 1*).The following resources were searched from their start dates to the present day: Completed studies (systematic reviews): Database of Abstracts of Reviews of Effects (DARE); CRD; Cochrane Database of Systematic Reviews (CDSR), Health Technology Assessment (HTA) database. Completed studies (other study types): CENTRAL; MEDLINE, EMBASE, Bioscience Information Service (BIOSIS); Google Scholar; Allied and Complimentary Medicine Database (AMED). Ongoing studies: National Research Register archive; United Kingdom Clinical Research Network (UKCRN) Portfolio; Current Controlled Trials; ClinicalTrials.gov. Grey literature: Conference Proceedings Citation Index-Science (1990–present); The British Library's Electronic Table of Contents (Zetoc) conference search; Scientific Advisory Committee on Nutrition (SACN) website; Department of Health website; The King's Fund library database; Trip database; HTA website; Health Management Information Consortium (HMIC) database. Bibliographies of selected papers were hand-searched. First authors and other experts in several fields including metabolic bone disease, obstetrics, infant nutrition, child development, and allergy were contacted for unpublished findings. Identification of unpublished research was considered important in order to avoid publication bias. Unpublished observational evidence may be difficult to find since observational studies are not registered in the way that RCTs are. All relevant studies (published or unpublished) that satisfied selection criteria for the review were considered. There was also a possibility that inclusion of those identified may itself introduce bias, due to over-representation of the findings of groups known to reviewers. This was assessed at the analysis stage of the review. The initial search strategy included articles up to 3 January 2011. A subsequent additional search from 3 January 2011 to 18 June 2012 was also performed to look for studies published more recently.

Screening of abstracts

When applying selection criteria, all abstracts and potentially relevant papers were independently assessed by two reviewers (CH, and PC or RM) and decisions shown to be reproducible. Disagreements over inclusion were resolved through consensus and, where necessary, following discussion with a third member of the review team (NCH).

Data extraction

Data extraction was carried out by two reviewers. Disagreements were resolved in the same way as for screening of abstracts. Separate forms were used to mark or correct errors or disagreements and a database kept for potential future methodological work.

Data were abstracted onto an electronic form. This contained the following items: general information (e.g. date of data extraction, reviewer ID); study characteristics (e.g. study design, inclusion/exclusion

criteria); study population characteristics; method of assessment of vitamin D status; baseline data (e.g. age, sex, ethnicity, measures of vitamin D status/supplementation); quality criteria; outcomes (what they were and how they were ascertained); confounding factors; analysis (statistical techniques, sample size based on power calculation, adjustment for confounding, losses to follow-up); results (direction of relationship, size of the effect and measure of precision of effect estimate such as 95% CI or standard error). The data extraction forms for different study types are included in *Appendix 2*.

Effect modifiers/confounders

The effect modifiers and confounding factors considered included ethnicity, skin covering, season, sunlight exposure, alcohol intake, smoking, dietary calcium, physical activity, comorbidity (e.g. diabetes mellitus), current medication, maternal body mass index (BMI), infant feeding, infant supplementation and maternal postnatal supplementation if breastfeeding. Inclusion of these factors was recorded for each study and used as a marker of quality. Where meta-analysis was performed to generate a pooled effect size, inclusion and adjustment for these factors in individual studies was again recorded and used in quality assessment.

Study quality assessment

Quality assessment of studies took place (1) during data extraction and (2) in the analysis of review findings. The quality of included studies was assessed by the two reviewers, using a checklist of questions. The questions used, although based initially on CRD guidelines, were refined through piloting and agreement with the advisory group. Aspects of quality assessed included appropriateness of study design, ascertainment of exposure and outcome, consideration of the effects of important confounding factors, rigour of analysis, sample size and response rates. Quality assessment also incorporated specific issues related to vitamin D. Quality criteria are summarised in *Appendix 3*. Quality data were used in narrative descriptions of study quality, and to produce composite validity scores with which to assign a quality level to each study such that studies could be stratified during synthesis of evidence. Quality assessment tools were agreed by the advisory group and refined during piloting. Each study was allocated a score for each quality criterion to estimate the overall risk of bias: +1 indicated a low risk of bias, 0 a medium risk of bias and −1 a high risk of bias. These scores were then added to give a composite score, indicating bias in relation to the review question for each study. This score was between −16 and +16 for intervention and case–control studies; cohort and cross-sectional studies were allocated a score of between −13 and +13. A total composite score < 0 indicated a high risk of bias, a score between 0 and 4 indicated a medium risk of bias and scores of ≥ 5 indicated a low risk of bias. Vitamin D-specific issues are summarised below:

- How is 'vitamin D' assessed (dietary intake, supplement use, blood levels of 25(OH)D, blood levels of 1,25(OH)D, PTH concentration)?
- Are season and sunlight exposures including sunscreen use and skin covering considered?
- Are ethnicity and skin pigmentation considered?
- How is 25(OH)D blood level assessed?
- What assay is used?
- Are D_2 and D_3 forms adequately measured and are quality data (e.g. DEQAS) given?
- What definition of 'normal range' for 25(OH)D is used?
- Is the concentration treated as categorical (e.g. deficient, insufficient, replete) or continuous?
- Has infant postnatal vitamin D intake (breast, bottle feeding, supplementation) and sunlight exposure been considered?
- Has maternal compliance with supplementation been assessed?

Synthesis of extracted evidence

The aim of this part of the review was to investigate whether or not effects were consistent across studies and to explore reasons for apparent differences. We used both descriptive (qualitative) and quantitative synthesis; our capacity for the latter was determined by the evidence available. Where meta-analysis was possible, we used standard analytical procedures.[79] Only independent studies were meta-analysed. Thus, where a study contained two treatment arms, these were not included in the same analysis.

It was, therefore, not possible to include all treatment arms from all RCTs in the same analysis. Two main approaches were employed: first, a meta-analysis of low-dose studies (total dose < 120,000 IU vitamin D), including relevant single treatment arm studies, and the low-dose and placebo arms of studies with more than one treatment arm; and, second, a similar approach but including those studies/study arms with high dose (total > 120,000 IU). Inevitably, the observed estimates of the effects reported in the studies included in the meta-analysis varied. Some of this variation is due to chance alone, as no study can be large enough to completely remove the random error. However, the reported effects may also vary due not only to chance but also to methodological differences between studies. This variation between studies defines statistical heterogeneity. Statistical analysis was performed using Stata v12.1 (StataCorp LP, College Station TX, USA). Between-study statistical heterogeneity was assessed by *Q*-statistic and quantified by I^2 test;[80,81] values of I^2 index of 25%, 50% and 75% indicated the presence of low, moderate and high between-trials heterogeneity, respectively, while a *p*-value of < 0.10 was considered to denote statistical significance of heterogeneity. Differences in mean birthweight and serum calcium between supplemented and unsupplemented groups in RCTs were analysed using weighted mean difference and 95% CIs. Results from observational studies were also synthesised. Pooled regression coefficients and odds ratios (ORs) and the 95% CIs were calculated for continuous and dichotomous outcomes respectively. For all analyses performed, if no significant heterogeneity was noted, fixed-effect model analysis using the Mantel–Haenszel method was presented; otherwise, results of the random-effects model (REM) analysis using the DerSimonian–Laird method were presented.[82]

Studies included in the review

A total of 22,961 citations were identified from the initial database search up to 3 January 2011. A subsequent additional database search from 3 January 2011 to 18 June 2012 identified another 2448 citations, yielding a total of 25,409 citations. A further 66 citations were identified from other sources (e.g. grey literature, bibliographies). After duplicate citations were removed, 16,842 citations were screened. Of these, 16,669 were excluded on the basis of the content of the title and/or the abstract (if available). A further eight papers could not be found despite thorough searching; thus, 16,677 records were excluded. A total of 165 full-text articles were retrieved for detailed assessment and, of these, 76 papers were included in the review. A flow diagram of this selection process is included in *Appendix 4*.

Studies excluded from the review

A total of 89 papers retrieved for assessment were excluded. Around one-third of these ($n = 34$) were abstracts. Twenty-one papers had no relevant maternal or offspring outcome; 11 papers had no estimate of maternal vitamin D status; 10 papers used data from other papers included in the review; eight papers were either review articles, letters, editorials or commentaries with no new results; one paper was of a non-human study; and four papers reported on an outcome not assessed in any other paper (maternal breast cancer, offspring schizophrenia, offspring multiple sclerosis and offspring influenza A).

Quality assessment of included studies

Summary tables of the quality assessment scores for each included study can be found in *Appendix 5*. Studies are divided according to design (case–control, cohort, cross-sectional, intervention study) and listed in alphabetical order of first author.

Chapter 5 Results of the review

The majority of the results relate to study questions 2 and 3 [What adverse maternal and neonatal health outcomes are associated with low maternal circulating 25(OH)D?; Does maternal supplementation with vitamin D in pregnancy lead to an improvement in these outcomes (including assessment of compliance and effectiveness)?]. These are presented in detail below. Significant associations between maternal vitamin D and outcomes are described as either positive or negative. Effect sizes, if available from the original paper, are presented in the supplementary tables for each outcome (see *Appendix 6*, *Tables 8–31*). Very few studies were identified which could directly inform the other questions. These are discussed in *Chapter 6, Summary discussion*.

Offspring birthweight

Observational studies (see Appendix 6, Table 8*)*

Nineteen observational studies[24,40,41,83–98] linking maternal vitamin D status to offspring birthweight were identified. These were all of either cross-sectional ($n = 5$) or cohort ($n = 14$) design. Maternal vitamin D status was assessed by maternal serum 25(OH)D concentration in 14 studies, dietary intake in four studies and ambient UVB radiation during the last trimester of pregnancy in one. Sample sizes ranged from 84 to 13,904. Few studies considered all confounding factors of relevance to the review question. Composite bias scores ranged from –2 to +8, with 7 of the 19 studies scored as having a low risk of bias. Of the 14 studies relating maternal serum 25(OH)D concentration to offspring birthweight, only three studies[83–85] demonstrated a significant positive association; one study[89] found a significant negative association. In contrast, three[86–88] of the four studies assessing the influence of maternal vitamin D intake during pregnancy on offspring birthweight found a significant positive association. One study[41] found no significant association between ambient UVB exposure in pregnancy and offspring birthweight.

Armirlak *et al.*[83] (composite bias score 2, medium risk) found a positive association between maternal 25(OH)D at delivery and offspring birthweight in a cross-sectional study of 84 healthy Arab and South Asian women with uncomplicated deliveries. Maternal 25(OH)D was generally low, with a mean of 18.5 nmol/l. A large Australian study (Bowyer *et al.*,[84] composite bias score 4, medium risk) of 971 pregnant women found that offspring birthweight was significantly lower in those women with 25(OH)D deficiency (< 25 nmol/l) even after adjusting for gestational age, maternal age and overseas maternal birthplace. Similarly, the Amsterdam Born Children and their Development (ABCD) study (Leffelaar *et al.*,[85] composite bias score 4, medium risk) incorporated 3730 pregnant women and found that early pregnancy maternal 25(OH)D < 30 nmol/l was significantly associated with a lower offspring birthweight, even after adjusting for multiple confounding factors. However, when serum 25(OH)D was analysed as a continuous variable a significant association with birthweight was no longer seen. Mannion *et al.*[86] (Canada, composite bias score 1, medium risk), Scholl and Chen[87] (USA, composite bias score 2, medium risk) and Watson and McDonald[88] (New Zealand, composite bias score 3, medium risk) attempted to assess maternal vitamin D intake during pregnancy via Food Frequency Questionnaires (FFQs) at various stages of gestation. Mannion *et al.*[86] and Scholl and Chen[87] found that maternal vitamin D intake was positively associated with offspring birthweight. Similar findings were made by Watson and McDonald[88] assessing maternal vitamin D intake at 4 months; however, a relationship was no longer observed when maternal vitamin D intake was measured again at 7 months.

Only one study found a negative association between offspring birthweight and maternal 25(OH)D. Weiler *et al.*[89] (composite bias score 3, medium risk) found that offspring birthweight was significantly lower in women with adequate vitamin D status [defined by the study group as 25(OH)D ≥ 37.5 nmol/l]. However, the number of participants in this study was low overall and only 18 women had 25(OH)D < 37.5 nmol/l. In addition, of those women with serum 25(OH)D concentration < 37.5 nmol/l, a significantly higher

percentage were of non-white race (67%) compared with those with an adequate concentration of 25(OH)D (25%).

Twelve observational studies reported no significant association between maternal vitamin D status and offspring birthweight. Four of these studies were from Asia (Ardawi *et al.*,[90] Sabour *et al.*,[91] Maghbooli *et al.*[92] and Farrant *et al.*[93]), three from the UK (Gale *et al.*,[24] Harvey *et al.*[40] and Sayers and Tobias[41]), two from Australia (Morley *et al.*[94] and Clifton-Bligh *et al.*[95]), one from the USA (Dror *et al.*[96]), one from Finland (Viljakainen *et al.*[97]) and one from Africa (Prentice *et al.*[98]). Ten studies[24,40,90,92–97] had measured maternal 25(OH)D during pregnancy or at delivery, one[91] had assessed vitamin D intake during pregnancy and the largest study[41] of 13,904 pregnant women had assessed maternal UV sun exposure in the last trimester as a proxy measure of vitamin D status.

Evidence synthesis

Results from studies that analysed log-transformed vitamin D were synthesised separately from results of studies that analysed vitamin D in its original units. The studies included in the first meta-analytic model were Harvey *et al.*,[40] Gale *et al.*[24] and Farrant *et al.*,[93] using log-transformed units. The combined estimate of the unadjusted regression coefficients for changes in birthweight (grams) per 10% increase in vitamin D was positive but did not reach statistical significance (pooled regression coefficient 0.47, 95% CI −3.12 to 4.05; see *Appendix 7*, *Figure 2*). In contrast, when adjusted estimates were synthesised (with adjustments being gestational age, maternal age, maternal BMI, ethnicity and parity where possible), there were significant differences in birthweight (grams) for each 10% increase in vitamin D (pooled regression coefficient 5.63, 95% CI 1.11 to 10.16; see *Appendix 7*, *Figure 3*). Amirlak *et al.*,[83] Prentice *et al.*,[98] Leffelaar *et al.*[85] and Dror *et al.*[96] analysed vitamin D in its original units. All four studies provided adjusted estimates, and all but Amirlak also provided unadjusted regression coefficients. No significant differences in birthweight (grams) per 25 nmol/l increase in vitamin D were found in either combined unadjusted associations (pooled regression coefficient 0.47, 95% CI −1.14 to 2.09; see *Appendix 7*, *Figure 4*) or combined adjusted (as per paper) associations (pooled regression coefficient 0.12, 95% CI −1.84 to 2.08; see *Appendix 7*, *Figure 5*).

Intervention studies (see Appendix 6, Table 9*)*

Nine intervention trials[3–7,21,99–101] were identified, only two[99,100] of which were carried out in the last 20 years and the earliest of which was from 1980.[3] Sample sizes ranged from 40 to 350. Seven of these studies were rated as having a high chance of bias on the composite score (−2 to −9); only the most recent studies by Yu *et al.*[99] and Hollis *et al.*[100] were assessed as having a low risk of bias (composite bias score 5 and 10 respectively). Eight studies[3–7,99–101] reported randomisation, although only one study (Brooke *et al.*[3]) was of a double-blind design and this was also the only study that was placebo-controlled. In eight[3–7,21,99,101] of the studies, intervention took place in the last trimester of pregnancy; one study[101] intervened in months 6 and 7 of pregnancy and one study[100] supplemented from weeks 12–16 onwards. Interventions were highly variable, including 1000 IU daily of ergocalciferol, two doses of 60,000 IU cholecalciferol, two doses of 600,000 IU cholecalciferol, a single oral dose of 200,000 IU and 1200 IU cholecalciferol in combination with 375 mg calcium daily. Change in maternal serum 25(OH)D concentration before and after supplementation was given in three studies only. Three[4,5,101] of the eight studies (all from India) demonstrated a statistically significantly greater birthweight in offspring of supplemented than unsupplemented mothers. The remainder showed no difference in infant birthweight regardless of supplementation.[3,6,7,21,99,100]

Two Indian studies, both by Marya *et al.*[4,5] (composite bias scores −6 and −2, respectively, high risk), demonstrated significantly higher birthweights in infants born to women supplemented with high-dose cholecalciferol (given as two doses of 600,000 IU in months 7 and 8 of gestation). The earlier of these studies also had a third arm of women supplemented with 1200 IU vitamin D plus 375 mg calcium throughout the third trimester of pregnancy. Birthweights of infants in this group were also significantly higher than in the unsupplemented group, but not by as much as in the high-dose supplement group. The third study reporting a positive association between maternal vitamin D supplementation and offspring

birthweight was also from India (Kaur *et al.*,[101] composite bias score −7, high risk). Again, significantly higher infant birthweight was found in the supplemented group (two doses of 60,000 IU cholecalciferol in months 6 and 7) than in the unsupplemented group, although the number of participants in this study was low ($n = 25$ in each arm). Of note, none of the three studies measured maternal 25(OH)D at any point during pregnancy, and all were assessed to have a high risk of bias.

Three UK studies had investigated the effect on offspring birthweight of maternal vitamin D supplementation in the third trimester of pregnancy. Brooke *et al.*[3] (composite bias score −2, high risk) and Congdon *et al.*[21] (composite bias score −9, high risk) recruited only Asian women residing in the UK, whereas Yu *et al.*[99] (composite bias score 5, low risk) included equal numbers of four ethnic groups (black, Caucasian, Asian, Middle Eastern). None of the studies reported a significant difference in offspring birthweight between the supplemented and unsupplemented groups, even despite Brooke *et al.*[3] demonstrating significantly higher maternal 25(OH)D concentrations in the supplemented group at term. Two studies, both from France (Delvin *et al.*,[6] composite bias score −2, high risk; Mallet *et al.*,[7] composite bias score −3, high risk), also failed to demonstrate a significant difference in offspring birthweight with maternal vitamin D supplementation. The most recent, and largest, study (Hollis *et al.*,[100] composite bias score 10, low bias risk) randomised 350 pregnant women residing in the USA to either 400 IU per day, 2000 IU per day or 4000 IU per day of oral vitamin D_3 from 12 to 16 weeks' gestation until delivery. Although maternal serum 25(OH)D at delivery was higher in those women receiving the higher dose supplement regimes, there was no significant difference in offspring birthweight among the three groups.

Evidence synthesis

Two meta-analyses were performed to combine the published evidence of an effect of vitamin D supplementation on birthweight. The first included Brooke *et al.*,[3] Marya *et al.*[4] (low dose of vitamin D), Congdon *et al.*,[21] Mallet *et al.*[7] (low dose of vitamin D) and Kaur *et al.*[101] (see *Appendix 7*, *Figure 6*). Owing to statistically significant heterogeneity in the results ($I^2 = 86.3\%$, $p < 0.001$), a REM was fitted. The combined estimate showed a non-significant difference in birthweight between the unsupplemented and supplemented groups (mean weighted difference 116.23 g, 95% CI −57.0 g to 289.5 g). The second meta-analytical model included Brooke *et al.*,[3] Marya *et al.*[4] (high dose of vitamin D), Congdon *et al.*,[21] Mallet *et al.*[7] (high dose of vitamin D), Marya *et al.*[5] and Kaur *et al.*[101] (see *Appendix 7*, *Figure 7*). Again, here, owing to statistically significant heterogeneity ($I^2 = 96\%$, $p < 0.001$), a REM was fitted and the combined results did not show a significant difference in birthweight between the supplemented and the non-supplemented groups (mean weighted difference 147.3 g, 95% CI −112.5 g to 407.15 g).

Discussion

The results of the included studies were conflicting, with some demonstrating positive associations between 25(OH)D concentration and birthweight and some no relationship. The observation studies were, on the whole, of greater quality than the intervention studies, with almost all of the latter assessed as having a high risk of bias. Meta-analysis revealed weak positive associations across three observational studies, after adjustment for potential confounders, between log-transformed 25(OH)D concentrations and offspring birthweight. However, confounding factors considered varied across the studies, and the potential for residual confounding is large. Despite these caveats, the relationships were generally positive, albeit not statistically significant, across the majority of identified studies, suggesting that further exploration in a well-designed, randomised, placebo-controlled, double-blind trial might be appropriate.

Offspring birth length

Observational studies (see Appendix 6, Table 10*)*

Thirteen observational studies[24,41,85,86,90–98] including maternal vitamin D status and offspring birth length were identified; nine of the these were cohort in design, with the remaining three being cross-sectional studies. The number of participants in each study ranged from 120 to 10,584. Maternal vitamin D status was

assessed by serum 25(OH)D concentration in 10 studies[24,85,90,92,93–98] and by dietary intake in two;[86,91] in the remaining study[41] maternal ambient UVB exposure during late pregnancy was used as a surrogate marker of vitamin D status. One study[91] was assessed as having a high risk of bias (composite score −2, high risk), with the others demonstrating composite scores between +1 and +8. Consideration of potential confounding factors was variable. Two studies[41,91] identified a positive relationship between maternal vitamin D status and offspring birth length, neither of which directly measured maternal 25(OH)D. The remaining 10 studies[24,85,90,92–98] showed no relationship. We did not identify any studies that demonstrated an inverse relationship between maternal vitamin D status in pregnancy and offspring birth length.

Sabour *et al.*[91] (composite bias score −2, high risk), in a cross-sectional study of 449 pregnant women in Iran, found that offspring birth length was significantly higher in mothers with adequate vitamin D intake (defined by the authors as > 200 IU vitamin D per day). This study was assessed to have a high risk of bias and maternal serum 25(OH)D was not measured, as vitamin D status was estimated from a FFQ of dietary intake. The second study showing a positive relationship came from Sayers and Tobias[41] (composite bias score 3, medium risk) using data from the large UK cohort (ALSPAC). In this study, again, maternal serum 25(OH)D was not directly measured but estimated using maternal UVB exposure in the last 98 days before birth as a surrogate. Maternal UVB exposure in late pregnancy was positively associated with offspring birth length. Additionally, Leffelaar *et al.*[85] measured offspring length at 1 month and found that infants born to mothers with 25(OH)D < 30 nmol/l (the threshold used by the authors for vitamin D deficiency) had a significantly lower length at 1 month even after adjusting for multiple confounders (including gestational age, season of blood sample, maternal height, maternal age, smoking pre-pregnancy, smoking in pregnancy, educational level, ethnicity and parity).

The remaining 10 studies[24,86,90,92–98] found no significant relationship between maternal vitamin D status and offspring birth length. Of these studies, nine used maternal 25(OH)D as the predictor and six were assessed to have a low risk of bias. Two studies were from the Middle East (Ardawi *et al.*,[90] composite bias score 5, low risk; Maghbooli *et al.*,[92] composite bias score 1, medium risk), two from Australia (Morley *et al.*,[94] composite bias score 8, low risk; Clifton-Bligh *et al.*,[95] composite bias score 6, low risk), two from North America (Mannion *et al.*,[86] composite bias score 1, medium risk; Dror *et al.*,[96] composite bias score 7, low risk) and the remainder from the UK (Gale *et al.*,[24] composite bias score 4, medium risk), Finland (Viljakainen *et al.*,[97] composite bias score 3, medium risk), India (Farrant *et al.*,[93] composite bias score 5, low risk) and Africa (Prentice *et al.*,[98] composite bias score 5, low risk).

Intervention studies (see Appendix 6, Table 11*)*

Two RCTs of vitamin D supplementation in pregnancy included birth length as an outcome; both were assessed to have a high risk of bias (composite bias score of both −2, high risk). A double-blind placebo-controlled trial (Brooke *et al.*[3]) found no significant difference in offspring birth length in UK Asian women supplemented with 1000 IU ergocalciferol per day in the last trimester compared with the control group. In contrast, a larger Indian study by Marya *et al.*[5] found that birth length was significantly higher in women supplemented with a much higher dose of vitamin D (two doses of 600,000 IU cholecalciferol in the seventh and eighth month of gestation) than in unsupplemented women.

Discussion

Again, the majority of the observational studies suggested no relationship between maternal 25(OH)D status and offspring birth length. One[41] of the studies which showed a significant association was large and prospective, but used ambient UVB radiation rather than a direct measure of vitamin D status. Of the two randomised trials[3,5] to investigate birth length, one found a statistically significant relationship and the other did not. Thus, the results are mixed but do not support the use of maternal vitamin D supplementation to reduce the risk of low birth length.

Offspring head circumference

Observational studies (see Appendix 6, Table 12*)*

Eleven observational studies[24,86,90–98] assessed the relationship between maternal vitamin D status in pregnancy and offspring head circumference. Eight[24,86,90,93–95,97,98] of the studies were of cohort design, with the remaining three[91,92,96] being cross-sectional studies. Participant numbers ranged from 120 to 559. Maternal vitamin D status was assessed by serum 25(OH)D concentration in nine studies;[24,90,92–98] the remainder used dietary intake (Sabour *et al.*[91] and Mannion *et al.*[86]). Composite bias scores ranged from −2 to +8, with six studies[90,93–96,98] having a low risk of bias. Of those relating maternal serum 25(OH)D to offspring head circumference at birth, no study found a statistically significant relationship, regardless of when during pregnancy 25(OH)D was measured.

Three studies were from the Middle East: Ardawi *et al.*[90] and Maghbooli *et al.*[92] found no association with offspring head circumference at birth and maternal 25(OH)D measured at delivery. Likewise, Sabour *et al.*[91] observed no difference in offspring head circumference in women taking < 200 IU vitamin D per day compared with those taking > 200 IU vitamin D per day. Two Australian studies (Morley *et al.*[94] and Clifton-Bligh *et al.*[95]) measured maternal vitamin 25(OH)D in the third trimester of pregnancy and also found no significant association between maternal 25(OH)D concentration and offspring head circumference. Morley *et al.*[94] also measured 25(OH)D in early pregnancy and again a relationship was not demonstrated. Similar findings were made by Mannion *et al.*[86] (a Canadian study using estimated dietary intake of vitamin D in pregnancy as the predictor), Gale *et al.*[24] [UK, 25(OH)D measured in the third trimester], Farrant *et al.*[93] [India, 25(OH)D measured in the third trimester], Prentice *et al.*[98] [The Gambia, Africa, 25(OH)D measured in the second and third trimesters], Viljakainen *et al.*[97] [Finland, mean of early pregnancy and postpartum 25(OH)D concentration used] and Dror *et al.*[96] (USA, measured perinatally).

Intervention studies (see Appendix 6, Table 13*)*

Offspring head circumference at birth was an outcome in two RCTs[3,5] of vitamin D supplementation in pregnancy, both of which were assessed as having a high risk of bias (composite bias score −2 in both). Brooke *et al.*[3] included 126 Asian patients and randomised in a double-blind fashion to either placebo or 1000 IU daily ergocalciferol in the last trimester. Head circumference did not differ between the treatment and placebo groups. In contrast, Marya *et al.*[5] randomised 200 Indian women to either no supplement or to two doses of 600,000 IU cholecalciferol in the last trimester and found that head circumference at birth was significantly higher in the supplemented group than in the unsupplemented group.

Discussion

Thus, the majority of the observational studies demonstrated no association between maternal 25(OH)D status in pregnancy and offspring head circumference at birth. One[5] of the intervention studies found a positive relationship between supplement use and head circumference. It should be noted that this study generally found statistically significant relationships for most of the measured outcomes and was considered to be of high risk of bias. The evidence base is insufficient to recommend vitamin D supplementation for the optimisation of, or prevention of, low head circumference.

Offspring bone mass

Observational studies (see Appendix 6, Table 14*)*

Eight observational studies[1,41,89,96–98,102,103] that included offspring bone mass outcomes were identified. Five of these were cohort studies, with the remaining three being cross-sectional in design. All studies were assessed as being of medium to low risk of bias, with composite bias scores ranging from 3 to 7. The age at which offspring were assessed ranged from within 24 hours of birth to 9.9 years. Bone outcome measures also varied across the studies and included whole-body, lumbar spine, radial midshaft, tibial and femoral BMC, whole-body and lumbar spine BA, whole-body and tibial bone mineral density (BMD), tibial cross-sectional area (CSA) and whole-body BMC adjusted for BA (areal bone mineral density;

aBMD). Most studies (six[1,41,89,96,98,103] of eight) used DEXA to assess bone mass; two studies[97,102] used peripheral quantitative computed tomography (pQCT) and one study[98] used SPA in addition to DEXA. Seven studies[1,89,96–98,102,103] measured maternal 25(OH)D during pregnancy or at delivery, one study[41] used UVB exposure in the third trimester of pregnancy as a measure of maternal vitamin D status. Five studies[1,41,89,97,102] demonstrated a positive relationship between maternal vitamin D status and offspring bone health; three studies[96,98,103] showed no relationship.

Weiler *et al.*[89] (composite bias score 3, medium risk, $n = 50$) found that neonates born to mothers with adequate maternal 25(OH)D at delivery (defined by the authors as > 37.5 nmol/l) had significantly higher whole-body and femoral BMC per unit body weight than neonates born to mothers with insufficient maternal vitamin D concentration (< 37.5 nmol/l), even after adjustment for multiple confounders. However, there was no significant difference in infant lumbar spine, femoral or whole-body BMC between the two groups. Viljakainen *et al.*[97] (composite bias score 3, medium risk) also measured neonatal bone mass in a Finnish cohort of 125 primiparous Caucasian women. Tibial bone mass was assessed by pQCT and those with maternal 25(OH)D above the median (42.6 nmol/l) had significantly higher tibial BMC and CSA than those with maternal 25(OH)D below the median, even after adjusting for confounders including maternal height and birthweight. No relationship was seen between maternal 25(OH)D and tibial BMD. A subsample of 55 children was also assessed again at 14 months (Viljakainen *et al.*[102]) and tibial BMC was no longer significantly different by maternal 25(OH)D status. Tibial CSA, however, remained significantly lower in those with maternal 25(OH)D below the median. Two cohort studies from the UK also demonstrated significant associations between maternal vitamin D status and offspring bone mass measured later in childhood. Javaid *et al.*[1] measured maternal 25(OH)D in late pregnancy and offspring bone mass by DEXA at mean 8.9 years in a cohort of 198 pregnant women. Positive associations were observed between maternal 25(OH)D and offspring whole-body and lumbar spine BMC, lumbar spine BA and whole-body and lumbar spine BMD after adjustments were made for offspring gestational age at delivery and offspring age at DEXA. Sayers and Tobias[41] found that maternal UVB exposure in late pregnancy was positively associated with offspring BMC, BA and BMD in 6955 children at mean age 9.9 years. No relationship was seen between aBMD and maternal UVB exposure.

Three studies found no associations between maternal 25(OH)D and offspring bone mass. Two studies (Akcakus *et al.*[103] and Dror *et al.*[96]), both cross-sectional in design, and with a similar number of participants, measured maternal 25(OH)D at delivery and used DEXA to assess offspring bone mass up to the first month of life. A third study (Prentice *et al.*[98]) measured mid- and late-pregnancy 25(OH)D in a cohort of 125 pregnant Gambian women taking part in a larger clinical trial of vitamin supplementation. Offspring underwent assessment of BMC and BA using SPA of the midshaft radius; a subset also underwent whole-body DEXA at ages 2, 13 and 52 weeks. Again, no statistically significant relationship between maternal 24(OH)D and offspring BMC at any time point was observed. It should be noted that mean maternal 25(OH)D levels in this cohort were much higher than any other study with an average of 103 nmol/l for mid-pregnancy and 111 nmol/l for late pregnancy, and none of the women in the study was considered vitamin D deficient.

Intervention studies (see Appendix 6, Table 15*)*

One clinical trial of maternal vitamin D supplementation and its effect on offspring bone mass was identified. Congdon *et al.*[21] randomised 64 Asian women in the UK to either no supplement or 1000 IU vitamin D plus calcium daily in the third trimester. Forearm BMC was measured in offspring within 5 days of birth, although the type of equipment used to measure this was not recorded. No difference in offspring radial BMC was observed between the two groups. This study was assessed to have a high risk of bias (composite bias score –9) and maternal serum vitamin D concentration in pregnancy was not recorded at any time point.

Discussion

Five[1,41,89,97,102] of the eight observational studies relating maternal 25(OH)D status to offspring bone outcomes demonstrated positive associations. The one small intervention study[21] identified did not, but the methodology is unclear and a statistically significant result is unlikely based on the sample size. Thus, observational studies suggest that maternal 25(OH)D status may influence offspring bone development, but do not allow public health recommendations to be made. Further high-quality intervention studies are required here, such as the ongoing Maternal Vitamin D Osteoporosis Study (MAVIDOS).[104]

Offspring anthropometric and body composition measures

Observational studies (see Appendix 6, Table 16*)*

Six observational studies[24,41,89,94,105,106] (five cohort and one cross-sectional) have examined the relationships between maternal vitamin D status and a variety of anthropometric measures in the offspring. Composite bias scores ranged from 3 to 8, indicating a medium to low risk of bias. Five studies[24,89,94,105,106] had measured maternal serum 25(OH)D in pregnancy (four in the third trimester and one at delivery); one study[41] used maternal UVB exposure during the last trimester of pregnancy as a surrogate estimate of maternal vitamin D status. Anthropometric measurements of the offspring ranged across the studies and included skinfold thickness, limb circumference and muscle area. Five studies[24,41,89,105,106] used DEXA to measure offspring fat and/or lean mass. Four studies[41,94,105,106] demonstrated a significant relationship between offspring anthropometry and maternal 25(OH)D; the remaining two[24,89] showed no relationship.

Morley *et al.*[94] measured offspring subscapular, triceps and suprailiac skinfold thickness using Harpenden callipers (British Indicators, Burgess Hill, UK), along with mid-upper-arm and calf circumferences using measuring tape in 374 Australian neonates. Although there no was significant association between maternal 25(OH)D at 11 weeks' gestation and any of the neonatal outcome measures, a weak inverse association was observed between maternal 25(OH)D measured at 28–32 weeks and neonatal subscapular and triceps skinfold thickness. This association was weakened further but still remained statistically significant after adjustments were made for offspring sex, maternal height, whether or not the offspring was a first child, maternal smoking and season of blood sample. No significant association with maternal 25(OH)D was found with the other offspring anthropometric outcomes assessed. Krishnaveni *et al.*[105] also assessed offspring subscapular and triceps skinfolds, using callipers, in addition to arm muscle area, waist circumference, fat mass, per cent body fat, fat-free mass and per cent fat-free mass, using a combination of measuring tape and bioimpedence, in an older cohort of Indian children aged 5 years ($n = 506$) and again at age 9.5 years ($n = 469$). Children born to mothers with late-pregnancy vitamin D deficiency [25(OH)D concentration < 50 nmol/l] had significantly reduced arm–muscle area in comparison with children born to mothers with adequate levels. No significant relationship was observed with the other anthropometric measurements at either time point.

Of the four studies using DEXA to measure offspring fat and/or lean mass, two reported no relationship with maternal vitamin D status. Weiler *et al.*[89] used DEXA to measure whole-body fat in a group of 50 neonates in Canada. No significant difference was observed between those born to mothers with 25(OH)D concentration < 37.5 nmol/l at delivery and those born to mothers with 25(OH)D > 37.5 nmol/l. Gale *et al.*[24] found no significant association between maternal 25(OH)D in late pregnancy and offspring fat mass or lean mass in 178 UK children aged 9 years. Fat and lean mass tended to be lower in children born to mothers in the lowest quarter of 25(OH)D distribution, but this did not achieve significance. In contrast, Sayers and Tobias[41] using maternal UVB exposure in late pregnancy as a surrogate measure for vitamin D status found that offspring lean mass at mean age 9.9 years was positively associated with maternal UVB exposure. However, no significant association was seen with fat mass. In contrast, Crozier *et al.*[106] (composite bias score 8, low risk) found that maternal serum 25(OH)D in late pregnancy was positively associated with offspring fat mass at birth, measured by DEXA, after adjusting for confounders. Interestingly, no significant relationship was seen between maternal 25(OH)D and offspring

fat mass at 4 years, and a negative relationship was seen at 6 years of age. No significant relationship was observed between maternal 25(OH)D and offspring's fat-free mass at any time point.

Intervention studies (see Appendix 6, Table 17*)*

Two intervention studies were identified and have been described earlier. Both studies were assessed to have a high risk of bias (composite bias score −2 for both). Brooke *et al.*[3] found no difference in neonatal triceps skinfold thickness or forearm length between those born to supplemented mothers and placebo group mothers. Marya *et al.*[5] found significantly greater mid-upper-arm circumference and triceps and subscapular skinfold thicknesses in neonates of supplemented than in those born to unsupplemented mothers (all $p < 0.01$).

Discussion

The identified observational studies demonstrated a variety of modest relationships between maternal 25(OH)D status and offspring anthropometric measures, with some finding positive relationships between maternal 25(OH)D status and measures of offspring muscle and fat mass. Consistent with other anthropometric outcomes in their study, Marya *et al.* found greater skinfold thicknesses in the supplemented group than in the unsupplemented group. The evidence base is therefore insufficient to warrant recommendation of maternal vitamin D supplementation to optimise childhood anthropometric measures.

Offspring asthma and atopy

Observational studies (see Appendix 6, Table 18*)*

Ten studies[24,26,107–114] were identified that examined the relationships between maternal vitamin D intake during pregnancy, maternal serum 25(OH)D level in pregnancy, or cord blood 25(OH)D concentration and markers of atopy in the offspring. These were all observational cohort studies, ranging in size from 178 to 1724 mother–child pairs. Eight studies[24,26,107–112] reported the outcome wheeze or asthma as determined by parental questionnaires at between 16 months and 9 years of age.

