Vitamin D supplementation in pregnancy: A systematic review

HTA Evidence Synthesis: 10/33/04 Diagnosis and treatment of vitamin D deficiency during pregnancy

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Declared competing interests: Authors have completed the unified competing interest form at www.icmje.org/coi\_disclosure.pdf (available on request from the corresponding author) and declare (1) no financial support for the submitted work from anyone other than their employer; (2) no financial relationships with commercial entities that might have an interest in the submitted work; (3) no spouses, partners, or children with relationships with commercial entities that might have an interest in the submitted work; and (4) no non-financial interests that may be relevant to the submitted work, other than NCH who has received speaker fees from Amgen, Servier, Shire and Eli Lilly, and acted as a consultant to Consilient; KMG, who has received speaker fees from, and acted as a consultant to, Abbott Nutrition, and has received reimbursement for education from Nestle Nutrition and travel expenses from ILSI Europe; NJB, who has received speaker fees from Danone; acted as a consultant for Alexion, GSK, Merck and Amgen and support for studies from Alexion; and CC who has acted as a consultant to Amgen, ABBH, Eli Lilly, Medtronic, Merck, Novartis and Servier.

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List of Abbreviations

Alb: Albumin

aBMC: Areal Bone Mineral Density

ABCVitamin D: Vitamin D Supplementation During Pregnancy for Prevention of Asthma in Childhood trial

ALP: Alkaline Phosphatase

ALSPAC: Avon Longitudinal Study of Parents and Children

AMED: Allied and Complementary Database

ATP: Adenosine Tri-Phosphate

BA: Bone Area

BMC: Bone Mineral Content

BMD: Bone Mineral Density

BMUS: British Medical Ultrasound Society

BRU: Biomedical Research Unit

BW: Birth weight

Ca: Calcium

COMA: Committee on Medical Aspects of Food and Nutrition Policy

CSA: Cross sectional Area

CD4: Cluster Differentiation 4

CDSR: Cochrane Database of Systematic Reviews

CRD: Centre for Reviews and Dissemination

DARE: Database of Abstracts of Reviews of Effects

DBP: Vitamin D Binding Protein

DEQAS: Vitamin D External Quality Assessment Scheme

DNA: Deoxyribonucleic Acid

DMC: Data Monitoring Committee

DXA: Dual-Energy X-ray Absorptiometry

FEV1: Forced Expiratory Volume in 1 Second

FVC: Forced Vital Capacity

GCP: Good Clinical Practice

GC-MS: Gas Chromatography-Mass Spectroscopy

GMP: Good Manufacturing Practice

GnRH: Gonadotrophin Releasing Hormone

HIV: Human Immunodeficiency Virus

HLA: Human Leucoctye Antigen

HMIC: Health Management Information Consortium

HMSO: Her Majesty’s Stationery Office

HPLC: High Performance Liquid Chromatography

HTA: Health Technology Assessment

ISRCTN: International Standard Randomised Controlled Trial Number

IMP: Investigational Medicinal Product

IOV: Inter-Operator Variation

IQ: Intelligence Quotient

ITT: Intention to Treat

LMP: Last Menstrual Period

MAVIDOS: Maternal Vitamin D Osteoporosis Study

MHRA: Medicines and Healthcare products Regulatory Agency

MRC: Medical Research Council

mRNA: messenger Ribonucleic Acid

NHS: National Health Service

NIHR: National Institute for Health Research

RCT: Randomised Controlled Trial

RIA: Radio-Immuno Assay

pQCT: Peripheral Quantitative Computed Tomography

PTH: Parathyroid Hormone

NICE: National Institute for Health and Clinical Excellence

SACN: Scientific Advisory Committee on Nutrition

SGA: Small for Gestational Age

SPA: Single Photon Absorptiometry

SWS: Southampton Women’s Survey

UK: United Kingdom

UKCRN: United Kingdom Clinical Research Network

UHS: University Hospital Southampton NHS Foundation Trust

USA: United States of America

UVB: Ultra-Violet B

VDARRT: Vitamin D Antenatal Asthma Reduction Trial

VDR: Vitamin D Receptor

WMD: Weighted Mean Difference

1. Abstract

Background

It is unclear whether the current evidence base allows definite conclusions to be made regarding the optimal maternal circulating concentration of 25(OH)-vitamin D during pregnancy, and how this might best be achieved. CRD42011001426.

Aim/ 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)-vitamin 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 (D2 or D3), 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 systematic review and where possible combined study results using meta-analysis to estimate the combined effect size.

Major electronic databases were searched 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.

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)-vitamin D concentration) or supplementation of participants with vitamin D or vitamin D containing food e.g. oily fish.

*Outcomes:* Offspring: Birth weight, birth length, head circumference, bone mass, anthropometry and body composition, risk of asthma and atopy, small for gestational dates, preterm birth, type 1 diabetes, low birth weight, serum calcium concentration, blood pressure and rickets. Mother: Preeclampsia, gestational diabetes, risk of caesarean section and bacterial vaginosis.

**Results**

76 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)-vitamin D and 1) offspring birth weight in meta-analysis of 3 observational studies using log-transformed 25(OH)-vitamin D concentrations after adjustment for potential confounding factors (pooled regression coefficient 5.63g/10% change maternal 25(OH)D, 95% CI 1.11,10.16), but not in those 4 studies using natural units, or across intervention studies; 2) offspring cord blood or postnatal calcium concentrations in a meta-analysis of 6 intervention studies (all found to be at high risk of bias; mean difference 0.05mmol/l, 95% CI 0.02, 0.05); 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)-vitamin D status and offspring birth weight, bone mass and serum calcium concentrations, these findings were limited by their observational nature (birth weight, bone mass) or risk of bias and low quality (calcium concentrations). High quality randomised trials are now required.

1. Executive Summary

Background

Low levels of serum 25(OH)-vitamin D have been observed in many populations, including pregnant women. Studies have demonstrated associations between low levels of serum 25(OH)-vitamin D during pregnancy and maternal/offspring health outcomes. However, many of these studies are observational in nature and it is unclear whether the current evidence base allows definite conclusions to be made regarding the optimal maternal circulating concentration of 25(OH)-vitamin 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)-vitamin 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)-vitamin 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 (D2 or D3), 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):* DARE (Database of Abstracts of Reviews of Effects) (Centre for Reviews and Dissemination (CRD)), CDSR (Cochrane Database of Systematic Reviews), HTA (Health Technology Assessment database (CRD)); *Completed studies (other study types):* CENTRAL (Cochrane Register of Controlled Trials), Medline, Embase, Biosis, Google scholar, AMED (Allied and Complementary Database; *Ongoing studies:* National Research Register archive, UKCRN (United Kingdom Clinical Research Network) Portfolio, Current Controlled Trials, Clinical Trials.gov; *Grey literature:* Conference Proceedings Citation Index- Science (1990-present), Zetoc conference search, Scientific Advisory Committee on Nutrition website, Department of Health website, King’s Fund Library database, Trip database, HTA website, HMIC (Health Management Information Consortium 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.

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)-vitamin D concentration) or supplementation of participants with vitamin D or vitamin D containing food e.g. oily fish.

*Outcomes:*

Primary: Maternal osteomalacia; Neonatal hypocalcaemia, rickets and reduced bone mass.

Secondary: Maternal quality of life; Neonatal body composition and bone mass, later offspring health outcomes (including asthma, diabetes, 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. 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 effect and measure of precision of effect estimate such as 95% confidence interval or standard error).

Assessment of validity and quality

Quality assessment of studies occurred initially during data extraction and secondly 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, while 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 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 procedures1. 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 I2 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, if significant heterogeneity was detected, or as fixed effects models if minimal heterogeneity was detected. All analysis was performed using Stata v11.0 (Statacorp, Texas, USA).

Results

*Included/ excluded studies:* 22,961 citations were identified from the initial database search up to 3rd January 2011. A subsequent additional search from 3rd January 2011 to 18th June 2012 identified another 2,448 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 8 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. 21 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; 8 papers were either review articles, letters, editorials or commentaries with no new results; 1 paper was of a non-human study and 4 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, either in terms of biochemical relationships, or disease outcomes.

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

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

These results relevant to these two study questions are itemised by individual health outcome below:

*Birth weight*

Nineteen observational studies were identified. Composite bias scores ranged from -2 to +8, with seven of the nineteen studies scored as having a low risk of bias. Six studies demonstrated a significant positive relationship between maternal vitamin D status and offspring birth weight; 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 5 and 10 respectively). Sample sizes ranged from 40 to 350 and interventions were highly variable. Three studies demonstrated significantly greater birth weight in offspring of supplemented mothers. The remainder showed no significant difference in infant birth weight regardless of supplementation (birth weight was non-significantly higher in the supplemented group in 2 of these, non-significantly lower in the supplemented group in one; birth weight was not presented in the remaining two.

Meta-analysis of 3 observational studies found weak positive associations between log-transformed maternal 25(OH)-vitamin D concentrations and offspring birth weight after adjustment for potential confounders (pooled regression coefficient 5.63g/10% change maternal 25(OH)D, 95% CI 1.11,10.16).

*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)-vitamin 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 one 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, offspring birth length of women supplemented with vitamin D was greater than for un-supplemented 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 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 five days of birth) between supplemented and un-supplemented 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)-vitamin D and offspring lung function at aged 6-7 years. All but one study was judged to be at moderate to high risk of bias, and no intervention studies were identified.

*Offspring born small for gestational age (SGA)*

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 SGA if maternal 25(OH)-vitamin D <30 nmol/l. A second study found a U-shaped relationship between SGA and maternal 25(OH)-vitamin D concentration in white women only, with the lowest risk between 60-80 nmol/l. No relationship was seen in black women. A third study of pregnant women with early onset preeclampsia found significantly lower serum 25(OH)D in those women with SGA infants compared to 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 and the other high risk of bias, and neither of which found a significant difference in SGA risk in women supplemented with vitamin D compared to un-supplemented mothers. There was however a non-significant trend towards higher SGA risk in the un-supplemented 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)-vitamin 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 in the offspring with lower maternal concentration of 25(OH)-vitamin D in late pregnancy. The remaining studies found no significant relationship. No intervention studies were identified.

*Offspring low birth weight (LBW)*

Three observational studies were identified, with composite bias scores ranged from -2 to 3 indicating a medium to high risk of bias. One study found a significantly reduced risk of LBW 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)-vitamin 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: 0.05mmol/l, 95% CI 0.02, 0.05). 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)-vitamin D concentration and offspring blood pressure. No intervention trials were identified.

*Preeclampsia*

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 preeclampsia, the remaining six studies found no significant relationship. Meta-analysis was possible for four studies, suggesting an inverse relationship between 25(OH)D and preeclampsia risk, but which did not achieve statistical significance. One intervention trial was identified; no difference in risk of preeclampsia was seen in mothers supplemented with vitamin D compared with un-supplemented women.

*Gestational diabetes*

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 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)-vitamin D was significantly associated with an increased risk of bacterial vaginosis in pregnancy. No intervention trials were identified.

*What is the optimal type (D2 or D3), 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 birth weight (meta-analysis of observational studies), neonatal calcium concentrations (meta-analysis of randomised controlled trials) 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 clearly further high-quality research is 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 randomised controlled trials. 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.

1. Background
   1. 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(OH)-vitamin D concentrations are common in this group. In one cohort in Southampton, composed of white Caucasians, 31% had concentrations of circulating 25(OH)-vitamin D lower than 50 nmol/l and 18% less than 25 nmol/l.2 A recent US study of a population representative of the national demographic distribution revealed that 80% of black pregnant women had levels less than 50 nmol/l; the figures for Hispanic and white pregnant women were 45% and 13% respectively3. In Asian cohorts in the northern hemisphere the burden is even higher.4-8 possibly reaching 90% or greater: 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)-vitamin D ≤37.5 nmol/l and 26% ≤12.5 nmol/l9; a survey of the UK (non-pregnant) population revealed low levels of 25(OH)-vitamin D in 50%10. As the main source of vitamin D is synthesis in the skin under the influence of UVB radiation from sun light exposure, ethnicity (dark skin), covering and northerly latitudes (as in UK) are all major risk factors for low concentrations.11 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)-vitamin D concentrations are accompanied by increased parathyroid hormone levels. Maternal vitamin D deficiency in pregnancy has been associated with neonatal hypocalcaemia12 and other adverse birth outcomes, such as craniotabes and widened growth plates, suggestive of rachitic (rickets-like) change.13 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)-vitamin D, implying a relatively early effect,14 findings confirmed in a further cohort.15 Infants of mothers with low vitamin D intake may have lower calcium levels at day four post-delivery.16 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 post-natally.17-20 However accurate population-wide epidemiological data are lacking, and the 25(OH)-vitamin D concentration, below which an individual is considered deficient, is the subject of much debate (see section 1.7).

* 1. Intervention studies

There have been several, mainly small, intervention studies examining this issue (Table 1). Thus in one study 506 women were supplemented at 12 weeks gestation to 400 IU/day vs. 633 placebo.21 Levels of 25(OH)-vitamin D were higher in maternal, umbilical cord, and infant serum (day 3 and 6) in the supplemented group. This was not a randomised trial, but supplemented women from one clinic vs. placebo in another clinic. Another study compared 59 Asian women, supplemented with 1000 IU in the last trimester of pregnancy4, with 67 controls. Calcium levels were higher in the supplemented mothers, and there was a lower incidence of symptomatic neonatal hypocalcaemia and growth retardation amongst babies of supplemented mothers. Again in an Asian population5, 25 mothers were randomised to 1200 IU vitamin D per day, 20 mothers to 600,000 IU twice (7th and 8th month), and 75 mothers to placebo. In this study there was no difference in calcium and alkaline phosphatase levels between mothers taking 1200 IU/day and those taking placebo. However, those taking 600,000 IU twice had higher maternal and cord calcium and lower alkaline phosphatase than placebo. In a second study6 the same group supplemented 100 Asian-Indian women with 600 000 IU twice (again at 7th and 8th months) vs. 100 controls and found again, higher maternal and cord calcium and lower alkaline phosphatase. There have been two studies in French populations: 15 women were randomised to receive 1000 IU per day from 3rd trimester vs. 15 controls.7 Day 4 neonatal calcium and 25(OH)-vitamin 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 7th month and 29 acted as controls8. Here neonatal calcium at day 2 and 6 was similar in all groups, but maternal serum 25(OH)-vitamin D was greater in both intervention groups than in the controls. In the one study, measuring bone mineral at birth22 there was no difference in radial BMC in offspring of 19 Asian mothers who had taken 1000 IU vitamin D per day compared with 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 in measuring the tiny amount of bone mineral in the baby’s distal radius.