Four of these seven studies used maternal vitamin D intake during pregnancy as the exposure and had composite bias scores of between −1 and 2 (Erkkola *et al.*,[107] Devereux *et al.*,[26] Miyake *et al.*[108] and Camargo *et al.*[109]). These four studies all reported a lower risk of wheeze in offspring of mothers with higher vitamin D intakes during pregnancy, although the definitions used for wheeze varied between studies. Miyake *et al.*[108] included 763 mother–offspring pairs in a prospective cohort study in Osaka, Japan (bias score −1, high risk). Vitamin D intake was measured by FFQs between 5 and 39 weeks of pregnancy and the children followed up between 16 and 24 months of age using the International Study of Asthma and Allergy in Childhood (ISAAC) questionnaire. In this study, consumption of ≥ 172 IU per day vitamin D was associated with a reduced risk of both wheeze and eczema. Camargo *et al.*[109] reported in a prospective cohort study in Massachusetts, USA, which included 1194 mother–offspring pairs, that children born to mothers in vitamin D intake quarters 2 (446–562 IU/day), 3 (563–658 IU/day) and 4 (659–1145 IU/day) had a reduced risk of recurrent wheeze (two or more episodes of wheeze in children with a personal diagnosis of eczema or parental history of asthma) at 3 years compared with those born to mothers in the lowest quarter of vitamin D intake, but, in contrast to Miyake *et al.*,[108] there was no difference in the incidence of eczema. Erkkola *et al.*[107] found a lower risk of persistent asthma (physician diagnosis and a requirement for asthma medication in the preceding 12 months) at 5 years in children born to mothers with higher vitamin D intake, but, similarly to Camargo *et al.*,[109] there was no reduced risk of atopic eczema. However, this Finnish study included only children who had human leucocyte antigen (HLA) *HLA-DQB1*-conferred susceptibility to type 1 diabetes mellitus. The composite bias score was −1, indicating a high risk of bias. Finally, Devereux *et al.*[26] also reported a lowered risk of reported wheeze in the preceding year in 5-year-old children born to mothers with the highest quintile of vitamin D intake at 32 weeks' gestation (189–751 IU/day) compared with the lowest quintile (46–92 IU/day). There was no

statistically significant reduction in the OR for wheeze when quintiles 2, 3 and 4 were compared with quintile 1 but a significant overall trend ($p = 0.009$).

Two studies assessed the associations between cord blood 25(OH)D and parental report of wheeze and/or asthma. These studies had composite bias scores of 2 and 3 (medium risk of bias). Camargo *et al.*[110] found that in 823 children in New Zealand the OR for wheeze at 5 years of age decreased across categories of cord 25(OH)D, but there was no association with incident asthma. Similarly, Rothers *et al.*[111] found no association between cord 25(OH)D and asthma (physician diagnosed and medication requirement in preceding year) at 5 years. Two studies, by Gale *et al.*[24] and Morales *et al.*,[112] assessed the association between maternal 25(OH)D measured in pregnancy and parental-reported wheeze or diagnosis of asthma. Gale *et al.*[24] (composite bias score 4, medium bias risk) assessed the association between maternal 25(OH)D in late pregnancy and parental report of asthma in 178 children. Exposure to the highest quarter of maternal concentrations of 25(OH)D was associated with an increased risk of reported asthma at age 9 years compared with children whose maternal 25(OH)D concentration had been in the lowest quarter of the distribution. In addition, the risk of offspring eczema at 9 months (assessed by either physical examination or parental report) was also higher in children in the highest quarter of maternal 25(OH)D distribution than in those in the bottom quarter. By 9 years of age, however, although offspring in the highest quarter of maternal 25(OH)D still tended to have a higher risk of reported eczema than those in the lowest quarter, the difference was no longer significant. In this study, the number of cases of asthma or eczema per maternal 25(OH)D quarter was low, ranging from 2 to 15. Conversely, Morales *et al.*[112] (composite bias score 3, medium bias risk) found no significant association between maternal 25(OH)D measured at mean (standard deviation; SD) 12.6 (2.5) weeks and parent-reported offspring wheeze at 1 year or 4 years, or asthma (defined as parental report of doctor diagnosis of asthma or receiving treatment for asthma) at age 4–6 years.

Four studies[26,111,113,114] utilised other outcome markers of asthma and/or atopic disease; these studies were subject to less potential bias (composite bias scores −1 to 3). Two studies[26,113] measured offspring spirometry: Cremers *et al.*[113] (bias score 3, medium risk) found no associations between maternal plasma 25(OH)D at 36 weeks' gestation and offspring forced expiratory volume in 1 second (FEV_1) ($p = 0.99$) or forced vital capacity (FVC) ($p = 0.59$) at 6–7 years in 415 mother–offspring pairs. Similarly, Devereux *et al.*[26] (bias score −1, high risk) did not identify any differences in lung function at 5 years of age across quintiles of maternal vitamin D intake at 32 weeks' gestation. Two studies also undertook skin prick testing as a measure of atopic sensitisation. Devereux *et al.*[26] found that maternal vitamin D intake at 32 weeks' gestation was not associated with differences in atopic sensitisation to cat, timothy grass, egg or house dust mite at 5 years of age. Conversely, Rothers *et al.*[111] (bias score 2, medium risk) found that children with cord blood 25(OH)D ≥ 100 nmol/l, when compared with those with cord 25(OH)D 50–74.9 nmol/l, had a greater risk of a positive response to a skin prick testing battery that included 17 aeroallergens common to the geographical area. Finally, two studies included offspring IgE concentration as a measure of atopy. Rothers *et al.*[111] reported a non–linear relationship between cord 25(OH)D and total and allergen-specific IgE for six inhalant allergens. The highest levels of IgE were identified in children with cord 25(OH)D concentration < 50 nmol/l and ≥ 100 nmol/l. Conversely, Nwaru *et al.*[114] found increasing maternal vitamin D intake determined by FFQ was inversely associated with sensitisation (IgE > 0.35 ku/l) to food allergens (IgE > 0.35 ku/l) but not inhaled allergens at 5 years of age.

Intervention studies

No intervention studies examining the influence of vitamin D supplementation in pregnancy on offspring risk of asthma or atopy were identified.

Discussion

The studies on asthma were all observational; no intervention studies were identified. The investigations were marked by substantial heterogeneity in terms of study design, outcome definition and exposure definition, and gave a variety of conflicting results. It is difficult to conclude any definitive relationship between maternal 25(OH)D status and offspring asthma and no recommendation can be made.

Further high-quality intervention studies are required here, such as the ongoing Vitamin D Antenatal Asthma Reduction Trial [VDAART; International Standard Randomised Controlled Trial Number (ISRCTN) NCT00920621] and Vitamin D Supplementation During Pregnancy for Prevention of Asthma in Childhood trial (ABCVitamin D; ISRCTN NCT00856947).

Offspring born small for gestational age

Observational studies (see Appendix 6, Table 19*)*

Seven observational studies[85,103,115–119] assessing the relationship between maternal 25(OH)D and the risk of offspring being born SGA were identified. Of these, two were case–control studies,[115,116] one was cross-sectional[103] and four were cohort studies.[85,117–119] All achieved a composite bias score of between +1 and +7, indicating a medium–low risk of bias. Five studies[85,103,115,118,119] defined SGA as birthweight below the 10th percentile according to nomograms based on sex and gestational age. Three studies reported how gestational age was assessed (known dates of last menstrual period (LMP) and/or fetal ultrasound in early pregnancy), with the remainder giving no explanation. All studies measured serum maternal 25(OH)D concentration. The time of sampling ranged from 11 weeks' gestation to delivery. One study[117] defined SGA as birthweight below the third percentile. Three studies[85,115,116] (two nested case–control and one cohort study) reported a significant association between maternal 25(OH)D and risk of SGA; the remaining four studies[103,117–119] did not demonstrate a significant relationship.

Leffelaar *et al.*[85] measured maternal 25(OH)D concentration in women at 11–13 weeks' gestation taking part in the large ABCD study. Of the 3730 women in the cohort, 9.2% delivered SGA infants. Women with a serum 25(OH)D concentration < 30 nmol/l had a significantly higher risk of giving birth to SGA infants than women with 25(OH)D concentrations > 50 nmol/l; this relationship remained even after adjusting for gestational age, season of blood collection, sex of infant and maternal parity, age, smoking, pre-pregnancy BMI, educational level and ethnicity. No significant risk was observed, however, in women with 25(OH)D concentration between 30.0 and 49.9 nmol/l. Bodnar *et al.*[115] (composite bias score 7, low risk) found that the relationship between maternal 25(OH)D and SGA varied according to race. In this nested case–control study from an overall cohort of 1198 nulliparous women, 111 cases were identified and compared with 301 randomly selected controls; all had 25(OH)D measured before 22 weeks' gestation. Among black mothers, no relationship between SGA risk and maternal 25(OH)D concentration was observed. However, in white women, a U-shaped relationship was observed between the odds of delivering a SGA infant and maternal 25(OH)D concentration. Significantly higher odds for SGA were observed in those with 25(OH)D concentrations < 37.5 and > 75 nmol/l, with the lowest odds of SGA in women with 25(OH)D concentrations of 60–80 nmol/l. These relationships remained significant even after adjusting for pre-pregnancy BMI, smoking, socioeconomic score, season, maternal age, gestational age at blood sample, marital status, insurance status, conceptual multivitamin use and preconception physical activity. Finally, Robinson *et al.*[116] (composite bias score 0; medium risk), in a case–control study of pregnant women, all of whom had early-onset severe pre-eclampsia (as defined by the American Congress of Obstetrics and Gynaecology), found that maternal serum vitamin D was significantly lower in cases with SGA infants than with controls. This study did not present an OR or define SGA, and it was not clear at what stage of gestation maternal vitamin D was measured.

A cross-sectional Turkish study of 100 pregnant women (Akcakus *et al.*,[103] composite bias score 4, medium risk), 30 of whom gave birth to SGA infants, found no difference in maternal mean 25(OH)D at delivery in cases of SGA [maternal 25(OH)D concentration 21.8 nmol/l] compared with mothers of infants born at a size appropriate for gestational age [maternal 25(OH)D concentration 21.5 nmol/l]. Average maternal concentrations of 25(OH)D in this study were low, a reflection of the fact that most women in the study were veiled. A similar finding was observed by Mehta *et al.*[119] (composite bias score 3, medium risk) in the African cohort study of 1078 women all infected with human immunodeficiency virus (HIV). Seventy-four SGA infants were identified. Again, no difference in mean maternal 25(OH)D concentration measured in mid-pregnancy was observed between cases and normal deliveries. Shand *et al.*[117] observed similar

findings in a cohort study of Canadian women with biochemical or clinical risk factors for pre-eclampsia. No significantly increased odds of SGA were observed in women with 25(OH)D concentrations < 75 nmol/l compared with concentrations > 75 nmol/l. In this study, cases of SGA were low ($n = 13$). Finally, a Spanish cohort study from Fernandez-Alonso *et al.*[118] (composite bias score 3, medium risk) identified 46 cases of SGA out of a cohort of 466. No significant relationship between maternal 25(OH)D and SGA infants was observed. Neither mean 25(OH)D concentrations nor an OR were reported.

Intervention studies (see Appendix 6, Table 20*)*

Two clinical trials of maternal vitamin D supplementation evaluated the relationship between maternal 25(OH)D and risk of SGA infants. Both defined SGA as birthweight below the 10th percentile, although neither reported how gestational age was assessed. Neither observed a significant relationship. Brooke *et al.*,[3] in a double-blind, placebo-controlled randomised trial, allocated 67 pregnant women to either placebo ($n = 67$) or vitamin D_2 1000 IU per day in the last trimester of pregnancy ($n = 59$). Both groups were similar in terms of maternal age, height, parity, offspring sex and length of gestation. In this British study, all participants were Asian, with the majority of Indian ethnicity. Although the mean maternal 25(OH)vitamin D concentration was significantly higher in the supplemented group at delivery than in the unsupplemented group, the percentage of SGA infants did not differ significantly between groups (19 in the placebo group vs. 9 in the supplemented group). The composite bias score of this study was −2 indicating a high risk of bias. Yu *et al.*[99] (composite bias score 5, low risk) reported similar findings in a more recent British clinical trial. Pregnant women were randomised to one of three arms: no supplement ($n = 59$); oral vitamin D_2 800 IU per day from 27 weeks onwards ($n = 60$); or a single bolus dose of 200,000 IU vitamin D_2 at 27 weeks' gestation ($n = 60$). Each group contained equal numbers of four ethnic groups (black, Caucasian, Asian, Middle Eastern). No significant difference in the incidence of SGA was observed across the three groups.

Discussion

There was substantial variation in the methodology, exposure and outcome definitions for studies investigating the relationship between maternal 25(OH)D status and risk of offspring being SGA. Outcomes were conflicting. The two intervention studies[3,99] which included this outcome, the more recent of which was deemed of reasonable quality, found that supplementation with vitamin D during pregnancy was not associated with reduced risk. There appears to be no evidence base with which to recommend maternal vitamin be supplemented for the prevention of offspring being SGA neonatal.

Offspring preterm birth

Observational studies (see Appendix 6, Table 21*)*

Six observational studies[117–122] relating maternal 25(OH)D to the risk of premature birth were identified (three cohort, one cross-sectional, two case–control). One further cross-sectional study[123] assessing the risk of threatened premature birth was also included. Two studies were case–control,[120,121] three cohort[117–119] and two cross-sectional.[122,123] There was some disparity in the definition of preterm birth between studies. Most studies[117–119,122] defined preterm birth as spontaneous delivery before 37 weeks' gestation; one study[121] used a threshold of < 35 weeks. Only three studies reported how gestational age was measured: two studies used a combination of LMP and/or fetal ultrasound and one used the scoring system of Dubowitz *et al.*[124] (based on examination of the neonate and scored on neurological and physical examination features). All studies measured maternal serum 25(OH)D at some point during pregnancy or at delivery. Only one study[123] found a significant relationship between maternal 25(OH)D and risk of premature delivery.

Shibata[123] (composite bias score 4, medium risk), in a cross-sectional study of 93 Japanese pregnant women attending hospital for a routine medical check-up in Toyoake, Japan, found that maternal 25(OH)D measured after 30 weeks' gestation was significantly lower in the 14 cases of threatened premature delivery [mean 25(OH)D concentration 30.0 nmol/l] than in normal pregnancies [mean 25(OH)D

concentration 37.9 nmol/l]. Threatened premature delivery was defined as progressive shortening of cervical length (< 20 mm) as detected by transvaginal ultrasound before the 34th week of gestation and/or elevation of granulocyte elastase level in the cervical mucus before 32 weeks' gestation plus two or more uterine contractions every 30 minutes (before the 32nd week of gestation).

In contrast, six studies[117,118,119–122] did not demonstrate a significant relationship between maternal 25(OH)D and premature delivery. A small case–control study by Delmas *et al.*[120] found no difference in mean maternal 25(OH)D concentration measured at delivery in the 10 cases of preterm birth [mean maternal 25(OH)D concentration 44.9 nmol/l] compared with the nine controls [mean maternal 25(OH)D concentration 47.4 nmol/l]. This study achieved a low composite bias score of –4, suggesting a high risk of bias. No adjustment or considerations for potential confounders were made. Similarly, a prospective cohort study from Tanzania of 1078 pregnant African women infected with HIV and taking part in a clinical trial of vitamin use (Mehta *et al.*,[119] composite bias score 2, medium risk) found no increased relative risk of preterm or severe preterm birth (defined as spontaneous delivery before 34 weeks' gestation) in women with a serum 25(OH)D concentration measured at 12–27 weeks' gestation < 80 nmol/l compared with those with levels > 80 nmol/l. A nested case–control study in North Carolina, USA (Baker *et al.*,[121] composite bias score 5, low risk), identified 40 cases and 120 controls matched by race/ethnicity in a 1 : 3 ratio and compared maternal 25(OH)D measured at 11–14 weeks' gestation. Again, no significant difference in the OR for preterm birth was found in women with 25(OH)D < 75 nmol/l compared with those with 25(OH)D concentration > 75 nmol/l. Shand *et al.*[117] in a cohort study of 221 pregnant women in Vancouver, Canada, with either clinical or biochemical risk factors for pre-eclampsia found no significant relationship between maternal 25(OH)D, measured between 10 weeks' and 20 weeks 6 days' gestation, and risk of preterm birth using three different thresholds of maternal 25(OH)D (< 37.5 nmol/l, < 50 nmol/l, < 75 nmol/l) after adjustment for maternal age, BMI, season, multivitamin use and smoking. The risk factors for pre-eclampsia included an obstetric history of early-onset or severe pre-eclampsia, unexplained elevated α-fetoprotein ≥ 2.5 multiples of the median (MoMs), unexplained elevated human chorionic gonadotropin, or low pregnancy-associated plasma protein A (≤ 0.6 MoM). Hossain *et al.*,[122] in a cross-sectional study of 75 pregnant women in Pakistan (composite bias score 4, medium risk), found that mean maternal 25(OH)D_3 at delivery tended to be higher in those who delivered preterm [mean 25(OH)D_3 concentration 42.2 nmol/l] than in those with full term deliveries [mean 25(OH)D_3 concentration 32.9 nmol/l], but this did not achieve statistical significance and no adjustments for confounders were made. Finally, in a Spanish cohort study (Fernandez-Alfonso *et al.*,[118] composite bias score 3, medium risk) there was no significant difference in mean maternal 25(OH)D concentration measured at 11–14 weeks in those who delivered preterm ($n = 33$) and those who delivered at term ($n = 433$); again, no consideration for confounding factors was made.

Intervention studies

No intervention studies were identified.

Discussion

The data relating maternal 25(OH)D status to risk of offspring preterm birth are all observational. The results of the studies are varied but do not support the use of maternal supplementation to prevent this obstetric outcome.

Offspring type 1 diabetes mellitus

Observational studies (see Appendix 6, Table 22*)*

Three observational studies (two case–control and one cohort), all from Scandinavia, were identified, relating maternal 25(OH)D status to the risk of type 1 diabetes mellitus in the offspring.[125–127] Only one of these studies used 25(OH)D concentration; the other two attempted to estimate vitamin D intake. Sorensen *et al.*[125] (composite bias score 8, low risk) performed a case–control study of 109 children with type I diabetes mellitus (mean age 9 years) and 219 controls within a cohort of 29,072 individuals.

25(OH)D concentration had been measured at a median of 37 weeks' gestation. The mean 25(OH)D concentration in the mothers of cases was 65.8 nmol/l and in the mothers of controls was 73.1 nmol/l. Compared with children of mothers whose levels were > 89 nmol/l, children of mothers whose 25(OH)D concentrations in late pregnancy were ≤ 54 nmol/l were at increased risk of developing type 1 diabetes mellitus. Stene and Joner[126] (composite bias score 2, medium risk) performed a case–control study comparing 545 children with type 1 diabetes mellitus (mean age 10.9 years) with 1668 matched controls. Maternal use of vitamin D supplementation during pregnancy was assessed retrospectively by questionnaire and no association was found between maternal vitamin D supplementation in pregnancy and risk of offspring type 1 diabetes mellitus. Marjamaki *et al.*[127] (composite bias score 6, low risk) studied a prospective cohort of 3723 children who were at an increased genetic risk of developing diabetes mellitus. Among this cohort 74 children developed type 1 diabetes mellitus over the mean observation period of 4.3 years. Maternal vitamin D intake was assessed retrospectively from a FFQ completed 1–3 months after delivery and which was focused on food and supplements taken in the eighth month of pregnancy. There was no statistically significant relationship observed between maternal vitamin D intake either from food or supplements, and risk of offspring type 1 diabetes mellitus.

A further study by Krishnaveni *et al.*[105] (composite bias score 4, medium risk), using a cohort of 506 Indian children aged 5 years (469 of whom were also followed up to 9.5 years), did not measure rates of type 1 diabetes mellitus per se, but measured fasting glucose, fasting insulin, insulin resistance and insulin increment 30 minutes after a glucose tolerance test in the children. No significant association was found between any of these offspring measurements at age 5 years and maternal 25(OH)D concentration, measured at 28–32 weeks' gestation. At age 9 years, however, a significant inverse relationship was observed between maternal 25(OH)D concentration and offspring fasting insulin and insulin resistance after adjustment for child sex and age, maternal BMI, gestational diabetes mellitus, socioeconomic score, parity and religion.

Intervention studies

No intervention studies were identified.

Discussion

The three observational studies[125–127] relating maternal serum 25(OH)D status to risk of offspring type 1 diabetes mellitus were assessed to be of moderate to low risk of bias and were generally consistent in suggesting an inverse relationship. However, one[127] used vitamin D dietary intake and there were no intervention studies. Thus, maternal vitamin D supplementation to prevent offspring type 1 diabetes mellitus cannot be recommended; however, high-quality intervention studies are warranted.

Offspring low birthweight

Observational studies (see Appendix 6, Table 23*)*

Three observational studies[91,92,119] (two cross-sectional studies and one cohort study) examining the relationship between low-birthweight infants and maternal 25(OH)D concentration were identified. All studies were from the developing world (Iran and Tanzania) and composite bias scores ranged from –2 to 3, indicating a high–medium risk of bias. The definition of low birthweight (< 2500 g) was consistent across all three studies. Two studies[92,119] directly measured maternal serum 25(OH)D and reported no association with low-birthweight infants. In one study, by Sabour *et al.*,[91] maternal vitamin D intake during pregnancy was estimated from FFQs completed by 449 Iranian pregnant women at delivery. The incidence of low birthweight was lower in the offspring of women with adequate intake of calcium and vitamin D (100 mg calcium, 200 IU vitamin D per day) than in the offspring of those with inadequate intake (numbers not given). This study achieved the lowest composite bias score (composite bias score –2) of these studies, indicating the highest risk of bias; no consideration for potential confounders was made.

Two studies reported no significant relationship between maternal 25(OH)D and risk of offspring low birthweight. Maghbooli *et al.*[92] (composite bias score 1, medium risk), in a second cross-sectional study from Iran, measured maternal 25(OH)D at delivery in 552 Iranian women. The study reported that 5.4% (approximately $n = 30$) of the cohort had low-birthweight offspring. No significant difference in mean 25(OH)D was observed between mothers of low-birthweight offspring and mothers of normal-weight offspring [mean 25(OH)D concentration in each group not given]. Similarly, Mehta *et al.*[119] (composite bias score 3, medium risk), in a cohort study of 1078 HIV-infected women taking part in a vitamin supplement trial, found no significantly increased odds of low-birthweight infants ($n = 80$) in mothers with a 25(OH)D concentration < 80 nmol/l compared with those with a concentration > 80 nmol/l. In this study a threshold of 80 nmol/l was used to divide maternal 25(OH)D concentration into adequate or low. Adjusting the analysis for maternal multivitamin supplementation, age at baseline, cluster differentiation 4 (CD4) count at baseline and HIV disease stage did not alter the findings.

Intervention studies

No intervention studies were identified.

Discussion

Of the three observational studies relating maternal 25(OH)D status to risk of low birthweight in the offspring, only one[91] demonstrated a positive result, suggesting that low birthweight was less likely where women took at least 100 mg of calcium and 200 IU vitamin D daily. However, this study was judged to be at high risk of bias; the remaining two studies[92,119] demonstrated no relationship and, therefore, maternal vitamin D supplementation cannot be recommended to prevent low birthweight. Larger prospective observational studies in several different populations would be sensible before moving to an intervention study.

Offspring serum calcium concentration

Observational studies (see Appendix 6, Table 24*)*

One observational study examining the relationship between maternal vitamin D status and offspring serum calcium concentration was identified. In a cross-sectional study of 264 women in Saudi Arabia, Ardawi *et al.*[90] found no significant correlation between maternal 25(OH)D measured at delivery and offspring venous umbilical cord blood calcium concentration. A relationship was still not observed even if the group was divided using a maternal 25(OH)D concentration of 20 nmol/l as a threshold. This study was assessed to have a low risk of bias (composite bias score 5); however, no adjustments were made for potential confounding factors.

Intervention studies (see Appendix 6, Table 25*)*

Seven clinical trials[4–7,20,21] of maternal vitamin D supplementation were identified; all measured venous umbilical cord calcium concentration at delivery and three[3,6,20] went on to measure offspring venous calcium again within the first week of life. None of the trials was within the last 20 years and all were found to have a high risk of bias (composite bias score −9 to −1). Sample sizes ranged from 40 to 1139. Five studies[4–7,136] reported adequate randomisation; however, only two trials[3,20] were placebo controlled and only one[3] was of double-blind design. Supplementation strategies were highly variable: six trials[3–7,21] supplemented pregnant women with vitamin D in the last trimester; one study[20] supplemented from 12 weeks onwards. There was also much diversity with regards to the type of supplementation used, ranging from 1000 IU ergocalciferol daily (with or without calcium) in the last trimester[3,6,7,21] to bolus oral dosing of 600,000 IU cholecalciferol twice in the last trimester.[4,5] Six studies[3–6,20,21] reported higher offspring calcium concentrations in the supplemented group than in the unsupplemented group; one trial[7] showed no difference in offspring venous calcium regardless of maternal vitamin D supplementation strategy.

Brooke *et al.*[3] (composite bias score −2, high risk), in a trial of ergocalciferol supplementation in the last trimester of pregnancy of Asian women living in the UK, found no difference in umbilical cord calcium concentration between groups, but neonatal serum calcium was greater in offspring of supplemented mothers than in the offspring of mothers who had received placebo at 3 and 6 days postnatally. There were five cases of symptomatic hypocalcaemia in the control group but none in the treatment group. Higher rates of breastfeeding were observed in the treatment group which in itself was positively associated with offspring venous calcium concentration and was not controlled for in analysis. Similar findings were noted in a larger ($n = 1139$) British study by Cockburn *et al.*[20] (composite bias score −1, high risk) and in a French study by Delvin *et al.*[6] (composite bias score −2, high risk). Neither study found a difference in venous cord calcium concentrations between the supplemented and unsupplemented groups, but both found higher infant venous calcium concentrations in the supplemented group, at days 6[20] and 4.[6] The third, and most recent, British study (Congdon *et al.*[21]) found that cord calcium was significantly higher in the offspring of Asian women supplemented with daily 1000 IU vitamin D plus calcium in the last trimester than in the offspring of those who received no supplement. This study was assessed to have the highest risk of bias with a composite bias score of −9. The number of subjects in this trial was low, with only 19 receiving supplement, and no information about randomisation or whether or not blinding was implemented were reported. These findings are in agreement with two Indian studies, both by Marya *et al.*[4,5] (1981, composite bias score −6, high risk; 1989, composite bias score −2, high risk). Both studies found that cord calcium concentrations were significantly higher in those pregnant women supplemented with two doses of 600,000 IU cholecalciferol in months 7 and 8 of gestation than in the unsupplemented group.

In contrast, a French study (Mallet *et al.*[7] composite bias score −3, high risk) found no effect of maternal vitamin D supplementation in the third trimester on cord calcium concentration, regardless of whether supplementation was provided at 1000 IU per day for 3 months or as a single high dose of 200,000 IU in the seventh month of gestation.

Evidence synthesis

The available published results were combined in two separate models. The first meta-analysis included the studies of Cockburn *et al.*,[20] Brooke *et al.*,[3] Marya *et al.*[4] (low dose of vitamin D), Mallet *et al.*[7] (low dose of vitamin D) and Delvin *et al.*[6] (see *Appendix 7*, *Figure 8*). Owing to statistically significant heterogeneity in the results ($I^2 = 67.6\%$, $p = 0.015$), a REM was fitted. Serum calcium concentration in the supplemented group did not differ from that in the unsupplemented group (mean difference 0.01 mmol/l, 95% CI −0.02 mmol/l to 0.04 mmol/l). The second meta-analytic model included the studies by Cockburn *et al.*,[20] Brooke *et al.*,[3] Marya *et al.*[4] (high dose of vitamin D), Mallet *et al.*[7] (high dose of vitamin D), Delvin *et al.*[6] and Marya *et al.*[5] (see *Appendix 7*, *Figure 9*). As in the previous model, a REM was fitted owing to significant heterogeneity ($I^2 = 90\%$, $p < 0.001$). The combined results showed that the mean difference of serum calcium concentration between the supplemented and the unsupplemented groups was significantly different from 0 (mean difference 0.05 mmol/l, 95% CI 0.02 mmol/l to 0.05 mmol/l).

Discussion

The majority of the intervention studies and the one observational study consistently demonstrated positive relationships between maternal 25(OH)D status and offspring serum calcium concentrations measured either in venous umbilical cord serum or from postnatal venesection. Some also found a reduced risk of hypocalcaemia in the neonate. Meta-analysis of higher-dose intervention studies also suggested a positive effect. However, these intervention studies were all felt to be at high risk of bias and none of them was published within the last 20 years. Assay technology has improved dramatically over recent decades and the reliability of the relationships must be open to question. Given the known physiology of the vitamin D axis in adults, a positive association between maternal 25(OH)D and offspring calcium concentration might not be a surprising finding; however, little is known about relationships between 25(OH)D and fetal calcium concentrations in utero. Furthermore, none of the identified studies addressed postnatal factors such as mode of feeding (breast vs. formula) as potential risk modifiers. A positive relationship between maternal 25(OH)D status and offspring calcium concentrations does not justify intervention unless the increased calcium concentration brings a benefit. Symptomatic hypocalcaemia did not appear to be found

in all studies and is likely to be much more common in high-risk populations. It seems reasonable, on the basis of the current evidence, to suggest that maternal vitamin D supplementation is likely to reduce the risk of neonatal hypocalcaemia, but that the dose required, duration and target group is currently unclear (e.g. by skin colour, ethnicity, or mode of infant feeding), and might usefully form the basis of further investigation.

Offspring blood pressure

Observational studies (see Appendix 6, Table 26*)*

Two cohort studies were identified which examined the relationship between maternal serum 25(OH)D concentration in pregnancy and offspring blood pressure. Both studies were of cohort design and measured maternal serum 25(OH)D in late pregnancy. Composite bias score was 4 for both, indicating a medium risk of bias. Gale *et al.*[24] measured blood pressure in 178 children aged 9 years in the Princess Anne Cohort study, UK. No association was observed between maternal 25(OH)D and offspring blood pressure. Krishnaveni *et al.*,[105] using a larger Indian cohort of 338 mother–offspring pairs, measured blood pressure in the offspring at two time points: age 5 and 9.5 years. Similarly, no significant difference in blood pressure was observed between those children born to mothers with vitamin D deficiency (defined by the authors as < 37.5 nmol/l) and those born to mothers without vitamin D deficiency. Adjustments for offspring sex and age, maternal BMI, gestational diabetes mellitus, socioeconomic score, parity and religion made little difference to the results.

Intervention studies

No intervention studies were identified.

Discussion

Neither of the two observational studies relating maternal 25(OH)D status to offspring blood pressure demonstrated a statistically significant relationship and therefore no treatment recommendation can be made.

Offspring rickets

Observational studies

No observational studies of maternal vitamin D status and offspring rickets were identified.

Intervention studies

No intervention studies of maternal vitamin D supplementation and offspring rickets were identified. A UK trial, by Congdon *et al.*,[21] found no difference in the incidence of offspring craniotabes between the supplemented group ($n = 4$) and the unsupplemented group ($n = 3$). This study was assessed to have a high risk of bias, with a composite bias score of –9.

Discussion

It is interesting that there are so few data relating maternal 25(OH)D status to offspring rickets. However, rickets does not tend to manifest until the first year of life, in contrast to neonatal hypocalcaemia, and therefore it is likely that the determinant is the child's own sun exposure and vitamin D intake. If the child is wholly breastfed and receives little sun exposure then increased risk of rickets might be expected. However, this scenario does not fall within the remit of the current review.

Maternal pre-eclampsia

Observational studies (see Appendix 6, Table 27*)*

Eleven observational studies were identified, comprising six case–control,[128–133] four cohort[117,118,134,135] and one cross-sectional study.[122] The case–control studies were generally of small size with the minimum number of 12 cases and maximum 55 cases and the number of control subjects ranging from 24 to 220. The definition of pre-eclampsia was similar across studies: new-onset gestational hypertension after 20 weeks [systolic blood pressure persistently (two or more occasions) ≥ 140 mmHg and/or diastolic blood pressure ≥ 85 or ≥ 90 mmHg] and proteinuria (either 300 mg protein excreted in the urine in 24 hours, or a random sample of between 1+ and 2+ protein on urine dipstick, or a protein–creatinine ratio > 0.3). Two of the case–control studies[129,130] identified cases of severe pre-eclampsia only, using the American Congress of Obstetrics and Gynaecology 2002 definition [systolic blood pressure ≥ 160 mmHg and/or a diastolic blood pressure ≥ 110 mmHg on at least two occasions plus proteinuria (≥ 300 mg in a 24-hour collection or 1+ on urine dipstick), or systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg plus 5 g proteinuria in a 24-hour period after 20 weeks' gestation]. All six case–control studies, the cross-sectional study and three of the five cohort studies used serum 25(OH)D concentration as the marker of maternal vitamin D status,[117,118,122,128–133] with the other two cohort studies[134,135] using dietary intake. The timing of serum measurements varied across the studies with some measuring in the first trimester[118,132] and others in the last,[128,131] and one study[133] at three time points. Composite bias scores ranged from 2 to 9, indicating that studies were considered low to medium risk of bias. Confounding factors were variably included and there was also variation in the criteria for matching to controls.

Of the included studies, three (one case–control, one cross-sectional and one cohort) reported statistically significant inverse associations between maternal vitamin D status and risk of pre-eclampsia. A further two case–control studies demonstrated a similar association between maternal 25(OH)D and risk of severe pre-eclampsia. A nested case–control study (55 cases and 220 randomly selected, unmatched controls from a cohort of 1198) from Bodnar *et al.*[128] (composite bias score 8, low risk) measured 25(OH)D in nulliparous pregnant women living in Pittsburgh, USA, at two time points (before 22 weeks' gestation and pre-delivery). A significant inverse relationship was observed at both time points. At < 22 weeks' gestation a 50 nmol/l reduction in maternal 25(OH)D was associated with an over twofold increased risk of pre-eclampsia after adjusting for maternal race, ethnicity, pre-pregnant BMI, education, season and gestational age at blood sample. A cross-sectional study from Pakistan (Hossain *et al.*,[122] composite bias score 4, medium risk) measured maternal 25(OH)D_3 at delivery in 75 women (76% of whom covered their face, arms, hands and head). Although the number of pre-eclampsia cases is not given, when the group was divided into thirds, a significantly increased risk of pre-eclampsia was observed for those in the lowest and middle tertile compared with the highest. The relationship between maternal 25(OH)D and pre-eclampsia was only observed in individuals with serum 25(OH)D < 50 nmol/l. In contrast to other studies, women were classified as having pre-eclampsia based on blood pressure alone (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg). The largest study to date (Haugen *et al.*,[134] composite bias score 2, medium risk) followed up a cohort of 23,425 pregnant women enrolled in the Norwegian Mother and Child Cohort Study. Maternal 25(OH)D was not directly measured, but estimated from a FFQ at 22 weeks. A total of 1267 cases of pre-eclampsia were identified. Lower total vitamin D intake was associated with a significantly increased risk of pre-eclampsia.

Both studies examining the relationship between severe pre-eclampsia and maternal 25(OH)D demonstrated significant inverse associations. Both were US-based case–control studies with a comparable number of cases and controls, and assessed to have a low risk of bias. Baker *et al.*[129] (composite bias score 9, low risk) identified 44 cases and 201 randomly selected controls matched by race/ethnicity from a cohort of 3992 women. Significantly higher odds of severe pre-eclampsia were found in those with maternal 25(OH)D < 50 nmol/l than in those with 25(OH)D > 50 nmol/l, even after adjusting for season of blood sampling, maternal age, multiparity, BMI, gestational age at blood sample. Similarly, Robinson *et al.*[130] (composite bias score 5, low risk), in a study of 50 cases and 100 controls matched for race and gestational age at the time of sample, found that the odds of severe pre-eclampsia significantly

reduced as maternal 25(OH)D increased even after adjusting for maternal BMI, maternal age, African American race and gestational age at sample collection.

Six studies, however, found no association between maternal vitamin D status and pre-eclampsia risk. Seely *et al.*[131] (composite bias score 2, medium risk) observed no significant difference in late-pregnancy mean maternal 25(OH)D in 12 women with pre-eclampsia and 24 control women of similar age, gestation, height, weight, parity (primiparous or not) and ethnicity (Caucasian or not). A second US nested case–control study from Powe *et al.*[132] (composite bias score 4, medium risk) drew similar conclusions. In this study of 39 cases and 131 unmatched controls from an overall cohort of 9930, the odds of pre-eclampsia were not related to first-trimester maternal 25(OH)D concentration. Adjusting for maternal BMI, non-white race and summer blood collection made no difference to the results. A significant relationship was still not seen even when the analysis was restricted to mothers with a serum 25(OH)D concentration < 37.5 nmol/l. A further US nested case–control study from Azar *et al.*[133] (composite bias score 5, low risk) assessed pre-eclampsia risk in only white women, all with type 1 diabetes mellitus, who had serum 25(OH)D measured at three time points during their pregnancy (early, mid and late pregnancy). Twenty-three cases were identified and compared with 24 controls, matched for age, diabetes mellitus duration, glycated haemoglobin (HbA_{1c}) level and parity, out of a cohort of 151. Again, no statistically significant relationship between maternal 25(OH)D, measured at any time point, and pre-eclampsia risk was observed. A Canadian study of 221 pregnant women with clinical or biochemical risk factors for pre-eclampsia (Shand *et al.*,[117] composite bias score 6, low risk) found no significantly increased odds of pre-eclampsia in pregnant women with mid-pregnancy 25(OH)D concentrations < 37.5, < 50 or <75 nmol/l compared with those with 25(OH)D concentrations > 75 nmol/l. However, only 28 cases of pre-eclampsia were identified. The most recent study by Fernandez-Alonso *et al.*[118] (composite bias score 3, medium risk), again, found no difference in mean early pregnancy maternal 25(OH)D between those who developed pre-eclampsia and those with normal pregnancies. This study included the lowest number of cases ($n = 7$). Finally, Oken *et al.*[135] (composite bias score 5, low risk) identified 58 cases of pre-eclampsia from the US Project Viva Cohort Study of 1718 women. Maternal serum 25(OH)D was not measured directly, but estimated from a FFQ at mean 10.4 weeks' gestation. No significant relationship between pre-eclampsia risk and vitamin D intake was seen.

Evidence synthesis

Usable results for meta-analysis of the risk of pre-eclampsia with increased vitamin D were available from four studies: Bodnar *et al.*,[128] Powe *et al.*,[132] Robinson *et al.*[130] and Azar *et al.*[133] (early pregnancy visit). All but Bodnar *et al.*[128] provided unadjusted ORs. The unadjusted estimates were synthesised in a REM owing to statistically significant heterogeneity ($I^2 = 78.4\%$, $p = 0.01$). The pooled estimate showed no significant risk of pre-eclampsia with increased vitamin D (pooled OR 0.78, 95% CI 0.59 to 1.05; see *Appendix 7*, *Figure 10*). Synthesising the available adjusted ORs from all four studies the result was very similar; there was no statistically significant increased risk of pre-eclampsia with decreased vitamin D status (pooled OR 0.75, 95% CI 0.48 to 1.19; see *Appendix 7*, *Figure 11*).

Intervention studies (see Appendix 6, Table 28*)*

One clinical trial that included maternal pre-eclampsia as an outcome measure was identified. Marya *et al.*[136] randomised 400 pregnant women attending an antenatal clinic in India to either a trial of vitamin D plus calcium (375 mg/day calcium plus 1200 IU vitamin D) from 20 to 24 weeks until delivery or to no supplement ($n = 200$ in each arm). Serum 25(OH)D concentrations were not measured during the study. There were 12 cases of pre-eclampsia in the supplemented group compared with 18 cases of pre-eclampsia in the non-supplemented group, a result which did not achieve statistical significance. Systolic and diastolic blood pressure were significantly lower in the supplemented than in the unsupplemented group at 32 and 36 weeks' gestation, but no difference was observed at 24–28 weeks' gestation. This study had a composite bias score of –2, indicating a high risk of bias, and clearly could not separate an effect of vitamin D from that of calcium supplementation.