Table 1: Trials of vitamin D supplements in pregnancy

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Trial** | **No.** | **Location** | **Intervention** | **Outcome** |  |
| Cockburn (1980) | 1139 | Scotland | 400 IU/day or | 25(OH)D maternal | ↑ |
|  |  |  | or placebo | Cord | ↑ |
|  |  |  |  | Infant | ↑ |
| Brooke (1980) | 126 | UK Asian | 1,000 IU/day | Ca maternal | ↑ |
|  |  |  | or placebo | Cord | → |
|  |  |  |  | Neonatal | ↑ |
|  |  |  |  | Maternal weight | ↑ |
| Marya (1981) | 120 | Asian | 600,000 IU (x2); | Ca maternal | ↑ |
|  |  | Indian | 1,200 IU/day | Cord | ↑ |
|  |  |  | or placebo | ALP maternal | ↓ |
|  |  |  |  | Cord | ↓ |
| Marya (1988) | 200 | Asian | 600,000 IU (x2); | Ca/P maternal | ↑ |
|  |  | Indian | or placebo | Cord | ↑ |
|  |  |  |  | ALP maternal | ↓ |
|  |  |  |  | Cord | ↓ |
| Delvin (1986) | 34 | France | 1,000 IU/day; | 25(OH)D cord | ↑ |
|  |  |  | or no vit D | Neonatal | ↑ |
| Mallet (1986) | 68 | France | 200,000 IU (x1); | 25(OH)D maternal | ↑ |
|  |  |  | 1,000 IU/day; | with both regimes |  |
|  |  |  | or no vit D |  |  |

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

* 1. Safety of vitamin D supplementation in pregnancy

None of these studies listed above has 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 rat studies using the equivalent of 15,000,000 IU per day have resulted in extra-skeletal calcifications, there is no evidence that doses below 800,000 IU per day have any adverse effect. Two studies23;24 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 greater than 30 ng/ml.25 However, in this study the numbers were small with only 6 cases of atopy (asthma, eczema) by 9 years in the top quartile of maternal vitamin D, 4 each in the middle quartiles and 2 in the bottom. These numbers, even in the highest quartile, were actually lower than the figure for the general population. Additionally, in the Southampton Women’s Survey, there was no association between maternal 25(OH)-vitamin D status and atopic or non-atopic eczema at 9 months of age26. 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.

* 1. Maternal vitamin D status, offspring wheezing and diabetes

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.27 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.28 Hypponen found, in an adult population cohort, that circulating IgE levels (a marker of atopic tendency) were positively related to concentrations of 25(OH)-vitamin D but that this was only apparent at very high concentrations (>125nmol/l).29 Animal studies have implicated 1,25(OH)-vitamin D as a modulator of immune balance between a tendency to autoimmunity and atopy, but these studies have again suggested influences in both directions.30 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), or with 25 or 1,25(OH)-vitamin 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 in children who had been supplemented with vitamin D as infants.31

* 1. 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)-vitamin D insufficiency may lead to sub-optimal bone size and density in the offspring post-natally, 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: (a) those evaluating bone mineral and fracture risk in cohorts of adults for whom birth and/or childhood records are available; and (b) those studies relating the nutrition, body build and lifestyle of pregnant women to the bone mass of their offspring.32 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 mass33-36 and a reduced risk of fracture37 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, intra-pair differences in birth weight were associated with bone mineral content in middle age, even among monozygous pairs.38 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.39

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 study40 showing that premature babies who were supplemented with vitamin D had an increased whole body bone mass at age 12 years, 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)-vitamin D was measured by radio-immunoassay in late pregnancy2. Whole body, hip and lumbar spine bone area, BMC and BMD were measured in the healthy, term offspring at age 9 years. 31% of the mothers had reduced (insufficient or deficient) circulating concentrations of 25(OH)-vitamin D in late pregnancy. There was a positive association between maternal 25(OH)-vitamin D concentration in late pregnancy and whole body bone mineral content (r=0.21, p=0.0088) and 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 ultraviolet B (UVB) radiation during late pregnancy and use of vitamin D supplements predicted maternal 25(OH)-vitamin 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)-vitamin D concentration with lower offspring bone mass, have come from the Southampton Women’s Survey (SWS)41. In this ongoing prospective cohort study of women aged 20-34 years, characterised before and during pregnancy, maternal 25(OH)-vitamin D status was measured by radio-immunoassay in late pregnancy and 556 healthy term neonates underwent whole body dual energy X-ray absorptiometry (DXA) within 20 days of birth. Offspring of mothers who were insufficient or deficient (<40 nmol/l) in vitamin D in late pregnancy had lower bone mass than those of mothers who were replete. Thus the mean whole body bone area of the female offspring of deficient mothers was 112 cm2 *vs.* 120 cm2 in offspring of replete mothers (p=0.045). The mean whole body bone mineral content of offspring of deficient *vs.* replete mothers was 59g *vs.* 64g (p=0.046) respectively. There were weaker associations in the boys and there was no association with maternal alkaline phosphatase. Additionally, maternal UVB exposure during pregnancy was positively associated with whole body bone mineral content in the offspring aged 9 years in the Avon Longitudinal Study of Parents and Children (ALSPAC).42

* 1. 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)-vitamin 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.

* 1. Considerations for appraisal of data

There are several factors which make any study of evidence surrounding vitamin D problematic. Firstly, 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. Secondly, 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. Thirdly the definition of a normal range is difficult, even in non-pregnant populations, and techniques used to measure 25(OH)-vitamin D concentrations have widely different characteristics. Fourthly, dose-response and differences between use of vitamin D2 and vitamin D3 are unclear. Fifthly post-natal vitamin D intake by the offspring may confound any pregnancy relationships, and 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 growth43. Around 95% is acquired via photosynthesis in the skin, with the minority from the diet44. There are two dietary forms: D2, from plants, and D3, from animals; the latter mainly found in oily fish and fortified margarines and breakfast cereals44. Vitamin D is synthesised from the action of sunlight (wavelengths 290-315nm) on cutaneous 7-dehydrocholesterol, converting it to pre-vitamin D3.11;43 Once formed, pre-vitamin D3 undergoes membrane-enhanced temperature-dependent isomerisation to vitamin D343, which is translocated into the circulation where it binds to vitamin D-binding protein (DBP).11 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 D44. At latitudes of 48.5° (Paris, France), the skin is unable to form vitamin D between the months of October through to March.43 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 winter11. 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 levels11. Circulating vitamin D is converted in the liver to 25(OH)-vitamin 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)-vitamin D in the circulation11;45, bound to DBP. Excess 25(OH)-vitamin D is converted to 24,25(OH)-vitamin D which is thought be relatively metabolically inactive11. The 25(OH)-vitamin 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 compound45. Although the kidney is the primary site for conversion of circulating 25(OH)-vitamin D, many tissues, such as macrophages, osteoblasts, keratinocytes, prostate, colon and breast express the 1-α-hydroxylase enzyme43;46;47. Since anephric patients have very low levels of 1,25(OH)2-vitamin D in the blood, it seems likely that these extra-renal sites function at the paracrine level, and do not play a major role in calcium homeostasis44.

Food sources, recommended intakes and dose response

Few foods contain significant amounts of vitamin D. The most effective sources are oily fish (for example 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. (100g).11 There is much controversy over the recommended daily intake of vitamin D. Older guidance has suggested 200 IU per day for children and adults up to 50 years old and 400–600 IU for older adults.48 However, humans have evolved to synthesise much higher levels of vitamin D in the skin: 30 minutes exposure at midday 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 persons49. 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)-vitamin D over winter months, let alone replenish stores in somebody who is frankly vitamin D deficient.50 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.51

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 vitamin D binding protein.52 This rise is associated with an increase in intestinal calcium absorption (to around 80% intake), and an absorptive hypercalciuria.52 There does not seem to be a rise in maternal parathyroid hormone or 25(OH)-vitamin D during pregnancy, suggesting that the rise in 1,25(OH)2-vitamin D may be due to another factor, such as parathyroid hormone-related peptide, which may be secreted by the placenta.53 Studies of maternal bone mass in pregnancy have been conflicting, but most suggest a probable decrease, with a possibly greater decrease in lactation.54-58 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.59 The role of vitamin D in utero is uncertain, although 25(OH)-vitamin D does cross the placenta.60 In a mouse model, lack of VDR did not significantly affect placental calcium transport or skeletal mineralisation59; conversely in the rat, 1,25(OH)2-vitamin D did seem to influence placental calcium flux.61 Additionally chondrocytes are an extrarenal source of 1a-hydroxylase activity (and so conversion of 25(OH)-vitamin D to 1,25(OH)2-vitamin D.62 This observation therefore suggests a possible mechanism by which maternal 25(OH)-vitamin D status might influence bone size in the fetus. Further evidence to support this notion comes from mouse models in which the gene for 1α-hydroxylase (Cyp27b1) was either knocked out or over-expressed in chondrocytes leading to altered growth plate morphology.63 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)-vitamin D concentrations positively predicted offspring bone mass at birth64, and at 9 years old2, with umbilical cord calcium concentrations and placental calcium transporters65 implicated in the mechanisms.

Normal range and measurement of vitamin D

Circulating 25(OH)-vitamin 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 meaningful44. The concept of what is the normal range for 25(OH)-vitamin 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 to 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 US has recently reiterated the 50 nmol/l threshold as the desirable level of circulating 25(OH)-vitamin D66. The distinction between replete and insufficient/ deficient has been made on the basis of whether there is a secondary rise in parathyroid hormone. 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)-vitamin 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 5967. Where a threshold was detected, this varied between 25 and 125 nmol/l. Studies of fractional calcium absorption are similarly heterogeneous68. Furthermore, in an autopsy-based study of 675 cadavers69, although bone mineralisation defects (osteomalacia) were not observed in any individual with 25(OH)-vitamin D > 75 nmol/l, in those with levels below 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)-vitamin D concentration alone to disease is difficult at the level of the individual.

There are several different methods available to measure 25(OH)-vitamin D. The gold standard is seen to be gas chromatography-mass spectrometry (GC-MS), but this technique is slow, expensive and time-consuming. Most labs use commercial kit assays, which are usually radio-immunometric assays (RIA; for example, IDS, Diasorin, Nicholls), although a chemi-luminescence assay also exists (Diasorin Liaison). The assays tend to be less accurate than GC-MS and high-performance liquid chromatography (HPLC), and also discriminate less well between the D2 and D3 forms.70 Comparison of the Diasorin RIA kits with HPLC showed good correlation for D3, but D2 tended to be slightly underestimated71. A national system now exists to standardise measurement of 25(OH)-vitamin across laboratories in the UK (Vitamin D External Quality Assessment Scheme <http://www.deqas.org/>), and the US National Institutes of Health are leading a global programme aimed at standardisation of 25(OH)-vitamin D assays across both platform and laboratory (<http://ods.od.nih.gov/Research/VitaminD.aspx#vdsp>).

Infant post-natal vitamin D intake

Infant feeding, supplementation and sunlight exposure are strong determinants of post-natal infant 25(OH)-vitamin D levels and bone health.72 Concentrations of 25(OH)-vitamin 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 post-natal vitamin D supplementation of either the mother (whilst breast feeding) 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 There are very few studies which 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)-vitamin D level below which osteomalacic changes on bone biopsy are always seen.74

1. Existing evidence synthesis

Two previous systematic reviews have been performed in this area. The most recent (Mahomed and Gulmezoglu75) from the Cochrane group, asked the question “What are the effects of vitamin D supplementation on pregnancy outcome?”, and although published in 2009, the actual searches and conclusions were established in 1999. The authors searched for intervention studies registered on the Cochrane Pregnancy and Childbirth Group trials register (October 2001) and the Cochrane Controlled Trials Register (Issue 3, 2001). Thus more recent work and observational data, plus unpublished evidence were not included. We believe that a further Cochrane review is underway. Two trials of vitamin D supplementation in pregnancy (Mallet et al, 19868 and Brooke et al, 19804; see table 1) were assessed worthy of inclusion but the authors concluded that there was insufficient evidence on which to base any recommendations. NICE (National Institute for Health and Clinical Excellence) produced guidelines for antenatal care in 2008 (CG62 <http://www.nice.org.uk/nicemedia/live/11947/40115/40115.pdf>). 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:(<http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/@ps/@sta/@perf/documents/digitalasset/dh_107667.pdf>). Most recently, Aghajafari et al76 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, pre-eclampsia and bacterial vaginosis, small for gestational age infants and lower birth weight infants, but not delivery by caesarean section.

1. 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)-vitamin 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 (D2 or D3), dose, regimen and route for vitamin D supplementation in pregnancy?

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

1. Review methods
   1. 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 (<http://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;

[http://www.crd.york.ac.uk/PROSPERO/display\_record.asp?ID=CRD42011001426](http://webapp.doctors.org.uk/Session/3105326-3Ula8V8Mvq9nLrJ5uwyO-aokxazj/Message.wssp?Mailbox=INBOX&MSG=18995) .

* 1. 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)-vitamin D concentration) or supplementation of participants with vitamin D or vitamin D containing food e.g. oily fish.

Outcomes:

Primary: Neonatal hypocalcaemia, rickets in the offspring and offspring bone mass; maternal osteomalacia;

Secondary: Offspring body composition (including offspring birth weight, birth length, head circumference, anthropometry, risk of being born small for gestational age, risk of low birth weight); offspring preterm birth and later offspring health outcomes (including asthma and atopy, blood pressure and Type 1 diabetes); maternal quality of life (including pre-eclampsia, gestational diabetes, 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 [www.cebm.net/index.aspx?o=1025](http://www.cebm.net/index.aspx?o=1025)) are shown below:

Level 1a Systematic review (with homogeneity) of randomised controlled trials;

Level 1b Individual randomised controlled trial (with narrow confidence interval);

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.

* 1. Exclusion criteria

Studies were excluded if they were not written in English, 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 assessed. Systematic reviews were not included in the narrative, but used as a source of references through hand-searching.

* 1. 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): DARE (Database of Abstracts of Reviews of Effects) (Centre for Reviews and Dissemination (CRD)), CDSR (Cochrane Database of Systematic Reviews), HTA (Health Technology Assessment database (CRD)); Completed studies (other study types): CENTRAL (Cochrane Register of Controlled Trials), Medline, Embase, Biosis, Google scholar, AMED (Allied and Complimentary Database; Ongoing studies: National Research Register archive, UKCRN (UK Clinical Research Network) Portfolio, Current Controlled Trials, Clinical Trials.gov; Grey literature: Conference Proceedings Citation Index- Science (1990-present), Zetoc conference search, Scientific Advisory Committee on Nutrition website, Department of Health website, King’s Fund Library database, Trip database, HTA website, HMIC (Health Management Information Consortium 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 randomised control trials (RCT) 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 3rd January 2011. A subsequent additional search from 3rd January 2011 to 18th 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 (NH).