Discussion

As with many other outcome measures, results of the various observational studies were conflicting, with some demonstrating an inverse association between maternal vitamin D status and risk of pre-eclampsia[122,128–130,134] and others no relationship.[117,118,131–133,135] Both studies looking at the risk of severe pre-eclampsia found statistically significant inverse relationships with maternal 25(OH)D concentration.[129,130] There was, however, significant heterogeneity between studies in terms of gestational age at which maternal vitamin D status was assessed, confounding factors adjusted for and the definition of pre-eclampsia used. Most observational studies were case–control and included only small numbers of women with pre-eclampsia ($n = 7$[118] to 55[128]). Only one intervention study[136] was identified. This was of reasonable size; however, the study was assessed to have a high risk of bias and the supplemented group received calcium and vitamin D together, rather than vitamin D alone. No difference in the risk of pre-eclampsia was identified in the unsupplemented group. Thus, it is difficult to make any treatment recommendations based on the current evidence. Further high-quality intervention studies are needed.

Maternal gestational diabetes mellitus

Observational studies (see Appendix 6, Table 29*)*

Eight observational studies (four case–control, one cross-sectional and three prospective cohort) examined relationships between maternal 25(OH)D status and risk of gestational diabetes mellitus.[93,95,118,137–141] One study, by Maghbooli *et al.*,[137] found, in a cross-sectional cohort of 741 Iranian women, that mean 25(OH)D concentrations (measured at 24–28 weeks) were lower in the 52 subjects who had gestational diabetes mellitus (16.5 nmol/l) than in the 527 women who did not (23 nmol/l). There was no adjustment for confounding factors in this analysis and the overall bias score was 3, indicating a medium risk for bias. A further study from Iran, of case–control design (Soheilykhah *et al.*,[138] composite bias score 3, medium risk), found significantly increased odds of gestational diabetes mellitus in those with 25(OH)D concentrations < 37.5 nmol/l (measured between 24 and 28 weeks). Thus, the mean 25(OH)D concentration was 24 nmol/l in those with gestational diabetes mellitus and was 32.3 nmol/l in those without gestational diabetes mellitus. Clifton-Bligh *et al.*,[95] in a prospective cohort of 307 women in New South Wales, Australia, found that the mean 25(OH)D concentration (measured at a mean of 28.7 weeks) was 48.6 nmol/l in 81 women with gestational diabetes mellitus compared with 55.3 nmol/l in women without. They also found that serum 25(OH)D concentration was negatively associated with fasting glucose after adjustment for age, BMI and season. This study was found to be of low risk of bias with a score of 6. Zhang *et al.*[139] performed a nested case–control study within a US cohort ($n = 953$), containing 57 women with gestational diabetes mellitus (70% white ethnicity) and 114 controls (84% white ethnicity). Controls were frequency matched to cases by the estimated season of conception. After adjustment for maternal age, ethnicity, family history of type 2 diabetes mellitus and pre-pregnant BMI, 25(OH)D concentration < 50 nmol/l was associated with increased odds of gestational diabetes mellitus, compared with women with concentrations > 75 nmol/l. This study, again, achieved a low risk of bias, with composite score of 8.

In contrast, an Indian prospective cohort study (Farrant *et al.*,[93] composite bias score 5, low risk) found no difference in 25(OH)D concentrations between those with gestational diabetes mellitus [$n = 34$, mean 25(OH)D concentration 38.8 nmol/l] and those without [$n = 525$, mean 25(OH)D concentration 37.8 nmol/l] ($p = 0.8$). No associations were found by three further studies: Makgoba *et al.*[140] (composite bias score 7, low risk), in a nested case–control study of 90 women with gestational diabetes mellitus and 158 controls, within an overall cohort of 1200 women, found no difference in serum 25(OH)D concentration (47.2 nmol/l in cases vs. 47.6 nmol/l in controls, measured at 11–13 weeks' gestation). An inverse relationship was found between the serum 25(OH)D concentration and fasting glucose, glucose concentration 2 hours after a glucose tolerance test, and HbA_{1c} at 28 weeks' gestation. However, after adjustment for BMI, gestation at the time of blood sampling, smoking, ethnicity, parity, maternal age, conception status, previous gestational diabetes mellitus and season, only the relationship with 2-hour glucose concentration remained statistically significant. A nested case–control study (Baker *et al.*,[141] composite bias score 7, low risk), this

time set within a US cohort of 4225 women in whom serum 25(OH)D concentration was assessed at 11–14 weeks' gestation, found that among the 60 cases of gestational diabetes mellitus and 120 controls, after adjustment for maternal age, insurance status, BMI, gestational age at sample collection and season, there was no association between serum 25(OH)D concentration and gestational diabetes mellitus. Finally, in a Spanish prospective cohort of 466 women (Fernandez-Alonso *et al.*,[118] composite bias score 3, medium risk) in whom 25(OH)D concentrations were measured at 11–14 weeks, there was no statistically significant relationship between baseline 25(OH)D concentration and development of gestational diabetes mellitus.

Intervention studies

No intervention studies were identified.

Discussion

Several large studies, of low to moderate risk of bias, found no relationship between maternal 25(OH)D status and risk of gestation diabetes mellitus. Although two Iranian studies[137,138] did find an increased risk of gestational diabetes mellitus in women with low levels of 25(OH)D, these seem at odds with the majority of investigations from elsewhere and thus there appears to be no consistent evidence on which to base a recommendation of vitamin D supplementation to prevent gestational diabetes mellitus.

Maternal caesarean section

Observational studies (see Appendix 6, Table 30*)*

Six observational studies[90,118,142–145] were identified, one of which was case–control[144] and the others cohort designs.[90,118,142,143,145] Two studies[142,143] found inverse relationships between 25(OH)D status and risk of caesarean section, with the remaining studies demonstrating no statistically significant associations.[90,118,144,145] Scholl *et al.*[142] (composite bias score 5, low risk) studied 290 women who delivered by caesarean section, out of a cohort of 1153 pregnant women. 25(OH)D concentration was assessed at a mean of 13.7 weeks' gestation. Compared with women who had serum 25(OH)D concentrations between 50 and 125 nmol/l in early pregnancy, those who had levels < 30 nmol/l appeared at increased risk of caesarean section, and this association persisted after adjustment for age, parity, ethnicity, gestation at entry to study, season and BMI. Merewood *et al.*[143] (composite bias score 6, low risk), in a cross-sectional study of US women, found increased odds of caesarean section if maternal 25(OH)D concentration was < 37.5 nmol/l in 67 cases of caesarean section compared with 277 controls, after adjustment for ethnicity, alcohol use in pregnancy, educational status, insurance status and age.

Ardawi *et al.*[90] (composite bias score 5, low risk) studied a cohort of 264 women in Jeddah, Saudi Arabia. Among women with serum 25(OH)D status < 20 nmol/l the frequency of caesarean section was 12.5%, compared with a frequency of 9.6% in those with serum concentrations above this level, a difference which did not achieve statistical significance. A Pakistani study (Brunvand *et al.*,[144] composite bias score 1, medium risk) of nulliparous Pakistani women of low social class found that the median 25(OH)D concentration in 37 women who delivered by caesarean section (measured just before delivery) was 26 nmol/l, compared with 19 nmol/l in 80 controls who delivered vaginally. This did not, however, achieve statistical significance. A UK cohort study of 1000 pregnancies yielded 199 caesarean sections (Savvidou *et al.*,[145] composite bias score 7, low risk) and found no relationship between 25(OH)D concentration measured between 11 and 13 weeks' gestation and risk of caesarean section, after adjustment for maternal age, racial origin, smoking, method of conception and season. Finally, in the Spanish study of Fernandez-Alonso *et al.*[118] (composite bias score 3, medium risk), 105 of the cohort of 466 women underwent caesarean section. There was no relationship between 25(OH)D concentration, measured between 11 and 14 weeks' gestation, and risk of caesarean section.

Intervention studies

No intervention studies were identified.

Discussion

The data relating to caesarean section are all observational and conflicting. Given that many other factors will influence risk of caesarean section, including physician preference, local policy and pre-existing morbidity, it seems likely that any relationships between maternal 25(OH)D concentration and caesarean section risk will be difficult to extricate from the surrounding noise. The current evidence base does not support use of vitamin D supplementation to reduce risk of caesarean section and a well-designed, prospective observational study is warranted before moving to intervention studies.

Maternal bacterial vaginosis

Observational studies (see Appendix 6, Table 31)

Three studies[146–148] were identified (two cohort, one cross-sectional) which examined relationships between maternal 25(OH)D status and bacterial vaginosis. All three studies elucidated statistically significant relationships although at very different thresholds of 25(OH)D concentration. Bodnar *et al.*[146] (composite bias score 5, low risk) studied 469 women, all of whom were non-Hispanic and white or black. 25(OH)D concentration was measured at a mean of 9.5 weeks' gestation. Among the 192 cases of bacterial vaginosis, median 25(OH)D concentration was 29.5 nmol/l, compared with 40.1 nmol/l in the non-diseased women. At 25(OH)D concentrations < 80 nmol/l there was an inverse association between frequency of bacterial vaginosis and early pregnancy serum 25(OH)D concentration ($p < 0.0001$). Above this threshold no relationship was observed. Results were adjusted for the presence of sexually transmitted diseases. Using the National Health and Nutrition Examination Survey (NHANES) cohort, Hensel *et al.*[147] (composite bias score 4, medium risk) found a statistically significantly increased risk of bacterial vaginosis in those women whose serum 25(OH)D concentration was < 75 nmol/l. However, it is unclear at what stage 25(OH)D concentration was measured, and the mean 25(OH)D concentrations, together with the unadjusted analyses, are not presented. Dunlop[148] (composite bias score 2, medium risk) sampled 160 non-Hispanic white/non-Hispanic black women from a total of 1547 women participating in the Nashville Birth Cohort. In this cross-sectional analysis, risk of bacterial vaginosis was higher in women whose serum 25(OH)D concentration at delivery was < 30 nmol/l than in those whose levels were above this threshold, after adjustment for race, age, smoking, BMI, gestational age at delivery and health-care funding source.

Intervention studies

No intervention studies of maternal vitamin D supplementation on risk of bacterial vaginosis were identified.

Discussion

Although reasonably large, only three studies[146–148] were identified that reported bacterial vaginosis as an outcome. Each study differed in methodology, using differing thresholds for low serum vitamin D, and there remains a strong possibility of residual confounding which may account for the relationships between bacterial vaginosis and maternal vitamin D. Thus, the evidence base does not currently warrant the recommendation of vitamin D supplementation to reduce the risk of bacterial vaginosis, and further high-quality prospective observational studies are required before moving to an intervention study.

Other study questions

Given the altered physiology during pregnancy, it is difficult to define a normal 25(OH)D concentration in relation to PTH or fractional intestinal calcium absorption, as has been done in non-pregnant individuals. However, even in these non-pregnant situations, widely disparate estimates of normality have been obtained.[65] A better approach might be to define a level at which adverse influences on the mother and offspring are minimised. However, it is apparent, from the results presented above, that the evidence base is extremely heterogeneous in this regard; where thresholds have been defined, they differ markedly between studies, and many studies find no relationships at all. Thus, on the basis of the identified studies,

it is not possible to answer the study question 'What are the clinical criteria for vitamin D deficiency in pregnant women?' or to rigorously define an optimal level of serum 25(OH)D during pregnancy.

Similarly, the studies are extremely heterogeneous with regard to dose, use of vitamin D_2 or D_3, route and timing; there is a dearth of high-quality interventional evidence. It was therefore also not possible to answer the study question 'What is the optimal type (D_2 or D_3), dose, regimen and route for vitamin D supplementation in pregnancy?. Furthermore, no health economic evaluation was identified. Thus, it is not possible to make a rigorously evidence-based recommendation regarding optimal vitamin D supplementation in pregnancy.

Chapter 6 Summary discussion

Specific discussion of the findings in relation to each outcome is given in the relevant sections above. There was some evidence to support a positive relationship between maternal vitamin D status and offspring birthweight (meta-analysis of observational studies) and offspring bone mass (observational studies); meta-analysis of RCTs suggested a positive effect of maternal vitamin D supplementation on neonatal calcium concentrations, but the dose required, duration and target group are currently unclear, and might usefully form the basis of further investigation. Recurring themes in each disease area included marked heterogeneity between studies in terms of design, definition of exposure and outcome, dose, timing, route, statistical analysis and treatment of potential confounding factors. The overall effect of these considerations undoubtedly contributed to the statistically significant measures of heterogeneity in the meta-analyses, but it is difficult to identify individual factors which might predominate. In no single disease area did the evidence base unequivocally support the use of vitamin D supplementation during pregnancy. Although a systematic search for evidence of harm from vitamin D supplementation in pregnancy was not undertaken (as this was not part of the commissioned brief), no studies documenting adverse effects associated with such a strategy were identified. However, it was clear that follow-up of participants was almost always of short duration, and the current evidence base is therefore also insufficient to allow the potential identification of more protracted adverse effects.

The strengths of our review include comprehensive coverage of the available literature with exhaustive searching of databases, hand searching of reference lists and contact with authors. CRD methods were followed, with two reviewers executing each stage of the review process. Additionally, the review and interpretation of evidence has been based on an understanding of vitamin D physiology, together with possible sources of bias particularly important for this exposure. The overall objectives comprehensively addressed the issue of vitamin D in pregnancy, in terms of normal levels, maternal and child health outcomes, potential interventions and health economic assessments.

Limitations in this review were identified at both study and outcome level, and at the level of the overall review. There was considerable heterogeneity between all of the studies included in the review. Study methodology varied widely in terms of design, population, maternal vitamin D assessment, exposure measures and outcome definition. For example, measures of maternal vitamin D status included serum concentration, estimated dietary intake and UV sunlight exposure. Even when serum 25(OH)D concentration was measured, the assay and technique varied widely. Although we included comparability and standardisation of assay results in the quality criteria, these issues were not commonly considered or documented by study authors. Clearly, given the multiplicity of both laboratory techniques [e.g. RIA, HPLC, liquid chromatography-mass spectrometry (LC-MS)] and different operators, standardisation of assays across technique and laboratory is essential, and currently the subject of a global initiative by the US National Institutes of Health.[71] A further issue was the frequent lack of documentation of the gestational age at which sampling occurred, ranging from early pregnancy through to delivery. Confounding factors considered varied widely from study to study. Only a small number of intervention studies were identified, most of which were not blinded or placebo controlled; all varied in terms of the dose and duration of vitamin D supplementation (e.g. doses ranged from 800 IU daily to two bolus doses of 600,000 IU in the last trimester). Offspring outcomes were also assessed at varying time points, ranging from birth through to 9 years of age. The potential for residual confounding and reverse causality in studies of vitamin D is a very important consideration and also difficult to address methodologically. For example, maternal obesity is a risk factor for adverse birth outcomes, and is also associated with reduced 25(OH)D concentrations because of sequestration in adipose tissue. Increasing physical activity might be associated with better maternal health, but also greater 25(OH)D concentrations because of greater sun exposure.

Limitations were also identified at the review level. Although our search strategy was comprehensive, non-English articles were excluded and we were unable to obtain copies of some listed articles, despite requesting them from our local Health Services library and The British Library, or direct from authors. There is the possibility that we did not identify all the relevant studies in this field; however, this risk was minimised by a comprehensive electronic search strategy complemented by hand-searching and contacting authors and other specialists in this field. Although we did not detect evidence of publication bias, this remains a possibility, such that studies showing null results may not receive priority for publication. In addition, some of the studies identified did not present all necessary summary data, especially if the result was null. In such cases, we did attempt to contact authors for missing data, but this was not possible in all cases.

We set out to answer a number of research questions as described in *Chapter 1*. The first of these addressed normal levels of vitamin D in pregnancy. Such a value is controversial in non-pregnant adult populations, and *Chapter 1*, *Considerations for appraisal of data* sets out the reasons why current definitions are lacking in biological support. For many biochemical measurements, the definition of normality may be derived from assessment of a cohort representative of the general population and defining a lower cut-off (e.g. the lowest 2.5%). We did not identify any such study in pregnant women, and indeed, for vitamin D, which is largely determined by sunshine exposure and skin colour, such an approach may not be appropriate: one hypothesis is that white skin is an adaptation to low sun exposure in northern hemisphere countries and that this adaptation has not gone far enough to achieve optimal levels. Thus, it may be that 'normality' (in the sense of what is actually observed in the population) is actually suboptimal.

It may, therefore, be more appropriate to attempt to define 'healthy' levels based on relationships between maternal serum 25(OH)D concentration and maternal/offspring disease outcomes. Unfortunately, although there are plenty of studies which attempt to investigate such associations, it is difficult to use them to inform a cut-off below which disease is likely. Typical caveats within studies include small numbers, pre-determined rather than study-derived thresholds, poor disease definition, lack of attention to potential confounding and reverse causality. Between studies, these include variable populations, variable ascertainment of vitamin D status and outcome definitions, together with the use of different thresholds. All of these issues make it impossible to make a truly reliable evidence-based judgement as to the normal (or 'healthy') level of 25(OH)D in pregnancy. Furthermore, it is very likely that the optimal level relating to one outcome may not be the same for another; there is also no reason to suppose that increasing levels of 25(OH)D will lead to universally positive effects on all diseases. Studies describing the long-term safety of vitamin D supplementation are conspicuous by their non-existence.

We did find evidence of offspring outcomes associated with maternal vitamin D status in pregnancy. Thus, there was some evidence to support a positive relationship between maternal vitamin D status and offspring birthweight (meta-analysis of observational studies), neonatal calcium concentrations (meta-analysis of RCTs) and offspring bone mass (observational studies). However, it was not possible to deduce thresholds at which risk of these outcomes increased, or whether indeed there is a threshold at all.

The next aim was to elucidate whether or not supplementation with vitamin D in pregnancy would lead to improvements with offspring health, and to identify specific dose requirements. Again, the data do not allow definite conclusions to be made. The majority of the RCTs of vitamin D supplementation aimed at optimising offspring outcomes are small, of poor methodology and date from around 20 years ago, when assay technology was much less well advanced. In several areas (offspring birthweight, calcium concentration, bone mass) the evidence is sufficient to warrant the instatement of properly conducted

large RCTs, but, for other areas, better-quality observational evidence should be obtained. A further consideration is how women will feel about potentially taking higher doses of vitamin D during pregnancy than is currently recommended, a subject that is being assessed as part of the MAVIDOS trial. The lack of good evidence linking maternal vitamin D status to offspring disease, and to maternal outcomes, means that it is difficult to obtain a reliable health economic assessment of the potential impact of maternal vitamin D supplementation in pregnancy. Indeed, we were unable to identify any studies which attempted to make such an estimate. Clearly, it would be appropriate to confirm that maternal vitamin D supplementation does actually lead to an improvement in maternal and/or offspring health before going on to estimate its health-economic impact.

Chapter 7 Conclusions (implications for health care; recommendations for research)

The fundamental conclusion is that the current evidence base does not allow the study questions to be definitively answered. It is, therefore, not possible to make rigorously evidence-based recommendations regarding maternal vitamin D supplementation during pregnancy.

Further high-quality research is needed. In many areas, large, well-designed, prospective cohort studies are most appropriate as the next step. In others (e.g. birthweight, serum calcium concentration, bone mass), the evidence base is sufficient to suggest RCTs. Additionally, a critical underlying issue is to ensure that 25(OH)D measurements are comparable between studies, through global standardisation programmes. Specific recommendations are given below:

- Long-term follow-up of mothers and children who have taken part in the vitamin D supplementation trials is required. Although vitamin D supplementation at modest doses appears safe in the short term, the long-term effects are unknown.
- Key issues for all vitamin D research are the requirement for standardisation of exposures and outcomes, inclusion and standardisation of potential confounding factors, and adequate length of follow-up. Work aimed at standardising 25(OH)D measurements across the globe should be supported, such as the programme led by the US National Institutes of Health,[71] and which incorporates UK centres.
- There is a need to optimise the biochemical assessment of vitamin D status, whether this is simply 25(OH)D concentration, or should incorporate other indices such as DBP or albumin, and whether it should be related to PTH or calcium concentrations.
- 25(OH)D concentrations should be surveyed in a large population-based pregnancy cohort representative of the UK as a whole to enable acquisition of high-quality descriptive epidemiological data on the prevalence of low levels of circulating 25(OH)D. This work would need to take into account potential confounding factors, particularly season, latitude, skin pigmentation, covering and ethnicity.
- High-quality large prospective cohort studies are required to investigate the relationship between maternal 25(OH)D status and the following outcomes: maternal caesarean section, bacterial vaginosis, offspring birth length, anthropometric measures and risk of low birthweight. These studies should take account of potential confounding factors and include measures of vitamin D status early in pregnancy as well as at delivery. Such studies should be performed in several different populations of varying ethnicity, and outcomes and exposures should be standardised, as should potential confounding factors.
- Large well-designed RCTs with double-blind, placebo-controlled methodology are warranted to investigate the relationship between maternal vitamin D supplementation during pregnancy and offspring birthweight, calcium concentrations, bone mass, with a weaker recommendation (compared with the appropriateness of high-quality prospective observational studies) for offspring asthma, type 1 diabetes mellitus and maternal pre-eclampsia. There are currently several large RCTs under way which may help to address the study questions. Examples of these include MAVIDOS[104] (ISRCTN 82927713), which is investigating the effects of maternal vitamin D supplementation on offspring bone mass, VDAART (ISRCTN 00920621) and ABCvitaminD (ISRCTN 00856947), both of which are investigating the effects of maternal vitamin D supplementation on asthma and wheeze.

Without such a rigorous approach, there is a risk that public health policy will be made on the basis of optimistic evaluations of conflicting and heterogeneous studies. Although modest doses of vitamin D in pregnancy might well be relatively safe, at least in the short term, there are no long-term data to inform their potential long-term effects on offspring health. As with most interventions, it is probably optimistic to expect that there will be no risk of adverse events.

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All authors were involved in writing the manuscript.

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Appendix 1 Search strategy

Sources

Completed studies (systematic reviews)

- DARE (CRD).
- CDSR.
- HTA database (CRD).

Completed studies (other study types)

- CENTRAL.
- MEDLINE.
- EMBASE.
- BIOSIS.
- Google Scholar.
- AMED.

Hand searching of reference lists from papers identified

Ongoing studies

- National Research Register archive.
- UKCRN Portfolio.
- Current Controlled Trials.
- ClinicalTrials.gov.

Grey literature

- Conference Proceedings Citation Index-Science (1990–present).
- Zetoc conference search.
- SACN website.
- Department of Health website.
- The King's Fund library database.
- Trip database.
- HTA website.
- HMIC database.

Databases and years searched	Terms	Number retrieved	Number of relevant hits
Systematic reviews			
The Cochrane Library: CDSR, current Issue, 2010 URL: www.thecochranelibrary.com/view/0/index.html			
DARE (CRD) 2000–10 URL: www.crd.york.ac.uk/crdweb/			
HTA database (CRD) URL: www.crd.york.ac.uk/crdweb/			
National Coordinating Centre for HTA website URL: www.nets.nihr.ac.uk/programmes/hta			
Other study types			
The Cochrane Library: CENTRAL, current Issue, 2010 URL: www.thecochranelibrary.com/view/0/index.html			
MEDLINE (OVID) 1950–2010, June, week 1 (15 June 2010)	Pregnan$.ti,ab. 295,057 Preconception$.ti,ab. 1752 preconceptual.ti,ab. 135 pre-concept$.ti,ab. 250 Fetal.ti,ab. 157,883 Foetal.ti,ab. 11,957 Fetus.ti,ab. 43,868 Foetus.ti,ab. 4543 Newborn$.ti,ab. 104,312 Neonat$.ti,ab. 154,612 Baby.ti,ab. 21,290 Babies.ti,ab. 22,884 Infant.ti,ab. 99,951 Infancy.ti,ab. 29,601 Premature.ti,ab. 68,207 Toddler$.ti,ab. 3913 Offspring.ti,ab. 33,494 Child$.ti,ab. 770,655 Postnatal.ti,ab. 61,090 Postpartum.ti,ab. 25,159 Maternal.ti,ab. 126,587 Maternity.ti,ab. 10,210 Mother.ti,ab. 58,088 small-for-gestational age.ti,ab. 4212 pre-natal.ti,ab. 573 prenatal.ti,ab. 52,711 ante-natal.ti,ab. 267 post-partum.ti,ab. 6959 post-natal.ti,ab. 3777 puerperium.ti,ab. 4552 childbear$.ti,ab. 6830 birthweight.ti,ab. 9667 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 1,557,322	6501 hits	First 500 references saved [Reference IDs: 82–581 in Reference Manager database (version 12; Thomson ResearchSoft, San Francisco, CA, USA)]

Databases and years searched	Terms	Number retrieved	Number of relevant hits
	Pregnancy/ 609,281 Prenatal Nutritional Physiological Phenomena/ 695 Pregnancy, High-Risk/ 3586 Maternal Nutritional Physiological Phenomena/ 988 Pregnancy Complications/ 62,603 Pregnancy Outcome/ 29,721 Maternal Fetal exchange/ 26,212 Prenatal Exposure Delayed Effects/ 14,989 exp "Embryonic and Fetal Development"/ 163,222 Child Development/ 28,583 Preconception Care/ 981 Prenatal Care/ 16,979 Postpartum Period/ 14,439 exp infant/ 817,413 Postnatal Care/ 3095 49 exp Pregnancy Trimesters/ 27,623 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 34 or 35 or 36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 2,155,617 exp Vitamin D/ 34,004 "1406-16-2 (Vitamin D)".rn. 15,518 "25(OH)-vit D".ti,ab. 15 25OHD.ti,ab. 424 hypovitaminosis D.ti,ab. 440 "19356-17-3 (Calcifediol)".rn. 2398 "32222-06-3 (Calcitriol)".rn. 11,536 "64719-49-9 (25-hydroxyvitamin D)".rn. 1333 Vitamin D deficiency/ 5668 Vitamin D.ti,ab. 25,020 Vitamin D2.ti,ab. 862 Vitamin D3.ti,ab. 5527 Cacidiol.ti,ab. 0 calciol.ti,ab. 12 "67-97-0 (Cholecalciferol)".rn. 4441 Ergocalciferol.ti,ab. 288 Cholecalciferol.ti,ab. 1086 Colecalciferol.ti,ab. 21 Calciferol.ti,ab. 330 Calcitriol.ti,ab. 2923 Hydroxycholecalciferol.ti,ab. 1111 dihydroxycholecalciferol$.ti,ab. 1366 dihydroxyvitamin d.ti,ab. 3858 dihydrotachysterol$.ti,ab. 294 doxercalciferol$.ti,ab. 48 alfacalcidol$.ti,ab. 297 paricalcitol$.ti,ab. 180 Calcitriol/ 11,536 51 or 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 45,279 49 and 79 67 50 and 79 8116		

Databases and years searched	Terms	Number retrieved	Number of relevant hits
	Animals/ 4,579,351 Humans/ 11,255,304 82 and 83 1,175,867 82 not 84 3,403,484 **81 not 85 6501**		
EMBASE (OVID) 2000–4, week 21			
BIOSIS 1985–2010			
Ongoing studies			
National Research Register archive, 14 June 2010 URL: https://portal.nihr.ac.uk/Pages/NRRArchiveSearch.aspx	"Vitamin D" and pregnancy [All fields]	20	0
UKCRN Portfolio, 14 June 2010	Pregnancy [Title]	41	1, possible 2
URL: http://public.ukcrn.org.uk/Search/Portfolio.aspx	Pregnancy vitamin [research summary]	2	1
Current Controlled Trials including Medical Research Council Trials database, 14 June 2010 URL: http://controlled-trials.com/	Vitamin d AND pregnancy	207	13 (slight overlap with UKCRN)
ClinicalTrials.gov URL: http://clinicaltrials.gov/			
Conferences and grey literature			
Conference Proceedings Citation Index-Science (1990–present)			
Trip database URL: www.tripdatabase.com/search/advanced			
The King's Fund database, 14 June 2010 URL: www.kingsfund.org.uk/library/	Pregnancy	528	
	Vitamin d	15	Possible 2
SACN website, 14 June 2010 URL: www.sacn.gov.uk/reports_position_statements/index.html	Browse reports and position statements section	Figure 12 report	2 reports
Department of Health website, 14 June 2010 URL: http://webarchive.nationalarchives.gov.uk/20130107105354/http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4005936	Browse reports	Figure 2	
Zetoc (general and conferences) URL: http://zetoc.mimas.ac.uk/wzgw?id=23685659			

Databases and years searched	Terms	Number retrieved	Number of relevant hits
Guidelines			
SIGN URL: www.sign.ac.uk NICE URL: www.nice.org.uk National Guidelines Clearinghouse URL: www.ahcpr.gov/clinic/assess.htm			

Appendix 2 Data extraction forms

Data extraction forms: case–control studies

a. Study basic details
UIN/AN
Title
Reviewer
Date reviewed
Author
Journal and year
Source

AN, article number; UIN, unique identifier number.

b. Study description
1. Setting
2. Study design
3. Outcome measured
4. Statistical techniques used
5. Confounding factors adjusted for
6. Cohort size
7. Number of subjects studied for outcome
8. % follow-up (5 ÷ 6)

c. Inclusion criteria	d. Exclusion criteria

e. Quality assessment: enter a rating and justify with a brief comment

Criterion	Score	Comment
1. Case definition explicit and appropriate?		
2. How is maternal vitamin D measured?		
3. Participants grouped according to vitamin D status?		
4. Measurements of outcomes reliably ascertained?		
5. Measurement of later outcomes objective?		
6. Control selection appropriate?		
7. Measures of vitamin D intake/25(OH)D level, outcomes rounded?		
8. Setting and population appropriate?		
9. Outcome assessment blind to vitamin D status?		
10. Analysis rigorous and appropriate?		
11. Response rates for: a. cases b. controls (A separate score for each should be given)		
12. Information on representativeness and non-participants?		
13. Sample sizes for: a. cases b. controls (A separate score for each should be given)		
14. Adequate consideration for important confounding factors? (e.g. season, sunlight exposure, calcium intake, maternal compliance, infant feeding)		
Overall quality rating (sum of scores)		

f. Study results: free text, to consider cohort details, associations found, any additional quality comments

g. Screen of references: any additional studies listed which have not already been reviewed?

Data extraction forms: intervention studies

a. Study basic details
UIN/AN
Title
Reviewer
Date reviewed
Author
Journal and year
Source

AN, article number; UIN, unique identifier number.

b. Study description
1. Setting
2. Study design
3. Outcome measured
4. Statistical techniques used
5. Intention-to-treat analysis. Patients analysed according to the group they were randomised to?
5. Confounding factors adjusted for
6. Cohort size
7. Number of subjects studied for outcome
8. % follow-up (5 ÷ 6)
9. Age range (mean age + SD)
10. Treatment given/dose/route of administration/duration of treatment
11. Duration of follow-up

c. Inclusion criteria	d. Exclusion criteria

e. Quality assessment: enter a rating and justify with a brief comment

Criterion	Score	Comment
1. Study design appropriate?		
2. Are CONSORT guidelines followed?		
3. Adequate description of study participants?		
4. Is randomisation adequate?		
5. Is there placebo control and is blinding adequate?		
6. Are details of the study medication given		
7. Is change in maternal vitamin D status measured?		
8. Are details of the assay given?		
9. Measurements of outcomes reliably ascertained?		
10. Measurements of later outcomes objective?		
11. Measures of vitamin D intake/25(OH)D, bone outcomes, e.g. BMD rounded		
12. Consideration for the effects of important confounding factors? (e.g. season, sunlight exposure, calcium intake, maternal compliance, infant feeding)		
13. What proportion of the cohort completed the trial		
14. Information on non-participants?		
15. Analysis rigorous and appropriate?		
16. Sample size		
Overall quality rating (sum of scores)		

CONSORT, Consolidated Standards of Reporting Trials.

f. Study results: free text, to consider cohort details, associations found, any additional quality comments

g. Screen of references: any additional studies listed which have not already been reviewed?

Data extraction forms: cohort studies

a. Study basic details (automatically completed)
UIN/AN
Title
Author
Journal and year
Source

AN, article number; UIN, unique identifier number.

b. Study description
1. Setting
2. Study design
3. Outcome measured
4. Statistical techniques used
5. Confounding factors adjusted for
6. Cohort size
7. Number of subjects studied for outcome
8. % follow-up (e ÷ f)
9. Age range (mean age + SD)

c. Inclusion criteria	d. Exclusion criteria

e. Quality assessment: enter a rating and justify with a brief comment

Criterion	Score	Comment
1. Study design appropriate?		
2. Adequate description of study participants?		
3. Measurements of vitamin D reliably ascertained?		
4. Participants grouped according to vitamin D status?		
5. Measurements of later outcomes reliably ascertained?		
6. Measures of later outcomes objective		
7. Measures of vitamin D intake/25(OH)D, bone outcomes rounded?		
8. Consideration for the effects of important confounding factors (e.g. season, sunlight exposure, calcium intake, maternal compliance, physical activity)		
9. Outcome assessment blind to maternal vitamin D status?		
10. What proportion of the cohort was followed up?		
11. Information on non-participants		
12. Analysis rigorous and appropriate?		
13. Sample size		
Overall quality rating (sum of scores)		

f. Study results: free text, to consider cohort details, infant size/growth measures(s), muscle strength outcome(s), associations found, any additional quality comments

g. Screen of references: any additional studies listed which have not already been reviewed?

Appendix 3 Study quality assessment system

TABLE 2 Summary of case–control study quality assessment system

	Risk of bias (score)		
Criterion	**High (–1)**	**Medium (0)**	**Low (+1)**
1. Case definition explicit and appropriate?	Definition and/or inclusion/exclusion criteria not given, ambiguous or clearly unsuitable	Basic definition given; enough to satisfy that chosen cases (and the criteria used to select them) are suitable	Detailed definition and explanation; all suitable cases included
2. How is maternal vitamin D status measured?	Dietary intake only or insufficient information	Blood levels of 25(OH)D	Blood levels of circulating 25(OH)D, with details of precision, pick up of D_2 and D_3 and assay used
3. Participants grouped according to vitamin D status?	Subjects divided and analysed in groups based on pre-existing vitamin D thresholds	Subjects divided and analysed in groups according to vitamin D level based on group characteristics	Subjects not divided into groups according to Vitamin D level/or grouped according to at threshold generated from the study
4. Measurements of outcomes reliably ascertained?	Inadequately explained or obviously unsuitable	Adequate description and reliability/suitability of at least one of the following: instruments, technique/definition/protocol, people, place	Detailed description and reliability of one and at least adequate description of the others
5. Measurements of later outcomes objective?	Subjective measure, e.g. bone or muscle pain, wheezing	Ascertained from researcher examination	Objective measure, e.g. DEXA, bone biopsy, lung function tests
6. Control selection appropriate?	No information at all, ambiguous, or not selected from population of cases or otherwise clearly inappropriate to the study objectives	Selection is from population of cases, and is basically appropriate and similar to cases for all factors other than the outcome of interest, but not optimally, or with incomplete information	Selection is from population of cases in a manner wholly appropriate to the study objectives, and in such a way as to make them as similar as possible to cases in all respects except the outcome of interest
7. Measures of vitamin D intake/25(OH)D level, bone outcomes rounded?	Categorisation or very rough rounding, or if any clear evidence of rounding exists without explanation in the text	Measures are rounded, but not by much	No information given, and no obvious reason to suspect rounding has occurred Or explicitly stated that measurements were not rounded
8. Setting and population appropriate?	Ambiguously described, obviously bias inducing or unsuitable for the objectives and stated conclusions	Possibly restricting but reflected in the scope of the objectives and the stated conclusions	Planned to minimise bias and allow generalisability beyond the immediate scope of the objectives
9. Outcome assessment blind to vitamin D status?	N/A	No details given	Some details or statement given

continued

TABLE 2 Summary of case–control study quality assessment system (*continued*)

Criterion	Risk of bias (score)		
	High (–1)	Medium (0)	Low (+1)
10. Analysis rigorous and appropriate?	No statistical analyses carried out (just tables or description), or analysis badly carried out	Tables of means and differences given with statistical tests (e.g. *t*-tests), or some regression but without clear/valid measure of association	Regression (or similar technique) is used which gives a valid measure of association (e.g. ORs, hazard ratios, relative risks)
11. Response rates for: a. cases b. controls (A separate score for each should be given)	Low (< 70%)	Medium (70–90%) or not given	High (> 90%)
12. Information on representativeness and non-participants	Cases obviously unrepresentative of wider population alluded to in text	Some information on cases and controls lost or excluded, or no information but with no reason to suspect a detrimental lack of representativeness	Detailed information on cases and controls lost or excluded, with numbers and reasons
13. Sample sizes for: a. cases b. controls (A separate score for each should be given)	Extremely ambiguous, not given, or small (< 100)	Average (100–1000)	Large (> 1000)
14. Adequate consideration of important confounding factors? (e.g. season, sunlight exposure, calcium intake, maternal compliance, infant feeding)	One factor matched on or controlled for in tables; nothing for the others (NB: whether they were *measured* or not is irrelevant)	Most factors matched on or controlled for in tables, or fewer if one or more is adjusted for in regression	Most factors adjusted for in regression

N/A, not applicable.