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% confidence interval 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), current medication, maternal body mass index, infant feeding, infant supplementation and maternal post-natal supplementation if breast feeding. 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 occurred initially during data extraction and secondly 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, while 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 for a medium risk and -1 for 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)-vitamin D, blood levels of 1,25(OH)-vitamin 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)-vitamin D blood level assessed?

What assay is used?

Are D2 and D3 forms adequately measured and are quality data (e.g. DEQAS) given?

What definition of “normal range” for 25(OH)-vitamin D is used?

Is the concentration treated as categorical (e.g. deficient, insufficient, replete) or continuous?

Has infant post-natal 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 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 procedures1. 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 randomised controlled trials in the same analysis. Two main approaches were employed: Firstly 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 secondly 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, since no study can be large enough in order to completely remove the random error. However, the reported effects may also vary due not only to chance but due to methodological differences between studies. This variation between studies defines statistical heterogeneity. Statistical analysis was performed using STATA version 12.1. Between-study statistical heterogeneity was assessed by Q-statistic and quantified by I2 test77;78; values of I2 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 birth weight and serum calcium between supplemented and un-supplemented groups in randomised control trials were analysed using weighted mean difference (WMD) and 95% confidence intervals (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 (FEM) analysis using the Mantel-Haenszel method was presented; otherwise, results of the random-effects model (REM) analysis using the DerSimonian-Laird method were presented.79

1. Studies included in the review

22,961 citations were identified from the initial database search up to 3rd January 2011. A subsequent additional database search from 3rd January 2011 to 18th June 2012 identified another 2,448 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 8 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.

1. Studies excluded from the review

A total of 89 papers retrieved for assessment were excluded. Around a third of these (n=34) were abstracts. 21 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; 8 papers were either review articles, letters, editorials or commentaries with no new results; 1 paper was of a non-human study and 4 papers reported on an outcome not assessed in any other paper (maternal breast cancer, offspring schizophrenia, offspring multiple sclerosis and offspring influenza A).

1. 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.

1. Results of the review

The majority of the results relate to study questions two and three (what adverse maternal and neonatal health outcomes are associated with low maternal circulating 25(OH)-vitamin 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 (Appendix 6, Tables 8-31). Very few studies were identified which could directly inform the other questions. These are discussed in section 11.

* 1. Offspring birth weight

Observational studies (Appendix 6, Table 8)

Nineteen observational studies linking maternal vitamin D status to offspring birth weight 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)-vitamin D concentration in fourteen 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 seven of the nineteen studies scored as having a low risk of bias. Of the fourteen studies relating maternal serum 25(OH)-vitamin D concentration to offspring birth weight, only three studies demonstrated a significant positive association; one study found a significant negative association. In contrast, three of the four studies assessing the influence of maternal vitamin D intake during pregnancy on offspring birth weight found a significant positive association. One study found no significant association between ambient UVB exposure in pregnancy and offspring birth weight.

Armirlak80 (composite bias score 2, medium risk) found a positive association between maternal 25(OH)-vitamin D at delivery and offspring birth weight in a cross-sectional study of 84 healthy Arab and South Asian women with uncomplicated deliveries. Maternal 25(OH)-vitamin D was generally low with a mean of 18.5 nmol/l. A large Australian study (Bowyer81, composite bias score 4, medium risk) of 971 pregnant women found that offspring birth weight was significantly lower in those women with 25(OH)-vitamin D deficiency (<25 nmol/l) even after adjusting for gestational age, maternal age and overseas maternal birth place. Similarly, in the Amsterdam Born Children and their Development (ABCD) study incorporating 3,730 pregnant women, Leffelaar82 (composite bias score 4, medium risk) found that early pregnancy maternal 25(OH)-vitamin D less than 30 nmol/l was significantly associated with a lower offspring birth weight, even after adjusting for multiple confounding factors. However, when serum 25(OH)-vitamin D was analysed as a continuous variable a significant association with birth weight was no longer seen. Mannion83 (Canada, composite bias score 1, medium risk), Scholl84 (USA, composite bias score 2, medium risk) and Watson85 (New Zealand, composite bias score 3, medium risk) attempted to assess maternal vitamin D intake during pregnancy via food frequency questionnaires at various stages of gestation. Mannion and Scholl found that maternal vitamin D intake was positively associated with offspring birth weight. Similar findings were made by Watson 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 birth weight and maternal 25(OH)-vitamin D. Weiler86 (composite bias score 3, medium risk) found that offspring birth weight was significantly lower in women with adequate vitamin D status (defined by the study group as 25(OH)-vitamin D ≥37.5 nmol/l). However, the number of participants in this study was low overall and only 18 women had 25(OH)-vitamin D <37.5 nmol/l. In addition, of those women with serum 25(OH)-vitamin D concentration <37.5 nmol/l, a significantly higher percentage were of non-white race (67%) compared to those with an adequate concentration of 25(OH)-vitamin D (25%).

Twelve observational studies reported no significant association between maternal vitamin D status and offspring birth weight. Four of these studies were from Asia (Ardawi87, Sabour88, Magbooli89, Farrant90), three from the UK (Gale25, Harvey64, Sayers42), two from Australia (Morley91, Clifton-Bligh92, one from the US (Dror93), one from Finland (Viljakainen94) and one from Africa (Prentice95). Ten had measured maternal 25(OH)-vitamin D during pregnancy or at delivery, one had assessed vitamin D intake during pregnancy and the largest study 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 2008, Gale 2008 and Farrant 2009, using log-transformed units. The combined estimate of the unadjusted regression coefficients for changes in birth weight (grams) per 10% increase in vitamin D was positive but did not reach statistical significance (pooled regression coefficient 0.47, 95% CI -3.12,4.05; Appendix 7, Figure2)). 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 birth weight (grams) for 10% increase in vitamin D (pooled regression coefficient 5.63, 95% CI 1.11,10.16; Appendix 7, Figure 3). Amirlak, Prentice, Leffelaar and Dror analysed vitamin D in its original units. All four studies provided adjusted estimates, whereas all but Amirlak also provided unadjusted regression coefficients. No significant differences in birth weight (grams) per 25 nmol/l increase in vitamin D were found in either combined unadjusted associations (pooled regression coefficient 0.47, 95% CI -1.14,2.09; Appendix 7, Figure 4) or combined adjusted (as per paper) associations (pooled regression coefficient 0.12, 95% CI -1.84, 2.08; Appendix 7, Figure 5).

Intervention studies (Appendix 6, Table 9)

Nine intervention trials were identified, only two of which was within the last 20 years; the earliest from 1980. 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 Yu96 and Hollis97 were assessed as having a low risk of bias (composite bias score 5 and 10 respectively). Eight studies reported randomisation, although only one study (Brooke4) was of a double-blind design and this was also the only study that was placebo-controlled. In seven of the studies intervention took place in the last trimester of pregnancy; one study intervened in months 6 and 7 of pregnancy and one study 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 375mg calcium daily. Change in maternal serum 25(OH)-vitamin D concentration before and after supplementation was given in three studies only. Three of the eight studies (all from India) demonstrated a statistically significantly greater birth weight in offspring of supplemented than un-supplemented mothers. The remainder showed no difference in infant birth weight regardless of supplementation.

Two Indian studies, both by Marya et al5;6 (composite bias scores -6 and -2 respectively, high risk) demonstrated significantly higher birth weights in infants born to women supplemented with high dose cholecalciferol (given as two doses of 600,000 IU in months 7 and 8 gestation). The earlier of these studies also had a third arm of women supplemented with 1200 IU vitamin D plus 375mg calcium throughout the third trimester of pregnancy. Birth weights of infants in this group were also significantly higher than in the un-supplemented 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 birth weight was also from India (Kaur98, composite bias score -7, high risk). Again significantly higher infant birth weight was found in the supplemented group (2 doses of 60,000 IU cholecalciferol in months 6 and 7) compared to the un-supplemented 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)-vitamin D at any point during pregnancy, and were assessed to have a high risk of bias.

Three UK studies had investigated the effect of maternal vitamin D supplementation in the third trimester of pregnancy on offspring birth weight. Brooke4 (composite bias score -2, high risk) and Congdon22 (composite bias score -9, high risk) recruited only Asian women residing in the UK, whereas Yu96 (composite bias score 5, low risk) included equal numbers of four ethnic groups (Caucasian, Black, Asian, Middle Eastern). None of the studies reported a significant difference in offspring birth weight between the supplemented and un-supplemented groups, even despite Brooke demonstrating significantly higher maternal 25(OH)-vitamin D concentrations in the supplemented group at term. Two studies, both from France (Delvin7, composite bias score -2, high risk; Mallet8, composite bias score -3, high risk) also failed to demonstrate a significant difference in offspring birth weight with maternal vitamin D supplementation. The most recent, and largest study (Hollis97, composite bias score 10, low bias risk) randomised 350 pregnant women residing in the US to either 400 IU/day, 2000 IU/day or 4000 IU/day of oral vitamin D3from 12-16 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 between the three groups.

Evidence synthesis

Two meta-analyses were performed to combine the published evidence of an effect of vitamin D supplementation on birth weight. The first included Brooke 1980, Marya 1981 (low dose of vitamin D), Congdon 1983, Mallet 1986 (low dose of vitamin D) and Kaur 1991 (Appendix 7, Figure 6). Due to statistically significant heterogeneity in the results (I2 86.3%, p<0.001), a random-effects model was fitted. The combined estimate showed a non-significant difference in birth weight between the un-supplemented and supplemented group (mean weighted difference: 116.23g, 95% CI -57.0, 289.5). The second meta-analytical model included Brooke 1980, Marya 1981 (high dose of vitamin D), Congdon 1983, Mallet 1986 (high dose of vitamin D), Marya 1988 and Kaur 1991 (Appendix 7, Figure 7). Again, here, due to statistically significant heterogeneity (I2 96%, p<0.001) a random effects model was fitted and the combined results did not show a significant difference in birth weight between the supplemented and the non-supplemented groups (mean weighted difference: 147.3g, 95% CI -112.5, 407.15).

Discussion

The results of the included studies were conflicting, with some demonstrating positive associations between 25(OH)-vitamin D concentration and birth weight 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)-vitamin D concentrations and offspring birth weight. 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.

* 1. Offspring birth length

Observational studies (Appendix 6, Table 10)

Twelve observational studies 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)-vitamin D concentration in ten studies and by dietary intake in two; in the remaining study maternal ambient UVB exposure during late pregnancy was used as a surrogate marker of vitamin D status. 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. Consideration of potential confounding factors was variable. Two studies identified a positive relationship between maternal vitamin D status and offspring birth length, neither of which directly measured maternal 25(OH)-vitamin D. The remaining ten studies 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.

Sabour88 (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/day). This study was assessed to have a high risk of bias and maternal serum 25(OH)-vitamin D was not measured, as vitamin D status was estimated from a food frequency questionnaire of dietary intake. The second study showing a positive relationship came from Sayers42 (composite bias score 3, medium risk) using data from the large UK cohort, ALSPAC). In this study, again maternal serum 25(OH)-vitamin 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 Leffelaar82 measured offspring length at one month and found that infants born to mothers with 25(OH)-vitamin D <30 nmol/l (the threshold used by the authors for vitamin D deficiency) had a significantly lower length at one 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 ten studies found no significant relationship between maternal vitamin D status and offspring birth length. Of these studies nine used maternal 25(OH)-vitamin D as the predictor and six were assessed to have a low risk of bias. Two studies were from the Middle East (Ardawi87, composite bias score 5, low risk; Magbooli89, composite bias score 1, medium risk) two from Australia (Morley91, composite bias score 8, low risk; Clifton-Bligh92, composite bias score 6, low risk), two from North America (Mannion83, composite bias score 1, medium risk; Dror93, composite bias score 7, low risk) and the remainder from the UK (Gale25, composite bias score 4, medium risk), Finland (Viljakainen94, composite bias score 3, medium risk), India (Farrant90, composite bias score 5, low risk) and Africa (Prentice95, composite bias score 5, low risk).

Intervention studies (Appendix 6, Table 11)

Two randomised controlled trials 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 (Brooke4) found no significant difference in offspring birth length in UK Asian women supplemented with 1000 IU ergocalciferol per day in the last trimester compared to the control group. In contrast, a larger Indian study by Marya6 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 7th and 8th month of gestation), compared to un-supplemented women.

Discussion

Again, the majority of the observational studies suggested no relationship between maternal 25(OH)-vitamin D status and offspring birth length. One 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 2 randomised trials 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.

* 1. Offspring head circumference

Observational studies (Appendix 6, Table 12)

Eleven observational studies assessed the relationship between maternal vitamin D status in pregnancy and offspring head circumference. Eight of the studies were of cohort design, with the remaining three being cross-sectional studies. Participant numbers ranged from 120 to 559. Maternal vitamin D status was assessed by serum 25(OH)-vitamin D concentration in nine studies; the remainder used dietary intake (Sabour88 and Mannion83). Composite bias scores ranged from -2 to +8, with six studies having a low risk of bias. Of those relating maternal serum 25(OH)-vitamin D to offspring head circumference at birth, no study found a statistically significant relationship, regardless of when during pregnancy 25(OH)-vitamin D was measured.

Three studies were from the Middle East: Ardawi87 and Magbooli89 found no association with offspring head circumference at birth and maternal 25(OH)-vitamin D measured at delivery. Likewise, Sabour88 observed no difference in offspring head circumference in women taking <200 IU vitamin D per day compared to those taking >200 IU vitamin D today. Two Australian studies (Morley91 and Clifton-Bligh92) measured maternal vitamin 25(OH)-vitamin D in the third trimester of pregnancy and also found no significant association between maternal 25(OH)-vitamin D concentration and offspring head circumference. Morley also measured 25(OH)-vitamin D in early pregnancy and again a relationship was not demonstrated. Similar findings were made by Mannion83 (a Canadian study using estimated dietary intake of vitamin D in pregnancy as the predictor), Gale25 (UK, 25(OH)-vitamin D measured in the 3rd trimester), Farrant90 (India, 25(OH)-vitamin D measured in the 3rd trimester), Prentice95 (The Gambia, Africa,25(OH)-vitamin D measured in the 2nd and 3rd trimester), Viljakainen94 (Finland, mean of early pregnancy and postpartum 25(OH)-vitamin D concentration used) and Dror93 (USA, measured perinatally).

Intervention studies (Appendix 6, Table 13)

Offspring head circumference at birth was an outcome in two randomised controlled trials of vitamin D supplementation in pregnancy, both of which were assessed as having a high risk of bias (composite bias score -2 in both). Brooke4 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, Marya6 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 compared to the un-supplemented group.