TABLE 3 Summary of cohort/cross-sectional study quality assessment system

	Risk of bias (score)		
Criterion	High (–1)	Medium (0)	Low (+1)
1. Study design appropriate?	Ambiguously described, obviously bias inducing or unsuitable for the objectives and stated conclusions	Possibly restricting but reflected in the scope of the objectives and the stated conclusions	Planned to minimise bias and allow generalisability beyond the immediate scope of the objectives
2. Adequate description of study participants?	Little or no information given	Including/excluding and other criteria such as term/preterm/SGA baby given in some way; at least two useful measures including measure of vitamin D status, ethnicity	Including/excluding and other criteria such as term/preterm/SGA baby given in some way; at least three useful measures including measure of vitamin D status, ethnicity with measures of precision
3. How is maternal vitamin D status measured?	Dietary intake only or insufficient information	Blood levels of circulating 25(OH)D	Blood levels of circulating 25(OH)D, with details of precision, pick up of D_2 and D_3 and assay used
4. Participants grouped according to vitamin D status?	Subjects divided and analysed in groups based on pre-existing vitamin D thresholds	Subjects divided and analysed in groups according to Vitamin D level based on group characteristics	Subjects not divided into groups according to Vitamin D level/or grouped according to at threshold generated from the study
5. Measurements of outcomes reliably ascertained?	Inadequately explained or obviously unsuitable	Adequate description and reliability/suitability of at least one of the following: instruments, technique/definition/protocol, people, place	Detailed description and reliability of one and at least adequate description of the others
6. Measurements of later outcomes objective?	Subjective measure, e.g. bone or muscle pain, wheezing	Ascertained from researcher examination	Objective measure, e.g. DEXA, bone biopsy, lung function tests
7. Measures of vitamin D intake/25(OH)D level, bone outcomes rounded?	Measures categorised or rounded very roughly, or if any clear evidence of rounding exists without explanation in the text	Yes, but not by much	No information given and no obvious reason to suspect rounding has occurred; or explicitly stated that measurements were not rounded
8. Consideration for the effects of important confounding factors (e.g. season, sunlight exposure, calcium intake, maternal compliance, infant feeding)?	One factor controlled for in tables, nothing for the others (NB whether they were *measured* or not is irrelevant)	Most factors controlled for in tables, or fewer if one or more is adjusted for in regression	Most factors adjusted for in regression
9. Outcome assessment blind to maternal vitamin D status?	N/A (cannot score –1 in this category)	No details given	Some details or statement given
10. What proportion of the cohort was followed up?	% follow-up is not given, unclear, or low (< 70%)	% follow-up is low to average (70–90%)	% follow-up is high (> 90%)

continued

TABLE 3 Summary of cohort/cross-sectional study quality assessment system (*continued*)

Criterion	Risk of bias (score)		
	High (–1)	Medium (0)	Low (+1)
11. Information on non-participants	Very little or no information, or information given that is adequate but suggests a serious potential for bias	Adequate information given, or information given that is very clear but suggests a moderate potential for bias	Above average information given, none of which suggests a potential for bias
12. Analysis rigorous and appropriate?	No statistical analyses carried out (just tables or description)	Tables of means and differences given with statistical tests (e.g. *t*-tests), or some regression but without clear/valid measure of association	Regression (or similar technique) used which gives a valid measure of association (e.g. ORs, hazard ratios, relative risks)
13. Sample size	Extremely ambiguous, not given, or small (< 100)	Average (100–1000)	Large (> 1000)

N/A, not applicable.

TABLE 4 Summary of intervention study quality assessment system

Criterion	Risk of bias (score)		
	High (–1)	Medium (0)	Low (+1)
1. Study design appropriate?	Ambiguously described, obviously bias inducing or unsuitable for the objectives and stated conclusions	Possibly restricting but reflected in the scope of the objectives and the stated conclusions	Planned to minimise bias and allow generalisability beyond the immediate scope of the objectives
2. Are CONSORT guidelines followed?	Not described, not followed or poorly adherent	CONSORT report presented but some data missing	Full adherence to CONSORT guidelines
3. Adequate description of study participants?	Little or no information given	Including/excluding and other criteria such as term/preterm/SGA baby given in some way; at least two useful measures including measure of vitamin D status, ethnicity	Including/excluding and other criteria such as term/preterm/SGA baby given in some way; at least three useful measures including measure of vitamin D status, ethnicity with measures of precision
4. Is randomisation adequate?	No randomisation or not discussed	Some attempt at randomisation	Robust randomisation
5. Is there placebo control and is blinding adequate?	Not controlled, not adequate or not discussed	Placebo control, either not blinded or single blinded	Placebo control, double blinded
6. Are details of the study medication given?	No details	Some detail, e.g. 'vitamin D 1000 IU/day'	Full details including D_2 or D_3, manufacturer, GMP compliant, full regimen
7. Is change in maternal vitamin D status measured?	N/A	No	Yes
8. Are details of the assay given?	No details	Some details, e.g. DiaSorin RIA	Fully detailed – type, manufacturer, precision, D_2/D_3 pick up
9. Measurements of outcomes reliably ascertained?	Inadequately explained or obviously unsuitable	Adequate description and reliability/suitability of at least one of the following: instruments, technique/definition/protocol, people, place	Detailed description and reliability of one and at least adequate description of the others
10. Measurements of later outcomes objective?	Subjective measure, e.g. bone or muscle pain, wheezing	Ascertained from researcher examination	Objective measure, e.g. DEXA, bone biopsy, lung function tests
11. Measures of vitamin D intake/25(OH)D level, bone outcomes, e.g. BMC rounded?	Measures categorised or rounded very roughly, or if any clear evidence of rounding exists without explanation in the text	Yes, but not by much	No information given and no obvious reason to suspect rounding has occurred; or explicitly stated that measurements were not rounded
12. Consideration for the effects of important confounding factors? (e.g. season, sunlight exposure, calcium intake, maternal compliance, infant feeding)	One factor controlled for in tables, nothing for the others (NB whether they were *measured* or not is irrelevant)	Most factors controlled for in tables, or fewer if one or more is adjusted for in regression	Most factors adjusted for in regression

continued

TABLE 4 Summary of intervention study quality assessment system (*continued*)

Criterion	Risk of bias (score)		
	High (–1)	Medium (0)	Low (+1)
13. What proportion of the cohort completed the trial?	% follow-up is not given, unclear, or low (< 70%)	% follow-up is low to average (70–90%)	% follow-up is high (> 90%)
14. Information on non-participants	Very little or no information, or information given that is adequate but suggests a serious potential for bias	Adequate information given, or information given that is very clear but suggests a moderate potential for bias	Above average information given, none of which suggests a potential for bias
15. Analysis rigorous and appropriate?	No statistical analyses carried out (just tables or description)	Appropriate statistical techniques but no mention of whether intention to treat or pre protocol	Appropriate statistical techniques and intention to treat primary analysis
16. Sample size	Extremely ambiguous, not given, or small (< 100)	Average (100–250)	Large (> 250)

CONSORT, Consolidated Standards of Reporting Trials; GMP, good manufacturing practice; N/A, not applicable.

Appendix 4 Flow diagram of study selection

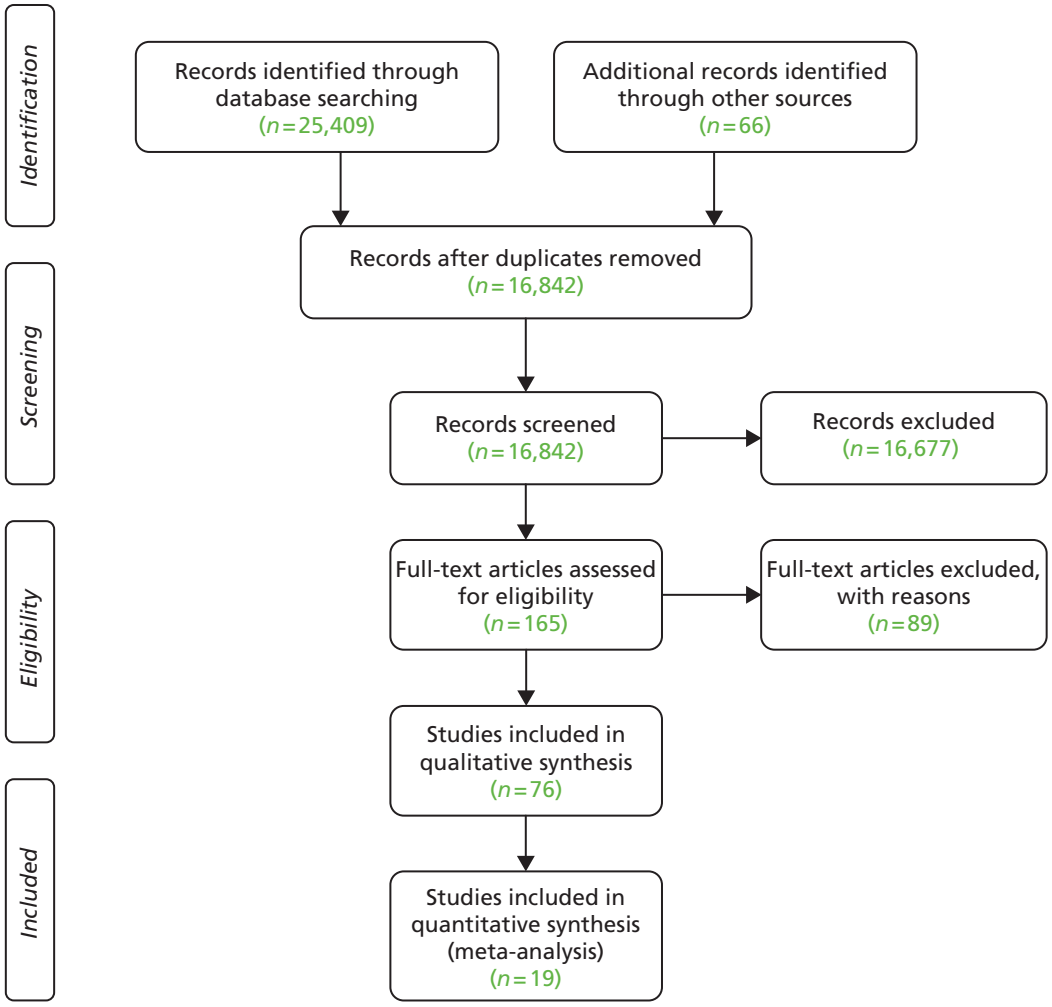

FIGURE 1 Flow diagram of study selection.

Appendix 5 Summary of quality assessment scores

TABLE 5 Summary of scoring results in terms of risk of bias (low, medium or high) of all case–control studies included in the review

First author	1. Design	2. Vitamin D measurement	3. Grouping of participants by vitamin D status	4. Outcomes reliably ascertained	5. Outcomes objective	6. Controls	7. Rounding	8. Setting
Azar 2011[133]	Low	Low	Low	Medium	Low	Medium	Medium	Medium
Baker 2010[129]	Low	Low	Low	Medium	Low	Low	Medium	Low
Baker 2011[121]	Low	Low	High	Medium	Low	Medium	Medium	Low
Baker 2012[141]	Low	Low	Medium	Low	Low	Medium	Medium	Medium
Bodnar 2007[128]	Low	Low	Low	Medium	Low	Medium	Medium	Low
Bodnar 2010[115]	Medium	Low	Low	Medium	Low	Medium	Medium	Low
Brunvand 1998[144]	Medium	Low	Low	Low	Medium	High	Medium	Medium
Delmas 1987[120]	High	Medium	Low	High	Medium	High	Medium	Low
Makgoba 2011[140]	Low	Low	Medium	Low	Low	Medium	Medium	Low
Powe 2010[132]	Low	Low	Low	Medium	Low	Medium	Medium	Low
Robinson 2010[130]	Low	Low	Low	Medium	Low	High	Medium	Low
Robinson 2011[116]	Medium	Low	Medium	Medium	Medium	Medium	Medium	Medium
Seely 1992[131]	Low	Medium	Low	Medium	Low	Medium	Medium	Medium
Soheilykhah 2010[138]	Low	Low	High	Low	Low	Medium	Medium	Medium
Sorensen 2012[125]	Low	Low	Low	Medium	Medium	Medium	Medium	Low
Stene 2003[126]	Low	High	High	Medium	Low	Medium	Medium	Medium
Zhang 2008[139]	Low	Low	Low	Low	Low	Medium	Medium	Medium

a Numbers represent an estimate of the overall risk of bias, totalling the risk for each question defined as −1 for a 'high' risk of bias, 0 for a 'medium' risk of bias, and +1 for a 'low' risk of bias.

		11. Response rates			13. Sample size				
9. Blinding	10. Analysis	Cases	Controls	12. Non-participants	Cases	Controls	14. Confounding	Overall total[a]	Overall classification
Medium	Low	Low	Low	High	High	High	Low	5	Low
Medium	Low	Medium	Low	Low	High	Medium	Low	9	Low
Medium	Low	Low	Low	Medium	High	Medium	Low	5	Low
Medium	Low	Low	Low	Medium	High	Medium	Low	7	Low
Medium	Low	Low	Low	Medium	High	Medium	Low	8	Low
Medium	Low	Low	Low	Medium	Medium	Medium	Low	7	Low
Medium	Low	Medium	Medium	Medium	High	High	Medium	1	Medium
Medium	Medium	Medium	Medium	Medium	High	High	High	–4	High
Medium	Low	Medium	Medium	Medium	High	Medium	Low	6	Low
Medium	Low	High	High	Medium	High	Medium	Low	4	Medium
Medium	Low	Medium	Medium	Medium	High	Medium	Low	5	Low
Medium	Medium	Medium	Medium	Medium	Medium	Medium	Medium	1	Medium
Medium	Low	Medium	High	Medium	High	High	Medium	2	Medium
Medium	Low	Medium	Medium	Medium	High	Medium	Medium	3	Medium
Medium	Low	Low	Low	Medium	Medium	Medium	Low	8	Low
Medium	Low	Medium	High	Medium	Medium	Low	Low	2	Medium
Medium	Low	Low	Low	Medium	High	Medium	Low	6	Low

TABLE 6 Summary of scoring results in terms of risk of bias (low, medium or high) of all cohort/cross-sectional studies included in the review

First author	1. Design	2. Participant	3. Vitamin D measurement	4. Grouping of participant by vitamin D status	5. Outcomes reliably ascertained	6. Outcomes objective	7. Rounding
Akcakus 2006[103]	Medium	Low	Low	Low	Medium	Low	Medium
Amirlak 2009[83]	Medium	Low	Medium	Low	Medium	Low	Medium
Ardawi 1997[90]	Medium	Low	Low	Low	Low	Low	Medium
Bodnar 2009[146]	High	Low	Low	Low	Low	Low	Medium
Bowyer 2009[84]	Low	Low	Low	High	Medium	Low	Medium
Camargo 2007[109]	Low	Low	High	Low	Medium	High	Medium
Camargo 2011[110]	Low	Low	Low	High	High	High	Medium
Clifton-Bligh 2008[95]	Medium	Low	Low	Low	Low	Low	Medium
Cremers 2011[113]	High	Low	Medium	Medium	Low	Low	Medium
Crozier 2012[106]	Low	Low	Low	Low	Low	Low	Medium
Devereux 2007[26]	Medium	Medium	High	Medium	Medium	High	Medium
Dror 2012[96]	Low	Medium	Medium	Low	Low	Low	Medium
Dunlop 2011[148]	Medium	Medium	Medium	High	Low	Low	Medium
Erkkola 2009[107]	Medium	Medium	High	Medium	Medium	High	Medium
Farrant 2009[93]	Medium	Low	Low	Low	Low	Low	Medium
Fernandez-Alonso 2012[118]	Low	Medium	Low	High	Low	Low	Medium
Gale 2008[24]	Medium	Low	Low	High	Low	Low	Medium
Hensel 2011[147]	Medium	High	Low	High	Low	Low	Medium
Haugen 2009[134]	Medium	Low	High	High	Medium	Low	Medium
Hossain 2011[122]	Medium	Low	Low	Low	Medium	Medium	Medium
Javaid 2006[1]	Low	Low	Low	Medium	Low	Low	Medium
Krishnaveni 2011[105]	Medium	Medium	Low	Low	Low	Low	Medium
Leffelaar 2010[85]	Low	Low	Low	High	Medium	Low	Medium
Maghbooli 2007[92]	Medium	High	Low	Low	Medium	Medium	Low
Maghbooli 2008[32]	Medium	Low	Low	Medium	Low	Low	High
Mannion 2006[86]	Medium	Low	High	Low	Medium	Medium	Medium
Marjamaki 2010[127]	Medium	Low	High	Low	Low	Low	Medium
Mehta 2009[119]	Medium	Medium	Medium	Medium	Medium	Medium	Medium
Merewood 2009[143]	Medium	Low	Medium	High	Low	Low	Medium
Miyake 2010[108]	Medium	Medium	High	Medium	Medium	High	Medium
Morales 2012[112]	Low	Low	Medium	Low	High	High	Medium
Morley 2006[94]	Medium	Low	Low	Low	Low	Low	Medium
Nwaru 2010[114]	Medium	Medium	High	Low	Low	Low	Medium
Oken 2007[135]	Medium	Low	High	Low	Medium	low	Medium
Prentice 2009[98]	Medium	Low	Low	Low	Low	Low	Medium

8. Confounding	9. Blinding	10. % follow-up	11. Non-participants	12. Analysis	13. Sample size	Overall total[a]	Overall classification
High	Medium	Medium	Medium	Low	Medium	4	Medium
Medium	Medium	High	High	Low	High	2	Medium
High	Medium	Low	Medium	Medium	Medium	5	Low
High	Medium	Low	Medium	Low	Medium	5	Low
Medium	Medium	High	Low	Low	Medium	4	Medium
Low	Medium	High	High	Low	Low	2	Medium
Low	Medium	Low	Medium	Low	Medium	3	Medium
Low	Medium	Medium	High	Low	Medium	6	Low
Low	Medium	High	Medium	Low	Medium	3	Medium
Low	Medium	High	Low	Low	Medium	8	Medium
Low	Medium	High	High	Low	Low	−1	High
Low	Medium	Medium	Low	Low	Medium	7	Low
Low	Medium	High	Medium	Low	Medium	2	Medium
Medium	Medium	High	Medium	Low	Low	−1	High
Medium	Medium	High	Medium	Low	Medium	5	Low
High	Medium	Low	Medium	Medium	Medium	3	Medium
Medium	Medium	Medium	Medium	Low	Medium	4	Medium
Low	Medium	Low	Medium	Low	Medium	4	Medium
Low	Medium	Medium	High	Low	Low	2	Medium
Medium	Medium	Medium	Medium	Low	Medium	4	Medium
Medium	Medium	High	Medium	Low	Medium	5	Low
Medium	Medium	Medium	High	Low	Medium	4	Medium
Low	Medium	High	Medium	Low	Low	5	Low
High	Medium	Low	High	Medium	Medium	1	Medium
High	Medium	Low	High	Medium	Medium	3	Medium
Medium	Medium	High	High	Low	Medium	1	Medium
Medium	Medium	Medium	Low	low	Low	6	Low
Medium	Medium	Medium	Low	Low	Medium	2	Medium
Low	Medium	Low	Low	Low	Medium	6	Low
Low	Medium	Medium	High	Low	Medium	−1	High
Low	Medium	High	Medium	Low	Low	3	Medium
Low	Medium	Medium	Low	Low	Medium	8	Low
Low	Medium	Medium	High	Low	Medium	3	Medium
Low	Medium	Medium	Low	Low	Low	6	Low
Low	Medium	High	High	Low	Medium	5	Low

TABLE 6 Summary of scoring results in terms of risk of bias (low, medium or high) of all cohort/cross-sectional studies included in the review (*continued*)

First author	1. Design	2. Participant	3. Vitamin D measurement	4. Grouping of participant by vitamin D status	5. Outcomes reliably ascertained	6. Outcomes objective	7. Rounding
Rothers 2011[111]	Low	Medium	Medium	High	Low	Low	Medium
Sabour 2006[91]	Med	Low	High	High	Medium	Medium	Medium
Savvidou 2012[145]	Low	Low	Low	Medium	Low	Low	Medium
Sayers 2009[41]	Low	Medium	High	Low	Low	Low	Low
Scholl 2009[87]	Medium	Low	High	Medium	Low	Medium	Low
Scholl 2012[142]	Medium	Low	Low	High	Low	Low	Medium
Shand 2010[117]	Medium	Low	Low	High	Medium	Low	Medium
Shibata 2011[123]	Low	Medium	Low	Low	Medium	Medium	Medium
Viljakainen 2010[97]	Medium	Low	Low	Medium	Low	Low	Medium
Viljakainen 2011[102]	Medium	Medium	Low	Medium	Low	Low	Medium
Watson 2010[88]	Medium	Low	High	Low	Medium	Low	Medium
Weiler 2005[89]	Low	Medium	Low	High	Low	Low	Medium

a Numbers represent an estimate of the overall risk of bias, totalling the risk for each question defined as –1 for a 'high' risk of bias, 0 for a 'medium' risk of bias, and +1 for a 'low' risk of bias.

8. Confounding	9. Blinding	10. % follow-up	11. Non-participants	12. Analysis	13. Sample size	Overall total[a]	Overall classification
Medium	Medium	High	Med	Low	Medium	2	Medium
High	Medium	Medium	High	Low	Medium	–2	High
Low	Medium	Low	Medium	Medium	Medium	7	Low
High	Medium	High	High	Low	Low	3	Medium
Medium	Medium	High	High	Low	Low	2	Medium
Low	Medium	High	Medium	Low	Low	5	Low
Low	Medium	Low	Low	Low	Medium	6	Low
Medium	Medium	Medium	Medium	Low	Medium	4	Medium
Medium	Medium	High	High	Low	Medium	3	Medium
Low	Medium	High	Low	Low	High	4	Medium
Low	Medium	Medium	High	Low	Medium	3	Medium
Low	Medium	High	Medium	Low	High	3	Medium

TABLE 7 Summary of scoring results in terms of risk of bias (low, medium or high) of all intervention studies included in the review

First author	1. Design	2. CONSORT guidance followed	3. Participant	4. Randomisation	5. Placebo control and blinding	6. Study medication details	7. Maternal 25(OH)D	8. Assay detail
Brooke 1980[3]	Medium	High	Medium	Medium	Low	Medium	Low	Medium
Cockburn 1980[20]	Medium	High	High	High	Medium	Medium	Low	Medium
Congdon 1983[21]	Medium	High	High	High	High	Medium	High	Medium
Delvin 1986[6]	Low	High	High	Medium	High	Medium	Low	Medium
Hollis 2011[100]	Low	Low	Medium	Medium	Medium	Low	Low	Low
Kaur 1991[101]	Medium	High	Medium	Medium	High	Medium	Medium	Medium
Marya 1981[4]	Medium	High	High	Medium	High	Medium	Medium	High
Marya 1987[136]	Medium	High	High	Medium	High	Medium	Medium	Medium
Marya 1988[5]	Medium	High	Low	Medium	High	Medium	Medium	High
Mallet 1986[7]	Medium	High	High	Medium	High	Medium	Medium	Low
Yu 2009[99]	Low	Low	Medium	Low	High	Medium	Low	High

CONSORT, Consolidated Standards of Reporting Trials.

a Numbers represent an estimate of the overall risk of bias, totalling the risk for each question defined as –1 for a 'high' risk of bias, 0 for a 'medium' risk of bias, and +1 for a 'low' risk of bias.

5. Outcomes reliably ascertained	6. Outcome objective	7. Rounding	8. Confounding	10. % follow-up	11. Non-participant	12. Analysis	13. Sample size	Overall total[a]	Overall classification
Medium	Medium	Medium	Medium	High	High	Medium	High	–2	High
Low	Low	Medium	Low	High	High	Medium	Medium	–1	High
High	Medium	Medium	Medium	High	High	Medium	High	–9	High
Low	Low	Medium	Medium	High	High	Medium	High	–2	High
Low	Low	Medium	Low	Low	Medium	Low	Medium	10	Low
High	Medium	Medium	High	High	High	Medium	High	–7	High
Medium	Low	Medium	High	High	High	Medium	Medium	–6	High
Medium	Low	Medium	High	Low	High	Medium	Low	–2	High
Medium	Medium	Low	Low	High	High	Medium	Medium	–2	High
Medium	Low	Medium	Medium	High	High	Low	high	–3	High
Medium	Low	Medium	High	Low	Medium	Medium	Medium	3	Medium

Appendix 6 Study assessments

TABLE 8 The association between maternal vitamin D status in gestation and offspring birthweight: observational studies

First author, year	Bias score	Study details	Study type	Confounders/ adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)
Ardawi, 1997[90]	5 (low)	Jeddah, Saudi Arabia Cohort size $n = 264$ women	Cohort	Nil	Delivery	47.71 (15.77) 25(OH)D < 20 nmol/l in 23% 25(OH)D > 20 nmol/l in 77%
Weiler, 2005[89]	3 (medium)	Winnipeg, MB, Canada Sample size for analysis $n = 50$ women	Cross-sectional	Nil, but no significant difference in terms of offspring sex, season of birth, gestational age at birth in mothers with 25(OH)D ≥ 37.5 nmol/l compared with those with 25(OH)D < 37.5 nmol/l Significant difference in race between the two groups ($p = 0.010$)	Within 48 hours of delivery	Overall mean not given Mean in adequate 25(OH)D group (≥ 37.5 nmol/l, $n = 32$) = 61.6 (24.7) Mean in the deficient group (< 37.5 nmol/l, $n = 18$) = 28.6 (7.8)
Mannion, 2006[86]	1 (medium)	Calgary, AB, Canada $n = 279$ women 207 women restricted milk intake (≤ 250 ml milk) which equates to ≤ 90 IU vitamin D and 72 women did not restrict milk intake	Cohort	Gestational weight gain, maternal age, height, education, BMI put into regression	Not measured directly Repeat 24-hour dietary telephone recall. Three or four times during pregnancy (one cup of milk = 90 IU vitamin D)	In those not restricting milk, vitamin D intake = 524 (180) IU/day In those restricting milk, < 2.25 mcg/day, vitamin D intake = 316 (188) IU/day
Morley, 2006[94]	8 (low)	Melbourne, VIC, Australia $n = 374$ women (232 recruited in winter, 127 in summer)	Cohort	Sex, maternal height, whether or not first child, smoking, season of blood sample	11 weeks and 28–32 weeks	Winter recruitment, geometric mean at 11 weeks = 49.2; 26–32 weeks = 48.3 Summer recruitment geometric mean at 11 weeks = 62.6; 26–32 weeks = 68.9
Sabour, 2006[91]	–2 (high)	Tehran, Islamic Republic of Iran $n = 449$ women	Cross-sectional	Nil	Not measured directly Estimated from validated dietary FFQ at delivery (unclear when assessed)	Not measured Mean vitamin D intake = 90.4 (74.8) IU/day
Maghbooli, 2007[92]	1 (medium)	Tehran, Islamic Republic of Iran $n = 552$ women	Cross-sectional	None	Delivery[a]	27.82 (10.86)[a]

Birthweight (g) mean (SD) or median (IQR)					Unadjusted regression coefficient β (95% CI) for birthweight (g) per 1 nmol/l increase in 25(OH)D	Adjusted regression coefficient β (95% CI) for birthweight (g) per 1 nmol/l increase in 25(OH)D	Conclusion
	25(OH)D < 20 nmol/l (n = 24)			25(OH)D > 20 nmol/l (n = 240)	Not given	Not given	No difference in offspring birthweight in mothers with 25(OH)D < 20 nmol/l at delivery compared with those with 25(OH)D > 20 nmol/l
Birthweight	3323 (439)			3481 (410)			
	25(OH)D < 37.5 nmol/l (n = 18)			25(OH)D ≥ 37.5 nmol/l (n = 32)	Not given	Not given	Offspring birthweight in mothers with 25(OH)D ≥ 37.5 nmol/l significantly lower than in mothers with 25(OH)D < 37.5 nmol/l
Birthweight	3698 (380)			3399 (451)			p = 0.022
In those not restricting milk, birthweight = 3530 (466)					Not given	Not given	Vitamin D intake in pregnancy is positively associated with offspring birthweight
In those restricting milk, birthweight = 3410 (475)						β for each 40 IU/day increase in vitamin D intake = 10.97 (1.19 to 20.75)	
p-value (difference between groups) = 0.07						p = 0.029	
3540 (520)					At 28–32 weeks β for every $\log_2$ increase in 25(OH)D = 40 (–39 to 119)	At 28–32 weeks β for every $\log_2$ increase in 25(OH)D = 31 (–51 to 112)	No significant association seen between log-25(OH)D at 11 weeks (data not given) or 28–32 weeks and offspring birthweight
	25(OH)D < 28 nmol/l at 28–32 weeks	25(OH)D > 28 nmol/l at 28–32 weeks	Difference	Adjusted difference			
Birthweight	3397 (57)	3555 (52)	–157	–153			
Overall group mean (SD)		3190 (450)			Not given	Not given	No significant association seen between vitamin D intake and birthweight
Vitamin D intake < 200 IU/day		3150 (480)					
Vitamin D intake > 200 IU/day		3190 (440)					p = 0.53
3190 (225)					Not given	Not given	No significant association seen between serum 25(OH)D_3 and birthweight
							p-value not given

TABLE 8 The association between maternal vitamin D status in gestation and offspring birthweight: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/ adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)
Clifton-Bligh, 2008[95]	6 (low)	New South Wales, Australia *n* = 307 women (included 81 women with gestational diabetes mellitus)	Cohort	Gestational age	Mean (SD) 28.7 (3.3) weeks	53.8 (23.9)
Harvey, 2008[40]		SWS, UK *n* = 604 women	Cohort	Gestational age, maternal age, maternal BMI, parity	34 weeks	
Gale, 2008[24]	4 (medium)	Princess Anne cohort, UK *n* = 466 women	Cohort	Gestational age, maternal age, maternal BMI, ethnicity and parity	Late pregnancy, median (IQR) 32.6 (32–33.4) weeks	50 (30, 75.3) 50.4% had 25(OH)D > 50 nmol/l 28.3% had levels 27.5–50 nmol/l 21.1% had levels < 27.5 nmol/l
Farrant, 2009[93]	5 (low)	Mysore Parthenon Study, India *n* = 559 women (included 34 women with gestational diabetes mellitus)	Cohort	Maternal age, fat mass, diabetes mellitus status	30 (± 2) weeks	37.8 (24.0–58.5) 60% of women had 25(OH)D < 50 nmol/l, 31% had 25(OH)D < 28 nmol/l
Scholl, 2009[87]	2 (medium)	The Camden Study, NJ, USA *n* = 2251 low income minority pregnant women (47% Hispanic, 37% African American, 15% white)	Cohort	Energy intake, calcium, folate, iron, zinc, protein, age, parity, BMI, ethnicity and gestational age	Not measured directly. Estimated from FFQ at 20 and 28 weeks to calculate daily intake during pregnancy	412.4 (3.56) IU/day
Amirlak, 2009[83]	2 (medium)	United Arab Emirates *n* = 84 healthy Arab and South Asian women with uncomplicated term deliveries	Cross-sectional	Cord blood vitamin A, maternal serum ferritin	Delivery	18.5 (11.0–25.4)

Birthweight (g) mean (SD) or median (IQR)	Unadjusted regression coefficient β (95% CI) for birthweight (g) per 1 nmol/l increase in 25(OH)D	Adjusted regression coefficient β (95% CI) for birthweight (g) per 1 nmol/l increase in 25(OH)D	Conclusion
Not given	Not given	Not given	No association between maternal 25(OH)D and offspring birthweight $p > 0.4$
3506 (441)	β per log-25(OH)D increase = 31.59 (–44.19 to 107.36) $p = 0.42$	β per log-25(OH)D increase = 68.27 (–7.16 to 143.71) $p = 0.08$	No significant association seen between maternal serum log-25(OH)D and offspring birthweight
Divided into quarters according to maternal 25(OH)D (nmol/l): < 30: 3380 (460) 30–50: 3400 (560) 50–75: 3490 (570) > 75: 3430 (510)	β per log-25(OH)D increase = 1.45 (–31.4 to 21.7) $p = 0.247$	β per log-25(OH)D increase = 52.9 (–14.4 to 120.3) $p = 0.123$	No significant association seen between maternal serum log-25(OH)D and offspring birthweight
Geometric mean (SD) = 2900 (400)	β per log-25(OH)D increase = –26.82 (–79.28 to 25.65) $p = 0.32$	β per log-25(OH)D increase = –72.47 (–195.82 to 50.88) $p = 0.25$	No association seen between late pregnancy maternal log-serum 25(OH)D and offspring birthweight when data analysed both continuously or dividing the group into categories using 25(OH)D < 50 nmol/l as a threshold $p = 0.8$
3196 (12.77) Vitamin D intake (IU/day) — Birthweight < 285 — 3163 (21) 285–368 — 3187 (20) 368–440 — 3193 (19) 440–535 — 3207 (19) > 535 — 3228 (23)	Not given	Not given	Positive association seen between vitamin D intake and birthweight *p*-value for trend = 0.043 (after adjustments) When comparing birthweight in those with intake of < 200 IU/day (inadequate intake) to those > 200 IU/day (adequate intake) $p = 0.0270$ (after adjustments)
3317 (510)	Unadjusted β not given Unadjusted r = 0.23 $p < 0.05$	11.6 (3.0 to 20.1) $p = 0.009$	Positive correlation seen between maternal 25(OH)D at delivery and birthweight For every 1 unit increase in 25(OH)D, birthweight increased by 11.6 g

TABLE 8 The association between maternal vitamin D status in gestation and offspring birthweight: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/ adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)
Bowyer, 2009[84]	4 (medium)	Sydney, NSW, Australia *n* = 971 women	Cohort	Gestation, maternal age, overseas maternal birthplace	30–32 weeks	52.0 (17–174) Median vitamin D concentration according to group: vitamin D ≤ 25 nmol/l (*n* = 144) = 18 (17–22); vitamin D 26–50 nmol/l (*n* = 317) = 39 (32–45); vitamin D > 50 nmol/l (*n* = 510) = 73 (60–91)
Prentice, 2009[98]	5 (low)	Gambia, Africa Subset of pregnant Gambian women participating in a calcium supplementation trial *n* = 125 women	Cohort	Season, maternal height, weight, weight gain, infant sex and whether or not received calcium supplement	20 weeks and 36 weeks	20 weeks = 103 (25) 36 weeks = 111 (27)
Sayers, 2009[41]	3 (medium)	ALSPAC, UK *n* = 13,904 women		Nil	Not directly measured Ambient UVB measured during 98 days preceding birth	
Leffelaar, 2010[85]	4 (medium)	ABCVitamin D, Netherlands *n* = 3730 women, all term offspring (37 weeks)	Cohort	Gestational age, season of blood sampling, sex, maternal height, maternal age, smoking, pre-pregnancy BMI, educational level, ethnicity, smoking, parity	Early pregnancy (mean 13 weeks)	54.4 (32–78) Group divided by serum vitamin D concentration as follows: > 50 nmol/l (median 73.3); 30–49.9 nmol/l (median 40.4); < 29.9 nmol/l (median 19.9)

Birthweight (g) mean (SD) or median (IQR)			Unadjusted regression coefficient β (95% CI) for birthweight (g) per 1 nmol/l increase in 25(OH)D	Adjusted regression coefficient β (95% CI) for birthweight (g) per 1 nmol/l increase in 25(OH)D	Conclusion
25(OH)D nmol/l	Unadjusted birthweight	Adjusted birthweight	Not given	Not given	Offspring birthweight significantly lower in women with 25(OH)D deficiency (≤ 25 nmol/l) $p < 0.001$
≤ 25	3254 (545)	Not given			
> 25	3453 (555)	Not given			
Difference (95% CI)	195 (90 to 305)	151 (50 to 250)			
	2990 (360)		At 36 weeks = –0.70 (± 2.35) $p = 0.55$	At 36 weeks = –0.12 (± 2.16) $p = 0.91$	No significant association seen between maternal 25(OH)D and offspring birthweight when analysed both continuously and categorically [25(OH)D > 80 nmol/l vs. < 80 nmol/l]
	Boys ($n = 7192$) = 3429 (608) Girls ($n = 6722$) = 3327 (550)		1.46 (–8.14 to 11.06) $p = 0.77$		No association between UVB exposure in third trimester and birthweight
	Overall = 3515.6 (489.1)		1.404 (0.893 to 1.916)	0.068 (–0.483 to 0.619)	When analysed continuously, no significant relationship observed between maternal early pregnancy 25(OH)D and offspring birthweight When analysed according to categories of 25(OH)D status, deficient vitamin D status (< 29.9 nmol/l) was significantly associated with a lower birthweight Adjusted = –64 (–107.1 to –20.9) Insufficient vitamin D (30–49.9 nmol/l) was not significantly associated with birthweight Adjusted β = 1 (–35.1 to 37.2) (All β adjusted)
	≤ 29.9 nmol/l	3418.4 (510.3)			
	30–49.9 nmol/l	3505.6 (496.2)			
	≥ 50 nmol/l	3559.8 (471.3)			

TABLE 8 The association between maternal vitamin D status in gestation and offspring birthweight: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/ adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)
Watson, 2010[88]	3 (medium)	Northern New Zealand *n* = 439 women: European (75%), Maori (18%) and Pacific Polynesian (7%) women	Cohort	Gestational age, sex, maternal height, weight, smoking, number of preschooler's, number of other adults in the house	Not measured directly 24-hour recall and 3-day dietary FFQ at 4 months and 7 months	Mean vitamin D intake at 4 and 7 months = 84 IU/day
Viljakainen, 2010[97]	3 (medium)	Helsinki, Finland *n* = 125 women recruited during last trimester (October–December). All Caucasian, non-smokers, primiparous	Cohort	Parental size, maternal weight gain in pregnancy, solar exposure, total intake of vitamin D and initial 25(OH)D concentration	First trimester (8–10 weeks) and 2 days postpartum. Mean of two values used to calculate 'vitamin D status'	At 8–10 weeks = 41.0 (13.6) Postpartum = 45.1 (11.9) Overall mean = 44.8 (11.9) Overall median 'vitamin D status' used to categorise group = 42.6
Dror, 2012[96]	7 (low)	Oakland, CA, USA *n* = 120 women	Cross-sectional	Gestational age, maternal age, maternal BMI, maternal height, ethnicity, parity, gestational diabetes mellitus	Perinatal	75.5 (32.3)

IQR, interquartile range.
a Measured 25(OH)D_3.