Discussion

Thus the majority of the observational studies demonstrated no association between maternal 25(OH)-vitamin D status in pregnancy and offspring head circumference at birth. One 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 optimization of, or prevention of low, head circumference.

* 1. Offspring bone mass

Observational studies (Appendix 6, Table 14)

Eight observational studies 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 mid-shaft, tibial and femoral bone mineral content (BMC), whole body and lumbar spine bone area, whole body and tibial bone mineral density, tibial cross-sectional area (CSA) and whole body BMC adjusted for bone area (aBMC). Most studies (six of eight) used DXA to assess bone mass; two studies used peripheral quantitative computed tomography (pQCT) and one study used single photon absorptiometry (SPA) in addition to DXA. Seven studies measured maternal 25(OH)-vitamin D during pregnancy or at delivery, one study used UVB exposure in the third trimester of pregnancy as a measure of maternal vitamin D status. Five studies demonstrated a positive relationship between maternal vitamin D status and offspring bone health; three studies showed no relationship.

Weiler86 (composite bias score 3, medium risk, n=50) found that neonates born to mothers with adequate maternal 25(OH)-vitamin D at delivery (defined by the authors as >37.5 nmol/l) had significantly higher whole body and femoral BMC per unit body weight compared to those with insufficient maternal vitamin D concentration (<37.5 nmol/l) even after adjustment for multiple confounders. There was no significant difference in infant lumbar spine, femoral or whole body BMC between the two groups however. Viljakainen94 (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)-vitamin D above the median (42.6 nmol/l) had significantly higher tibial BMC and cross-sectional area (CSA) than those below the median, even after adjusting for confounders including maternal height and birth weight. However, when the age of the offspring at pQCT was included in the regression model, a significant relationship between maternal 25(OH)-vitamin D and offspring tibial BMC was no longer seen. No relationship was seen between maternal 25(OH)-vitamin D and tibial bone mineral density (BMD). A subsample of 55 children were also assessed again at 14 months (Viljakainen, 201199. Tibial BMC was no longer significantly different by maternal 25(OH)-vitamin D status. Tibial CSA however, remained significantly lower in those with maternal 25(OH)-vitamin 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. Javaid2 2006 measured maternal 25(OH)-vitamin D in late pregnancy and offspring bone mass by DXA at mean 8.9 years in a cohort of 198 pregnant women. Positive associations were observed between maternal 25(OH)-vitamin D and offspring whole body and lumbar spine BMC, lumbar spine bone area (BA) and whole body and lumbar spine BMD after adjustments were made for offspring gestational age at delivery and offspring age at DXA. Sayers42 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 with aBMC and maternal UVB exposure.

Three studies found no associations between maternal 25(OH)-vitamin D and offspring bone mass. Two studies (Akcakus100 and Dror93), both cross-sectional in design and with a similar number of participants, measured maternal 25(OH)-vitamin D at delivery and used DXA to assess offspring bone mass up to the first month of life. A third study (Prentice95) measured mid and late pregnancy 25(OH)-vitamin D in a cohort of 125 pregnant Gambian women taking part in a larger clinical trial of vitamin supplementation. Offspring underwent assessment of bone mineral content and bone area using single photon absorptiometry of the midshaft radius; a subset also underwent whole body DXA at ages 2, 13 and 52 weeks. Again, no statistically significant relationship between maternal 24(OH)-vitamin D and offspring BMC at any time-point was observed. It should be noted that mean maternal 25(OH)-vitamin D levels in this cohort were much higher than any other study with an average at 103 nmol/l for mid-pregnancy and 111 nmol/l for late pregnancy and none of the women in the study were considered vitamin D deficient.

Intervention studies (Appendix 6, Table 15)

One clinical trial of maternal vitamin D supplementation and its effect on offspring bone mass was identified. Congdon22 randomised 64 Asian women in the UK to either no supplement or 1000 IU vitamin D plus calcium daily in the third trimester. Offspring had their forearm BMC measured 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 of the eight observational studies relating maternal 25(OH)-vitamin D status to offspring bone outcomes demonstrated positive associations. The one small intervention study 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)-vitamin 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 MAVIDOS Maternal Vitamin D Osteoporosis Study.101

* 1. Offspring anthropometric and body composition measures

Observational studies (Appendix 6, Table 16)

Six observational studies (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 had measured maternal serum 25(OH)-vitamin D in pregnancy (four in the third trimester and one at delivery); one study 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 used DXA to measure offspring fat and/or lean mass. Four studies demonstrated a significant relationship between offspring anthropometry and maternal 25(OH)-vitamin D; the remaining two showed no relationship.

Morley91 measured offspring subscapular, triceps and suprailiac skinfold thickness using Harpenden callipers, 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)-vitamin D at 11 weeks gestation and any of the neonatal outcome measures, a weak inverse association was observed between maternal 25(OH)-vitamin 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 the offspring was a first child, maternal smoking and season of blood sample. No significant association with maternal 25(OH)-vitamin D was found with the other offspring anthropometric outcomes assessed. Krishnaveni102 also assessed offspring subscapular and triceps skinfolds, using callipers, in addition to arm muscle area, waist circumference, fat mass, percent body fat, fat-free mass and percent 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)-vitamin 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 DXA to measure offspring fat and/or lean mass, two reported no relationship with maternal vitamin D status. Weiler86 used DXA 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)-vitamin D concentration <37.5 nmol/l at delivery and those born to mothers with 25(OH)-vitamin D >37.5 nmol/l. Gale25 found no significant association between maternal 25(OH)-vitamin 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)-vitamin D distribution but this did not achieve significance. In contrast, Sayers42 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. No significant association was seen with fat mass however. In contrast, Crozier103 (composite bias score 8, low risk) found that maternal serum 25(OH)-vitamin D in late pregnancy was positively associated with offspring fat mass at birth, measured by DXA, after adjusting for confounders. Interestingly no significant relationship was seen between maternal 25(OH)-vitamin 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)-vitamin D and offspring’s fat-free mass at any time-point.

Intervention studies (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). Brooke4 found no difference in neonatal triceps skinfold thickness or forearm length between those born to supplemented mothers and placebo group mothers. Marya6 found significantly greater mid-upper arm circumference, and triceps and subscapular skinfold thicknesses in neonates of supplemented than un-supplemented mothers (all p<0.01).

Discussion

The identified observational studies demonstrated a variety of modest relationships between maternal 25(OH)-vitamin D status and offspring anthropometric measures, with some finding positive relationships between maternal 25(OH)-vitamin 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 than un-supplemented group. The evidence base is therefore insufficient to warrant recommendation of maternal vitamin D supplementation to optimise childhood anthropometric measures.

* 1. Offspring asthma and atopy

Observational studies (Appendix 6, Table 18)

Ten studies were identified that examined the relationships between maternal vitamin D intake during pregnancy, maternal serum 25(OH)-vitamin D level in pregnancy or cord blood 25(OH)-vitamin 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 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 (Erkkola104; Devereux27; Miyake105; Camargo106 2007). 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; Miyake105 included 763 mother-offspring pairs in a prospective cohort study in Osaka, Japan (bias score -1, high risk). Vitamin D intake was measured by FFQ 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/day vitamin D was associated with a reduced risk of both wheeze and eczema. Camargo106 2007 reported in a prospective cohort study in Massachusetts, USA which included 1194 mother-offspring pairs, that children born to mothers in vitamin D intake quartiles two (446-562 IU/day), three (563-658 IU/day) and four (659-1145 IU/day) had a reduced risk of recurrent wheeze (≥2 episodes of wheeze in children with a personal diagnosis of eczema or parental history of asthma) at 3 years compared to those born to mothers in the lowest quartile of vitamin D intake, but in contrast to Miyake 2010, there was no difference in the incidence of eczema. Erkkola104 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 2007, there was no reduced risk of atopic eczema. However, this Finnish study only included children who had HLA-DQB1 conferred susceptibility to type 1-diabetes. The composite bias score was -1 indicating a high risk of bias. Finally, Devereux27 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 to the lowest quintile (46-92 IU/day). There was no statistically significant reduction in the odds ratio for wheeze when quintiles two, three and four were compared to quintile one, but a significant overall trend (p=0.009).

Two studies assessed the associations between cord blood 25(OH)-vitamin D and parental report of wheeze and/or asthma. These studies had composite bias scores of 2 and 3 (medium risk of bias). Camargo107 2011 found in 823 children in New Zealand that the odds ratio for wheeze at 5 years of age decreased across categories of cord 25(OH)-vitamin D, but there was no association with incident asthma. Similarly, Rothers108, found no association between cord 25(OH)-vitamin D and asthma (physician diagnosed and medication requirement in preceding year) at 5 years. Two studies, Gale25 and Morales109 assessed the association between maternal 25(OH)-vitamin D measured in pregnancy and parental reported wheeze or diagnosis of asthma. Gale25 (composite bias score 4, medium bias risk) assessed the association between maternal 25(OH)-vitamin D in late pregnancy and parental report of asthma in 178 children. Exposure to the highest quarter of maternal concentrations of 25(OH)-vitamin D was associated with an increased risk of reported asthma at age 9 years compared with children whose maternal 25(OH)-vitamin D concentration had been in the lowest quarter of the distribution. In addition, the risk of offspring eczema at nine months (assessed by either physical examination or parental report) was also higher in children in the highest quarter of maternal 25(OH)-vitamin D distribution compared to those in the bottom quarter. By 9 years of age however, although offspring in the highest quarter of maternal 25(OH)-vitamin 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)-vitamin D quartile were low however, ranging from 2-15. Conversely, Morales109 (composite bias score 3, medium bias risk) found no significant association between maternal 25(OH)-vitamin D measured at mean (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 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 measured offspring spirometry; Cremers110 2011(bias score 3, medium risk) found no associations between maternal plasma 25(OH)-vitamin D at 36 weeks gestation and offspring Forced Expiratory Volume in 1 second (FEV1) (p=0.99) or Forced Vital Capacity (FVC) (p=0.59) at 6-7 years in 415 mother-offspring pairs. Similarly Devereux27 (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 sensitization. Devereux27 found 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, Rothers108 (bias score 2, medium risk) found that those with cord blood 25(OH)-vitamin D ≥100 nmol/l, when compared to children with cord 25(OH)-vitamin 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, 2 studies included offspring IgE concentration as a measure of atopy. Rothers108 reported a non-linear relationship between cord 25(OH)-vitamin D and total and allergen-specific IgE for 6 inhalant allergens. The highest levels of IgE were identified in children with cord 25(OH)-vitamin D concentration <50 nmol/l and ≥100 nmol/l. Conversely, Nwaru111 2010 found increasing maternal vitamin D intake determined by FFQ was inversely associated with sensitisation (IgE>0.35ku/l) to food allergens (IgE>0.35ku/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)-vitamin D status and offspring asthma and no recommendation can be made. Further high-quality intervention studies are required here, such as the ongoing VDAART (Vitamin D Antenatal Asthma Reduction Trial, ISRCTN NCT00920621) and ABCVitamin D (Vitamin D Supplementation During Pregnancy for Prevention of Asthma in Childhood ISRCTN NCT00856947) trials.

* 1. Offspring born small for gestational age (SGA)

Observational studies (see Appendix 6, Table 19)

Seven observational studies assessing the relationship between maternal 25(OH)-vitamin D and the risk of offspring being born small for gestational age (SGA) were identified. Of these, two were case-control studies, one was cross-sectional and four were cohort studies. All achieved a composite bias score of between +1 and +7 indicating a medium-low risk of bias. Five studies defined SGA as infants born below the 10th percentile of birth weight according to nomograms based on gender and gestational age. Three studies reported how gestational age was assessed (known dates of last menstrual period and/or fetal ultrasound in early pregnancy), with the remainder giving no explanation. All studies measured serum maternal 25(OH)-vitamin D concentration. The number of week’s gestation when the sample was taken ranged from 11 weeks to delivery. One study defined SGA as infants born below the 3rd percentile of birth weight. Three studies (one nested case-control and one cohort study) reported a significant association between maternal 25(OH)-vitamin D and risk of SGA; the remaining four studies did not demonstrate a significant relationship.

Leffelaar82 measured maternal 25(OH)-vitamin D concentration in women at 11-13 weeks gestation taking part in the large Amsterdam Born Children and their Development (ABCD) study. Of the 3,730 women in the cohort, 9.2% delivered SGA infants. Women with a serum 25(OH)-vitamin D concentration less than 30 nmol/l had a significantly higher risk of SGA infants compared to women with 25(OH)-vitamin D concentrations greater than 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)-vitamin D concentration between 30-49.9 nmol/l. Bodnar112 (composite bias score 7, low risk) found that the relationship between maternal 25(OH)-vitamin 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 to 301 randomly selected controls; all had 25(OH)-vitamin D measured before 22 weeks gestation. Amongst black mothers, no relationship between SGA risk and maternal 25(OH)-vitamin D concentration was observed. However, in white women, a U-shaped relationship was observed between the odds of delivering an SGA infant and maternal 25(OH)-vitamin D concentration. Significantly higher odds for SGA were observed in those with 25(OH)-vitamin D concentrations <37.5 and >75 nmol/l, with the lowest odds of SGA in women with 25(OH)-vitamin D concentrations 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 multi-vitamin use and preconception physical activity. Finally, Robinson113 (composite bias score 0; medium risk), in a case-control study of pregnant women, all of whom had early onset severe preeclampsia (as defined by the American College of Obstetrics and Gynecology), found that maternal serum vitamin D was significantly lower in cases with SGA infants compared to controls. This study did not present an odds ratio, nor 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 (Akcakus100, composite bias score 4, medium risk), 30 of whom had SGA infants, found no difference in maternal mean 25(OH)-vitamin D at delivery in cases of SGA (maternal 25(OH)-vitamin D concentration 21.8 nmol/l) compared to infants appropriate for gestational age (maternal 25(OH)-vitamin D concentration 21.5 nmol/l). Average maternal concentrations of 25(OH)-vitamin 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 (composite bias score 3, medium risk) in the African cohort study of 1,078 women all infected with HIV. 74 cases of SGA were identified. Again no difference in mean maternal 25(OH)-vitamin D concentration measured in mid-pregnancy was observed between cases and normal deliveries. Shand114 observed similar findings in a cohort study of Canadian women all with biochemical or clinical risk factors for preeclampsia. No significantly increased odds of SGA were observed in women with 25(OH)-vitamin D concentrations less than 75 nmol/l compared to over 75 nmol/l. In this study, cases of SGA were low (n=13). Finally a Spanish cohort study from Fernadez-Alonso115 (composite bias score 3, medium risk) identified 46 cases of SGA out of a cohort of 466. No significant relationship between maternal 25(OH)-vitamin D and SGA infants was observed. Neither mean 25(OH)-vitamin D concentrations nor an odds ratio were reported.

Intervention studies (See Appendix 6, Table 20)

Two clinical trials of maternal vitamin D supplementation evaluated the relationship between maternal 25(OH)-vitamin D and risk of SGA infants. Both defined SGA as infants born below the 10th percentile for birth weight, although neither reported how gestational age was assessed. Neither observed a significant relationship. Brooke4, in a double-blind placebo controlled randomised trial, allocated 67 pregnant women to either placebo (n=67) or vitamin D2 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 compared to the un-supplemented group, the percentage of SGA infants did not differ significantly between groups (19 in the placebo group versus 9 in the supplemented group). The composite bias score of this study was -2 indicating a high risk of bias. Yu96 (composite bias score 5, low risk) reported similar findings in a more recent British clinical trial. Pregnant women was randomised to one of three arms; either no supplement (n=59), or oral vitamin D2 800 IU/day from 27 weeks onwards (n=60), or a single bolus dose of 200,000 IU vitamin D2 at 27 weeks gestation (n=60). Each group contained equal numbers of four ethnic groups (Caucasian, Black, 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)-vitamin D status and risk of offspring being small for gestation age. Outcomes were conflicting. The 2 intervention studies 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 small for gestational age neonatal.

* 1. Offspring preterm birth

Observational studies (Appendix 6, Table 21)

Seven observational studies relating maternal 25(OH)-vitamin D to the risk of premature birth were identified. (Three cohort, one cross-sectional, two case-control) One further cross-sectional study assessing the risk of threatened premature birth was also included. Two studies were case-control, three cohort and two cross-sectional. There was some disparity in the definition of preterm birth between studies. Most studies defined preterm birth as spontaneous delivery before 37 weeks gestation; one study used a threshold of less than 35 weeks. Only three studies reported how gestational age was measured: two studies used a combination of last menstrual period and/or fetal ultrasound; one study used the scoring system of Dubowitz, (based on examination of the neonate and scored on neurological and physical examination features). All studies measured maternal serum 25(OH)-vitamin D at some point during pregnancy or at delivery. Only one study found a significant relationship between maternal 25(OH)-vitamin D and risk of premature delivery.

Shibata116 (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)-vitamin D measured after 30 weeks gestation was significantly lower in the 14 cases of threatened premature delivery (mean 25(OH)-vitamin D concentration 30.0 nmol/l) compared to normal pregnancies (mean 25(OH)-vitamin D concentration 37.9 nmol/l). Threatened premature delivery was defined as progressive shortening of cervical length (<20mm) 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 the number of uterine contractions equal to or more than twice per 30 minutes (before the 32nd week of gestation).

In contrast, six studies did not demonstrate a significant relationship between maternal 25(OH)-vitamin D and premature delivery. A small case-control study by Delmas117 found no difference in mean maternal 25(OH)-vitamin D concentration measured at delivery in the 10 cases of preterm birth (mean maternal 25(OH)-vitamin D concentration 44.9 nmol/l) compared to the 9 controls (mean maternal 25(OH)-vitamin 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 1,078 pregnant African women infected with HIV and taking part in a clinical trial of vitamin use (Mehta118, 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)-vitamin D concentration measured at 12-27 weeks gestation less than 80 nmol/l compared to those with levels greater than 80 nmol/l. A nested case-control study in North Carolina, USA (Baker119, 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)-vitamin D measured at 11-14 weeks gestation. Again no significant difference in the odds ratio for preterm birth was found in women with 25(OH)-vitamin D less than 75 nmol/l compared to those with 25(OH)-vitamin D concentration greater than 75 nmol/l. Shand114 in a cohort study of 221 pregnant women in Vancouver, Canada with either clinical or biochemical risk factors for preeclampsia found no significant relationship between maternal 25(OH)-vitamin D, measured between 10 weeks and 20 weeks 6 days gestation, and risk of preterm birth using three different thresholds of maternal 25(OH)-vitamin 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 preeclampsia included a past obstetric history of early-onset or severe preeclampsia, unexplained elevated α-fetoprotein ≥ 2.5 multiples of the median (MoM), unexplained elevated human chorionic gonadatrophin, or low pregnancy-associated plasma protein A ≤ 0.6 MoM. Hossain120 2011, in a cross-sectional study of 75 pregnant women in Pakistan (composite bias score 4, medium risk), found that mean maternal 25(OH)-vitamin D3 at delivery tended to be higher in those who delivered preterm (mean 25(OH)-vitamin D3 concentration 42.2 nmol/l) than those with full term deliveries (mean 25(OH)-vitamin D3 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-Alfonso115 (composite bias score 3, medium risk)) there was no significant difference in mean maternal 25(OH)-vitamin 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)-vitamin 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.

* 1. Offspring Type I diabetes

Observational studies (Appendix 6, Table 22)

Three observational studies (two case-control and one cohort), all from Scandinavia, were identified, relating maternal 25(OH)-vitamin D status to the risk of type I diabetes mellitus in the offspring. Only one of these studies used 25(OH)-vitamin D concentration; the other two attempted to estimate vitamin D intake. Sorensen121 (composite bias score 8, low risk) performed a case-control study of 109 children with type I diabetes (mean age 9 years) and 219 controls within a cohort of 29,072 individuals. 25(OH)-vitamin D concentration had been measured at a median of 37 weeks gestation. The mean 25(OH)-vitamin D concentration in the mothers of cases was 65.8 nmol per litre and in the mothers of controls was 73.1 nmol per litre. Compared with children of mothers whose levels were greater than 89 nmol per litre, children of mothers whose 25(OH)-vitamin D concentrations in late pregnancy were less than or equal to 54 nmol per litre were at increased risk of developing type I diabetes mellitus. Stene122 (composite bias score 2, medium risk) performed a case-control study comparing 545 children with type I diabetes (mean age 10.9 years) with 1,668 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 I diabetes mellitus. Marjamaki123 (composite bias score 6, low risk) studied a prospective cohort of 3,723 children who were at an increased genetic risk of developing diabetes. Amongst this cohort 74 children developed type I diabetes over the mean observation period of 4.3 years. Maternal vitamin D intake was assessed retrospectively from a food frequency questionnaire completed 1 to 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 I diabetes mellitus.

A further study by Krishnaveni102, (composite bias score 4, medium risk) using a cohort of 506 Indian children age 5 years (469 of whom were also followed-up to 9.5yrs.) 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)-vitamin D concentration, measured at 28-32 weeks gestation. At age 9 years however a significant inverse relationship was observed between maternal 25(OH)-vitamin D concentration and offspring fasting insulin and insulin resistance after adjustment for child sex and age, maternal BMI, gestational diabetes, socioeconomic score, parity and religion.

Intervention studies

No intervention studies were identified.

Discussion

The 3 observational studies relating maternal serum 25(OH)-vitamin D status to risk of offspring type I diabetes were assessed to be of moderate to low risk of bias and were generally consistent in suggesting an inverse relationship. However one used vitamin D dietary intake and there are no intervention studies. Thus maternal vitamin D supplementation to prevent offspring type I diabetes cannot be recommended, however high-quality intervention studies are warranted.

* 1. Offspring low birth weight

Observational studies (Appendix 6, Table 23)

Three observational studies (two cross-sectional, one cohort) examining the relationship between infants born with low birth weight and maternal 25(OH)-vitamin 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 birth weight (offspring birth weight less than 2500g) was consistent across all three studies. Two studies directly measured maternal serum 25(OH)-vitamin D and reported no association with low birth weight infants. One study estimated vitamin D intake from a food frequency questionnaire and observed a significant relationship between vitamin D intake and offspring risk of low birth weight. This study from Sabour88 used a food frequency questionnaire in 449 Iranian pregnant women completed at delivery to estimate maternal vitamin D intake during pregnancy. The incidence of low birth weight infants (n not given) was lower in women with adequate intake of calcium and vitamin D (100mg calcium, 200 IU vitamin D/day compared to those with inadequate intake. 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)-vitamin D and offspring low birth weight risk. Maghbooli89 (composite bias score 1, medium risk) in a second cross-sectional study from Iran, measured maternal 25(OH)-vitamin D at delivery in 552 Iranian women. 5.4% (approx. n= 30) of the cohort had low birth weight offspring. No significant difference in mean maternal 25(OH)-vitamin D was observed between cases of low birth weight offspring and normal weight offspring (mean 25(OH)-vitamin D concentration in each group not given). Similarly Mehta118 (composite bias score 3, medium risk) in a cohort study of 1,078 HIV infected women taking part in a vitamin supplement trial, found no significantly increased odds of low birth weight infants (n=80) in mothers with a 25(OH)-vitamin D concentration <80 nmol/l compared to those with a concentration >80 nmol/l. In this study a threshold of 80 nmol/l was used to divide maternal 25(OH)-vitamin D concentration into adequate or low. Adjusting the analysis for maternal multivitamin supplementation, age at baseline, CD4 count at baseline and HIV disease stage did not alter the findings.

Intervention studies

No intervention studies were identified.

*Discussion*

Of the 3 observational studies relating maternal 25(OH)-vitamin D status to risk of low birth weight in the offspring, only one demonstrated a positive result, suggesting that low birth weight was less likely where women took at least 100mg of calcium and 200 IU vitamin D daily. However this was judged to be at high risk of bias; the remaining 2 studies demonstrated no relationship and therefore maternal vitamin D supplementation cannot be recommended to prevent low birth weight. Larger prospective observational studies in several different populations would be sensible before moving to an intervention study.

* 1. Offspring serum calcium concentration

Observational studies (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, Ardawi87 found no significant correlation between maternal 25(OH)-vitamin 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)-vitamin 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 (Appendix 6, Table 25)

Seven clinical trials of maternal vitamin D supplementation were identified; all measured venous umbilical cord calcium concentration at delivery and three went on to measure offspring venous calcium again within the first week of life. None of the trials were 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 1,139. Five studies reported adequate randomisation, however only two trials were placebo-controlled and only one was of double-blind design. Supplementation strategies were highly variable: six trials supplemented pregnant women with vitamin D in the last trimester; one study 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 to bolus oral dosing of 600,000 IU cholecalciferol twice in the last trimester. Six studies reported higher offspring calcium concentrations in the supplemented group compared to the un-supplemented group; one trial showed no difference in offspring venous calcium regardless of maternal vitamin D supplementation strategy.

Brooke4 (composite bias score -2, high risk), in a trial of ergocalciferol supplementation of Asian women living in the UK in their last trimester of pregnancy, found no difference in umbilical cord calcium concentration between groups, but neonatal serum calcium was greater in offspring of supplemented mothers than mothers who had received placebo at three and six 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 Cockburn21 (composite bias score -1, high risk) and in a French study by Delvin7 (Composite bias score -2, high risk). Neither study found a difference in venous cord calcium concentrations between the supplemented and un-supplemented groups, but both found higher infant venous calcium concentrations at days 6 and 4 respectively in the supplemented group. The third, and most recent, British study (Congdon22) found that offspring cord calcium was significantly higher in Asian women supplemented with daily 1000 IU vitamin D plus calcium in the last trimester compared to Asian women 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 details of whether randomisation or blinding occurred were not reported. These findings are in agreement with two Indian studies, both by Marya et al5;6(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 mothers supplemented with two doses of 600,000 IU cholecalciferol in months 7 and 8 of gestation compared to the un-supplemented group.

In contrast, a French study (Mallet8, 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 supplement was 1000 IU per day for 3 months or as a single high dose of 200,000 IU in the 7th month of gestation.

Evidence synthesis

The available published results were combined in two separate models. The first meta-analysis included Cockburn, Brooke, Marya 1981 (low dose of vitamin D), Mallet (low dose of vitamin D) and Delvin (Appendix 7, Figure 8). Owing to statistically significant heterogeneity in the results (I2=67.6%, p=0.015), a random – effects model was fitted. Serum calcium concentration in supplemented group did not differ from that in the un-supplemented group (mean difference: 0.01mmol/l, 95% CI -0.02,0.04). The second meta-analytic model included the studies Cockburn, Brooke, Marya 1981 (high dose of vitamin D), Mallet (high dose of vitamin D), Delvin 1986 and Marya 1988 (Appendix 7, Figure 9). As in the previous model, a random-effects model was fitted due to significant heterogeneity (I2=90%, p<0.001). The combined results showed that the mean difference of serum calcium concentration between the supplemented and the un-supplemented groups was significantly different from 0 (Mean difference: 0.05mmol/l, 95% CI 0.02, 0.05).

Discussion

The majority of the intervention studies and the one observational study consistently demonstrated positive relationships between maternal 25(OH)-vitamin 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)-vitamin D and offspring calcium concentration might not be a surprising finding; however little is known about relationships between 25(OH)-vitamin 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)-vitamin 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 (for example by skin colour, ethnicity, or mode of infant feeding), and might usefully form the basis of further investigation.

* 1. Offspring blood pressure

Observational studies (Appendix 6, Table 26)

Two cohort studies were identified which examined the relationship between maternal serum 25(OH)-vitamin D concentration in pregnancy and offspring blood pressure. Both studies were of cohort design and measured maternal serum 25(OH)-vitamin D in late pregnancy. Composite bias score was 4 for both, indicating a medium risk of bias. Gale25 measured blood pressure in 178 children aged 9 years in the Princess Anne Cohort, UK. No association was observed between maternal 25(OH)-vitamin D and offspring blood pressure. Krishnaveni102, 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 in those children born to mothers with vitamin D deficiency (defined by the authors as <37.5 nmol/l) compared with those born to mothers without vitamin D deficiency. Adjustments for offspring sex and age, maternal BMI, gestational diabetes, socioeconomic score, parity and religion made little difference to the results.

Intervention studies

No intervention studies were identified.

Discussion

Neither of the 2 observational studies relating maternal 25(OH)-vitamin D status to offspring blood pressure demonstrated a statistically significant relationship and therefore no treatment recommendation can be made.

* 1. 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, Congdon22, found no difference in the incidence of offspring craniotabes in the supplemented (n=4) group compared to the un-supplemented 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)-vitamin 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 it 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.