Birthweight (g) mean (SD) or median (IQR)				Unadjusted regression coefficient β (95% CI) for birthweight (g) per 1 nmol/l increase in 25(OH)D	Adjusted regression coefficient β (95% CI) for birthweight (g) per 1 nmol/l increase in 25(OH)D	Conclusion
3551 (544)				Not given	Not given	Vitamin D intake at 4 months is positively associated with log-(vitamin D) $p = 0.015$ No significant association seen at 7 months *p*-value not given
	25(OH)D below median (42.6 nmol/l)	25(OH)D above median (42.6 nmol/l)	*p*-value (difference between means)	Not given	Not given	No significant difference in offspring birthweight or *z*-score birthweight if maternal 25(OH) status below median compared with above median (median = 42.6 nmol/l) A weak inverse correlation was observed with postpartum 25(OH)D and birthweight *z*-score ($r = -0.193$, $p = 0.068$). This was further weakened after adjustment for confounders ($p = 0.07$)
Birthweight (g)	3700 (400)	3520 (440)	0.052			
Birthweight *z*-score	0.12 (0.81)	−0.23 (1.09)	0.082			
3420 (542)				−0.63 (−3.68 to 2.43) $p = 0.69$	−1.79 (−4.57 to 0.98) $p = 0.20$	No association seen between maternal serum 25(OH)D and offspring birthweight

TABLE 9 The effect of vitamin D supplementation in gestation on offspring birthweight: intervention studies

First author, year	Risk of bias	Setting	Randomisation	Adjustments/confounders accounted for	Number of weeks' gestation when 25(OH)D was measured
Brooke, 1980[3]	–2 (high)	London, UK $n = 126$, all Asian women	Double blinded Randomised to either placebo ($n = 67$) or 1000 IU/day of vitamin D_2 in last trimester ($n = 59$)	Nil, but groups of similar age, height, parity, offspring sex, length of gestation	28–32 weeks and at birth
Marya, 1981[4]	–6 (high)	Rohtak, India $n = 120$ women	Three arms Randomised to either no supplement ($n = 75$); 1200 IU vitamin D + 375 mg calcium/day[b] throughout the third trimester ($n = 25$); or oral 600,000 IU vitamin D_2; two doses in seventh and eighth months of gestation ($n = 20$)	Nil	Not measured
Congdon, 1983[21]	–9 (high)	Leeds, UK $n = 64$, all Asian women	Either 1000 IU vitamin D plus calcium (calcium dose not given) daily in the third trimester ($n = 19$) or no supplement ($n = 45$)	Nil, but groups similar in terms of maternal age, infant sex, gestation length, birthweight	Not measured
Delvin, 1986[6]	–2 (high)	Lyon, France $n = 40$ women	Randomised to either no supplement ($n = 20$) or 1000 IU vitamin D_3/day during third trimester ($n = 20$)	Nil, but groups similar in terms of maternal age and parity. All deliveries occurred in the same month (June)	At recruitment and at delivery
Mallet, 1986[7]	–3 (high)	Rouen, France $n = 77$, all white women	Three arms Randomised to either no supplement ($n = 29$); 1000 IU vitamin D/day[b] in the last 3 months of pregnancy ($n = 21$); or single oral dose of vitamin D[b] 200,000 IU in the seventh month ($n = 27$)	Nil, but groups of similar maternal age, parity, calcium intake and frequency of outdoors outings	During labour (February and March)
Marya, 1988[5]	–2 (high)	Rohtak, India $n = 200$ women	Randomised to either no supplement ($n = 100$) or oral 600,000 IU vitamin D_3; two doses in seventh and eighth months' gestation ($n = 100$)	Nil, but groups had similar maternal age, maternal height, maternal height, parity, haemoglobin, calcium intake and vitamin D intake	Not measured

Mean (SD)/mean (SE)[a] or median (IQR) maternal 25(OH)D concentration (nmol/l)			Mean (SD) or mean (SE)[a] birthweight (g) in unsupplemented group	Mean (SD) or mean (SE)[a] birthweight (g) in supplemented group	Conclusion
At allocation 25(OH)D = 20.1 (1.9)[a] At term, controls 25(OH)D = 16.2 (2.7)[a] At term, supplemented group 25(OH)D = 168.0 (12.5)[a]			3034 (64)	3157 (61)	No significant difference in birthweight between groups $p > 0.05$
Not measured			2730 (360)	1200 vitamin D + 375 mg calcium= 2890 (320) 600,000 IU vitamin D_2 = 3140 (450)	Birthweight significantly higher in those taking supplements and highest in the 600,000 IU group $p = 0.05$ for unsupplemented vs. 1200 IU group $p = 0.001$ for non-supplemented vs. 600,000 IU group
Not measured			3056 (59)[a]	3173 (108)[a]	No significant difference in birthweight between the two groups (p-value not given)
	Mean (SD) 25(OH)D in supplement group	Mean (SD) 25(OH)D in un-supplemented group	Not given	Not given	No significant difference in birthweight between the two groups (p-value not given)
At recruitment	54.9 (10.0)	27.5 (10.0)			
Delivery	64.9 (17.5)	32.4 (20.0)			
Overall mean not given According to group: Unsupplemented = 9.4 (4.9) 1000 IU/day = 25.3 (7.7) 200,000 IU = 26.0 (6.4)			3460 (70)	1000 IU/day = 3370 (80) 200,000 IU = 3210 (90)	No significant difference in birthweight across the three groups (p-value not given)
Not measured directly, but mean daily vitamin D intake given as follows: Unsupplemented = 35.71 (6.17) IU/day Supplemented group = 35.01 (7.13) IU/day			2800 (370)	2990 (360)	Birthweight significantly higher in the supplemented group $p < 0.001$

TABLE 9 The effect of vitamin D supplementation in gestation on offspring birthweight: intervention studies (*continued*)

First author, year	Risk of bias	Setting	Randomisation	Adjustments/confounders accounted for	Number of weeks' gestation when 25(OH)D was measured
Kaur, 1991[101]	–7 (high)	Rohtak, India $n = 50$ women	Randomised to either no supplement ($n = 25$) or oral 60,000 IU vitamin D_3; two doses in sixth and seventh months' gestation ($n = 25$)	Nil, but groups had similar maternal age, maternal weight, length of gestation, parity and haemoglobin	Not measured
Yu, 2009[99]	5 (low)	London, UK $n = 179$ women	Three arms Randomised to either no supplement ($n = 59$); oral vitamin D_2 800 IU/day from 27 weeks onwards ($n = 60$); or a single 200,000 IU calciferol at 27 weeks' gestation ($n = 60$) Each group contained equal numbers of four ethnic groups (black, Caucasian, Asian, Middle Eastern)	Nil No significant difference in baseline characteristics across the three groups	Measured at 26–27 weeks and again at delivery
Hollis, 2011[100]	10 (low)	Charleston, SC, USA	Three arms Randomised to either oral vitamin D_3 400 IU/day ($n = 111$); 2000 IU/day ($n = 122$); or 4000 IU/day ($n = 117$) from 12–16 weeks' gestation until delivery	Nil	Measured at baseline, then monthly and at delivery

IQR, interquartile range; SE, standard error.
a Mean (SE).
b Not known whether supplementation was vitamin D_2 or vitamin D_3.

Mean (SD)/mean (SE)[a] or median (IQR) maternal 25(OH)D concentration (nmol/l)			Mean (SD) or mean (SE)[a] birthweight (g) in unsupplemented group	Mean (SD) or mean (SE)[a] birthweight (g) in supplemented group	Conclusion
Not measured			2756 (60)[a]	3092 (90)[a]	Birthweight significantly higher in the supplemented group $p < 0.001$
	27 weeks	Delivery	Not given	Not given	No significant difference in birthweight across the three groups
No supplement	25 (21–38)	27 (27–39)			
800 IU daily	26 (20–37)	42 (31–76)			
Single supplement	26 (30–46)	34 (30–46)			
	Mean of measurements between 20 and 36 weeks	Delivery	No unsupplemented group. All groups received some form of vitamin D_3 supplementation	400 IU/day = 3221.8 (674.9) 2000 IU/day = 3360.1 (585.0) 4000 IU/day = 3284.6 (597.6)	No significant difference in birthweight across the three groups $p = 0.23$
400 IU daily	79.1 (29.5)	78.9 (36.5)			
2000 IU daily	94.4 (26.1)	98.3 (34.2)			
4000 IU daily	110.8 (28.3)	111.0 (40.4)			

TABLE 10 The association between maternal vitamin D status in gestation and offspring birth length: observational studies

First author, year	Bias score	Study details	Study type	Confounders/ adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)
Ardawi, 1997[90]	5 (low)	Jeddah, Saudi Arabia Cohort size = 264 women	Cohort	Nil	Delivery	47.71 (15.77) 25(OH)D < 20 nmol/l in 23% 25(OH)D > 20 nmol/l in 77%
Sabour, 2006[91]	–2 (high)	Tehran, Islamic Republic of Iran *n* = 449 women	Cross-sectional	Nil	Not measured directly Estimated from validated dietary FFQ at delivery (unclear when assessed)	Not measured Mean vitamin D intake = 90.4 (74.8) IU/day
Mannion, 2006[86]	1 (medium)	Calgary, AB, Canada *n* = 279 women, 207 women restricted milk intake (≤ 250 ml milk which equates to ≤ 90 IU vitamin D) and 72 not restricting milk intake	Cohort		Not measured directly Repeat 24-hour dietary telephone recall. Three or four times during pregnancy (one cup of milk = 90 IU vitamin D)	In those not restricting milk, vitamin D intake = 524 (180) IU/day In those restricting milk, < 2.25 mcg/day, vitamin D intake = 316 (188) IU/day
Morley, 2006[94]	8 (low)	Melbourne, VIC, Australia *n* = 374 women (232 recruited in winter, 127 in summer)	Cohort	Sex, maternal height, whether or not first child, smoking, season of blood sample	11 weeks and 28–32 weeks	Winter recruitment, geometric mean at 11 weeks = 49.2; 26–32 weeks = 48.3 Summer recruitment geometric mean at 11 weeks = 62.6; 26–32 weeks = 68.9
Maghbooli, 2007[92]	1 (medium)	Tehran, Islamic Republic of Iran *n* = 552 women	Cross-sectional	None	Delivery[a]	27.82 (21.71)[a]
Clifton-Bligh, 2008[95]	6 (low)	New South Wales, Australia *n* = 307 women (included 81 women with gestational diabetes mellitus)	Cohort	Gestational age	Mean (SD) 28.7 (3.3) weeks	53.8 (23.9)
Gale, 2008[24]	4 (medium)	Princess Anne cohort, UK *n* = 466 women	Cohort	Gestational age, maternal age, maternal BMI, ethnicity and parity	Late pregnancy Median 32.6 weeks (32.0–31.4)	50 (30–75.3) 50.4% had 25(OH)D > 50 nmol/l 28.3% had levels 27.5–50 nmol/l 21.1% had levels < 27.5 nmol/l

Mean (SD) or median (IQR) birth length (cm)					Unadjusted regression coefficient β (95% CI) for birth length (cm) per 1 nmol/l increase in 25(OH)D	Adjusted regression coefficient β (95% CI) for birth length (cm) per 1 nmol/l increase in 25(OH)D	Conclusion
	25(OH)D < 20 nmol/l (n = 24)		25(OH)D > 20 nmol/l (n = 240)		Not given	Not given	No difference in offspring birth length between mothers with 25(OH)D < 20 nmol/l at delivery and those with 25(OH)D > 20 nmol/l
Birth length (cm)	51.7 (2.9)		51.0 (2.4)				
Overall group mean (SD)		34.81 (6.55)			Not given	Not given	Offspring birth length significantly higher in mothers with adequate dietary vitamin D intake than in those with inadequate intake p = 0.03
Vitamin D intake < 200 IU/day		49.5 (3.77)					
Vitamin D intake > 200 IU/day		50.37 (2.73)					
In those not restricting milk, unadjusted birth length = 51.4 (3.6)					Not given	Not given	No difference in offspring birth length between mothers restricting milk intake in pregnancy and those with unrestricted intake
In those restricting milk, unadjusted birth length = 51.1 (3.5)							
p-value (difference between groups) = 0.46							
	25(OH)D < 28 nmol/l at 28–32 weeks	25(OH)D > 28 nmol/l at 28–32 weeks	Difference (95% CI)	Adjusted difference (95% CI)	At 28–32 weeks β for every $\log_2$ increase in 25(OH)D = −0.3 (−0.08 to 0.6)	At 28–32 weeks β for every $\log_2$ increase in 25(OH)D = −0.3 (−0.1 to 0.6)	No significant association seen between log-25(OH)D at 11 weeks (data not given) or 28–32 weeks and offspring birth length
Birth length	49.8 (2.7)	50.4 (2.4)	−0.6 (−1.5 to 0.3)	−0.6 (−1.5 to 0.3)			
50.02 (1.58)					Not given	Not given	No significant association seen between serum 25(OH)D_3 and offspring birth length (p-value not given)
Not given					Not given	Not given	No association between maternal 25(OH)D and offspring birth length p > 0.4
Not given					β per log-25(OH)D increase = 0.23 (−0.09 to 0.54) p = 0.150	β per log-25(OH)D increase = 0.18 (−0.10 to 0.46) p = 0.215	No association seen between maternal serum 25(OH)D and offspring birth length

TABLE 10 The association between maternal vitamin D status in gestation and offspring birth length: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/ adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)
Farrant, 2009[93]	5 (low)	Mysore Parthenon Study, India *n* = 559 women (included 34 women with gestational diabetes mellitus)	Cohort	Maternal age, fat mass, diabetes mellitus status	30 (± 2) weeks	37.8 (24.0–58.5) 60% of women had 25(OH)D < 50 nmol/l, 31% had 25(OH)D < 28 nmol/l
Prentice, 2009[98]	5 (low)	Gambia, Africa Subset of pregnant Gambian women participating in a calcium supplement trial *n* = 125 women	Cohort	Season, maternal height, weight, weight gain, infant sex and whether or not received calcium supplement	20 weeks and 36 weeks	20 weeks = 103 (25) 36 weeks = 111 (27)
Sayers, 2009[41]	3 (medium)	ALSPAC, UK *n* = 10,587 women	Cohort	Nil	Not directly measured Ambient UVB measured during 98 days preceding birth	Not measured
[b]Leffelaar, 2010[85]	4 (medium)	ABCVitamin D, Netherlands *n* = 3730 women, all term offspring (≥ 37 weeks)	Cohort	Gestational age, season of blood sampling, sex, maternal height, maternal age, smoking, pre-pregnancy BMI, educational level, ethnicity, smoking, parity	Early pregnancy (mean 13 weeks)	54.4 (32–78) Group divided by serum vitamin D concentration as follows: Adequate ≥ 50 nmol/l (median 73.3) Insufficient 30–49.9 nmol/l (median 40.4) Deficient ≤ 29.9 nmol/l (median (19.9)
Viljakainen, 2010[97]	3 (medium)	Helsinki, Finland *n* = 125 women recruited during last trimester (October–December). All Caucasian, non-smokers, primiparous	Cohort	Parental size, maternal weight gain in pregnancy, solar exposure, total intake of vitamin D and initial 25(OH)D concentration	First trimester (8–10 weeks) and 2 days postpartum. Mean of two values used to calculate 'vitamin D status'	At 8–10 weeks = 41.0 (13.6) Postpartum = 45.1 (11.9) Overall mean = 44.8 (11.9) Overall median 'vitamin D status' used to categorise group = 42.6

Mean (SD) or median (IQR) birth length (cm)							Unadjusted regression coefficient β (95% CI) for birth length (cm) per 1 nmol/l increase in 25(OH)D	Adjusted regression coefficient β (95% CI) for birth length (cm) per 1 nmol/l increase in 25(OH)D	Conclusion
Geometric mean = 48.9 (2.2)							β per log-25(OH)D increase = −0.07 (−0.34 to 0.20) $p = 0.6$	β per log-25(OH)D increase = −0.27 (−0.80 to 0.26) $p = 0.3$	No association seen between late pregnancy maternal log-serum 25(OH)D and offspring birth length when data analysed both continuously or dividing the group into categories using 25(OH)D < 50 nmol/l as a threshold $p = 0.9$
50.5 (1.9)[a]							0.0634 (0.136) $p = 0.36$	0.0736 (0.138) $p = 0.30$	No significant association seen between maternal 25(OH)D and offspring birth length when analysed both continuously and categorically [25(OH)D > 80 nmol/l vs. < 80 nmol/l]
Boys ($n = 5447$) = 50.93 (2.61) Girls ($n = 5140$) = 50.19 (2.44)							β per 1 SD increase in UVB 0.10 (0.05 to 0.15) $p = 0.00004$	No adjustments made	Maternal UVB exposure in late pregnancy is positively associated with offspring birth length
	All			25(OH)D ≤ 29.9 nmol/l	25(OH)D 30–49.9 nmol/l	25(OH)D ≥ 50 nmol/l	Not given	Not given	Infants born to mothers with 25(OH)D ≤ 29.9 nmol/l (deficient) had lower length at 1 month No difference between birth length in mothers with insufficient and adequate 25(OH) levels in early pregnancy
Unadjusted Length at 1 month	54.8 (0.05)	54.2 (0.09)	54.8 (0.10)	55.1 (0.06)					
		25(OH)D below median (42.6 nmol/l)		25 (OH)D above median (42.6 nmol/l)		*p*-value (difference between means)	Not given	Not given	No significant difference in offspring birth length or *z*-score birth length if maternal 25(OH) status below median compared with above median (median = 42.6 nmol/l). An inverse correlation was observed with postpartum 25(OH)D and birth length *z*-score (r = −0.261, $p = 0.013$). This relationship was no longer significant after adjustment for confounders
Unadjusted birth length (cm)		51.0 (1.9)		50.5 (1.8)		0.140			
Unadjusted *z*-score birth length		0.14 (1.0)		−0.20 (0.96)		0.104			

TABLE 10 The association between maternal vitamin D status in gestation and offspring birth length: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/ adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)
Dror, 2012[96]	7 (low)	Oakland, CA, USA $n = 120$ women	Cross-sectional	Gestational age, maternal age, maternal BMI, maternal height, ethnicity, parity, gestational diabetes mellitus	Perinatal	75.5 (32.3)

IQR, interquartile range.
a Measured 25(OH)D_3.
b Measured when infant was 1 month old.

Mean (SD) or median (IQR) birth length (cm)	Unadjusted regression coefficient β (95% CI) for birth length (cm) per 1 nmol/l increase in 25(OH)D	Adjusted regression coefficient β (95% CI) for birth length (cm) per 1 nmol/l increase in 25(OH)D	Conclusion
Not given	−0.004 $p = 0.53$	−0.009 (−0.022 to 0.004) $p = 0.18$	No association seen between maternal serum 25(OH)D and offspring birth length

TABLE 11 The effect of vitamin D supplementation in gestation on offspring birth length: intervention studies

First author, year	Risk of bias	Setting	Randomisation	Adjustments/confounders accounted for	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)	Mean (SD) or mean (SE)[a] birth length (cm) in unsupplemented group	Mean (SD) or mean (SE)[a] birth length (cm) in supplemented group	Conclusion
Brooke, 1980[3]	–2 (high)	London, UK $n = 126$ women (all Asian)	Double blinded Randomised to either placebo ($n = 67$) or 1000 IU/day of vitamin D_2 in last trimester ($n = 59$)	Nil, but groups of similar age, height, parity, offspring sex, length of gestation	28–32 weeks and at birth	At allocation 25(OH)D = 20.1 (1.9) At term, controls 25(OH)D = 16.2 (2.7) At term, supplemented group 25(OH)D = 168.0 (12.5)	49.5 (0.4)[a]	49.7 (0.3)[a]	No significant difference in birth length between groups $p > 0.05$
Marya, 1988[5]	–2 (high)	Rohtak, India	Randomised to either no supplement ($n = 100$) or oral 600,000 IU vitamin D_3; two doses in seventh and eighth months' gestation ($n = 100$)	Nil, but groups had similar maternal age, maternal height, maternal height, parity, haemoglobin, calcium intake and vitamin D intake	Not measured	Not measured directly, but mean daily vitamin D intake given as follows: Unsupplemented group = 35.71 (6.17) IU/day Supplemented group = 35.01 (7.13) IU/day	48.45 (2.04)	50.06 (1.79)	Birth length significantly higher in the supplemented group $p < 0.001$

IQR, interquartile range; SE, standard error.
a Mean (SE).

TABLE 12 The association between maternal vitamin D status in gestation and offspring head circumference: observational studies

First author, year	Bias score	Study details	Study type	Confounders/ adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)
Ardawi, 1997[90]	5 (low)	Jeddah, Saudi Arabia Cohort size = 264 women	Cohort	Nil	Delivery	47.71 (15.77) 25(OH)D < 20 nmol/l in 23% 25(OH)D > 20 nmol/l in 77%
Mannion, 2006[86]	1 (medium)	Calgary, AB, Canada *n* = 279 women, 207 women restricted milk intake (≤ 250 ml milk which equates to ≤ 90 IU vitamin D) and 72 not restricting milk intake	Cohort	No adjustments made for HC	Not measured directly Repeat 24-hour dietary telephone recall. Three or four times during pregnancy (one cup of milk = 90 IU vitamin D)	In those not restricting milk, vitamin D intake = 524 (180) IU/day In those restricting milk, < 2.25 mcg/day, vitamin D intake = 316 (188) IU/day
Morley, 2006[94]	8 (low)	Melbourne, VIC, Australia *n* = 374 women (232 recruited in winter, 127 in summer)	Cohort	Sex, maternal height, whether or not first child, smoking, season of blood sample	11 weeks and 28–32 weeks	Winter recruitment, geometric mean at 11 weeks = 49.2; 26–32 weeks = 48.3 Summer recruitment geometric mean at 11 weeks = 62.6; 26–32 weeks = 68.9
Sabour, 2006[91]	–2 (high)	Tehran, Islamic Republic of Iran *n* = 449 women	Cross-sectional	Nil	Not measured directly Estimated from validated dietary FFQ at delivery (unclear when assessed)	Not measured Mean vitamin D intake = 90.4 (74.8) IU/day
Maghbooli, 2007[92]	1 (medium)	Tehran, Islamic Republic of Iran *n* = 552 women	Cross-sectional	None	Delivery	27.82 (21.71)
Clifton-Bligh, 2008[95]	6 (low)	New South Wales, Australia *n* = 307 women (included 81 women with gestational diabetes mellitus)	Prospective cohort	Gestational age	Mean (SD) 28.7 (3.3) weeks	53.8 (23.9)

Mean (SD) or median (IQR) HC (cm)					Unadjusted regression coefficient β (95% CI) for HC (cm) per 1 nmol/l increase in 25(OH)D	Adjusted regression coefficient β (95% CI) for HC (cm) per 1 nmol/l increase in 25(OH)D	Conclusion
	25(OH)D < 20 nmol/l (n = 24)	25(OH)D > 20 nmol/l (n = 240)			Not given	Not given	No difference in offspring HC between mothers with 25(OH)D < 20 nmol/l at delivery and those with 25(OH)D > 20 nmol/l
HC (cm)	34.8 (1.3)	34.11 (1.46)					
In those not restricting milk, unadjusted HC = 34.6 (1.5) In those restricting milk, unadjusted HC = 34.3 (1.5) p-value (difference between groups) = 0.19					Not given	Not given	No difference in offspring HC between mothers restricting milk intake in pregnancy and those with unrestricted intake
	25(OH)D < 28 nmol/l at 28–32 weeks	25(OH)D ≥ 28 nmol/l at 28–32 weeks	Difference	Adjusted difference	At 28–32 weeks β for every $\log_2$ increase in 25(OH)D = –0.02 (–0.2 to 0.2)	At 28–32 weeks β for every $\log_2$ increase in 25(OH)D = –0.05 (–0.3 to 0.2)	No significant association seen between log-25(OH)D at 11 weeks (data not given) or 28–32 weeks and offspring HC
HC (cm)	34.5 (1.5)	34.7 (1.5)	–0.2	–0.2			
Overall group mean (SD) Vitamin D intake < 200 IU/day Vitamin D intake > 200 IU/day			34.81 (6.55) 34.51 (2.66) 35.19 (10.38)		Not given	Not given	No significant association seen between maternal vitamin D intake and offspring HC p = 0.47
Not given					Not given	Not given	No significant association seen between serum 25(OH)D_3 and offspring HC (p-value not given)
Not given					Not given	Not given	No association between maternal 25(OH)D and offspring HC p = 0.4

TABLE 12 The association between maternal vitamin D status in gestation and offspring head circumference: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/ adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)
Gale, 2008[24]	4 (medium)	Princess Anne cohort, UK *n* = 466 women	Cohort	Gestational age, maternal age, maternal BMI, ethnicity and parity	Late pregnancy Median 32.6 weeks (32.0–31.4)	50 (30–75.3) 50.4% had 25(OH)D levels > 50 nmol/l 28.3% had 25(OH)D levels 27.5–50 nmol/l 21.1% had 25(OH)D levels < 27.5 nmol/l
Farrant, 2009[93]	5 (low)	Mysore Parthenon Study, India *n* = 559 women (included 34 women with gestational diabetes mellitus)	Cohort	Maternal age, fat mass, diabetes mellitus status	30 (± 2) weeks	37.8 (24.0–58.5) 60% of women had 25(OH)D < 50 nmol/l, 31% of women had 25(OH)D < 28 nmol/l
Prentice, 2009[98]	5 (low)	Gambia, Africa Subset of pregnant Gambian women participating in a calcium supplementation trial *n* = 125 women	Cohort	Season, maternal height, weight, weight gain, infant sex and whether or not received calcium supplement	20 weeks and 36 weeks	20 weeks = 103 (25) 36 weeks = 111 (27)
Viljakainen, 2010[97]	3 (medium)log-	Helsinki, Finland *n* = 125 women recruited during last trimester (October–December). All Caucasian, non-smokers, primiparous	Cohort	No adjustments made for HC	First trimester (8–10 weeks) and 2 days postpartum. Mean of two values used to calculate 'vitamin D status'	At 8–10 weeks = 41.0 (13.6) Postpartum = 45.1 (11.9) Overall median 'vitamin D status = 42.6'
Dror, 2012[96]	7 (low)	Oakland, CA, USA *n* = 120 women	Cross-sectional	Gestational age, maternal age, maternal BMI, maternal height, ethnicity, parity, gestational diabetes mellitus, infant age in days, infant feeding practice (breast, formula, mixed)	Perinatal	75.5 (32.3)

HC, head circumference; IQR, interquartile range.
a HC measured in infant at 2 weeks.
b HC measured in infant between 8 and 21 days old.

Mean (SD) or median (IQR) HC (cm)				Unadjusted regression coefficient β (95% CI) for HC (cm) per 1 nmol/l increase in 25(OH)D	Adjusted regression coefficient β (95% CI) for HC (cm) per 1 nmol/l increase in 25(OH)D	Conclusion
Not given				β per log-25(OH)D increase = 0.06 (−0.14 to 0.26) $p = 0.557$	β per log-25(OH)D increase = 0.06 (−0.13 to 0.25) $p = 0.530$	No association seen between maternal serum 25(OH)D and offspring HC
53.40 (1.53)				β per log-25(OH)D increase = −0.002 (−0.19 to 0.19) $p = 0.98$	β per log-25(OH)D increase = −0.01 (−0.41 to 0.39) $p = 0.96$	No association seen between late pregnancy maternal log-serum 25(OH)D and offspring HC at birth
35.5 (1.6)[a]				−0.0371 (0.112) $p = 0.52$	−0.0465 (0.113) $p = 0.42$	No significant association seen between maternal 25(OH)D and offspring HC when analysed both continuously and categorically [25(OH)D > 80 nmol/l vs. < 80 nmol/l] Still no association when HC measured again at 13 or 52 weeks
	25(OH)D below median (42.6 nmol/l)	25(OH)D above median (42.6 nmol/l)	*p*-value (difference between means)	Not given	Not given	No significant difference in offspring HC if maternal 25(OH)D below median compared with above (median = 42.6 nmol/l)
HC (cm)	35.7 (1.4)	35.5 (1.6)	0.511			
Not given[b]				−0.003 (−0.012 to 0.005) $p = 0.46$	0.005 (−0.013 to 0.003) $p = 0.23$	No association seen between maternal serum 25(OH)D and offspring HC

TABLE 13 The effect of vitamin D supplementation in gestation on offspring head circumference: intervention studies

First author, year	Risk of bias	Setting	Randomisation	Adjustments/confounders accounted for	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)	Mean (SD) or mean (SE)[a] HC (cm) in unsupplemented group	Mean (SD) or mean (SE)[a] HC (cm) in supplemented group	Conclusion
Brooke, 1980[3]	–2 (high)	London, UK $n = 126$ women (all Asian)	Double blinded Randomised to either placebo ($n = 67$) or 1000 IU/day of vitamin D_2 in last trimester ($n = 59$)	Nil, but groups of similar age, height, parity, offspring sex, length of gestation	28–32 weeks and at birth	At allocation 25(OH)D = 20.1 (1.9) At term, controls 25(OH)D = 16.2 (2.7) At term, supplemented group 25(OH)D = 168.0 (12.5)	34.3 (0.2)[a]	34.5 (0.1)[a]	No significant difference in HC between groups $p > 0.05$
Marya, 1988[5]	–2 (high)	Rohtak, India $n = 200$ women	Randomised to either no supplement ($n = 100$) or oral 600,000 IU vitamin D_3; two doses in seventh and eighth months' gestation ($n = 100$)	Nil, but groups had similar maternal age, maternal height, maternal height, parity, haemoglobin, calcium intake and vitamin D intake	Not measured	Not measured directly, but mean daily vitamin D intake given as follows: Unsupplemented group = 35.71 (6.17) IU/day Supplemented group = 35.01 (7.13) IU/day	33.41 (1.11)	33.99 (1.02)	HC at birth significantly higher in the supplemented group $p < 0.001$

HC, head circumferece; IQR, interquartile range.
a Mean (SE).

TABLE 14 The association between maternal vitamin D status in gestation and offspring bone mass: observational studies

First author, year	Bias score	Study type	Study details, age at which children were assessed and technique used	Offspring bone outcomes assessed (units)	Confounders/adjustments	Number of weeks' gestation when maternal 25(OH)D was measured
Weiler, 2005[89]	3 (medium)	Cross-sectional	Winnipeg, MB, Canada Overall cohort = 342 women Sample size for analysis = 50 Neonates delivered at term and assessed within 15 days of birth by DEXA	LS BMC (g) LS BMC/ body weight (g/kg) Femur BMC Femur BMC/body weight WB BMC WB BMC/ body weight	Infant weight, gestational age at birth, infant weight, gestational age at scan, infant vitamin D status, lean mass Infant sex, infant length and maternal ethnicity not included in the final model since they did not significantly predict infant BMC	Within 48 hours of delivery
Javaid, 2006[1]	5 (low)	Cohort	Princess Anne cohort, UK *n* = 198 women Children assessed at mean 8.9 years by DEXA	WB BMC (g), BA (cm^2) and BMD (g/cm^2) LS BMC (g), BA (cm^2) and BMD (g/cm^2)	Gestational age, offspring age at DEXA	34 weeks
Prentice, 2009[98]	5 (low)	Cohort	Gambia, Africa Subset of pregnant Gambian women participating in a calcium supplementation trial *n* = 125 women Children assessed at 2, 13 and 52 weeks by SPA for radial measurements and DEXA for WB measurements	Radial midshaft BMC (g) and bone width WB BMC (g/cm) WB BA (cm^2)	Season, maternal height, weight, weight gain, infant sex and whether or not received calcium supplement	20 weeks and 36 weeks

Mean (SD) or median (IQR) maternal 25(OH)D concentration (nmol/l)	Mean (SD) bone outcome according to maternal 25(OH)D category/unadjusted correlation coefficient (r) or regression coefficient (β) (95% CI)				Adjusted correlation coefficient (r) or regression coefficient (β) (95% CI)			Conclusion
Overall mean not given Mean in adequate 25(OH)D group (> 37.5 nmol/l, *n* = 32) = 61.6 (24.7) Mean in the deficient group (< 37.5 nmol/l, *n* = 18) = 28.6 (7.8)	25(OH)D (nmol/l)	< 35	> 35	*p*-value	Not given			No significant difference in LS BMC or LS BMC/body weight, femur BMC or WB BMC was observed between those with adequate and deficient maternal 25(OH)D Significantly higher femur BMC/body weight and WB BMC/body weight in those with adequate maternal 25(OH)D
	LS BMC (g)	2.3 (0.5)	2.3 (0.5)	> 0.99				
	LS BMC/body weight (g/kg)	0.59 (0.14)	0.66 (0.125)	0.08				
	Femur BMC (g)	2.8 (0.7)	2.9 (0.6)	0.60				
	Femur BMC/body weight (g/kg)	0.71 (0.17)	0.81 (0.15)	0.027				
	WB BMC (g)	76.4 (12.9)	75.7 (13.7)	0.86				
	WB BMC/body weight (g/kg)	19.49 (3.05)	21.33 (2.03)	0.017				
25(OH)D concentration (nmol/l) *n* (%)	Not given				Outcome	r for each 2.5 nmol/l increase in maternal 25(OH)D	*p*-value	Positive association found between maternal 25(OH)D in late pregnancy and offspring WB and LS BMC, WB BA, WB and LS BMD at aged 9 years
< 27.5 28 (18)					WB BMC	0.21	0.0088	
27.5–50 49 (31)					WB BA	0.17	0.0269	
					WB BMD	0.21	0.0063	
> 50 83 (52)					LS BMC	0.17	0.03	
					LS BA	0.07	0.3788	
					LS BMD	0.21	0.0094	
20 weeks = 103 (25) 36 weeks = 111 (27)	Not given				Not given			No association between maternal 25(OH)D and infant radial midshaft BMC and bone width, or WB BMC and WB BA at either time point

TABLE 14 The association between maternal vitamin D status in gestation and offspring bone mass: observational studies (*continued*)

First author, year	Bias score	Study type	Study details, age at which children were assessed and technique used	Offspring bone outcomes assessed (units)	Confounders/adjustments	Number of weeks' gestation when maternal 25(OH)D was measured
Sayers, 2009[41]	3 (medium)	Cohort	ALSPAC, UK $n = 6955$ women Children assessed at mean age 9.9 years by DEXA	WB less head BMC, (g), BA (cm^2), BMD (g/cm^2), aBMC (g)	BMC adjusted for area BA adjusted for height	Not directly measured Ambient UVB measured during 98 days preceding birth
Akcakus 2006[103]	4 (medium)	Cross-sectional	Turkey Cohort = 100 women Three groups: 30 SGA infants; 40 AGA infants; 30 LGA infants Most women veiled Children assessed within 24 hours of birth by DEXA	WB BMC (g) WB BMD (g/cm^2)	Nil	Delivery
Viljakainen, 2010[97]	3 (medium)	Cohort	Helsinki, Finland $n = 125$ women recruited during last trimester (October–December). All Caucasian, non-smokers, primiparous Children assessed when newborn by pQCT of tibia	Tibial BMC (g/cm), tibial CSA (mm^2) and tibial BMD (mg/cm^3)	Three models: 1. Adjusted for *z*-score birthweight 2. As above + maternal height 3. As above + log-(age of newborn at pQCT)	First trimester (8–10 weeks) and 2 days postpartum. Mean of two values used to calculate 'vitamin D status'

Mean (SD) or median (IQR) maternal 25(OH)D concentration (nmol/l)	Mean (SD) bone outcome according to maternal 25(OH)D category/unadjusted correlation coefficient (r) or regression coefficient (β) (95% CI)			Adjusted correlation coefficient (r) or regression coefficient (β) (95% CI)			Conclusion
Not measured	Outcome	β (change in outcome per 1 SD increase in UVB) (95% CI)	*p*-value	Not given			Maternal UVB exposure in pregnancy was positively associated with offspring BMC, BA and BMD. This remained with BA even after adjusting for height
	BMC (g)	9.6 (5.3 to 13.8)	< 0.0001				No relationship was observed with maternal UVB exposure and aBMC
	BA (cm^2)	8.1 (4.3 to 11.9)	< 0.0001				
	BMD (g/cm^2)	0.003 (0.001 to 0.004	< 0.0001				
	aBMC (g)	0.69 (0.22 to 1.60)	0.14				
Overall not given SGA = 21.8 (7.5) AGA = 21.5 (7.5) LGA = 19.3 (7.0) > 90% had 25(OH)D < 25 nmol/l	WB BMC r = –0.055 WB BMD r = 0.042			Not given			No relationship observed between maternal 25(OH)D at delivery and neonatal BMC and BMD
At 8–10 weeks = 41.0 (13.6) Postpartum = 45.1 (11.9) Overall median 'vitamin D status = 42.6'	Bone outcome		r for log-25(OH)D, *p*-value	r after adjustment 1, *p*-value	r after adjustment 2, *p*-value	r after adjustment 3, *p*-value	A positive significant association seen between maternal 25(OH)D status and offspring tibial BMC and tibial CSA
	Tibial BMC		0.149, 0.163	0.232, 0.034	0.230, 0.036	0.192, 0.085	Tibial BMC and CSA significantly higher in those with maternal 25(OH)D above median than those below even after adjustments
	Log (tibial CSA)		0.197, 0.05	0.214, 0.05	0.218, 0.048	0.226, 0.042	No association seen with tibial BMD

TABLE 14 The association between maternal vitamin D status in gestation and offspring bone mass: observational studies (*continued*)

First author, year	Bias score	Study type	Study details, age at which children were assessed and technique used	Offspring bone outcomes assessed (units)	Confounders/adjustments	Number of weeks' gestation when maternal 25(OH)D was measured
Viljakainen, 2011[102]	4 (medium)	Cohort	Helsinki, Finland *n* = 68 women Children assessed at 14 months by pQCT of tibia This was a follow-up study of same cohort as Viljakainen, 2010.[97] 55 children had bone data at both time points	Tibial BMC (g/cm), tibial CSA (mm^2) and tibial BMD (mg/cm^3)	Sex, birthweight *z*-score, walking age, exclusive breastfeeding and offspring 25(OH)D at 14 months	First trimester (8–10 weeks) and 2 days postpartum. Mean of two values used to calculate 'vitamin D status'
Dror, 2012[96]	7 (low)	Cross-sectional	Oakland, CA, USA *n* = 120 women Children assessed between 8 and 21 days old by DEXA	WB BMC WB aBMC	Maternal height, gestational diabetes mellitus, infant age at DEXA, feeding practice (breast, formula, mixed), infant weight-for-height *z*-score, infant height-for-age *z*-score, BA and size for gestational age	Perinatal

aBMC, bone mineral content adjusted for BA; AGA, appropriate for gestational age; IQR, interquartile range; LGA, large for gestational age; LS, lumbar spine; WB, whole body.

Mean (SD) or median (IQR) maternal 25(OH)D concentration (nmol/l)	Mean (SD) bone outcome according to maternal 25(OH)D category/unadjusted correlation coefficient (r) or regression coefficient (β) (95% CI)	Adjusted correlation coefficient (r) or regression coefficient (β) (95% CI)	Conclusion
Not given Overall median 'vitamin D status = 42.6'	Not given	Not given	No difference in tibial BMC or BMD between offspring with maternal 25(OH)D above median and those below CSA higher at 14 months in offspring with maternal 25(OH)D above median than those below This suggests that postnatal vitamin D supplementation only partly improved the differences in bone variables induced by maternal vitamin D status during pregnancy
75.5 (32.3)	WB BMC: β = –0.02 $p = 0.52$	WB aBMC: β = 0.0007 (–0.031 to 0.032) $p = 0.97$	No association seen between maternal 25(OH)D and offspring WB BMC or WB aBMC analysed either continuously or categorically

TABLE 15 The effect of vitamin D supplementation in gestation on offspring bone mass: intervention studies

First author, year	Risk of bias	Setting	Randomisation and study details, age at which children were assessed and technique used	Offspring bone outcomes assessed (units)	Adjustments/confounders accounted for	Number of weeks' gestation when 25 (OH)D was measured	Mean (SE) maternal 25 (OH)D concentration (nmol/l)	Mean (SE) offspring bone outcome (units) in unsupplemented group	Mean (SE) bone outcome (units) in supplemented group	Conclusion
Congdon, 1983[21]	–9 (high)	Leeds, UK *n* = 64, all Asian women	Either 1000 IU vitamin D plus calcium (calcium dose not given) daily in the third trimester (*n* = 19) or no supplement (*n* = 45) Offspring assessed within 5 days of birth	Forearm BMC (units not given)	Nil, but groups similar in terms of maternal age, infant sex, gestation length, birthweight	Not measured	Not measured	3.10 (0.10)[a]	3.19 (0.12)[a]	No difference in forearm BMC between groups (*p*-value not given)

SE, standard error.
a Results expressed in arbitrary units proportional to the mineral mass per unit length of the radius and ulna combined.

TABLE 16 The association between maternal vitamin D status in gestation and offspring anthropometry and body composition: observational studies

First author, year	Bias score	Study type	Study details, age at which children were assessed and technique used	Offspring outcome assessed (units)	Confounders/ adjustments	Number of weeks' gestation when maternal 25(OH)D was measured
Weiler, 2005[89]	3 (medium)	Cross-sectional	Winnipeg, MB, Canada Sample size for analysis = 50 women Neonates delivered at term and assessed within 15 days of birth by DEXA	Whole-body fat (%)	Nil, but no significant difference in terms of offspring sex, season of birth, gestational age at birth between mothers with 25(OH)D > 37.5 nmol/l and those with 25(OH)D < 37.5 nmol/l Significant difference in race between the two groups ($p = 0.010$)	Within 48 hours of delivery
Morley, 2006[94]	8 (low)	Cohort	Melbourne, VIC, Australia $n = 374$ women (232 recruited in winter, 127 in summer) Neonates assessed between 12 and 72 hours of age using calipers/encircling tape	Subscapular skinfold (mm) Triceps skinfold (mm) Suprailiac skin fold (mm) Mid-upper-arm circumference (cm) Calf circumference (cm)	Sex, maternal height, whether or not first child, smoking, season of blood sample	11 weeks and 28–32 weeks
Gale, 2008[24]	4 (medium)	Cohort	Princess Anne cohort, UK Children assessed at birth ($n = 466$), 9 months ($n = 440$) and 9 years ($n = 178$) using measuring tape with DEXA at 9 years only	Mid-upper-arm circumference (cm) at birth and 9 months Fat mass (kg) at 9 years Lean mass (kg) at 9 years	Adjusted for age of child at scan	Late pregnancy Median (IQR) 32.6 (32–33.4) weeks

Mean (SD) or median (IQR) maternal 25(OH)D concentration (nmol/l)	Mean (SD) offspring outcome according to maternal 25(OH)D category/unadjusted correlation coefficient (r) or regression coefficient (β) (95% CI)			Adjusted correlation coefficient (r) or regression coefficient (β) (95% CI)	Conclusion
Overall mean not given Mean in adequate 25(OH)D group (> 37.5 nmol/l, *n* = 32) = 61.6 (24.7) Mean in the deficient group (< 37.5 nmol/l, *n* = 18) = 28.6 (7.8)		Maternal 215(OH)D < 37.5 nmol	Maternal 25(OH)D > 37.5 nmol	Not given	No significant difference in offspring whole-body fat between those with maternal 25(OH)D < 37.5 nmol/l and those with maternal 25(OH)D > 37.5 nmol/l
	Mean (SD) neonatal whole-body fat (%)	12.7 (4.1)	10.6 (4.1)		
Winter recruitment, geometric mean at 11 weeks = 49.2; 26–32 weeks = 48.3 Summer recruitment geometric mean at 11 weeks = 62.6; 26–32 weeks = 68.9		β (95% CI) for every $\log_2$ increase in maternal 25(OH)D [i.e. doubling of 25(OH)D at 28–32 weeks]		Adjusted β (95% CI) for every $\log_2$ increase in maternal 25(OH)D [i.e. doubling of 25(OH)D at 28–32 weeks]	A weak inverse association seen between maternal 25(OH)D and offspring subscapular and triceps skinfold thickness. No significant association seen with suprailiac skinfold thickness, mid-upper-arm circumference or calf circumference after adjustment for confounders
	Subscapular skinfold (mm)	−0.2 (−0.4 to −0.02)		−0.2 (−0.4 to −0.06)	
	Triceps skinfold (mm)	−0.3 (−0.5 to −0.02)		−0.1 (−0.4 to 0.1)	
	Suprailiac skin fold (mm)	−0.06 (−0.4 to 0.1)		−0.06 (−0.4 to 0.2)	
	Mid-upper-arm circumference (cm)	0.08 (−0.07 to 0.2)		0.1 (−0.06 to 0.3)	
	Calf circumference (cm)	0.05 (−0.1 to 0.2)		0 (−0.2 to 0.2)	
50 (30–75.3) 50.4% had 25(OH)D levels > 50 nmol/l 28.3% had 25(OH)D levels 27.5–50 nmol/l 21.1% had 25(OH)D levels < 27.5 nmol/l	*p*-value for difference in offspring outcome according to quarter of maternal 25(OH)D			Not given	No significant association between maternal 25(OH)D concentration measured in late pregnancy and offspring's mid-upper-arm circumference at birth and 9 months At 9 years fat mass and lean mass tended to be lower in children born to mothers in the lowest of 25(OH)D distribution, but no statistically significant linear trends seen
		p-value			
	Mid-upper-arm circumference at birth	0.080			
	Mid-upper-arm circumference at 9 months	0.581			
	Fat mass at 9 years	0.090			
	Lean mass at 9 years	0.090			

TABLE 16 The association between maternal vitamin D status in gestation and offspring anthropometry and body composition: observational studies (*continued*)

First author, year	Bias score	Study type	Study details, age at which children were assessed and technique used	Offspring outcome assessed (units)	Confounders/ adjustments	Number of weeks' gestation when maternal 25(OH)D was measured
Sayers, 2009[41]	3 (medium)	Cohort	ALSPAC, UK $n = 6955$ women Children assessed at mean age 9.9 years by DEXA	Lean mass (kg) Fat mass (kg)	Nil	Not directly measured Ambient UVB measured during 98 days preceding birth
Krishnaveni, 2011[105]	4 (medium)	Cohort	Mysore Parthenon Study, Mysore, India Children assessed at 5 years ($n = 506$) and 9.5 years ($n = 469$) using measuring tape, calipers and bioimpedence	AMA (cm^2) Subscapular skinfold, thickness (mm) Triceps skinfold thickness (mm) Waist circumference Fat mass (kg) Per cent body fat (%) Fat-free mass (kg) Per cent fat-free mass (%)	Offspring sex and age, maternal BMI, gestational diabetes mellitus, socioeconomic score, parity and religion	28–32 weeks (at study entry)

Mean (SD) or median (IQR) maternal 25(OH)D concentration (nmol/l)	Mean (SD) offspring outcome according to maternal 25(OH)D category/unadjusted correlation coefficient (r) or regression coefficient (β) (95% CI)			Adjusted correlation coefficient (r) or regression coefficient (β) (95% CI)			Conclusion
Not measured		β (95% CI) change in outcome per 1-SD increase in UVB	*p*-value	Not given			Maternal UVB exposure in pregnancy is positively associated with offspring lean mass at age 9 years. No significant association seen with fat mass
	Lean mass (kg)	163 (89 to 237)	0.00002				
	Fat mass (kg)	73.9 (–44.2 to 191.9)	0.22				
39.0 (24–58) 67% of women had 25(OH)D < 50 nmol/l (the authors' definition of deficiency)	Not given			Comparing offspring of mothers with and without 25(OH)D deficiency (deficient = 0, non-deficient = 1)			At ages 5 and 9.5 years offspring born to women with 25(OH)D < 50 nmol/l in late pregnancy had significantly reduced AMA compared with those children born to mothers without deficient 25(OH)D No significant difference seen in any of the other anthropometric or body composition measurements
					β	*p*-value	
				5 years			
				AMA	0.4	0.01	
				Subscap	0.004	0.86	
				Triceps	0.01	0.55	
				Waist	0.07	0.81	
				Fat mass	–0.01	0.92	
				% fat mass	–0.4	0.48	
				Fat-free mass	0.1	0.33	
				% fat-free mass	0.3	0.51	
				9.5 years			
				AMA	0.7	0.02	
				Subscap	–0.009	0.80	
				Triceps	0.004	0.88	
				Waist	0.3	0.62	
				Fat mass	–0.07	0.77	
				% fat mass	–0.6	0.34	
				Fat-free mass	0.2	0.50	
				% fat-free mass	0.6	0.33	

TABLE 16 The association between maternal vitamin D status in gestation and offspring anthropometry and body composition: observational studies (*continued*)

First author, year	Bias score	Study type	Study details, age at which children were assessed and technique used	Offspring outcome assessed (units)	Confounders/ adjustments	Number of weeks' gestation when maternal 25(OH)D was measured
Crozier, 2012[106]	8 (low)	Cohort	SWS, UK Children assessed at birth ($n = 574$), 4 years ($n = 565$) and 6 years ($n = 447$) using DEXA	Fat mass (kg) Fat-free mass (kg)	Offspring sex, gestation, age at measurement, length/height, maternal educational attainment, smoking in pregnancy, pre-pregnancy BMI, maternal height, parity, social class, Institute of Medicine weight gain category, breastfeeding duration, vitamin D intake at 3 years, physical activity at 3 years	34 weeks

AMA, arm muscle area; IQR, interquartile range.

Mean (SD) or median (IQR) maternal 25(OH)D concentration (nmol/l)	Mean (SD) offspring outcome according to maternal 25(OH)D category/unadjusted correlation coefficient (r) or regression coefficient (β) (95% CI)			Adjusted correlation coefficient (r) or regression coefficient (β) (95% CI)		Conclusion
62 (43–89)	Outcome	Unadjusted β (95% CI)	*p*-value	Adjusted β (95% CI)	*p*-value	Positive association between late pregnancy maternal 25(OH)D and offspring fat mass at birth after adjusting for confounders
	Birth fat mass (SD)	0.06 (–0.01 to 0.12)	0.09	0.08 (0.02 to 0.15)	0.02	
	Birth fat-free mass (SD)	0.02 (–0.03 to 0.07)	0.44	0.04 (–0.02 to 0.09)	0.17	
	4-year fat mass (SD)	–0.09 (–0.16 to –0.02)	0.02	–0.01 (–0.08 to 0.07)	0.81	Negative association late pregnancy maternal 25(OH)D and fat mass at 6 years after adjusting for confounders
	4-year fat-free mass (SD)	0.03 (–0.02 to 0.08)	0.21	0.03 (–0.02 to 0.08)	0.30	
	6-year fat mass (SD)	–0.16 (–0.23 to –0.08)	< 0.001	–0.10 (–0.17 to –0.02)	0.01	No significant association seen at 4 years after adjustments for confounders
	6-year fat-free mass (SD)	0.01 (–0.04 to 0.06)	0.65	0.02 (–0.03 to 0.07)	0.43	

TABLE 17 The effect of vitamin D supplementation in gestation on offspring anthropometry and body composition: intervention studies

First author, year	Risk of bias	Setting	Randomisation and study details, age at which children were assessed and technique used	Offspring outcome assessed (units)	Adjustments/ confounders accounted for	Number of weeks' gestation when 25(OH)D was measured
Brooke 1980[3]	–2 (high)	London, UK *n* = 126, all Asian women	Double blinded Randomised to either placebo (*n* = 67) or 1000 IU/day of vitamin D2 in last trimester (*n* = 59) Offspring assessed within 48 hours of birth. Method of measurement not given	Triceps skinfold (mm) Forearm length (cm) Fontanelle area (cm^2)	Nil, but groups of similar age, height, parity, offspring sex, length of gestation	28–32 weeks and at birth
Marya, 1988[5]	–2 (high)	Rohtak, India *n* = 200 women	Randomised to either no supplement (*n* = 100) or oral 600,000 IU vitamin D3; two doses in seventh and eighth months' gestation (*n* = 100) Offspring measured within the first 24 hours of birth using calipers and measuring tape	Mid-arm circumference (cm) Triceps skinfold thickness (mm) Infrascascapular skinfold thickness (mm)	Nil, but groups had similar maternal age, maternal height, maternal height, parity, haemoglobin, calcium intake and vitamin D intake	Not measured

SE, standard error.
a Mean (SE).

Mean (SE) maternal 25(OH)D concentration (nmol/l)	Mean (SD)/mean (SE)[a] offspring outcome (units) in unsupplemented group		Mean (SD)/mean (SE)[a] offspring outcome (units) in supplemented group		Conclusion
At allocation 25(OH)D = 20.1 (1.9)*	Triceps skinfold (cm)	3.6 (0.1)[a]	Triceps skinfold (cm)	3.8 (0.1)[a]	Significantly greater fontanelle area in the supplemented group
	Forearm length (cm)	8.1 (0.1)[a]	Forearm length (cm)	8.1 (0.1)[a]	
At term, controls 25(OH)D = 16.2 (2.7)*	Fontanelle area (cm^2)	6.1 (0.7)[a]	Fontanelle area (cm^2)	4.1 (0.4)[a]	$p < 0.05$
At term, supplemented group 25(OH)D = 168.0 (12.5)[a]					No significant difference in forearm length or triceps skinfold thickness
Not measured directly, but mean daily vitamin D intake given as follows:	Mid-arm circumference (cm)	9.44 (0.85)	Mid-arm circumference (cm)	9.82 (0.72)	Significantly higher mid-arm circumference, triceps skinfold and infrascapular skinfold in the supplemented group
	Triceps skinfold (mm)	7.30 (0.83)	Triceps skinfold (mm)	7.72 (0.67)	
Unsupplemented group = 35.71 (6.17) IU/day	Infrascapular skinfold (mm)	7.49 (0.89)	Infrascapular skinfold (mm)	7.82 (0.67)	All $p < 0.01$
Supplemented group = 35.01 (7.13) IU/day					

TABLE 18 The association between maternal vitamin D status in gestation and offspring asthma and atopy: observational studies

First author, year	Bias score	Cohort details	Study type	Adjustments	When was maternal serum 25(OH)D measured
Camargo, 2007[109]	2 (medium)	Massachusetts, USA Cohort *n* = 2128 women 1194 (56%) studied for outcome	Cohort	Sex, birthweight, income, maternal age, pre-pregnancy BMI, passive smoking exposure, breastfeeding duration, number of children in household, maternal and paternal history of asthma, dietary intake of fish, fruit and vegetables	Not measured Based on modification to validated FFQ at initial prenatal visit and 26–28 weeks' gestation
Devereux, 2007[26]	−1 (high)	Aberdeen, Scotland Cohort *n* = 1924 mother–offspring pairs 1212 (63%) children included in questionnaire follow-up at 5 years; 797 (41%) children had lung function assessment and skin-prick testing at 5 years	Cohort	Adjusted for maternal atopy, age, smoking, education, social class, deprivation index based on area of residence, breastfeeding, infant sex, infant antibiotic use in first year, birthweight, birth order, season of LMP, maternal intakes of vitamin E, zinc and calcium	Not measured Estimated from FFQ at 32 weeks' gestation
Gale, 2008[24]	4 (medium)	Princess Anne cohort, UK *n* = 440 at 9 months *n* = 178 at 9 years	Cohort	Nil	Late pregnancy Median (IQR) = 32.6 (33–33.4) weeks

Mean (SD) or median (IQR) $25(OH)D_3$ concentration (nmol/l – unless other stated)	Risk of asthma/wheeze/eczema	Conclusion
Not measured Mean vitamin D intake (mean of early pregnancy and 26–28 weeks for each participant) was 548 (167) IU/day	In comparison with the lowest quarter, mothers in the highest quarter of daily vitamin D intake had a lower risk of having a child with recurrent wheeze at 3 years (OR 0.38, 95% CI 0.22 to 0.65)	A higher maternal intake of vitamin D during pregnancy was associated with a lower risk of recurrent wheeze in children at 3 years of age
Not measured Median maternal vitamin D intake 131 (102–173) IU/day	In models adjusted for potential confounders, including the children's vitamin D intake, compared with the lowest quintile, the highest quintile of maternal vitamin D intake displayed lower risk of 'ever wheeze' (OR 0.48, 95% CI 0.25 to 0.91), and 'wheeze in the previous year' (OR 0.35, 95% CI 0.15 to 0.83) at 5 years determined by parental questionnaire No differences in atopic sensitisation or spirometry	Low maternal vitamin D intakes during pregnancy are associated with increased wheezing symptoms in children at 5 years

50 (30–75.3) 50.4% had 25(OH)D > 50 nmol/l 28.3% had levels 27.5–50 nmol/l 21.1% had levels < 27.5 nmol/l		OR (95% CI) for eczema or asthma			
	25(OH)D	< 30 nmol/l	30–50 nmol/l	50–75 nmol/l	> 75 nmol/l
	Visible eczema on examination at 9 months	1.0	0.59 (0.14 to 2.50)	0.79 (0.21 to 3.00)	3.26 (1.15 to 9.29)
	Atopic eczema at 9 months (UK Working Party's criteria)	1.0	1.11 (0.43 to 2.84)	1.75 (0.73 to 4.17)	1.62 (0.67 to 3.89)
	Reported eczema at 9 years	1.0	0.71 (0.15 to 3.39)	0.49 (0.08 to 2.68)	1.89 (0.51 to 6.99)
	Reported asthma at 9 years	1.0	2.05 (0.36 to 11.80)	2.05 (0.36 to 11.80)	5.40 (1.09 to 26.65)

TABLE 18 The association between maternal vitamin D status in gestation and offspring asthma and atopy: observational studies (*continued*)

First author, year	Bias score	Cohort details	Study type	Adjustments	When was maternal serum 25(OH)D measured
Erkkola, 2009[107]	−1 (high)	Finland Three university hospitals Cohort $n = 4193$ women 1669 (40%) studied for outcome	Cohort	Adjusted for sex, area of birth, gestation, maternal age, maternal education, smoking during pregnancy, siblings, parental asthma, atopic eczema, pets in house before 1 year of age, maternal intake of vitamin C, vitamin E, selenium and zinc	Not measured Estimated from FFQ. Completed retrospectively after delivery for eighth month of pregnancy
Miyake, 2010[108]	−1 (high)	Osaka, Japan Cohort $n = 1002$ women 763 (76%) studied for outcome	Cohort	Adjusted for maternal age, gestation at baseline, residential municipality during pregnancy, family income, maternal and parental education, history of asthma, atopic eczema and allergic rhinitis, season, changes in diet, smoking, older siblings, sex, birthweight, age at child assessment	Not measured Self-administered validated questionnaire of dietary intake. Measured between 5 and 39 weeks of pregnancy
Nwaru, 2010[114]	3 (medium)	Finland Cohort $n = 1175$ women 931 (79%) studied for outcome	Cohort	Place and season of birth, sex, siblings, gestational age at birth, parental asthma and allergic rhinitis, maternal age at delivery, maternal smoking, and maternal education	Not measured Estimated from FFQ. Completed retrospectively after delivery for eighth month of pregnancy
Camargo, 2011[110]	3 (medium)	Wellington and Christchurch, New Zealand Cohort = 922 women 823 (89%) studied for outcome	Cohort	Season of birth, study site, maternal age, parental history of asthma, gestational age, birthweight, child's sex and ethnicity, smoking, number of children in household, during of exclusive breastfeeding	Not measured Cord blood 25(OH)D were measured

Mean (SD) or median (IQR) $25(OH)D_3$ concentration (nmol/l – unless other stated)	Risk of asthma/wheeze/eczema	Conclusion
Not measured Mean total maternal vitamin D intake 260 (152) IU/day	After adjustment, maternal total vitamin D intake associated with reduced risk of asthma (HR 0.76, 95% CI 0.59 to 0.99) and allergic rhinitis (HR 0.84, 95% CI 0.72 to 0.98) but not atopic eczema (OR 0.94, 95% CI 0.83 to 1.07) at 5 years	Maternal vitamin D intake during pregnancy inversely associated with the development of asthma and allergic rhinitis
Not measured Mean intake of vitamin D = 248 (148) IU/day	Consumption of ≥ 4.309 mcg/day vitamin D associated with a decreased risk of wheeze (adjusted OR 0.64, 95% CI 0.43 to 0.97) and eczema (adjusted OR 0.63, 95% CI 0.41 to 0.98) at 16–24 months of age	Higher consumption of vitamin D in pregnancy was associated with a lower risk of wheeze and eczema in infancy
The mean daily intake of vitamin D during pregnancy by the mothers was 208 (112) IU/day Of the women, 28% had taken vitamin D supplements during pregnancy with a mean intake of 44 (96) IU/day	Increasing maternal intake of vitamin D was inversely association with sensitisation (specific IgE ≥ 0.35 KU/l) to food allergens [adjusted OR 0.56 (95% CI 0.35 to 0.91), $p < 0.026$] but not inhaled allergens [adjusted OR 0.76 (95% CI 0.50 to 1.17)] at 5 years of age	Increasing maternal intake of vitamin D was inversely associated with sensitisation to food allergens
Not measured Median cord blood 25(OH)D = 44 nmol/l (IQR 29–78)	Adjusting for season, the OR for cumulative wheeze at 5 years increased across categories of 25(OH)D [1.00 (reference) for ≥ 75 nmol/l, 1.63 (95% CI 1.17 to 2.26) for 25–74 nmol/l, and 2.15 (95% CI 1.39 to 3.33) for < 25 nmol/l]. No association with incident asthma at 5 years	Cord-blood levels of 25(OH)D had inverse associations with childhood wheezing but no association with incident asthma

TABLE 18 The association between maternal vitamin D status in gestation and offspring asthma and atopy: observational studies (*continued*)

First author, year	Bias score	Cohort details	Study type	Adjustments	When was maternal serum 25(OH)D measured
Cremers, 2011[113]	3 (medium)	Netherlands Cohort $n = 2834$ women (2343 women with a conventional lifestyle; 491 women with an alternative lifestyle with regard to child rearing practices, diet and vaccination programmes) 415 (15%) studied for outcome	Cohort	Recruitment group (conventional or alternative lifestyle), maternal age, maternal education, maternal smoking, alcohol consumption, pre-pregnancy BMI, child's BMI at 2 years, birthweight, exposure to tobacco smoke, season of blood sampling, physical activity	36 weeks' gestation
Rothers, 2011[111]	2 (medium)	Tucson, AZ, USA Cohort $n = 482$ women 219 (45%) studied for outcome	Cohort	Maternal ethnicity, household smoking, birth season	Not measured Plasma levels of 25(OH)D measured in cord blood specimens
Morales 2012[112]	3 (medium)	Spain Cohort $n = 2860$ women enrolled in the Infancia y Medio Ambiente (INMA) project 1233 (43%) children studied for outcome	Cohort	Offspring sex, maternal pre-pregnancy BMI, maternal history of asthma, maternal educational level, maternal smoking in pregnancy, breastfeeding duration, day-care attendance in the first year of life, area of study	Between 12 and 23 weeks' gestation Mean (SD) = 12.6 (2.5) weeks

HR, hazard ratio; IQR, interquartile range.

Mean (SD) or median (IQR) $25(OH)D_3$ concentration (nmol/l – unless other stated)	Risk of asthma/wheeze/eczema	Conclusion
46.0 (18.2) nmol/l	No association between maternal plasma 25(OH)D at 36 weeks' gestation and offspring FEV_1 ($p = 0.99$) or FVC ($p = 0.59$) at 6–7 years	No association between maternal late pregnancy 25(OH)D levels and lung function in children aged 6–7 years
Not measured Median cord blood 25(OH)D = 64 nmol/l (IQR 49–81)	Both total and inhalant allergen specific IgE showed non-linear associations with cord blood 25(OH)D in that levels were highest in those with cord blood 25(OH)D < 50 nmol/l and > 100 nmol/l Greater risk of skin-prick testing positivity to aeroallergens at 5 years in children with cord 25(OH)D ≥ 100 nmol/l than in reference group [25(OH)D 50–74.9 nmol/l], OR 3.4, 95% CI 1.0 to 11.4 ($p = 0.046$)	Non-linear relationship between vitamin D status at both and markers of atopy at 5 years
Median = 73.6 (56.2–92.6) nmol/l	No significant association seen between maternal 25(OH)D and: Wheeze at 1 year (unadjusted $p = 0.453$, adjusted $p = 0.441$) Wheeze at 4 years (unadjusted $p = 0.559$, adjusted $p = 0.708$) Asthma at 4–6 years (unadjusted $p = 0.339$; adjusted $p = 0.481$)	No association seen between maternal 25(OH)D and offspring wheeze at 1 year and 4 years, or offspring asthma at 4–6 years

TABLE 19 The association between maternal vitamin D status in gestation and risk of offspring being born small for gestational age: observational studies

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured
Akcakus, 2006[103]	4 (medium)	Turkey Cohort $n = 100$ women Cases of SGA[a] $n = 30$ Most women veiled	Cross-sectional	Nil	Delivery
Mehta, 2009[119]	3 (medium)	Tanzania Overall cohort $n = 1078$ women Women all HIV infected taking part in a clinical trial of vitamin use Cases of SGA[a] $n = 74$ Cohort for analysis $n = 675$	Prospective cohort	Multivitamin supplementation, maternal age at baseline, CD4 count at baseline, HIV disease stage at baseline	12–27 weeks (at enrolment to trial)
Leffelaar, 2010[85]	5 (low)	ABCVitamin D, Netherlands Cohort $n = 3730$ women Cases of SGA[a] $n = 9.2\%$ (approximately 343)	Prospective cohort	Two models: OR1 adjusted for gestational age, season of collection, sex, maternal parity, maternal age, smoking, pre-pregnancy BMI, educational level OR2 additional adjustment for ethnic group, vitamin D status	Early pregnancy (mean 13 weeks)

Maternal mean (SD) 25(OH)D concentration (nmol/l) in cases of SGA infants	Maternal mean (SD) 25(OH)D concentration (nmol/l) in infants AGA	OR (95% CI) of offspring being SGA from univariate analysis	OR (95% CI) of offspring being SGA from multivariate analysis	Conclusion
21.75 (7.5)	21.5 (7.5)	Not given	Not given	No difference in maternal 25(OH)D at delivery between SGA infants and AGA infants
Mean not given 44.6% had 25(OH)D < 80 nmol/l 55.4% had 25(OH)D > 80 nmol/l	Mean not given	1.25 (0.81 to 1.91) $p = 0.31$	1.25 (0.82 to 1.90) $p = 0.31$	No relationship between SGA risk and maternal 25(OH)D among women with HIV
Not given	Not given	Crude OR adjusted for season of blood sample and gestational age		After adjusting for confounders, women with 25(OH)D < 30 have a significantly increased risk of SGA infant

25(OH)D (nmol/l)	Crude OR (95% CI)	OR1 (95% CI)	OR2 (95% CI)
< 30	2.4 (1.0 to 3.2)	1.8 (1.3 to 2.5)	1.9 (1.4 to 2.7)
30–49.9	1.5 (1.1 to 2.0)	1.2 (0.9 to 1.7)	1.2 (0.9 to 1.3)
≥ 50	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)

TABLE 19 The association between maternal vitamin D status in gestation and risk of offspring being born small for gestational age: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured
Bodnar, 2010[115]	7 (low)	Pittsburg, PA, USA Overall cohort size $n = 1198$ women Cases of SGA[a] $n = 111$ Controls $n = 301$	Nested case–control	Pre-pregnancy BMI, smoking during pregnancy, socioeconomic score Additional adjustments for season, maternal age, gestational age at blood sampling, marital status, insurance status, smoking pre-pregnancy, pre-conceptual multivitamin use, preconception physical activity had no meaningful impact on results	< 22 weeks
Shand, 2010[117]	6 (low)	Vancouver, BC, Canada All women had either clinical or biochemical risk factors for pre-eclampsia Cohort $n = 221$ women Cases of SGA[b] $n = 13$	Cohort	Maternal age, ethnicity, parity, BMI, season, multivitamin use, smoking	Between 10 and 20 weeks 6 days [mean 18.7 (1.88) weeks]
Robinson 2011[116]	1 (medium)	South Carolina, USA All women has EOSPE[c] Cases $n = 33$ Controls $n = 23$	Case–control	No significant differences between cases and controls in terms of maternal age, nulliparity, African American race, mean arterial blood pressure, BMI Cases had significantly higher age at gestation; therefore, all birthweights converted to percentile growth for gestational age	Not given

Maternal mean (SD) 25(OH)D concentration (nmol/l) in cases of SGA infants	Maternal mean (SD) 25(OH)D concentration (nmol/l) in infants AGA	OR (95% CI) of offspring being SGA from univariate analysis			OR (95% CI) of offspring being SGA from multivariate analysis		Conclusion
Geometric mean (95% CI) according to race White = 73.2 (69.7 to 76.8) Black = 39.8 (36.7 to 43.2)	Geometric mean (95% CI) according to race White = 71.5 (64.0 to 79.9) Black = 39.8 (33.6 to 47.0)	OR broken down according to race			Adjusted OR broken down according to race		No relationship between SGA risk and maternal 25(OH)D among black mothers No significant difference in the geometric means of 25(OH)D in white women with and without SGA infants A U-shaped relation was seen between SGA risk and maternal 25(OH)D among white mothers with the lowest risk between 60 and 80 nmol/l
		25(OH)D (nmol/l)	White	Black	White	Black	
		< 37.5	10.6 (2.6 to 42.5)	1.4 (0.5 to 3.1)	7.5 (1.8 to 31.9)	1.5 (0.6 to 3.5)	
		37.5–75	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	
		> 75	1.9 (1.1 to 3.4)	1.9 (1.1 to 3.4)	2.1 (1.2 to 6.8)	2.2 (0.5 to 5.5	
Not given	Not given	Unadjusted values not given			25(OH)D concentration	OR (95% CI)	No significant relationship seen between maternal 25(OH)D and risk of infant being SGA
					< 37.5	1.78 (0.52 to 6.03)	
					< 50	2.34 (0.65 to 8.49)	
					< 75	2.16 (0.26 to 18.2)	
41.9 (22.2–57.4)	63.1 (39.9–82.4)	Not given			Not given		Serum 25(OH)D significantly lower between women with EOSPE and SGA offspring and EOSPE controls with normal-sized offspring $p = 0.02$

TABLE 19 The association between maternal vitamin D status in gestation and risk of offspring being born small for gestational age: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured
Fernandez-Alonso, 2012[118]	3 (medium)	Almeria, Spain Cohort $n = 466$ women Cases of SGA[a] $n = 46$	Cohort	Nil	Between 11 and 14 weeks

AGA, appropriate for gestational age; EOSPE, early onset pre-eclampsia; OR1, odds ratio 1; OR2, odds ratio 2; Ref, reference group; SGA, small for gestational age.

a SGA defined as infants born below the tenth percentile of birthweight according to nomograms based on sex and gestational age.

b SGA defined as infants born below the third percentile of birthweight according to nomograms based on sex and gestational age.

c Defined as meeting the American Congress of Obstetrics and Gynaecology criteria for severe pre-eclampsia and having this diagnosis at < 34 weeks' gestation.

Maternal mean (SD) 25(OH)D concentration (nmol/l) in cases of SGA infants	Maternal mean (SD) 25(OH)D concentration (nmol/l) in infants AGA	OR (95% CI) of offspring being SGA from univariate analysis	OR (95% CI) of offspring being SGA from multivariate analysis	Conclusion
Overall mean not given	Not given	Not given	Not given	No significant relationship seen between maternal 25(OH)D and risk of infant being SGA $p = 0.78$

TABLE 20 The effect of vitamin D supplementation in gestation on risk of offspring being born small for gestational age in the offspring: intervention studies

First author, year	Risk of bias	Setting	Randomisation	Adjustments/confounders accounted for	Number of weeks' gestation when 25(OH)D was measured
Brooke, 1980[3]	–2 (high)	London, UK $n = 126$ women (all Asian)	Double blinded Randomised to either placebo ($n = 67$) or 1000 IU/day of vitamin D_2 in last trimester ($n = 59$)	Nil, but groups of similar age, height, parity, offspring sex, length of gestation	28–32 weeks and at birth
Yu, 2009[99]	5 (low)	London, UK $n = 119$ women	Three arms Randomised to either no supplement ($n = 59$); oral vitamin D_2 800 IU/day from 27 weeks onwards ($n = 60$); or a single 200,000 IU D_2 at 27 weeks' gestation ($n = 60$) Each group contained equal numbers of four ethnic groups (black, Caucasian, Asian, Middle Eastern)	Nil No significant difference in baseline characteristics across the three groups	Measured at 26–27 weeks and again at delivery

IQR, interquartile range; SGA, small for gestational age.
a SGA defined as infants born below the tenth percentile of birthweight.

Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)			Percentage of infants SGA[a] in unsupplemented group	Percentage of infants SGA[a] in supplemented group	Conclusion
At allocation 25(OH)D = 20.1 (1.9) At term, controls 25(OH)D = 16.2 (2.7) At term, supplemented group 25(OH)D = 168.0 (12.5)			28.6% (19 out of 67)	15.3% (9 out of 59)	No significant difference in risk of SGA between groups $p > 0.05$; $\chi^2 = 3.1$
	27 weeks	Delivery	17%	15% in daily dose group 13% in single-dose group	No significant difference in rate of SGA across the three groups $p = 0.7$
No supplement	25 (21–38)	27 (27–39)			
Daily supplement	26 (20–37)	42 (31–76)			
Single supplement	26 (30–46)	34 (30–46)			

TABLE 21 The association between maternal vitamin D status in gestation and preterm birth of the offspring: observational studies

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured
Delmas, 1987[120]	−4 (high)	Lyon, France Controls $n = 9$ women Cases of preterm birth[a] $n = 10$ women None of the women were taking supplemental vitamin D	Case–control	None	Delivery
Mehta, 2009[119]	2 (medium)	Tanzania Overall cohort $n = 1078$ women Women all HIV infected taking part in a clinical trial of vitamin use Cases of preterm birth[b] $n = 204$ Cases of severe preterm birth[c] $n = 70$ Cohort for analysis $n = 758$	Prospective cohort	Multivitamin supplementation, maternal age at baseline, CD4 count at baseline, HIV disease stage at baseline	12–27 weeks (at enrolment to trial)
Baker, 2011[121]	5 (low)	North Carolina, USA Overall cohort size $n = 4225$ women Cases of preterm birth[d] $n = 40$ Controls $n = 120$	Nested case–control	Controls matched by race ethnicity in a 3 : 1 ratio No significant difference in terms of maternal age, ethnicity, parity, private insurance, BMI, gestational age at delivery between cases and controls Season of blood draw did differ but not significantly ($p = 0.06$) Results adjusted for maternal age, insurance status, BMI, gestational age at serum collection, season of blood draw	11–14 weeks
Shand, 2010[117]	6 (low)	Vancouver, BC, Canada All women had either clinical or biochemical risk factors for pre-eclampsia[f]	Cohort	Maternal age, ethnicity, parity, BMI, season, multivitamin use, smoking	Between 10 and 20 weeks 6 days [mean 18.7 (1.88) weeks]

Maternal mean (SD) 25(OH)D concentration (nmol/l) in cases of infants born preterm	Maternal mean (SD) 25(OH)D concentration (nmol/l) in full-term infants	OR (95% CI) of offspring being preterm from univariate analysis	OR (95% CI) of offspring being preterm from multivariate analysis	Conclusion
44.9 (17.5)	47.4 (7.5)	Not given	Not given	No difference in maternal 25(OH)D at delivery between preterm and full-term births (*p*-value not given)
Mean not given 34% of preterm births and 37% of severe preterm births had 25(OH)D < 80 nmol/l 66% of preterm births and 63% of severe preterm births had 25(OH)D > 80 nmol/l	Not given	RR if maternal 25(OH)D < 80 nmol/l compared with > 80 nmol/l Preterm birth = 0.83 (0.65 to 1.07) *p* = 0.14 Severe preterm birth = 0.77 (0.49 to 1.19) *p* = 0.24	Adjusted RR if maternal 25(OH)D < 80 nmol/l compared with > 80 nmol/l Preterm birth = 0.84 (0.65 to 1.07) *p* = 0.15 Severe preterm birth = 0.77 (0.50 to 1.18) *p* = 0.23	No increased risk of preterm or severe preterm birth if maternal 25(OH)D < 80 nmol/l compared with > 80 nmol/l
25(OH)D (nmol/l)	*n* (%)	25(OH)D (nmol/l)	*n* (%)	25(OH)D (nmol/l)	OR (95% CI), *p*-value	25(OH)D (nmol/l)	Adjusted OR (95% CI), *p*-value	No significant association seen between maternal 25(OH)D and risk of preterm birth
< 50	3 (7.5)	< 50	8 (6.7)	< 50	1.14 (0.31 to 4.26), *p* = 0.61	< 50	0.82 (0.19 to 3.57), *p* = 0.79	
50–74.9	8 (20)	50–74.9	24 (20)	50–74.9	1.01 (0.42 to 2.46), *p* = 0.99	50–74.9	0.87 (0.34 to 2.25), *p* = 0.77	
≥75	29 (72.5)	≥75	88 (73.3)	≥75	1 (Ref)	≥75	1 (Ref)	
Not given		Not given		Unadjusted values not given		25(OH)D concentration (nmol/l) < 37.5	OR (95% CI) 0.97 (0.43 to 2.21)	No significant relationship seen between maternal 25(OH)D and risk of preterm birth using three different maternal 25(OH)D cut-offs

TABLE 21 The association between maternal vitamin D status in gestation and preterm birth of the offspring: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured
		Cohort $n = 221$ women Cases of preterm birth[b] $n = 18$			
Hossain, 2011[122]	4 (medium)	Karachi, Pakistan Cohort $n = 75$ women Cases of preterm birth[b] $n =$ not given 26% of women covered their arms, hands and head; 76% also covered their face	Cross-sectional	None	At delivery
Shibata, 2011[123]	4 (medium)	Toyoake, Japan Cohort size $n = 93$ women Deliveries spread equally across seasons Cases of threatened premature delivery[g] $n = 14$	Cross-sectional	Maternal age, serum albumin, serum corrected calcium, serum bone specific ALP, serum type 1 collagen N-terminal telopeptide, serum phosphate	At recruitment (> 30 weeks)
Fernandez-Alonso, 2012[118]	3 (medium)	Almeria, Spain Cohort $n = 466$ women Cases of preterm birth[b] $n = 33$	Cohort	Nil	Between 11 and 14 weeks

Ref, reference group; RR, relative risk.
a No threshold for preterm birth given. Gestational age determined by the scoring system of Dubowitz *et al.*[124] (based on examination of the neonate and scored on neurological and physical examination features).
b Preterm birth defined as delivery at < 37 weeks' gestation.
c Severe preterm birth defined as delivery at < 34 weeks' gestation.
d Preterm birth defined as delivery at > 23 weeks and < 35 weeks' gestation.
e 25(OH)D_3 measured.
f Defined as past obstetric history of early-onset or severe pre-eclampsia, unexplained elevated α-fetoprotein ≥ 2.5 MoMs, unexplained elevated human chorionic gonadatrophin, or low pregnancy-associated plasma protein A ≤ 0.6 MoM.
g This study assessed risk of threatened premature delivery. Defined as progressive shortening of cervical length (< 20 mm) as detected by transvaginal ultrasound before the 34th week of gestation, and/or elevation of granulocyte elastase level in the cervical mucus before 32 weeks' gestation; AND the number of uterine contractions equal to or more than twice per 30 minutes (before the 32nd week of gestation).