* 1. Maternal preeclampsia

Observational studies (Appendix 6, Table 27)

Eleven observational studies were identified, comprising six case-control, four cohort and one cross-sectional study. The case-control studies were generally of small size with the minimum number of cases 12 and the maximum 55 and the number of controls ranging from 24 to 220. The definition of preeclampsia was similar across studies: new onset gestational hypertension after 20 weeks (systolic blood pressure persistently (two or more occasions) ≥140mmHg and/or diastolic blood pressure ≥85 or ≥90mmHg) and proteinuria (either 300mg 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 more than 0.3). Two of the case-control studies identified cases of severe preeclampsia only, using the American College of Obstetrics and Gynecology 2002 definition (systolic blood pressure ≥160mmHg and/or a diastolic blood pressure ≥110mmHg on at least 2 occasions plus proteinuria (≥300mg in a 24 hour collection or 1+ on urine dipstick), or systolic blood pressure ≥140mmHg and/or diastolic blood pressure ≥90mmHg plus 5g 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)-vitamin D concentration as the marker of maternal vitamin D status, with the other two cohort studies using dietary intake. The timing of serum measurements varied across the studies with some measuring in the first trimester and others in the last and one study at three time points. Composite bias scores ranged from 2 to 9 indicating that studies were considered of 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 preeclampsia. A further two case-control studies demonstrated a similar association between maternal 25(OH)-vitamin D and risk of severe preeclampsia. A nested case-control study (55 cases and 220 randomly selected, unmatched controls from a cohort of 1198) from Bodnar124 (composite bias score 8, low risk) measured 25(OH)-vitamin 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)-vitamin D was associated with an over two-fold increased risk of preeclampsia after adjusting for maternal race, ethnicity, pre-pregnant BMI, education, season and gestational age at blood sample. A cross-sectional study from Pakistan (Hossain120, composite bias score 4, medium risk) measured maternal 25(OH)-vitamin D3 at delivery in 75 women (76% of whom covered their face, arms, hands and head). Although the number of preeclampsia cases is not given, when the group was divided into thirds, a significantly increased risk of preeclampsia was observed for those in the lowest and middle tertile compared to the highest. The relationship between maternal 25(OH)-vitamin and preeclampsia was only observed in individuals with serum 25(OH)-vitamin D less than 50 nmol/l. Unlike other studies, women were classified as having preeclampsia based on blood pressure alone (systolic blood pressure ≥140mmHg and/or diastolic blood pressure ≥90mmHg). The largest study to date (Haugen125 (composite bias score 2, medium risk)) followed up a cohort of 23,425 pregnant women enrolled in the Norwegian mother and child cohort. Maternal 25(OH)-vitamin D was not directly measured, but estimated from a food frequency questionnaire at 22 weeks. 1,267 cases of preeclampsia were identified. Lower total vitamin D intake was associated with a significantly increased risk of preeclampsia.

Both studies examining the relationship between severe preeclampsia and maternal 25(OH)-vitamin 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. Baker126 (composite bias score 9) identified 44 cases and 201 randomly selected controls matched by race/ethnicity from a cohort of 3,992 women. Significantly higher odds of severe preeclampsia were found in those with maternal 25(OH)-vitamin D less than 50 nmol/l compared to those with 25(OH)-vitamin D over 50 nmol/l even after adjusting for season of blood sampling, maternal age, multiparity, BMI, gestational age at blood sample. Similarly, Robinson127 (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 preeclampsia significantly reduced as maternal 25(OH)-vitamin 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 preeclampsia risk. Seely128 (composite bias score 2, medium risk) observed no significant difference in late pregnancy mean maternal 25(OH)-vitamin D in 12 cases of preeclampsia compared with 24 controls of similar maternal age, gestation, height, weight, whether primiparous or not and whether Caucasian or not. A second US nested case-control study from Powe129 (composite bias score 4, medium risk) drew similar conclusions. In this study of 39 cases and 131 unmatched controls from an overall cohort of 9,930, the odds of preeclampsia were not related to first trimester maternal 25(OH)-vitamin 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)-vitamin D concentration <37.5 nmol/l. A further US nested case-control study from Azar130 (composite bias score 5, low risk) assessed preeclampsia risk in only white women, all with Type 1 diabetes mellitus, who had serum 25(OH)-vitamin D measured at three time points during their pregnancy (early, mid and late pregnancy). 23 cases were identified and compared to 24 controls, matched for age, diabetes duration, HbA1c and parity, out of a cohort of 151. Again, no statistically significant relationship between maternal 25(OH)-vitamin D, measured at any time-point and preeclampsia risk was observed. A Canadian study of 221 pregnant women with clinical or biochemical risk factors for preeclampsia (Shand114, composite bias score 6, low risk) found no significantly increased odds of preeclampsia in pregnant women with mid-pregnancy 25(OH)-vitamin D concentrations <37.5, <50 or <75 nmol/l compared to those with 25(OH)-vitamin D concentrations >75nmol/l. However, only 28 cases of preeclampsia were identified. The most recent study by Fernandez-Alonso115 (composite bias score 3, medium risk) again found no difference in mean early pregnancy maternal 25(OH)-vitamin D in those who developed preeclampsia compared to those with normal pregnancies. This study included the lowest number of cases (seven). Finally, Oken131 (composite bias score 5, low risk) identified 58 cases of preeclampsia from the US Project Viva cohort of 1,718 women. Maternal serum 25(OH)-vitamin D was not measured directly, but estimated from a food frequency questionnaire at mean 10.4 weeks gestation. No significant relationship between preeclampsia risk and vitamin D intake was seen.

Evidence synthesis

Usable results for meta-analysis of the risk of preeclampsia with increased vitamin D were available from four studies: Bodnar, Powe, Robinson and Azar (early pregnancy visit). All but Bodnar provided unadjusted odds ratios. The unadjusted estimates were synthesised in a random effects model due to statistically significant heterogeneity (I*2*=78.4%, p=0.01). The pooled estimate showed no significant risk of preeclampsia with increased vitamin D (pooled OR 0.78, 95% CI 0.59, 1.05; Appendix 7, Figure 10). Synthesising the available adjusted odds ratios from all four studies the result was very similar; there was no statistically significant increased risk of preeclampsia with decreased vitamin D status (pooled OR 0.75, 95% CI 0.48, 1.19; Appendix 7, Figure 11).

Intervention studies (Appendix 6, Table 28)

One clinical trial that included maternal preeclampsia as an outcome measure was identified. Marya132 randomised 400 pregnant women attending an antenatal clinic in India to either a trial of vitamin D plus calcium (375mg/day calcium plus 1200 IU vitamin D) from 20-24 weeks until delivery or no supplement (n=200 in each arm). Serum 25(OH)-vitamin D concentrations were not measured during the study. There were 12 cases of preeclampsia in the supplemented group versus 18 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 un-supplemented 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 preeclampsia and others no relationship. Both studies looking at the risk of severe preeclampsia found statistically significant inverse relationships with maternal 25(OH)-vitamin D concentration. 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 preeclampsia used. Most observational studies were case-control and included only small numbers of cases of preeclampsia (n=7 to 55). Only one intervention study was identified. This was of reasonable size, however 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 preeclampsia was identified in the un-supplemented group. Thus, it is difficult to make any treatment recommendations based on the current evidence. Further high quality intervention studies are needed.

* 1. Maternal gestational diabetes

Observational studies (Appendix 6, Table 29)

Eight observational studies (four case-control, one cross-sectional and three prospective cohort) examined relationships between maternal 25(OH)-vitamin D status and risk of gestational diabetes. One study, Maghbooli133, found, in a cross-sectional cohort of 741 Iranian women, that mean 25(OH)-vitamin D concentrations (measured at 24-28 weeks) were lower in the 52 subjects who had gestational diabetes (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 (Soheilykhah134, composite bias score 3, medium risk), found significantly increased odds of gestational diabetes in those with 25(OH)-vitamin D concentrations less than 37.5 nmol/l (measured between 24 and 28 weeks). Thus the mean 25(OH)-vitamin D concentration in those with gestational diabetes was 24 nmol/l and in those without was 32.3 nmol/l. Clifton-Bligh92, in a prospective cohort of 307 women in New South Wales, Australia, found that mean 25(OH)-vitamin D concentrations (measured at a mean of 28.7 weeks) were 48.6 nmol/l in 81 women with gestational diabetes compared with 55.3 nmol/l in women without. They also found that serum 25(OH)-vitamin 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. Zhang135 performed a nested case-control study within a US cohort (n=953), containing 57 women with gestational diabetes (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 II diabetes and prepregnant BMI, 25(OH)-vitamin D concentration less than 50 nmol/l was associated with increased odds of gestational diabetes, compared with women with concentrations greater than 75 nmol/l. This study again achieved a low risk of bias with composite score of 8.

In contrast, an Indian prospective cohort study (Farrant90, composite bias score 5, low risk) found no difference in 25(OH)-vitamin D concentrations between those with gestational diabetes (n=34, mean 25(OH)-vitamin D concentration 38.8 nmol/l) those without (n=525, mean 25(OH)-vitamin D concentration 37.8 nmol/l), p=0.8. No associations were found by three further studies: Makgoba136 (composite bias score 7, low risk), in a nested case-control study of 90 women with gestational diabetes and 158 controls, within an overall cohort of 1,200 women, found no difference in serum 25(OH)-vitamin D concentration (47.2 nmol/l in cases versus 47.6 nmol/l in controls, measured at 11-13 weeks gestation). An inverse relationship was found between the serum 25(OH)-vitamin D concentration and fasting glucose, glucose concentration two hours after a glucose tolerance test, and HbA1c at 28 weeks gestation. However, after adjustment for BMI, gestation of blood sampling, smoking, ethnicity, parity, maternal age, conception status, previous gestational diabetes and season, only the relationship with two hour glucose concentration remained statistically significant. A nested case-control study (Baker137, composite bias score 7, low risk), this time set within a US cohort of 4,225 women in whom serum 25(OH)-vitamin D concentration was assessed at 11-14 weeks gestation, found that amongst the 60 cases of gestational diabetes and 120 controls, after adjustment for maternal age, insurance status, body mass index, gestational age at sample collection and season, there was no association between serum 25(OH)-vitamin D concentration and gestational diabetes. Finally, in a Spanish prospective cohort of 466 women (Fernandez-Alonso115, composite bias score 3, medium risk) in whom 25(OH)-vitamin D concentrations were measured at 11-14 weeks, there was no statistically significant relationship between baseline 25(OH)-vitamin D concentration and development of gestational diabetes.

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)-vitamin D status and risk of gestation diabetes. Although two Iranian studies did find an increased risk of gestational diabetes in women with low levels of 25(OH)-vitamin 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.

* 1. Maternal Caesarean section

Observational studies (Appendix 6, Table30)

Six observational studies were identified, one of which was case-control and the others cohort designs. Two studies found inverse relationships between 25(OH)-vitamin D status and risk of Caesarean section, with the remaining studies demonstrating no statistically significant associations. Scholl138 (composite bias score 5, low risk) studied 290 women who delivered by Caesarean section out of a cohort of 1,153 pregnant women. 25(OH)-vitamin D concentration was assessed at a mean of 13.7 weeks gestation. Compared with women who had serum 25(OH)-vitamin D concentrations between 50 and 125 nmol/l in early pregnancy, those who had levels less than 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 body mass index. Merewood139 (composite bias score 6, low risk), in a cross-sectional study of US women, found increased odds of Caesarean section if maternal 25(OH)-vitamin D concentration was less than 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.

Ardawi87 (composite bias score 5, low risk) studied a cohort of 264 women in Jeddah, Saudi Arabia. Amongst women with serum 25(OH)-vitamin D status less than 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 (Brunvand140, composite bias score 1, medium risk) of nulliparous Pakistani women of low social class found that the median 25(OH)-vitamin 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 1,000 pregnancies yielded 199 Caesarean sections (Savvidou141, composite bias score 7, low risk) and found no relationship between 25(OH)-vitamin 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-Alonso115 (composite bias score 3, medium risk), 105 of the cohort of 466 women underwent Caesarean section. There was no relationship between 25(OH)-vitamin 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, pre-existing morbidity, it seems likely that any relationships between maternal 25(OH)-vitamin 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.

* 1. Maternal bacterial vaginosis

Observational studies (Appendix 6, Table 31)

Three studies were identified (two cohort, one cross-sectional) which examined relationships between maternal 25(OH)-vitamin D status and bacterial vaginosis. All three studies elucidated statistically significant relationships although at very different thresholds of 25(OH)-vitamin D concentration. Bodnar142 (composite bias score 5, low risk) studied 469 women who were all non-Hispanic white or non-Hispanic black. 25(OH)-vitamin D concentration was measured at a mean of 9.5 weeks gestation. Amongst the 192 cases of bacterial vaginosis median 25(OH)-vitamin D concentration was 29.5 nmol/l compared with 40.1nmol/l in the non-diseased women. At 25(OH)-vitamin D concentrations below 80 nmol/l there was an inverse association between frequency of bacterial vaginosis and early pregnancy serum 25(OH)-vitamin 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, Hensel143 (composite bias score 4, medium risk) found a statistically significantly increased risk of bacterial vaginosis in those women whose serum 25(OH)-vitamin D concentration was less than 75 nmol/l. However it is unclear at what stage 25(OH)-vitamin D concentration was measured, and the mean 25(OH)-vitamin D concentrations, together with the unadjusted analyses, are not presented. Dunlop144 (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)-vitamin D concentration at delivery was less than 30 nmol/l compared with those whose levels were above this threshold, after adjustment for race, age, smoking, BMI, gestational age at delivery, healthcare 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 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.

1. Other study questions

Given the altered physiology during pregnancy, it is difficult to define a normal 25(OH)-vitamin D concentration in relation to parathyroid hormone 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 obtained145. 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)-vitamin D during pregnancy.

Similarly, the studies are extremely heterogeneous with regard to dose, use of vitamin D2 or D3, 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 (D2 or D3), 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.

1. 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 birth weight (meta-analysis of observational studies) and offspring bone mass (observational studies); meta-analysis of randomised controlled trials suggested a positive effect of maternal vitamin D supplementation on neonatal calcium concentrations, but the dose required, duration and target group is 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 assessment included serum concentration, estimated dietary intake and UV sunlight exposure. Even when serum 25(OH)-vitamin D concentration was measured, the assay and technique varied widely. Indeed we included comparability and standardisation of assay results in the quality criteria, but these issues were not commonly considered or documented by study authors. Clearly, given the multiplicity of both laboratory techniques (for example, radio-immunoassay, HPLC, 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 (http://ods.od.nih.gov/Research/VitaminD.aspx#vdsp). 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 (for example 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)-vitamin D concentrations because of sequestration in adipose tissue. Increasing physical activity might be associated with better maternal health, but also greater 25(OH)-vitamin D concentrations because of greater sun expose.

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, of the studies identified some 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 section 5. The first of these addressed normal levels of vitamin D in pregnancy. Such a value is controversial in non-pregnant adult populations and section 3.7 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 sub-optimal.

It may, therefore, be more appropriate to attempt to define “healthy” levels based on relationships between maternal serum 25(OH)-vitamin 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)-vitamin 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)-vitamin 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 birth weight (meta-analysis of observational studies), neonatal calcium concentrations (meta-analysis of randomised controlled trials) 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 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 randomised controlled trials of vitamin D supplementation aimed at optimising offspring outcomes are small and of poor methodology and date from around 20 years ago, when assay technology was much less well advanced. In several areas (offspring birth weight, calcium concentration, bone mass) the evidence is sufficient to warrant the instatement of properly conducted large randomised controlled trials, 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 actually led to an improvement in maternal and/or offspring health before going on to estimate its health-economic impact.

1. 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 well designed large prospective cohort studies are most appropriate as the next step. In others (e.g. birth weight, serum calcium concentration, bone mass), the evidence base is sufficient to suggest randomised controlled trials. Additionally, a critical underlying issue is to ensure that 25(OH)-vitamin 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)-vitamin D measurements across the globe should be supported, such as the programme led by the US National Institutes of Health (<http://ods.od.nih.gov/Research/VitaminD.aspx#vdsp>), and which incorporates UK centres.
* There is a need to optimize the biochemical assessment of vitamin D status, whether this is simply 25(OH)-vitamin D concentration, or should incorporate other indices such as vitamin D binding protein, albumin, and be related to parathyroid hormone or calcium concentrations.
* 25(OH)-vitamin 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)-vitamin D. This work would need to take into account potential confounding factors, particularly season, latitude and skin pigmentation/covering/ethnicity.
* High-quality large prospective cohort studies are required to investigate the relationship between maternal 25 (OH)-vitamin D status and the following outcomes: maternal Caesarean section and bacterial vaginosis, and offspring birth length, anthropometric measures, and risk of low birth weight. 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 randomised controlled trials with double-blind, placebo-controlled methodology are warranted to investigate the relationship between maternal vitamin D supplementation during pregnancy and the following outcomes: offspring birth weight, calcium concentrations, bone mass, with a weaker recommendation (compared with the appropriateness of high quality prospective observational studies) for offspring asthma and type I diabetes, and maternal pre-eclampsia. There are currently several large randomised controlled trials underway which may help address the study questions. Examples of these include MAVIDOS146 (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.

1. Acknowledgements

We thank Elizabeth Payne for undertaking the initial literature searches, and Shirley Simmonds, Gill Strange and Ruth Fifield for their help with the formatting and checking of the manuscript. We thank the UK Vitamin D in Pregnancy Working Group for their invaluable thoughts and comments. UK Vitamin D in Pregnancy Working Group: Faisal Ahmed (Glasgow), Jeremy Allgrove (Barts and the London), Nicholas Bishop (Chair, Sheffield), Mike Beresford (Liverpool), Christine Burren (Bristol), Chris Carroll (Sheffield), Justin Davies (Southampton), Richard Eastell (Sheffield), Robert Fraser (Sheffield), William Fraser (Norwich), Susan Lanham-New (Guildford), Zulf Mughal (Manchester), Julie Mytton (University of the West of England), Amaka Offiah (Sheffield), Suzy Paisley (Sheffield), Ann Prentice (Cambridge), David Reid (Aberdeen), Nick Shaw (Birmingham), Kate Ward (Cambridge).

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The UK Vitamin D in Pregnancy Working Group has advised on design, methodology, approach to presentation, paediatric and obstetric considerations, and vitamin D physiology.

1. Funding

This review was funded by the National Institute for Health Research Health Technology Assessment Programme (HTA). HTA had no direct involvement in the writing of the review.

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1. Appendices
   1. Appendix 1: Search strategy

* Sources

Completed studies (systematic reviews):

* + DARE (CRD)
  + Cochrane Database of Systematic Reviews (CDSR)
  + HTA database (CRD)

Completed studies (other study types):

* + Cochrane Register of Controlled Trials (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
  + Clinical Trials.gov

Grey literature:

* Conference Proceedings Citation Index- Science (1990-present)
* Zetoc conference search
* Scientific Advisory Committee on Nutrition website
* Department of Health website
* King’s Fund Library database
* Trip database
* HTA website
* HMIC (Health Management Information Consortium database)

|  |  |  |  |
| --- | --- | --- | --- |
| **Databases and years searched** | **Terms** | **Number retrieved** | **Number of relevant hits** |
| Systematic reviews |  |  |  |
| Cochrane Library: CDSR, current Issue, 2010  <http://www.thecochranelibrary.com/view/0/index.html> |  |  |  |
| DARE (CRD) 2000-2010  <http://www.crd.york.ac.uk/crdweb/> |  |  |  |
| HTA Database (CRD)  <http://www.crd.york.ac.uk/crdweb/> |  |  |  |
| National Coordinating Centre for Health Technology Assessment website <http://www.hta.nhsweb.nhs.uk> |  |  |  |
| Other study types |  |  |  |
| Cochrane Library: CENTRAL, current Issue, 2010  <http://www.thecochranelibrary.com/view/0/index.html> |  |  |  |
| Medline (OVID) 1950-2010, June Week 1  (15/6/10) | 1 Pregnan$.ti,ab. 295057  2 Preconception$.ti,ab. 1752  3 preconceptual.ti,ab. 135  4 pre-concept$.ti,ab. 250  5 Fetal.ti,ab. 157883  6 Foetal.ti,ab. 11957  7 Fetus.ti,ab. 43868  8 Foetus.ti,ab. 4543  9 Newborn$.ti,ab. 104312  10 Neonat$.ti,ab. 154612  11 Baby.ti,ab. 21290  12 Babies.ti,ab. 22884  13 Infant.ti,ab. 99951  14 Infancy.ti,ab. 29601  15 Premature.ti,ab. 68207  16 Toddler$.ti,ab. 3913  17 Offspring.ti,ab. 33494  18 Child$.ti,ab. 770655  19 Postnatal.ti,ab. 61090  20 Postpartum.ti,ab. 25159  21 Maternal.ti,ab. 126587  22 Maternity.ti,ab. 10210  23 Mother.ti,ab. 58088  24 small-for-gestational age.ti,ab. 4212  25 pre-natal.ti,ab. 573  26 prenatal.ti,ab. 52711  27 ante-natal.ti,ab. 267  28 post-partum.ti,ab. 6959  29 post-natal.ti,ab. 3777  30 puerperium.ti,ab. 4552  31 childbear$.ti,ab. 6830  32 birthweight.ti,ab. 9667  33 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 1557322  34 Pregnancy/ 609281  35 Prenatal Nutritional Physiological Phenomena/ 695  36 Pregnancy, High-Risk/ 3586  37 Maternal Nutritional Physiological Phenomena/ 988  38 Pregnancy Complications/ 62603  39 Pregnancy Outcome/ 29721  40 Maternal Fetal exchange/ 26212  41 Prenatal Exposure Delayed Effects/ 14989  42 exp "Embryonic and Fetal Development"/ 163222  43 Child Development/ 28583  44 Preconception Care/ 981  45 Prenatal Care/ 16979  46 Postpartum Period/ 14439  47 exp infant/ 817413  48 Postnatal Care/ 3095  49 exp Pregnancy Trimesters/ 27623  50 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 2155617  51 exp Vitamin D/ 34004  52 "1406-16-2 (Vitamin D)".rn. 15518  53 "25(OH)-vit D".ti,ab. 15  54 25OHD.ti,ab. 424  55 hypovitaminosis D.ti,ab. 440  56 "19356-17-3 (Calcifediol)".rn. 2398  57 "32222-06-3 (Calcitriol)".rn. 11536  58 "64719-49-9 (25-hydroxyvitamin D)".rn. 1333  59 Vitamin D deficiency/ 5668  60 Vitamin D.ti,ab. 25020  61 Vitamin D2.ti,ab. 862  62 Vitamin D3.ti,ab. 5527  63 Cacidiol.ti,ab. 0  64 calciol.ti,ab. 12  65 "67-97-0 (Cholecalciferol)".rn. 4441  66 Ergocalciferol.ti,ab. 288  67 Cholecalciferol.ti,ab. 1086  68 Colecalciferol.ti,ab. 21  69 Calciferol.ti,ab. 330  70 Calcitriol.ti,ab. 2923  71 Hydroxycholecalciferol.ti,ab. 1111  72 dihydroxycholecalciferol$.ti,ab. 1366  73 dihydroxyvitamin d.ti,ab. 3858  74 dihydrotachysterol$.ti,ab. 294  75 doxercalciferol$.ti,ab. 48  76 alfacalcidol$.ti,ab. 297  77 paricalcitol$.ti,ab. 180  78 Calcitriol/ 11536  79 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 45279  80 49 and 79 67  81 50 and 79 8116  82 Animals/ 4579351  83 Humans/ 11255304  84 82 and 83 1175867  85 82 not 84 3403484  **86 81 not 85 6501** | 6501 hits | First 500 refs saved  (Ref Ids: 82-581 in Ref Man database) |
| Embase (OVID) 2000-2004, Week 21 |  |  |  |
| BIOSIS 1985- |  |  |  |
| Ongoing studies |  |  |  |
| NRR archive (National Research Register)  <https://portal.nihr.ac.uk/Pages/NRRArchiveSearch.aspx>  (14/6/10) | “Vitamin D” and pregnancy [All fields] | 20 | 0 |
| UKCRN Portfolio  <http://public.ukcrn.org.uk/Search/Portfolio.aspx>  (14/6/10) | Pregnancy [Title]  Pregnancy vitamin [research summary] | 41  2 | 1, poss 2  1 |
| Current Controlled Trials including MRC Trials dB  <http://controlled-trials.com/>  (14/6/10) | vitamin d AND pregnancy | 207 | 13 (slight overlap with UKCRN) |
| Clinical Trials.gov  <http://clinicaltrials.gov/> |  |  |  |
| Conferences and grey literature |  |  |  |
| Conference Proceedings Citation Index- Science (1990-present) |  |  |  |
| Trip database  <http://www.tripdatabase.com/search/advanced> |  |  |  |
| King’s Fund database  <http://www.kingsfund.org.uk/library/>  (14/6/10) | Pregnancy  Vitamin d | 528  15 | Poss 2 |
| Scientific Advisory Committee on Nutrition website  <http://www.sacn.gov.uk/reports_position_statements/index.html>  (14/6/10) | Browse reports and position statements section | 1. 2 report | 2 reports |
| Department of Health website  <http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_4005936>  (14/6/10) | Browse reports |  |  |
| Zetoc (general & conferences)  <http://zetoc.mimas.ac.uk/wzgw?id=23685659> |  |  |  |
| Guidelines |  |  |  |
| SIGN <http://www.sign.ac.uk> |  |  |  |
| NICE  <http://www.nice.org.uk/nice-web/> |  |  |  |
| National Guidelines Clearinghouse <http://www.ahcpr.gov/clinic/assess.htm> |  |  |  |

* 1. Appendix 2: Data extraction forms

DATA EXTRACTION FORMS – CASE CONTROL STUDIES

|  |  |
| --- | --- |
| **a. Study basic details** | |
| UIN / AN |  |
| Title |  |
| Reviewer |  |
| Date reviewed |  |
| Author |  |
| Journal & year |  |
| Source |  |

|  |  |
| --- | --- |
| **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** |
|  |  |
|  |  |
|  |  |
|  |  |
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|  |  |  |
| --- | --- | --- |
| **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)- Vitamin 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:   1. cases 2. controls   (a separate score for each should be given) |  |  |
| 12. Info on representativeness and non-participants |  |  |
| 13. Sample sizes for:   1. cases 2. controls   (a separate score for each should be given) |  |  |
| 14. Adequate consideration for important confounding factors? (eg 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 | | |
|  | | |

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| --- |
| **g. Screen of references –** any additional studies listed which have not already been reviewed? |

DATA EXTRACTION FORMS – INTERVENTIONAL STUDIES

|  |  |
| --- | --- |
| **a. Study basic details** | |
| UIN / AN |  |
| Title |  |
| Reviewer |  |
| Date reviewed |  |
| Author |  |
| Journal & year |  |
| Source |  |

|  |  |
| --- | --- |
| **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 randomized 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 admin/ duration of treatment |  |
| 11. Duration of follow-up |  |

|  |  |
| --- | --- |
| **c. Inclusion criteria** | **d. Exclusion criteria** |
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| --- | --- | --- |
| **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)-vitamin D, bone outcomes eg 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. info on non-participants |  |  |
| 15. Analysis rigorous and appropriate? |  |  |
| 16. Sample size |  |  |
| **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 – CASE CONTROL STUDIES

|  |  |
| --- | --- |
| **a. Study basic details** | |
| UIN / AN |  |
| Title |  |
| Reviewer |  |
| Date reviewed |  |
| Author |  |
| Journal & year |  |
| Source |  |

|  |  |
| --- | --- |
| **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** |
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| --- | --- | --- |
| **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)- Vitamin 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:   1. cases 2. controls   (a separate score for each should be given) |  |  |
| 12. Info on representativeness and non-participants |  |  |
| 13. Sample sizes for:   1. cases 2. controls   (a separate score for each should be given) |  |  |
| 14. Adequate consideration for important confounding factors? (eg 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 | | |
|  | | |

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| --- |
| **g. Screen of references –** any additional studies listed which have not already been reviewed? |

* 1. 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 incl/excl 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)-vitamin D | Blood levels of circulating 25(OH)-vitamin D, with details of precision, pick up of D2 and D3 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, eg bone or muscle pain, wheezing | Ascertained from researcher examination | Objective measure e.g. DXA, 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)-vitamin 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 |
| 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. odds ratios, hazard ratios, relative risks) |
| 11. Response rates for:   1. cases 2. controls   (a separate score for each should be given) | Low (<70%) | Medium (70-90%) or not given | High (>90%) |
| 12. Info 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:   1. cases 2. controls   (a separate score for each should be given) | Extremely ambiguous, not given, or small (under 100) | Average (100 to 1000) | Large (over 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 |

**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 | Incl/excl and other criteria such as term/ pre-term/ small for gestational age baby given in some way; at least two useful measures including measure of vitamin D status, ethnicity | Incl/excl and other criteria such as term/ pre-term/ small for gestational age 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)-vitamin D | Blood levels of circulating 25(OH)-vitamin D, with details of precision, pick up of D2 and D3 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, eg bone or muscle pain, wheezing | Ascertained from researcher examination | Objective measure e.g. DXA, bone biopsy, lung function tests |
| 7. Measures of vitamin D intake/ 25(OH)-vitamin 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? | % FU is not given, unclear, or low (below 70%) | % FU is low to average (70-90%) | % FU is high (over 90%) |
| 11. Info 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 & 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. odds ratios, hazard ratios, relative risks) |
| 13. Sample size | Extremely ambiguous, not given, or small (under 100) | Average (100 to 1000) | Large (over 1000) |