Maternal mean (SD) 25(OH)D concentration (nmol/l) in cases of infants born preterm		Maternal mean (SD) 25(OH)D concentration (nmol/l) in full-term infants	OR (95% CI) of offspring being preterm from univariate analysis	OR (95% CI) of offspring being preterm from multivariate analysis		Conclusion
				< 50	1.02 (0.48 to 2.17)	
				< 75	0.79 (0.31 to 2.06)	
42.2 (19.5)[e]		32.9 (16.8)[e]	Not given	Not given		Maternal 25(OH)D tended to be higher in those who delivered pre term but did not achieve statistical significance $p = 0.057$
30.0 (8.0)		37.9 (12.7)	Not given	$\beta = -0.019$ $p = 0.023$		Significantly lower maternal 25(OH)D between women with threatened premature delivery and those with normal deliveries *p*-value for difference in means = 0.002
25(OH)D concentration (nmol/l)	*n* (%)	Not given	Not given	Not given		No significant relationship seen between maternal 25(OH)D and risk of preterm birth $p = 0.86$
< 50	7 (21)					
50–74.9	15 (45)					
≥ 75	11 (33)					

TABLE 22 The association between maternal vitamin D status in gestation and risk of type 1 diabetes mellitus in the offspring: observational studies

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured
Stene, 2003[126]	2 (medium)	Norway Cases of offspring type 1 DM *n* = 545 [Mean age 10.9 (3.4) years] Controls *n* = 1668	Case–control	Controls matched for period of birth (between 1 January 1985 and 31 December 1999) Maternal use of cod liver oil in pregnancy, child's use of cod liver oil or other vitamin D supplement during the first year of life, duration of exclusive breastfeeding, child's age at introduction of solids, maternal education, smoking in pregnancy, maternal age at delivery, child number of siblings, type 1 DM among child's siblings or parents, child's age, child's sex	Not measured. Retrospective questionnaire of maternal use of vitamin D supplements during pregnancy. Grouped into either 'no supplements'; 'yes, one to four times per week' or 'yes, five or more times per week'
Marjamaki, 2010[127]	6 (low)	Diabetes mellitus Prediction and Prevention (DIPP) study, Finland Cohort size *n* = 3723 women and their children with increased genetic risk of DM[a] Cases of offspring type 1 DM *n* = 74 (children observed for mean 4.3 (range 0.2–8.9) years	Prospective cohort	Two models: HR1 adjusted for genetic risk and familial type 1 DM HR2 adjusted for genetic risk, familial type 1 DM, sex, gestational age, maternal age, maternal education, delivery hospital, route of delivery, number of earlier deliveries, smoking during pregnancy	Not measured. Estimated from FFQ completed 1–3 months after delivery – focused on food taken in the eighth month of pregnancy and the use of supplements

Maternal mean (SD) 25(OH)D concentration (nmol/l) in cases of offspring DM	Maternal mean (SD) 25(OH)D concentration (nmol/l) in offspring without DM	OR (95% CI) of offspring developing type 1 DM from univariate analysis		OR (95% CI) of offspring developing type 1 DM from multivariate analysis		Conclusion
Not measured	Not measured	Vitamin D supplement in pregnancy	OR (95% CI)	Vitamin D supplement in pregnancy	Adjusted OR (95% CI)	Maternal use of vitamin D supplements in pregnancy were not associated with an increased risk of type 1 DM in the offspring
		No	1 (Ref)	No	1 (Ref)	
		Yes, one to four times per week	0.86 (0.63 to 1.18)	Yes, one to four times per week	1.09 (0.77 to 1.56)	
		Yes, five or more times per week	0.89 (0.69 to 1.13)	Yes, five or more times per week	0.98 (0.73 to 1.31)	
		p-value for trend	0.28	*p*-value for trend	0.94	
Not given	Not given	Not given		HR given: HR1 = 1.18 (0.74 to 1.87), $p = 0.49$ HR2 = 1.08 (0.65 to 1.79), $p = 0.77$		Maternal intake of vitamin D, from either food or supplements, is not associated with type 1 DM or advanced B cell autoimmunity in the offspring

TABLE 22 The association between maternal vitamin D status in gestation and risk of type 1 diabetes mellitus in the offspring: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured
Sorensen, 2012[134]	8 (low)	Norway Overall cohort *n* = 29,072 women Cases of offspring type 1 DM *n* = 109 [Mean age at diagnosis 9.0 (3.6) years] Controls *n* = 219	Nested case–control	No significant difference between cases and controls in terms of maternal age, parity, gestational week of blood sample, frequency of caesarean section or maternal DM pre pregnancy. Significantly more female offspring in cases than in controls Adjustments (two models): OR1 adjusted for sex of child and season of blood sample OR2 adjusted for age of child at diagnosis, offspring sex, mother's age at delivery, parity, gestational week of blood sample, pre-gestational DM, season of blood sample, region of residence, percentage undergoing caesarean section	Median (IQR) cases = 37 (22–38) weeks Median (IQR) controls = 37 (24–38) weeks

Cont., test for continuous trend; DM, diabetes mellitus; HR, hazard ratio; HR1, hazard ratio 1; HR2, hazard ratio 2; IQR, interquartile range; OR1, odds ratio 1; OR2, odds ratio 2; Ref, reference group.

a Increased genetic risk defined by genotype *HLA-DQB1**02*0302 for high risk and *HLA-DQB1**0302/x, where x = other than *03, *0301 or *0602 for moderate risk.

Maternal mean (SD) 25(OH)D concentration (nmol/l) in cases of offspring DM	Maternal mean (SD) 25(OH)D concentration (nmol/l) in offspring without DM	OR (95% CI) of offspring developing type 1 DM from univariate analysis		OR (95% CI) of offspring developing type 1 DM from multivariate analysis			Conclusion
65.8 (26.5)	73.1 (27.2)	25(OH)D concentration	OR	25(OH)D concentration	OR1	OR2	Trend towards higher risk of type 1 DM in the offspring with lower levels of maternal 25(OH)D in late pregnancy, especially in those with 25(OH)D < 54 nmol/l
		> 89	1.0 (Ref)	> 89	1.0 (Ref)	1.0 (Ref)	
		> 69–89	1.32 (0.63 to 2.76)	> 69–89	1.35 (0.63 to 2.89)	Not given	
		> 54–69	1.73 (0.86 to 3.48)	> 54–69	1.78 (0.85 to 3.74)	Not given	
		≤ 54	2.25 (1.14 to 4.46)	≤ 54	2.38 (1.12 to 5.07)	2.39 (1.07 to 5.11)	
		Test for trend	$p = 0.022$	Test for trend	0.031	0.032	
		Cont.		Cont.			

TABLE 23 The association between maternal vitamin D status in gestation and risk of low birthweight[a] in the offspring: observational studies

First author, year	Bias score	Study details	Study type	Confounders/adjustments
Sabour, 2006[91]	−2 (high)	Tehran, Islamic Republic of Iran $n = 449$ women Cases of LBW[a] not given	Cross-sectional	Nil
Maghbooli, 2007[92]	1 (medium)	Tehran, Islamic Republic of Iran $n = 552$ women Cases of LBW[a] = 5.4% ($n = 30$)	Cross-sectional	None
Mehta, 2009[119]	3 (medium)	Tanzania Overall cohort $n = 1078$ Women all HIV infected taking part in a clinical trial of vitamin use Cases of LBW[a] $n = 80$ Cohort for analysis $n = 675$	Prospective cohort	Multivitamin supplementation, maternal age at baseline, CD4 count at baseline, HIV disease stage at baseline

LBW, low birthweight.
a LBW defined as infants born < 2500 g.
b Measured $25(OH)D_3$.

Number of weeks' gestation when 25(OH)D was measured	Maternal mean (SD) 25(OH)D concentration (nmol/l) in cases of LBW infants	Maternal mean (SD) 25(OH)D concentration (nmol/l) in infants without LBW	OR (95% CI) of offspring having LBW from univariate analysis	OR (95% CI) of offspring having LBW from multivariate analysis	Conclusion
Not measured directly Estimated from validated dietary FFQ at delivery (unclear when assessed)	Not given	Not given	Not given	Not given	Incidence of LBW significantly lower with adequate maternal calcium and vitamin D intake (1000 mg calcium, 200 IU vitamin D) $p = 0.007$
Delivery[b]	Not given	Not given	Not given	Not given	No significant association seen between serum $25(OH)D_3$ and LBW (p-value not given)
12–27 weeks (at enrolment to trial)	Mean not given 35% of LBW had 25(OH)D < 80 nmol/l 65% of LBW had 25(OH)D > 80 nmol/l	Not given	0.85 (0.55 to 1.32)	0.84 (0.55 to 1.28)	No relationship between LBW risk and maternal 25(OH)D among women with HIV $p = 0.42$

TABLE 24 The association between maternal vitamin D status in gestation and offspring serum calcium concentration: observational studies

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured
Ardawi, 1997[90]	5 (low)	Jeddah, Saudi Arabia Cohort size $n = 264$ women	Cross-sectional	Nil	Delivery

IQR, interquartile range.

Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)	Mean (SD) offspring serum calcium (mmol/l)		Unadjusted regression coefficient β (95% CI) or correlation coefficient r (95% CI) for offspring serum calcium (mmol/) per 1 nmol/l increase in 25(OH)D	Adjusted regression coefficient β (95% CI) or correlation coefficient r (95% CI) for offspring serum calcium (mmol/) per 1 nmol/l increase in 25(OH)D	Conclusion
47.71 (15.77)	Mean cord calcium = 2.49 (0.19)		r = 0.02 ($p = 0.40$)	No adjustments made	No significant correlation between maternal 25(OH)D measured at delivery and offspring cord calcium
25(OH)D < 20 nmol/l (inadequate) in 23%	Maternal 25(OH)D	Mean (SD) cord calcium concentration (mmol/l)			
25(OH)D > 20 nmol/l (adequate) in 77%	< 20 ($n = 24$)	2.48 (0.18)			No difference in cord calcium if group divided according to maternal 25(OH)D using 20 nmol/l as a threshold
	> 20 ($n = 240$)	2.40 (0.22)			$p > 0.05$

TABLE 25 The effect of vitamin D supplementation in gestation on offspring serum calcium concentration: intervention studies

First author, year	Risk of bias	Setting	Randomisation	Adjustments/confounders accounted for	Number of weeks' gestation when 25(OH)D was measured
Brooke, 1980[3]	–2 (high)	London, UK $n = 126$ women (all Asian)	Double blinded Randomised to either placebo ($n = 67$) or 1000 IU/day of vitamin D_2 in last trimester ($n = 59$)	Nil, but groups of similar age, height, parity, offspring sex, length of gestation 27% of control group and 22% of treatment group bottle fed their infants	28–32 weeks (allocation) and at birth
Cockburn, 1980[20]	–1 (high)	Edinburgh, Scotland $n = 1139$ women	Either given placebo ($n = 633$) or 400 IU vitamin D_2 ($n = 506$) from week 12 of gestation Deliveries on one ward given placebo, deliveries on another ward given supplement	Nil, but groups similar in terms of social class, parity and maternal age All deliveries between September and May Maternal age, parity, type of delivery, offspring Apgar score at birth, social class, maternal pre-eclampsia, birthweight and gestational age were not associated with offspring 6-day calcium concentration	24 weeks, 34 weeks and delivery
Marya, 1981[4]	–6 (high)	Rohtak, India $n = 120$ women	Three arms Randomised to either no supplement ($n = 75$); 1200 IU vitamin D + 375mg calcium/day[b] throughout the third trimester ($n = 25$) or oral 600,000 IU vitamin D_2; two doses in seventh and eighth months' gestation ($n = 20$)	Nil	Not measured
Congdon, 1983[21]	–9 (high)	Leeds, UK $n = 64$ women (all Asian women)	Either 1000 IU vitamin D plus calcium (calcium dose not given) daily in the third trimester ($n = 19$) or no supplement ($n = 45$)	Nil, but groups similar in terms of maternal age, infant sex, gestation length, birthweight	Not measured
Mallet, 1986[7]	–3 (high)	Rouen, France $n = 77$ women	Three arms Randomised to either no supplement ($n = 29$); 1000 IU vitamin D/day[b] in last 3 months of pregnancy ($n = 21$) or single oral dose of vitamin D[b] 200,000 IU in seventh month ($n = 27$)	Nil, but groups of similar maternal age, parity, calcium intake and frequency of outdoor outings	During labour (February and March)

Mean (SD)/mean (SE)[a] or median (IQR) 25(OH)D concentration (nmol/l)			Mean (SD) or mean (SE)[a] offspring serum calcium concentration (mmol/l) in unsupplemented group		Mean (SD) or mean (SE)[a] serum calcium concentration (mmol/l) in supplemented group		Conclusion
At allocation 25(OH)D = 20.1 (1.9)[a] At term, placebo group = 25(OH)D = 16.2 (2.7)[a] At term, supplemented group 25(OH)D = 168.0 (12.5)*			Cord Day 3 Day 6	2.65 (0.02)[a] 2.18 (0.04)[a] 2.29 (0.02)[a]	Cord Day 3 Day 6	2.71 (0.02)[a] 2.30 (0.04)[a] 2.49 (0.04)	No significant difference in cord calcium between groups at birth, but significantly higher levels in the treatment group at days 3 and 6, but higher rates of breastfeeding in the treatment group, which in itself was positively associated with offspring calcium concentration compared with bottle feeding When groups considered separately, a weak correlation seen between maternal 25(OH)D and cord calcium in the treatment group r = 0.31, $p < 0.05$ Five cases of symptomatic hypocalcaemia in control group, none in treatment group $\chi^2 = 4.6$, $p < 0.01$
	25(OH)D in placebo group	25(OH)D in supplement group	Cord	2.69 (0.26) ($n = 452$)	Cord	2.66 (0.27) ($n = 262$)	No significant difference in cord blood serum calcium at delivery Significantly higher serum calcium in infants at day 6 in the supplemented group, independent of infant sex and effects of type of feeding (breast vs. formula) 6% of infants in the supplemented group were hypocalcaemic at day 6 (calcium < 1.85 mmol/l) compared with 13% in the placebo group
24 weeks	32.5 ($n = 82$)	39.0 ($n = 82$)					
34 weeks	38.5 ($n = 80$)	44.5 ($n = 80$)	Day 6	2.25 (0.3) ($n = 394$)	Day 6	2.34 (0.2) ($n = 233$)	
Delivery	32.5 ($n = 84$)	42.8 ($n = 80$)					
Not measured			2.52 (0.23) (value represents cord blood at delivery)		1200 IU + calcium = 2.55 (0.17) 600,000 IU = 2.67 (0.12) (Value represents cord blood at delivery)		No difference in cord calcium between unsupplemented and 1200 IU + 375 mg calcium/day supplementation Cord calcium significantly higher in those taking 600,000 IU supplement than in those unsupplemented $p = 0.001$
Not measured			2.50 (0.03)		2.64 (0.05)		Cord calcium significantly higher in the supplemented group $p < 0.025$
Overall mean not given According to group: Unsupplemented = 9.4 (4.9) 1000 IU/day = 25.3 (7.7) 200,000 IU = 26.0 (6.4)			2.37 (0.11) (Value represents cord blood at delivery)		1000 IU/day = 2.44 (0.14) 200,000 IU = 2.41 (0.21) (Value represents cord blood at delivery)		No significant difference in serum across the three groups One case of neonatal hypocalcaemia observed in the unsupplemented group (serum calcium 1.69 mmol/l)

TABLE 25 The effect of vitamin D supplementation in gestation on offspring serum calcium concentration: intervention studies (*continued*)

First author, year	Risk of bias	Setting	Randomisation	Adjustments/confounders accounted for	Number of weeks' gestation when 25(OH)D was measured
Delvin, 1986[6]	–2 (high)	Lyon, France *n* = 40 women	Randomised to either no supplement (*n* = 20) or 1000 IU vitamin D_3/day during third trimester (*n* = 20)	Nil Groups similar in terms of maternal age and parity. All deliveries occurred in the same month (June) All infants of similar gestational age and breastfed from the sixth hour of life	At recruitment (*n* = 50) and at delivery
Marya, 1988[5]	–2 (high)	Rohtak, India *n* = 200 women	Randomised to either no supplement (*n* = 100) or oral 600,000 IU vitamin D_3; two doses in seventh and eighth months' gestation (*n* = 100)	Nil, but groups had similar maternal age, maternal height, maternal height, parity, haemoglobin, calcium intake and vitamin D intake	Not measured

SE, standard error.
a Mean (SE).
b Not known whether supplementation was vitamin D_2 or vitamin D_3.
Table includes any studies that measured maternal vitamin D status in pregnancy and either cord calcium concentration of offspring serum calcium concentration.

Mean (SD)/mean (SE)[a] or median (IQR) 25(OH)D concentration (nmol/l)			Mean (SD) or mean (SE)[a] offspring serum calcium concentration (mmol/l) in unsupplemented group		Mean (SD) or mean (SE)[a] serum calcium concentration (mmol/l) in supplemented group		Conclusion
	25(OH)D in supplement group	25(OH)D in unsupplemented group	When measured	Mean infant serum calcium (SE) (mmol/l)	When measured	Mean infant serum calcium (SE) (mmol/l)	Significant correlation between maternal 25(H)D and cord blood total calcium concentration $p < 0.005$
At recruitment (185 days' gestation)	54.9 (10.0)[a]	27.5 (10.0)[a]	Cord at delivery, $n = 15$	2.63 (0.025)[a]	Cord at delivery, $n = 15$	2.55 (0.5)[a]	No significant difference in cord blood total calcium concentration at delivery between groups At day 4, infant calcium levels were significantly higher in those in the supplemented group $p < 0.025$ Infant calcium fell significantly more from delivery to day 4 in the unsupplemented group compared with the supplemented group $p < 0.05$
Delivery	64.9 (17.5)[a]	32.4 (20.0)[a]	Infant day 6, $n = 12$	2.1 (0.05)[a]	Infant day 6, $n = 13$	2.28 (0.5)[a]	
Not measured directly, but mean daily vitamin D intake given as follows: Unsupplemented group = 35.71 (6.17) IU/day Supplemented group = 35.01 (7.13) IU/day			2.57 (0.26) (Value represents cord blood at delivery)		2.77 (0.18) (Value represents cord blood at delivery)		Cord serum calcium concentration significantly higher in the supplemented group $p < 0.001$

TABLE 26 The association between maternal vitamin D status in gestation and offspring blood pressure: observational studies

First author, year	Bias score	Study type	Study details, age at which offspring blood pressure children was measured	Confounders/adjustments	Number of weeks' gestation when maternal 25(OH)D_3 was measured	Mean (SD) or median (IQR) maternal 25(OH)D concentration (nmol/l)
Gale, 2008[24]	4 (medium)	Cohort	Princess Anne cohort, UK $n = 178$ women Children assessed at 9 years	Nil	Late pregnancy Median (IQR) 32.6 (32–33.4) weeks	50 (30–75.3) 50.4% had 25(OH)D levels > 50 nmol/l 28.3% had 25(OH)D levels 27.5–50 nmol/l 21.1% had 25(OH)D levels < 27.5 nmol/l
Krishnaveni 2011[105]	4 (medium)	Cohort	Mysore Parthenon Study, Mysore, India Children assessed at 5 years ($n = 338$) and 9.5 years ($n = 312$)	Offspring sex and age, maternal BMI, gestational diabetes mellitus, socioeconomic score, parity and religion	28–32 weeks (at study entry)	39.0 (24–58) 67% of women had 25(OH)D < 50 nmol/l (the authors' definition of deficiency)

IQR, interquartile range.

Mean (SD) offspring blood pressure according to maternal 25(OH)D category/unadjusted correlation coefficient (r) or regression coefficient (β) (95% CI)						Adjusted correlation coefficient (r) or regression coefficient (β) (95% CI)	Conclusion
	Maternal 25(OH)D (nmol/l)				*p*-value	Not given	No significant association between maternal 25(OH)D concentration measured in late pregnancy and offspring blood pressure at age 9 years
	< 30	–50	–75	> 75			
Systolic blood pressure (mmHg)	103.4 (7.94)	102.2 (7.26)	101.9 (8.18)	102.9 (8.10)	0.47		
Diastolic blood pressure (mmHg)	59.8 (5.25)	60.1 (5.49)	60.2 (5.7)	59.9 (6.2)	0.75		
Maternal 25(OH)D						Comparing offspring of mothers with and without 25(OH)D deficiency (deficient = 0, non-deficient = 1)	No significant difference in offspring blood pressure at 5 and 9.5 years between those born to mothers with 25(OH)D deficiency in late pregnancy and those born to mothers without vitamin D deficiency
	< 50 nmol/l (deficient)		>50 nmol/l (non-deficient)		*p*-value		
Systolic blood pressure at 5 years (mmHg)	96.7 (8.4)		97.0 (8.1)		0.67		
Diastolic blood pressure at 5 years (mmHg)	58.3 (6.8)		57.9 (6.6)		0.54	5 years' systolic blood pressure β = 0.3 (–1.32 to 1.89; *p* = 0.72)	
Systolic blood pressure at 9.5 years (mmHg)	101.6 (8.7)		100.5 (8.3)		0.2	5 years' diastolic blood pressure β = –0.3 (–1.67 to 0.98; *p* = 0.61)	
Diastolic blood pressure at 9.5 years (mmHg)	58.3 (6.5)		58.7 (7.2)		0.5	9.5 years' systolic blood pressure β = –1.2 (–2.87 to 0.42; *p* = 0.15)	
						9.5 years' diastolic blood pressure β = 0.4 (–0.90 to 1.74; *p* = 0.53)	

TABLE 27 The association between maternal vitamin D status in gestation and maternal pre-eclampsia: observational studies

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or mean (SE)[a] or median (IQR) 25(OH)D concentration (nmol/l) in cases	
Seely, 1992[131]	2 (medium)	Boston, MA, USA Cases $n = 12$ Controls $n = 24$	Case–control	No adjustments, but cases and controls similar for age, gestation, number Caucasian, height, weight, number primiparous	Mean 35.5 (0.6) weeks for cases and 36 (0.4) weeks for controls	73.9 (7.5)[a]	
Bodnar, 2007[128]	8 (low)	Pittsburgh, PA, USA Cohort size $n = 1198$ women Cases $n = 55$ Controls $n = 220$ All women nulliparous	Nested case–control	Controls randomly selected and unmatched Adjusted for maternal race/ethnicity, pre-pregnant BMI, education, season, gestational age at collection	Two occasions: Before 22 weeks Pre delivery	Adjusted geometric mean (< 22 weeks): 45.4 (38.6–53.4) Adjusted geometric mean at delivery: 54.4 (45.1–65.7)	
Oken, 2007[135]	5 (low)	Project Viva, Eastern Massachusetts, USA $n = 1718$ women Cases $n = 59$	Cohort	Maternal age, BMI, first trimester systolic BP, ethnicity, education, parity, total energy intake	Not measured FFQ at mean 10.4 weeks	Not measured Mean intake (IU/day) = 466 (183)	
Azar, 2011[133]	5 (low)	Oklahoma, USA All white women with type 1 diabetes mellitus Cohort $n = 151$ women Cases $n = 23$ Controls $n = 24$	Nested case–control	Cases and controls matched for age, diabetes mellitus duration, HbA1c and parity Higher BMI and lower high-density lipoprotein cholesterol in the cases Adjusted for parameters that differed between groups (BMI and HDL cholesterol)	Three visits Mean 12.2 (1.9) weeks Mean 21.6 (1.5) weeks Mean 31.5 (1.7) weeks	Visit 1 Visit 2 Visit 3	44.4 (32.9–51.4) 44.2 (35.7–58.2) 47.2 (23.5–55.4)

Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in controls	OR/relative risk of pre-eclampsia from univariate analysis	OR/relative risk of pre-eclampsia from multivariate analysis	Conclusion
89.3 (11.7)[a]	Unadjusted OR not given	OR not given	No statistically significant relationship seen
Adjusted geometric mean (< 22 weeks): 53.1 (47.1–59.9) Adjusted mean at delivery: 64.7 (56.4–74.2)	Unadjusted OR not given	At < 22 weeks: Adjusted OR for pre-eclampsia Serum 25(OH)D OR (95% CI) < 37.5 5 (1.7 to 14.1) 50 nmol/l reduction in 25(OH)D increased risk of pre-eclampsia, OR 2.4 (95% CI 1.1 to 5.4) At delivery: 25(OH)D significantly lower in cases (15% reduction; $p < 0.05$)	At < 22 weeks a strong inverse relationship between pre-eclampsia and 25(OH)D was observed $p = 0.02$
Not measured Mean intake (IU/day) = 492 (210)	Unadjusted OR not given	OR (per 100 IU increase in vitamin D intake per day) of developing pre-eclampsia = 0.99 (0.87 to 1.13)	No significant relationship seen
Visit 1 47.2 (37.4–58.2) Visit 2 43.4 (30.0–61.4) Visit 3 44.9 (33.2–65.9)	Visit 1 (early pregnancy) 0.91 (0.88 to 0.95) Visit 2 (mid-pregnancy) 1.02 (0.98 to 1.06) Visit 3 (late pregnancy) 0.90 (0.73 to 1.11)	Visit 1 0.99 (0.77 to 1.30) Visit 2 1.02 (0.78 to 1.33) Visit 3 0.92 (0.75 to 1.14)	No statistically significant relationship seen at any time point (after adjusting for confounders)

TABLE 27 The association between maternal vitamin D status in gestation and maternal pre-eclampsia: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or mean (SE)[a] or median (IQR) 25(OH)D concentration (nmol/l) in cases
[b]Baker, 2010[129]	9 (low)	Boston, MA, USA Cohort size $n = 3992$ women Cases $n = 44$ Controls $n = 201$	Nested case–control	Controls matched by race/ethnicity Adjusted for season of blood sampling, maternal age, multiparity, BMI, gestational age at serum collection	Between 15 and 20 weeks	75 (47–107)
Haugen, 2009[134]	2 (medium)	Norwegian Mother and Child Cohort Study, Norway $n = 23{,}425$ women Cases $n = 1267$	Cohort	BMI, height, maternal age, maternal education, season of childbirth	Not measured Estimated from FFQ at 22 weeks	Median (5th, 95th percentile) total vitamin D intake (IU/day): Cases = 308 (60, 1200)
Powe, 2010[132]	4 (medium)	Massachusetts General Hospital Obstetric Maternal Study, MA, USA Cohort size $n = 9930$ women Cases $n = 39$ Controls $n = 131$	Nested case–control	Controls unmatched Adjusted for BMI, non-white race, summer blood collection	First trimester	68.5 (0.48)[a]
[b]Robinson, 2010[130]	5 (low)	South Carolina, USA Cases $n = 50$ Controls $n = 100$	Case–control	Controls matched by race and gestational age at sample collection Adjusted for BMI, maternal age, African American race, gestational age at sample collection	Time of diagnosis < 34 weeks	45 (32.5–77.5)

Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in controls	OR/relative risk of pre-eclampsia from univariate analysis			OR/relative risk of pre-eclampsia from multivariate analysis			Conclusion
98 (680–114)	OR for severe pre-eclampsia			Adjusted OR for severe pre-eclampsia			Lower 25(OH)D was associated with increased risk of severe pre-eclampsia
	25(OH)D (nmol/l)	OR (95% CI)	*p*-value	25(OH)D (nmol/l)	Adjusted OR (95% CI)	*p*-value	
	> 75	1 (Ref)	–	>	1 (Ref)	–	
	50–74.9	1.53 (0.67 to 3.49)	0.31	50–74.9	2.16 (0.86 to 5.40)	0.10	
	< 50	3.63 (1.52 to 8.65)	0.004	< 50	5.41 (2.02 to 14.52)	0.001	
Median (5th, 95th percentile) total vitamin D intake (IU/day): 336 (68, 1256)	OR for pre-eclampsia			OR for pre-eclampsia			Lower total vitamin D intake associated with an increased risk of pre-eclampsia $p < 0.001$
	Total vitamin D intake (IU/day)	OR		Total vitamin D intake (IU/day)	OR		
	< 200	1		< 200	1		
	200–399	0.93 (0.81 to 1.07)		200–399	0.99 (0.85 to 1.14)		
	400–599	0.81 (0.67 to 0.97)		400–599	0.87 (0.73 to 1.05)		
	600–799	0.69 (0.55 to 0.87)		600–799	0.77 (0.61 to 0.96)		
	> 800	0.78 (0.65 to 0.92)		> 800	0.89 (0.89 to 1.06)		
72.0 (2.0)[a] nmol/l	OR per 25 nmol/l increase in 25(OH)D = 0.86 (0.60 to 1.25) If vitamin D < 37.5 nmol/l OR = 2.49 (0.89 to 6.90)			OR per 25 nmol/l increase in 25(OH)D = 1.24 (0.78 to 1.98) If vitamin D < 37.5 nmol/l OR = 1.35 (0.4 to 4.5)			No significant relationship seen $p = 0.435$
80 (50–110)	OR per 25 nmol/l increase in 25(OH)D = 0.58 (0.43 to 0.77)			OR per 25 nmol/l increase in 25(OH)D = 0.37 (0.22 to 0.62)			Lower 25(OH)D associated with increased risk of severe early pre-eclampsia $p < 0.001$

TABLE 27 The association between maternal vitamin D status in gestation and maternal pre-eclampsia: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or mean (SE)[a] or median (IQR) 25(OH)D concentration (nmol/l) in cases
Shand, 2010[117]	6 (low)	Vancouver, BC, Canada All women had either clinical or biochemical risk factors for pre-eclampsia[c] Cohort $n = 221$ women Cases $n = 28$	Cohort	Maternal age, ethnicity, parity, BMI, season, multivitamin use, smoking	Between 10 and 20 weeks 6 days [mean 18.7 (1.88) weeks]	42.6 (32.7–72.4)
Hossain, 2011[122]	4 (medium)	Karachi, Pakistan Cohort $n = 75$ women Cases n = not given 26% of women covered their arms, hands and head; 76% also covered their face	Cross-sectional	Maternal age, level of exercise, attire, duration of gestation, newborn weight	At delivery	29.7 (13.7)[c]
Fernandez-Alonso, 2012[118]	3 (medium)	Almeria, Spain Cohort $n = 466$ women Cases $n = 7$	Cohort	Nil	Between 11 and 14 weeks	Overall mean not given 25(OH)D concentration — n < 50 — 2 50–75 — 3 > 75 — 2

IQR, interquartile range; Ref, reference group; SE, standard error.
a Mean (SEM).
b Severe pre-eclampsia.
c Defined as past obstetric history of early-onset or severe pre-eclampsia, unexplained elevated α-fetoprotein ≥ 2.5 MoMs, unexplained elevated human chorionic gonadatrophin, or low pregnancy-associated plasma protein A ≤ 0.6 MoM.
d $25(OH)D_3$ measured.

Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in controls	OR/relative risk of pre-eclampsia from univariate analysis	OR/relative risk of pre-eclampsia from multivariate analysis		Conclusion
50.4 (35.8–68.0)	Unadjusted values not given	25(OH)D (nmol/l)	OR for pre-eclampsia	No significant relationship seen
		< 37.5	0.91 (0.31 to 2.62)	
		< 50	1.39 (0.54 to 3.53)	
		< 75	0.57 (0.19 to 1.66)	
36.2 (18.4)[d]	Not given	$25(OH)D_3$ tertile	Adjusted OR (95% CI) for pre-eclampsia (systolic blood pressure ≥ 140 mmHg, and/or diastolic blood pressure ≥ 90 mmHg)	Women in the lowest and middle tertile for $25(OH)D_3$ more likely to meet criteria for pre-eclampsia than those in the highest tertile $25(OH)D_3$ of 50 nmol/l maximum identified as the threshold relating to increased risk for pre-eclampsia
		Highest tertile	1.0 (Ref)	
		Middle tertile	11.05 (1.15 to 106.04)	
		Lowest tertile	3.38 (0.40 to 28.37)	
Not given	Not given	Not given		No significant association between development pre-eclampsia as a function of first trimester 25(OH)D status $p = 0.51$

TABLE 28 The effect of vitamin D supplementation in gestation on pre-eclampsia: intervention studies

First author, year	Risk of bias	Setting	Randomisation	Adjustments/confounders accounted for	Number of weeks' gestation when 25(OH)D_3 measured	Mean (SD) 25(OH)D concentration (nmol/l – unless other stated)	No. of cases in unsupplemented group	No. of cases in supplemented group	Conclusion
Marya, 1987[136]	–2 (high)	Rohtak, India	Randomised to either no supplement ($n = 200$) or 375 mg/day calcium + 1200 IU vitamin D given at 20–24 weeks until birth ($n = 200$)	Nil	Not measured	Not measured	18	12	No significant difference in rates of pre-eclampsia in the two groups $p > 0.05$ Significantly reduced diastolic and systolic blood pressure in the supplemented group at 32 and 36 weeks $p < 0.001$ No significant difference at 24 or 28 weeks (p-value not given)

TABLE 29 The association between maternal vitamin D status in gestation and risk of gestational diabetes mellitus: observational studies

First author, year	Bias score	Study details	Study type	Confounders/ adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in cases of GDM
Maghbooli, 2008[137]	3 (medium)	Tehran, Islamic Republic of Iran Overall cohort size *n* = 741 women Cases of GDM *n* = 52 Controls *n* = 527	Cross-sectional	Nil Cases significantly older, higher parity and higher BMI	24–28 weeks[a]	16.49 (10.44)[a]
Clifton-Bligh, 2008[95]	6 (low)	New South Wales, Australia Cases of GDM *n* = 81 women Normal pregnancies *n* = 183 women	Prospective cohort	Age, BMI, ethnicity, season	Mean (SD) 28.7 (3.3) weeks	48.6 (24.9)
Zhang, 2008[139]	8 (low)	Omega Study, Seattle and Washington, USA Overall cohort size *n* = 953 women Cases of GDM *n* = 57 women (70% white) Controls *n* = 114 women (84% white)	Nested case–control	Controls frequency matched to cases for the estimated season of conception OR1: maternal age, race/ethnicity, family history of type 2 GDM OR2: as above plus pre-pregnant BMI Physical activity measured but not included in the analysis as did alter the OR by > 10%	16 weeks	24.2 (8.5)

Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in unaffected controls	OR (95% CI) of GDM from univariate analysis		OR of GDM from multivariate analysis			Conclusion
22.97 (18.25)[a]	Not given		Not given			25(OH)D significantly lower in individuals with GDM $p = 0.009$
55.3 (23.3)	Not given		OR if 25(OH)D < 50 nmol/l = 1.92 (0.89 to 4.17)			Significant difference in mean 25(OH)D between cases and controls ($p = 0.04$). However, no significant association between GDM and 25(OH)D deficiency [25(OH)D < 50 nmol/l] 25(OH)D significantly negatively associated with fasting glucose, fasting insulin and insulin resistance in unadjusted analysis. After adjustments, however, only significant relationship remaining was with fasting glucose [r = –0.11 (–0.26 to –0.01)]
30.1 (9.7)	25(OH)D concentration	Unadjusted OR (95% CI)	25(OH)D concentration	OR1 (95% CI)	OR2 (95% CI)	25(OH)D is early pregnancy is significantly associated with an elevated risk of GDM
	≥ 75	1 (Ref)	≥ 75	1 (Ref)	1 (Ref)	
	50–74	1.86 (0.86 to 4.01)	50–74	1.86 (0.84 to 4.09)	1.56 (0.69 to 3.52)	
	< 50	4.33 (1.78 to 10.5)	< 50	3.74 (1.47 to 9.50)	2.66 (1.01 to 7.02)	
	p-value for trend	0.001	*p*-value for trend	0.006	0.05	
	Per 12.5 nmol/l reduction	1.44 (1.16 to 1.69)	Per 12.5 nmol/l reduction	1.36 (1.11 to 1.69)	1.29 (1.05 to 1.60)	

TABLE 29 The association between maternal vitamin D status in gestation and risk of gestational diabetes mellitus: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/ adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in cases of GDM
Farrant, 2009[93]	5 (low)	Mysore Parthenon Study, India Cases of GDM $n = 34$ women Normal pregnancies $n = 525$ women	Prospective cohort	Maternal age, fat mass, diabetes mellitus status	30 weeks	38.8
Soheilykhah, 2010[138]	3 (medium)	Islamic Republic of Iran Cases of GDM $n = 54$ women Controls $n = 111$ women	Case–control	Nil Controls matched for gestational age, maternal age, maternal BMI	24–28 weeks	24.05 (20.65)[a]
Makgoba, 2011[140]	7 (low)	London, UK Overall cohort size = 1200 women Cases of GDM $n = 90$ women Controls $n = 158$ women	Nested case–control	Unclear how cases and controls were matched Cases had higher BMI, prior history of type 2 GDM and a family history of type 2 GDM, higher blood pressure. No difference in parity, smoking, method of conception Adjusted for BMI, gestation age at blood sampling, smoking, ethnicity, parity, maternal age, conception status, previous GDM, month of blood sampling	11–13 weeks (+6 days)	47.2 (26.7)

Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in unaffected controls	OR (95% CI) of GDM from univariate analysis		OR of GDM from multivariate analysis	Conclusion
37.8	Not given		Not given	No significant association between serum 25(OH)D at 30 weeks and GDM ($p = 0.8$ for difference in mean between GDM and normal) 25(OH)D positively related to fasting 32–33 split proinsulin concentration. Negative association between 30-minute glucose concentration following glucose tolerance test and 25(OH)D in those with 25(OH)D < 50 nmol/l
32.25 (35.8)[a]	$25(OH)D_3$ concentration	OR (95% CI) of GDM	No multivariate analysis performed	Significantly increased risk of GDM if $25(OH)D_3$ < 37.5 nmol
	< 50	2.02 (0.88 to 4.6)		
	< 37.5	2.66 (1.26 to 5.6)		
47.6 (26.7)	Not given		Not given	No significant association between serum 25(OH)D in first trimester and GDM $p = 0.863$ in univariate analysis and $p = 0.782$ in multivariate analysis 25(OH)D negatively associated with fasting glucose ($p = 0.0009$), 2-hour glucose following glucose tolerance test ($p = 0.002$) and HbA_{1c} ($p = 0.002$) at 28 weeks in univariate analysis. After adjustments, however, the only significant relationship remaining was with 2-hour glucose ($p = 0.048$)

TABLE 29 The association between maternal vitamin D status in gestation and risk of gestational diabetes mellitus: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/ adjustments	Number of weeks' gestation when 25(OH)D was measured	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in cases of GDM	
Baker, 2012[141]	7 (low)	North Carolina, USA Overall cohort *n* = 4225 women Cases of GDM *n* = 60 women Controls *n* = 120 women	Nested case–control	Controls matched by race/ethnicity Adjusted for maternal age, insurance status, BMI, gestational age at serum collection, season of blood test	11–14 weeks	Mean not given 25(OH)D concentration < 50 50–74.9 ≥ 75	*n* (%) 5 (8.3) 11 (18.3) 44 (73.3)
Fernandez-Alonso, 2012[118]	3 (medium)	Almeria, Spain Cohort *n* = 466 women Cases of GDM *n* = 36	Prospective cohort	Nil	11–14 weeks	Overall mean not given 25(OH)D concentration < 50 50–75 > 75	*n* 109 191 166

GDM, gestational diabetes mellitus; IQR, interquartile range; OR1, odds ratio 1; OR2, odds ratio 2; Ref, reference group.
a Measured 25(OH)D_3.

Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in unaffected controls		OR (95% CI) of GDM from univariate analysis	OR of GDM from multivariate analysis	Conclusion
Mean not given		1.25 (0.39 to 4.05) if 25(OH)D < 50 compared with those with 25(OH)D > 75	0.78 (0.22 to 2.78) if 25(OH)D < 50 compared with those with 25(OH)D > 75	No significant association between serum 25(OH)D in early pregnancy and GDM
25 (OH)D concentration	*n* (%)			
< 50	8 (6.7)			
50–74.9	24 (20)			
≥ 75	88 (73.3)			
Not given		Not given	Not given	No significant association between serum 25(OH)D in early pregnancy and GDM ($p = 0.84$ for difference in mean between GDM and normal)

TABLE 30 The association between maternal vitamin D status in gestation and risk of caesarean section: observational studies

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured
Ardawi, 1997[90]	5 (low)	Jeddah, Saudi Arabia Cohort size $n = 264$ women	Cohort	Nil	Delivery
Brunvand, 1998[144]	1 (medium)	Pakistan Cases $n = 37$ women Controls $n = 80$ women All nulliparous Pakistani women of low social class Cases all had emergency caesarean sections due to mechanical dystocia	Case–control	Cases had higher maternal age, lower maternal height, lower maternal weight, longer length of gestation and higher neonatal birthweight Maternal height and birthweight included in logistic regression model	Just before delivery[a]
Merewood, 2009[143]	6 (low)	Boston, MA, USA Cohort $n = 277$ women Cases $n = 67$ women All cases were women having primary caesarean sections	Cross-sectional	No significant difference in season of birth, maternal age, maternal BMI, maternal education, maternal insurance status, marital status, prenatal vitamin use and calcium supplementation, milk in pregnancy or sunscreen in pregnancy. Race/ethnicity, alcohol in pregnancy (yes/no), maternal educational status, maternal insurance status and maternal age included in multivariate analysis	Within 72 hours of delivery

Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in cases of caesarean section	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in vaginal deliveries	OR/relative risk of caesarean section from univariate analysis	OR of caesarean section from multivariate analysis	Conclusion
Not given Caesarean section incidence of 12.5% ($n = 3$) if 25(OH)D < 20 nmol/l Caesarean section rate of 9.59% ($n = 23$) if 25(OH)D > 20 nmol/l	Not given	Not given	Not given	25(OH)D < 20 nmol/l was associated with an increased rate of caesarean section but results not significant $p > 0.05$
26 (15–37)[a]	19 (11–27)[a]	Not given	1.03 (0.99 to 1.06)	No significant association seen between maternal $25(OH)D_3$ concentration and risk of emergency caesarean section due to obstructed labour
Unadjusted = 45.0 (36.5–62.0)	Unadjusted = 62.5 (57.4–68.2)	If 25(OH)D < 37.5 nmol/l, OR = 2.43 (1.20 to 4.92)	If 25(OH)D < 37.5 nmol/l, adjusted OR = 3.84 (1.71 to 8.62)	25(OH)D < 37.5 nmol/l is significantly associated with an increased risk of primary caesarean section

TABLE 30 The association between maternal vitamin D status in gestation and risk of caesarean section: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured
Scholl, 2012[142]	5 (low)	Camden Cohort Study, NJ, USA Cohort *n* = 1153 women Cases *n* = 290 women (173 primary caesarean sections)	Cohort	Age, parity, ethnicity, gestation at entry to study, season at entry to study used to calculate adjusted OR1. Adjusted OR2 used the same confounders with the addition of maternal BMI	At entry to study Mean (SD) 13.73 (5.6) weeks

Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in cases of caesarean section	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in vaginal deliveries	OR/relative risk of caesarean section from univariate analysis	OR of caesarean section from multivariate analysis			Conclusion
Not given	Overall mean not given	Not given	25(OH)D concentration	OR1 (95% CI)	OR2 (95% CI)	Serum 25(OH)D < 30 nmol/l was associated with a significantly increased risk of overall caesarean section in both regression models Regarding primary caesarean section, if BMI is not included in the model (OR1), serum 25(OH)D < 30 nmol/l was associated with a significantly increased risk of primary caesarean section When maternal BMI is included in the model (OR2) the trend remains but the relationship-value no longer remains significant $p = 0.054$ Risk of overall caesarean section and primary caesarean section due to prolonged labour was significantly higher if 25(OH)D < 30 nmol/l even after adjusting for maternal BMI [OR2 = 2.24 (95% CI 1.17 to 3.98) for primary caesarean section]
			< 30	1.70 (1.12 to 2.58)	1.66 (1.09 to 2.52)	
			30–49.9	0.89 (0.63 to 1.25)	0.83 (0.59 to 1.17)	
			50–125	Ref	Ref	
			> 125	0.59 (0.17 to 2.08)	0.90 (0.49 to 1.66)	

TABLE 30 The association between maternal vitamin D status in gestation and risk of caesarean section: observational studies (*continued*)

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured
Savvidou, 2012[145]	7 (low)	London, UK Cohort $n = 1000$ women Cases $n = 199$ women ($n = 111$ emergency)	Cohort	Maternal age, racial origin, smoking, method of conception, season of blood sampling	Between 11 and 13 weeks
Fernandez-Alonso, 2012[118]	3 (medium)	Almeria, Spain Cohort $n = 466$ women Cases $n = 105$ women ($n = 61$ emergency)	Cohort	Nil	Between 11 and 14 weeks[a]

OR1, odds ratio 1; OR2, odds ratio 2; Ref, reference group.
a Measured 25(OH)D_3.

Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in cases of caesarean section	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in vaginal deliveries	OR/relative risk of caesarean section from univariate analysis	OR of caesarean section from multivariate analysis		Conclusion
Elective = 58.40 (28.12–78.89) Emergency = 42.53 (22.91–72.1)	46.4 (28.25–69.01)	Not given	OR not given. Result presented as MoMs after adjustments		No significant association seen between maternal 25(OH)D concentration and risk of either elective or emergency caesarean section
			Indication	MoM (IQR)	
			Vaginal	0.99 (0.71–1.33)	
			Elective	0.96 (0.73–1.27)	
			Emergency (total)	0.99 (0.71–1.46)	
			Emergency due to failure to progress	0.95 (0.71–0.25)	
			Emergency due to fetal distress in labour	0.95 (0.71–1.27)	
Overall mean not given	Not given	Not given	Not given		No significant association between caesarean section rates as a function of first trimester 25(OH)D_3 status
25(OH)D concentration	*n*				
< 50	23				Overall caesarean section, $p = 0.65$
50–75	41				
> 75	41				Emergency caesarean section, $p = 0.47$
					Elective caesarean section, $p = 0.06$

TABLE 31 The association between maternal vitamin D status in gestation and risk of bacterial vaginosis: observational studies

First author, year	Bias score	Study details	Study type	Confounders/adjustments	Number of weeks' gestation when 25(OH)D was measured
Bodnar, 2009[146]	5 (low)	Pittsburgh, PA, USA Cohort $n = 469$ women (all non-Hispanic white or non-Hispanic black) Cases $n = 192$ (approximate)	Cohort	Presence of other sexually transmitted disease Other confounders: maternal age, parity, education, employment status, season, family income, pre-pregnant BMI, gestational age at enrolment, number of sexual partners and frequency of vaginal intercourse were not included as they did not satisfy the priori change-in-estimate criterion (> 10% change in PR)	Mean (SD) 9.5 (3.2) weeks
Hensel, 2011[147]	4 (medium)	NHANES, USA Cohort $n = 440$ women	Cohort	Maternal age, race/ethnicity, education, poverty index, marital status, age at first sex, number of lifetime partners, ever had a female sex partner, unprotected sex in the last 30 days, current oral contraceptive use, douching frequency, active smoking, BMI	Unclear
Dunlop, 2011[148]	2 (medium)	Sample of the Nashville Birth Cohort Study, USA Total cohort size $n = 1547$ women Sample size $n = 160$ women (all non-Hispanic white or non-Hispanic black) Cases $n = 14$	Cross-sectional	Race, age, smoking, BMI, gestational age at delivery, payer source	At delivery

IQR, interquartile range; PR, prevalence ratio; Ref, reference group.

Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in cases of bacterial vaginosis	Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in unaffected controls	OR of bacterial vaginosis from univariate analysis		OR of bacterial vaginosis from multivariate analysis		Conclusion
Unadjusted geometric mean = 29.5 (27.1–32.0)	Unadjusted geometric mean = 40.1 (37.0–43.5)	Not given		PR given 25(OH)D concentration (nmol/l) 20 (25th centile) 50 (75th centile) 75 (90th centile) 90 (97th centile)	 Adjusted PR (95% CI) 1.65 (1.01 to 2.69) 1.26 (1.10 to 1.57) Ref 1.32 (0.84 to 2.09)	A significant relationship observed between serum 25(OH)D and risk of bacterial vaginosis Prevalence of bacterial vaginosis declined as 25(OH)D increased until a plateau at 80 nmol/l was reached ($p < 0001$). At doses higher than this, no significant relationship was observed
Not given	Not given	Not given		Adjusted OR (95% CI) if vitamin D deficient (< 75 nmol/l) = 2.87 (1.13 to 7.28) $p = 0.03$		Serum 25(OH)D < 75 nmol/l is significantly associated with an increased risk of bacterial vaginosis
45.0 (20.35)	60.85 (29.93)	25(OH)D concentration (nmol/l) < 30 < 50	OR (95% CI) 7.58 (2.13 to 27.03) 1.4 (0.79 to 14.93)	25(OH)D concentration (nmol/l) < 30 < 50	Adjusted OR (95% CI) 5.11 (1.19 to 21.97) 1.2 (0.39 to 3.85)	A significant risk of bacterial vaginosis seen if 25(OH)D < 30 nmol/l No significant association seen if 25(OH)D < 50 nmol/l

Appendix 7 Forest plots

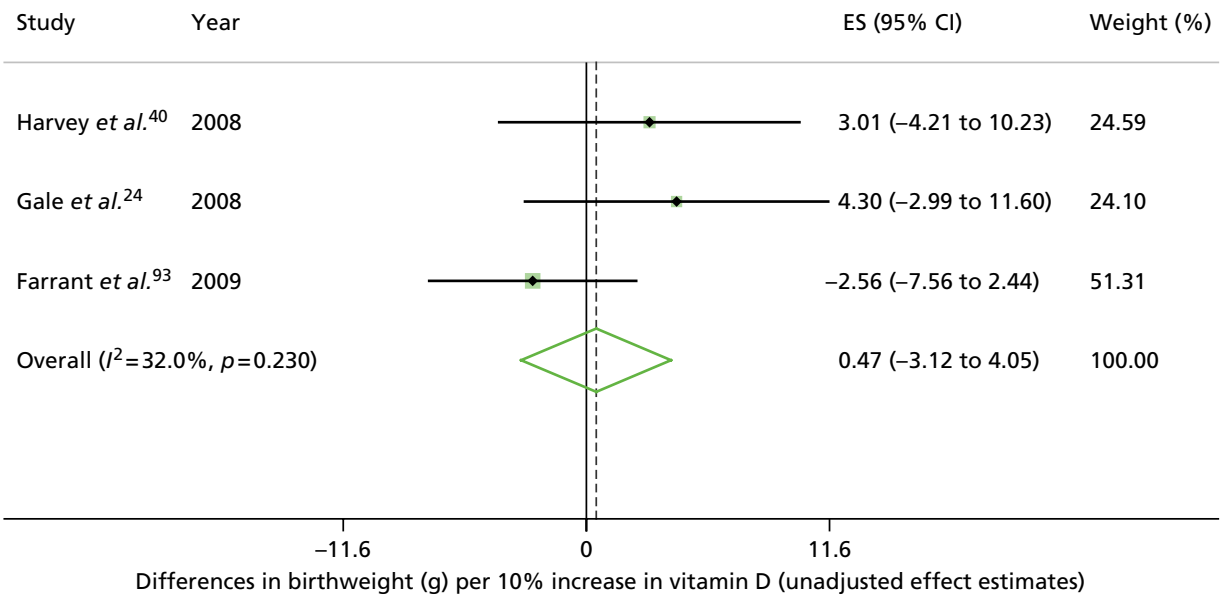

FIGURE 2 Forest plot of the association between maternal vitamin D status and offspring birthweight: observational studies using log-transformed 25(OH)D (unadjusted). ES, effect size.

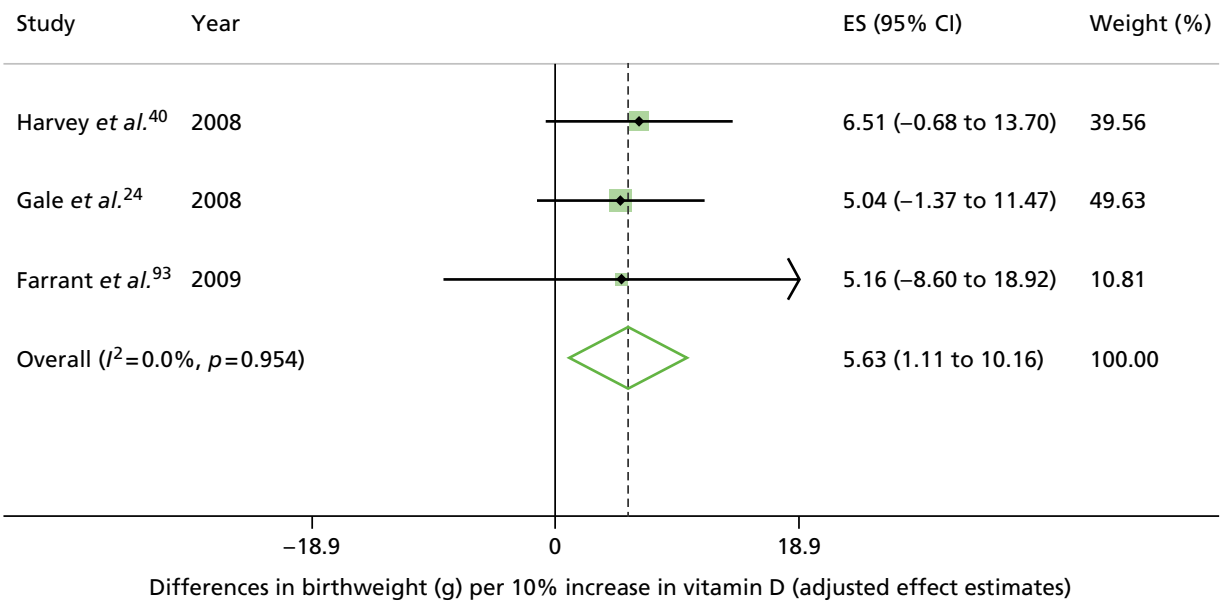

FIGURE 3 Forest plot of the association between maternal vitamin D status and offspring birthweight: observational studies using log-transformed 25(OH)D (adjusted). ES, effect size.

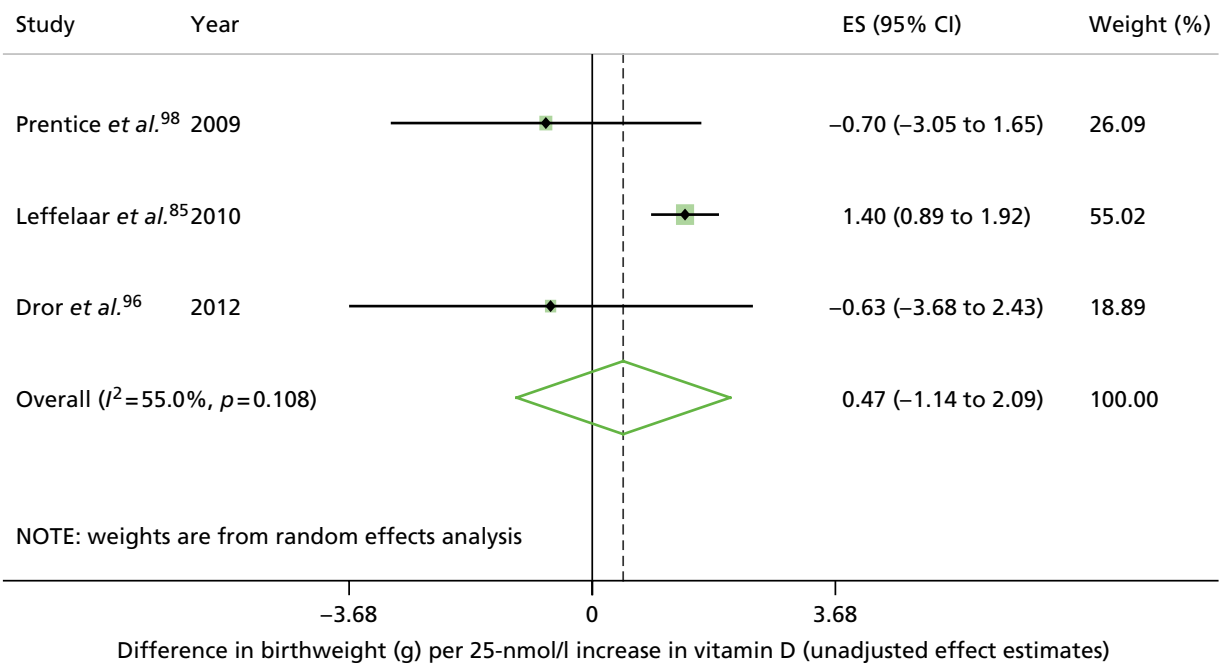

FIGURE 4 Forest plot of the association between maternal vitamin D status and offspring birthweight: observational studies (unadjusted). ES, effect size.

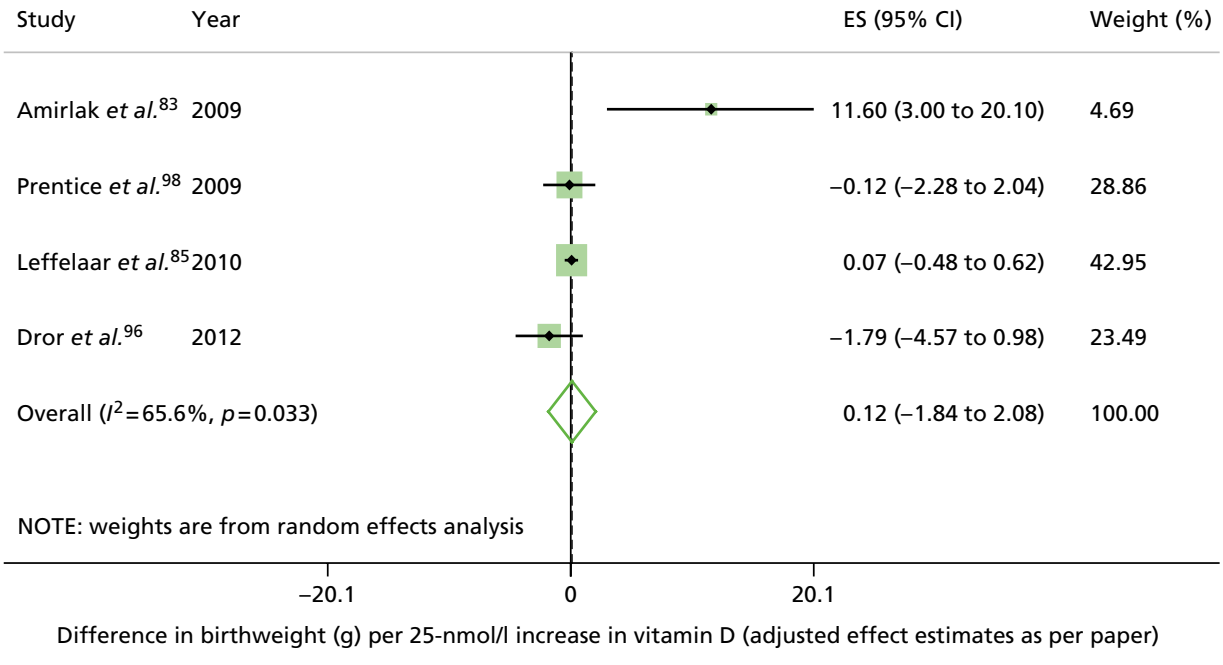

FIGURE 5 Forest plot of the association between maternal vitamin D status and offspring birthweight: observational studies (adjusted). ES, effect size.

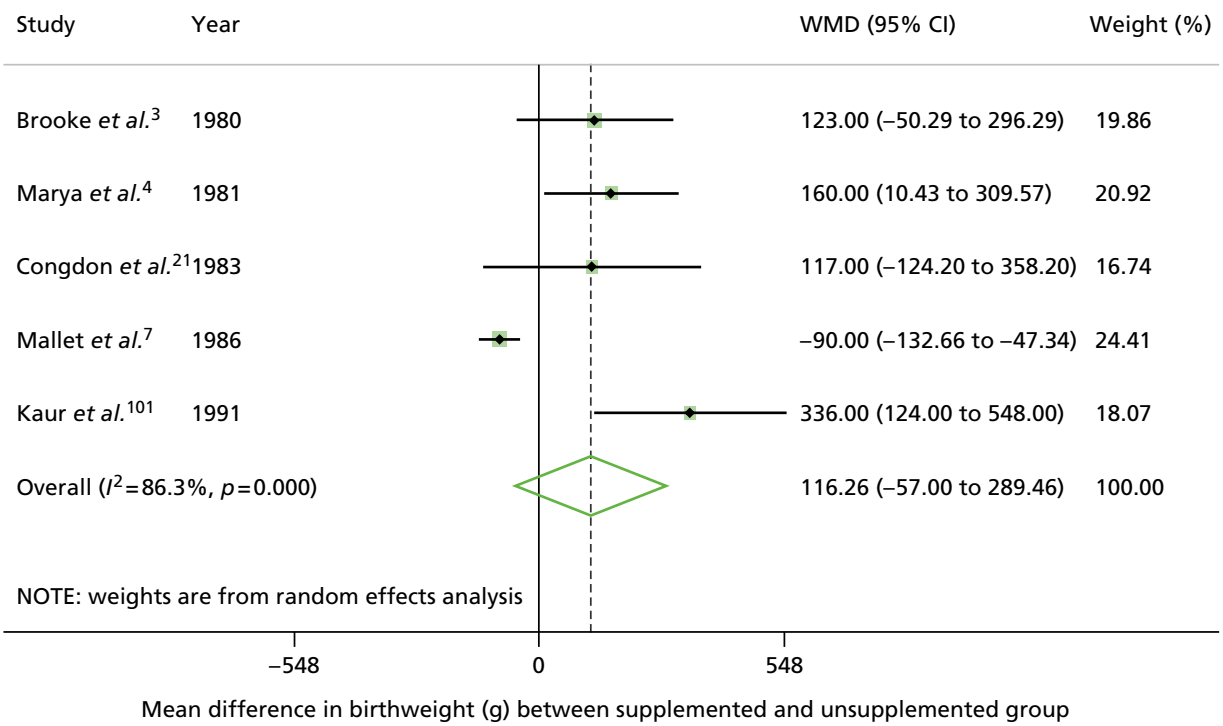

FIGURE 6 Forest plot of the effect of maternal vitamin D supplementation on offspring birthweight: intervention studies (low dose). WND, weighted mean difference.

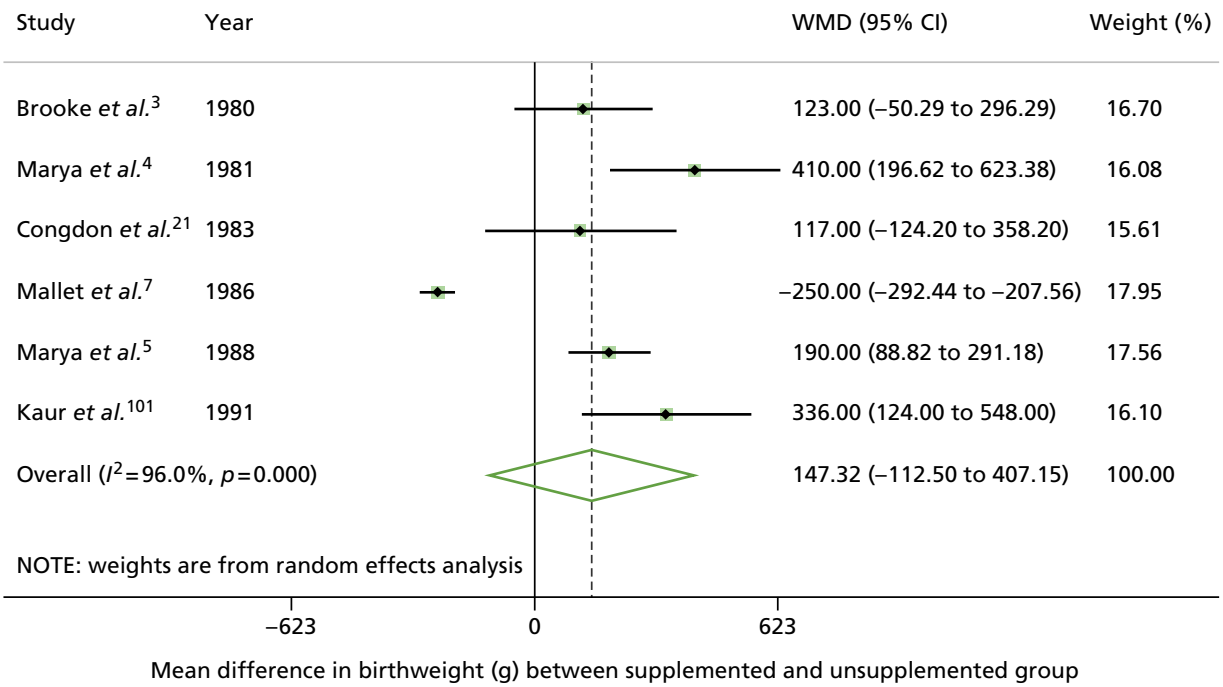

FIGURE 7 Forest plot of the effect of maternal vitamin D supplementation on offspring birthweight: intervention studies (high dose). WND, weighted mean difference.

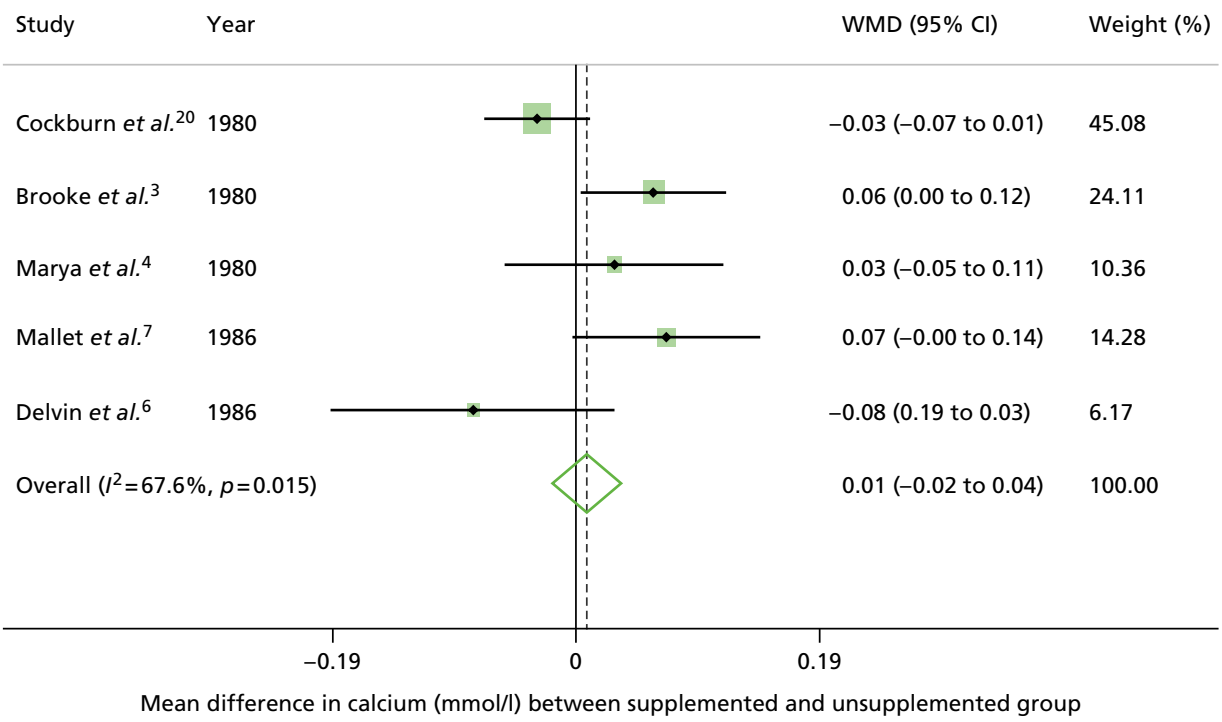

FIGURE 8 Forest plot of the effect of maternal vitamin D supplementation on offspring calcium concentration: intervention studies (low dose).

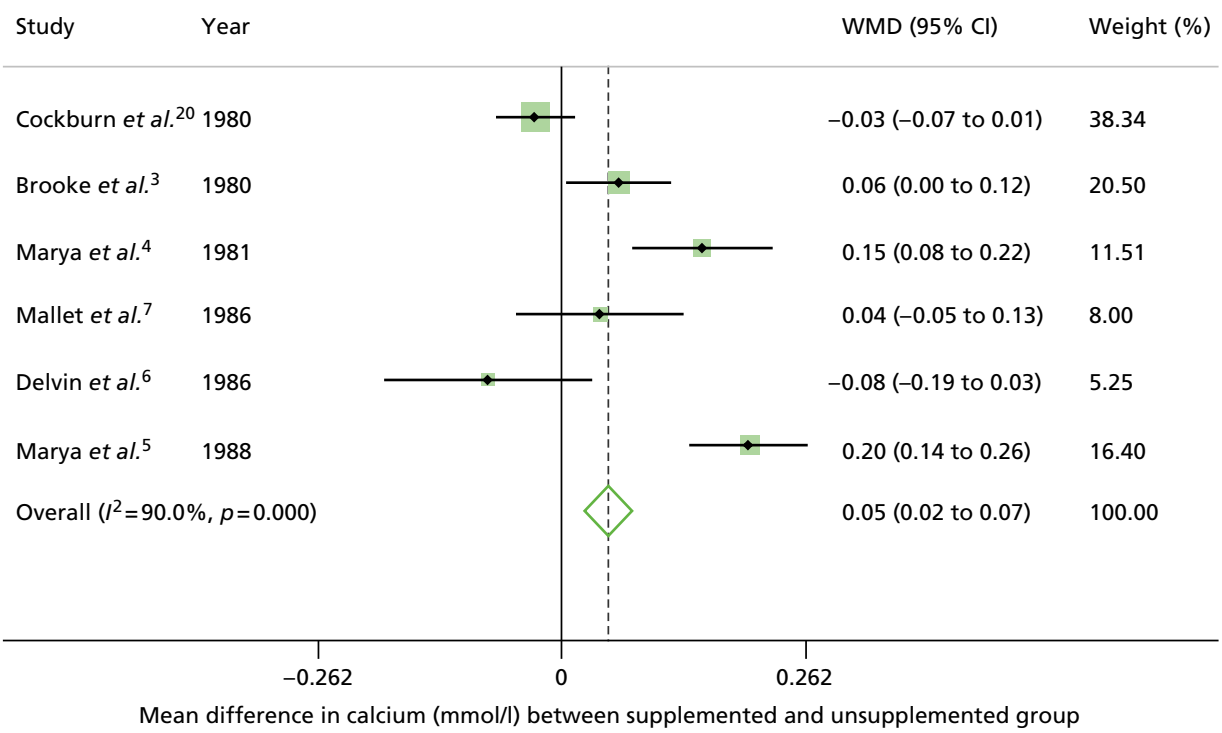

FIGURE 9 Forest plot of the effect of maternal vitamin D supplementation on offspring calcium concentration: intervention studies (high dose).

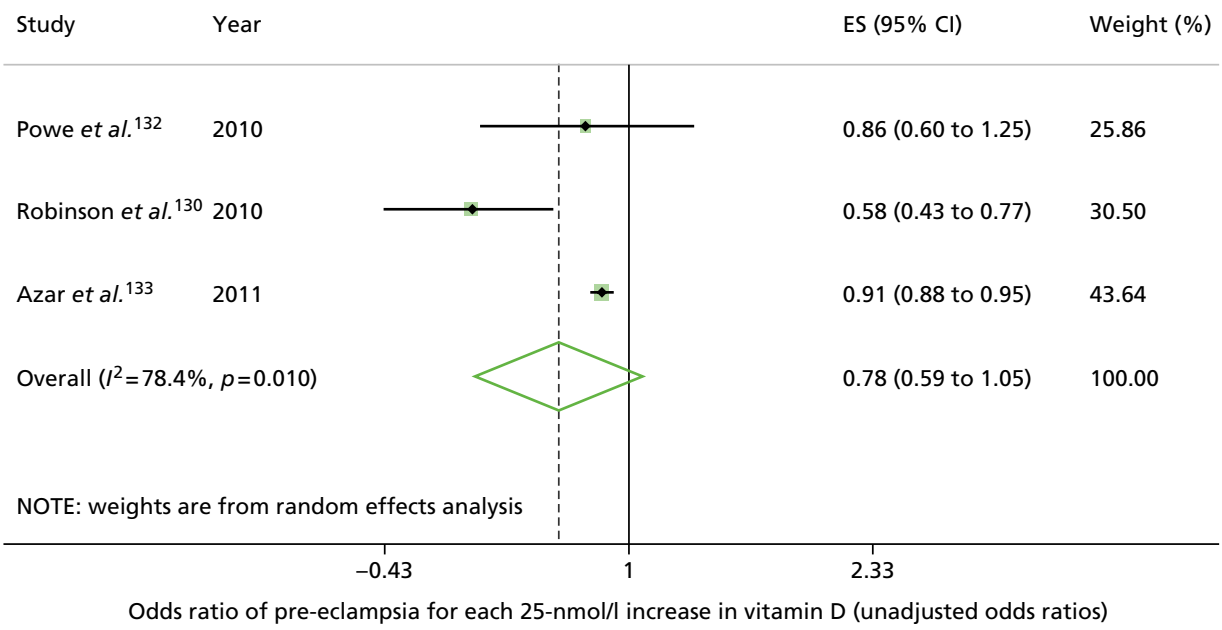

FIGURE 10 Forest plot of the association between maternal vitamin D status and risk of pre-eclampsia: observational studies (unadjusted). ES, effect size.

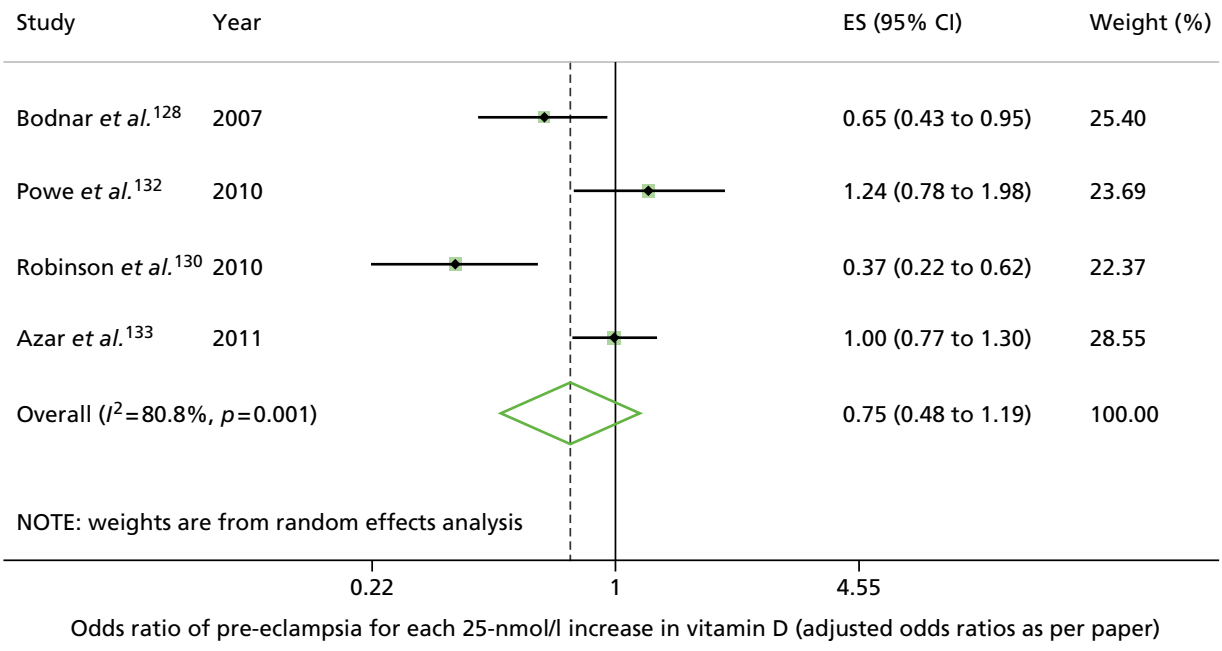

FIGURE 11 Forest plot of the association with maternal vitamin D status and risk of pre-eclampsia: observational studies (adjusted). ES, effect size.

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