**Table 4- Summary of intervention 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. Are CONSORT guidelines followed? | Not described, not followed or poorly adherent | CONSORT report presented but some data missing | Full adherence to CONSORT guidelines |
| 2. Adequate description of study participants? | Little or no information given | Incl/excl and other criteria such as term/ pre-term/ small for gestational age baby given in some way; at least two useful measures including measure of vitamin D status, ethnicity | Incl/excl and other criteria such as term/ pre-term/ small for gestational age 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 per day” | Full details including D2 or D3, 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 detail- type, manufacturer, precision, D2/D3 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, eg bone or muscle pain, wheezing | Ascertained from researcher examination | Objective measure e.g. DXA, bone biopsy, lung function tests |
| 11. Measures of vitamin D intake/ 25(OH)-vitamin 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 |
| 13. What proportion of the cohort completed the trial? | % FU is not given, unclear, or low (below 70%) | % FU is low to average (70-90%) | % FU is high (over 90%) |
| 14. Info 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 (under 100) | Average (100 to 250) | Large (over 250) |

* 1. Appendix 4: PRISMA Flow Diagram of Study Selection

Studies included in quantitative synthesis (meta-analysis)  
(n = 19)

Studies included in qualitative synthesis   
(n = 76)

Full-text articles excluded, with reasons   
(n =89)

Full-text articles assessed for eligibility   
(n = 165)

Records excluded   
(n = 16,677)

Records screened   
(n = 16,842)

Records after duplicates removed   
(n = 16,842)

Additional records identified through other sources   
(n = 66)

## Identification

## Eligibility

## Included

## Screening

Records identified through database searching   
(n = 25,409)

* 1. 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 m’ment** | **3.**  **Grouping of participants by vitamin D status** | **4.**  **Outcomes reliably ascertained** | **5.**  **Outcomes objective** | **6.**  **Controls** | **7.**  **Rounding** | **8.**  **Setting** | **9.**  **Blinding** | **10.**  **Analysis** | **11.**  **Response rates** | | **12.**  **Non-participants** | **13.**  **Sample size** | | **14. Confounding** | **Overall total** | **Reviewers’ judgement** |
| **cases** | **Controls** | **Cases** | **Controls** |
| **Azar**  **2011** | Low | Low | Low | Med | Low | Med | Med | Med | Med | Low | Low | Low | High | High | High | Low | 5 | **Low** |
| **Baker**  **2010** | Low | Low | Low | Med | Low | Low | Med | Low | Med | Low | Med | Low | Low | High | Med | Low | 9 | **Low** |
| **Baker**  **2011** | Low | Low | High | Med | Low | Med | Med | Low | Med | Low | Low | Low | Med | High | Med | Low | 5 | **Low** |
| **Baker**  **2012** | Low | Low | Med | Low | Low | Med | Med | Med | Med | Low | Low | Low | Med | High | Med | Low | 7 | **Low** |
| **Bodnar**  **2007** | Low | Low | Low | Med | Low | Med | Med | Low | Med | Low | Low | Low | Med | High | Med | Low | 8 | **Low** |
| **Bodnar**  **2010** | Med | Low | Low | Med | Low | Med | Med | Low | Med | Low | Low | Low | Med | Med | Med | Low | 7 | **Low** |
| **Brunvand 1998** | Med | Low | Low | Low | Med | High | Med | Med | Med | Low | Med | Med | Med | High | High | Med | 1 | **Medium** |
| **Delmas**  **1987** | High | Med | Low | High | Med | High | Med | Low | Med | Med | Med | Med | Med | High | High | High | -4 | **High** |
| **Makgoba 2011** | Low | Low | Med | Low | Low | Med | Med | Low | Med | Low | Med | Med | Med | High | Med | Low | 6 | **Low** |
| **Powe**  **2010** | Low | Low | Low | Med | Low | Med | Med | Low | Med | Low | High | High | Med | High | Med | Low | 4 | **Medium** |
| **Robinson**  **2010** | Low | Low | Low | Med | Low | High | Med | Low | Med | Low | Med | Med | ,ed | High | Med | Low | 5 | **Low** |
| **Robinson**  **2011** | Med | Low | Med | Med | Med | Med | Med | Med | Med | Med | Med | Med | Med | Med | Med | Med | 1 | **Medium** |
| **Seely**  **1992** | Low | Med | Low | Medlow | Low | Med | Med | Med | Med | Low | Med | High | Med | High | High | Med | 2 | **Medium** |
| **Soheilykkah 2010** | Low | Low | High | Low | Low | Med | Med | Med | Med | Low | Med | Med | Med | High | Med | Med | 3 | **Medium** |
| **Sorensen 2012** | Low | Low | Low | Med | Med | Med | Med | Low | Med | Low | Low | Low | Med | Med | Med | Low | 8 | **Low** |
| **Stene**  **2003** | Low | High | High | Med | Low | Med | Med | Med | Med | Low | Med | High | Med | Med | Low | Low | 2 | **Medium** |
| **Zhang 2008** | Low | Low | Low | Low | Low | Med | Med | Med | Med | Low | Low | Low | Med | High | Med | Low | 6 | Low |

**\*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**

**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 m’ment** | **4.**  **Grouping of participant by vitamin D status** | **5.**  **Outcomes reliably ascertained** | **6.**  **Outcomes objective** | **7.**  **Rounding** | **8.**  **Confounding** | **9.**  **Blinding** | **10.**  **% FU** | **11.**  **Non-participants** | **12.**  **Analysis** | **13.**  **Sample size** | **Overall total** | **Reviewers’ judgement** |
| **Akcakus 2006** | Med | Low | Low | Low | Med | Low | Med | High | Med | Med | Med | Low | Med | 4 | **Medium** |
| **Amirlak**  **2009** | Med | Low | Med | Low | Med | Low | Med | Med | Med | High | High | Low | High | 2 | **Medium** |
| **Ardawi 1997** | Med | Low | Low | Low | Low | Low | Med | High | Med | Low | Med | Med | Med | 5 | **Low** |
| **Bodnar 2009** | High | Low | Low | Low | Low | Low | Med | High | Med | Low | Med | Low | Med | 5 | **Low** |
| **Bowyer**  **2009** | Low | Low | Low | High | Med | Low | Med | Med | Med | High | Low | Low | Med | 4 | **Medium** |
| **Camargo 2007** | Low | Low | High | Low | Med | High | Med | Low | Med | High | High | Low | Low | 2 | **Medium** |
| **Camargo 2011** | Low | Low | Low | High | High | High | Med | Low | Med | Low | Med | Low | Med | 3 | **Medium** |
| **Clifton-Bligh**  **2008** | Med | Low | Low | Low | Low | Low | Med | Low | Med | Med | High | Low | Med | 6 | **Low** |
| **Cremers 2011** | High | Low | Med | Med | Low | Low | Med | Low | Med | High | Med | Low | Med | 3 | **Medium** |
| **Crozier 2012** | Low | Low | Low | Low | Low | Low | Med | Low | Med | High | Low | Low | Med | 8 | **Medium** |
| **Devereux 2007** | Med | Med | High | Med | Med | High | Med | Low | Med | High | High | Low | Low | -1 | **High** |
| **Dror**  **2012** | Low | Med | Med | Low | Low | Low | Med | Low | Med | Med | Low | Low | Med | 7 | **Low** |
| **Dunlop 2011** | Med | Med | Med | High | Low | Low | Med | Low | Med | High | Med | Low | Med | 2 | **Medium** |
| **Erkkola 2009** | Med | Med | High | Med | Med | High | Med | Med | Med | High | Med | Low | Low | -1 | **High** |
| **Farrant 2009** | Med | Low | Low | Low | Low | Low | Med | Med | Med | High | Med | Low | Med | 5 | **Low** |
| **Fernandez-Alonso**  **2012** | Low | Med | Low | High | Low | Low | Med | High | Med | Low | Med | Med | Med | 3 | **Medium** |
| **Gale**  **2008** | Med | Low | Low | High | Low | Low | Med | Med | Med | Med | Med | Low | Med | 4 | **Medium** |
| **Hensel**  **2011** | Med | High | Low | High | Low | Low | Med | Low | Med | Low | Med | Low | Med | 4 | **Medium** |
| **Haugen 2009** | Med | Low | High | High | Med | Low | Med | Low | Med | Med | High | Low | Low | 2 | **Medium** |
| **Hossain 2011** | Med | Low | Low | Low | Med | Med | Med | Med | Med | Med | Med | Low | Med | 4 | **Medium** |
| **Javaid**  **2006** | Low | Low | Low | Med | Low | Low | Med | Med | Med | High | Med | Low | Med | 5 | **Low** |
| **Krishnaveni 2011** | Med | Med | Low | Low | Low | Low | Med | Med | Med | Med | High | Low | Med | 4 | **Medium** |
| **Leffelaar**  **2010** | Low | Low | Low | High | Med | Low | Med | Low | Med | High | Med | Low | Low | 5 | **Low** |
| **Maghbooli**  **2007** | Med | High | Low | Low | Med | Med | Low | High | Med | Low | High | Med | Med | 1 | **Medium** |
| **Maghbooli 2008** | Med | Low | Low | Med | Low | Low | High | High | Med | Low | High | Med | med | 3 | **Medium** |
| **Mannion**  **2006** | Med | Low | High | Low | Med | Med | Med | Med | Med | High | High | Low | Med | 1 | **Medium** |
| **Marjameki 2010** | Med | Low | High | Low | Low | Low | Med | Med | Med | Med | Low | low | Low | 6 | **Low** |
| **Mehta 2009** | Med | Med | Med | Med | Med | Med | Med | Med | Med | Med | Low | Low | Med | 2 | **Medium** |
| **Merewood 2009** | Med | Low | Med | High | Low | Low | Med | Low | Med | Low | Low | Low | Med | 6 | **Low** |
| **Miyake 2010** | Med | Med | High | Med | Med | High | Med | Low | Med | Med | High | Low | Med | -1 | **High** |
| **Morales**  **2012** | Low | Low | Med | Low | High | High | Med | Low | Medium | High | Med | Low | Low | 3 | **Medium** |
| **Morley 2006** | Med | Low | Low | Low | Low | Low | Med | Low | Med | Med | Low | Low | Med | 8 | **Low** |
| **Nwaru 2010** | Med | Med | High | Low | Low | Low | Med | Low | Med | Med | High | Low | Med | 3 | **Medium** |
| **Oken**  **2007** | Med | Low | High | Low | Med | low | Med | Low | Med | Med | Low | Low | Low | 6 | **Low** |
| **Prentice 2009** | Med | Low | Low | Low | Low | Low | Med | Low | Med | High | High | low | med | 5 | **Low** |
| **Rothers 2011** | Low | Med | Med | High | Low | Low | Med | Med | Med | High | Med | Low | Med | 2 | **Medium** |
| **Sabour**  **2006** | Med | Low | High | High | Med | Med | Med | High | Med | Med | High | Low | Med | -2 | **High** |
| **Savvidou 2012** | Low | Low | Low | Med | Low | Low | Med | Low | Med | Low | Med | Med | Med | 7 | **Low** |
| **Sayers**  **2009** | Low | Med | High | Low | Low | Low | Low | High | Med | High | High | Low | Low | 3 | **Medium** |
| **Scholl**  **2008** | Med | Low | High | Med | Low | Med | Low | Med | Med | High | High | Low | Low | 2 | **Medium** |
| **Scholl**  **2012** | Med | Low | Low | High | Low | Low | Med | Low | Med | High | Med | Low | Low | 5 | **Low** |
| **Shand**  **2010** | Med | Low | Low | High | Med | Low | Med | low | Med | Low | Low | Low | Med | 6 | **Low** |
| **Shibata 2011** | Low | Med | Low | Low | Med | Med | Med | Med | Med | Med | Med | Low | Med | 4 | **Medium** |
| **Viljakainen 2010** | Med | Low | Low | Med | Low | Low | Med | Med | Med | High | High | Low | Med | 3 | **Medium** |
| **Viljakainen 2011** | Med | Med | Low | Med | Low | Low | Med | Low | Med | High | Low | Low | High | 4 | **Medium** |
| **Watson**  **2010** | Med | Low | High | Low | Med | Low | Med | Low | Med | Med | High | Low | Med | 3 | **Medium** |
| **Weiler 2005** | Low | Med | Low | High | Low | Low | Med | Low | Med | High | Med | Low | High | 3 | **Medium** |

**\*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**

**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.**  **Random-isation** | **5.**  **Placebo control and blinding** | **6.**  **Study med details** | **7.**  **Maternal 25(OH)D** | **8.**  **Assay detail** | **5.**  **Outcomes reliably ascertained** | **6.**  **Outcomeobjective** | **7.**  **Rounding** | **8.**  **Confounding** | **10.**  **% FU** | **11.**  **Non-participant** | **12.**  **Analysis** | **13.**  **Sample size** | **Overall total** | **Reviewers’ judgement** |
| **Brooke 1980** | Med | High | Med | Med | Low | Med | Low | Med | Med | Med | Med | Med | High | High | Med | High | -2 | High |
| **Cockburn 1980** | Med | High | High | High | Med | Med | Low | Med | Low | Low | Med | Low | High | High | Med | Med | -1 | High |
| **Congdon 1983** | Med | High | High | High | High | Med | High | Med | High | Med | Med | Med | High | High | Med | High | -9 | High |
| **Delvin 1986** | Low | High | High | Med | High | Med | Low | Med | Low | Low | Med | Med | High | High | Med | High | -2 | High |
| **Hollis**  **2011** | Low | Low | Med | Med | Med | Low | Low | Low | Low | Low | Med | Low | Low | Med | Low | Med | 10 | Low |
| **Kaur**  **1991** | Med | High | Med | Med | High | Med | Med | Med | High | Med | Med | High | High | High | Med | High | -7 | High |
| **Marya 1981** | Med | High | High | Med | High | Med | Med | High | Med | Low | Med | High | High | High | Med | Med | -6 | High |
| **Marya 1987** | Med | High | High | Med | High | Med | Med | Med | Med | Low | Med | High | Low | High | Med | Low | -2 | High |
| **Marya 1988** | Med | High | Low | Med | High | Med | Med | High | Med | Med | Low | Low | High | High | Med | Med | -2 | High |
| **Mallet**  **1986** | Med | High | High | Med | High | Med | Med | Low | Med | Low | Med | Med | High | High | Low | high | -3 | High |
| **Yu**  **2009** | Low | Low | Med | Low | High | Med | Low | High | Med | Low | Med | High | Low | Med | Med | Med | 3 | Medium |

**\*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**

* 1. Appendix 6: Study assessments

**Table 8: The effect of maternal Vitamin D status in gestation on offspring birth weight (BW) - Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and 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)** | **Birth weight (g) mean (SD) or median (IQR)** | | | | | | | | | | | | | **Unadjusted regression co-efficient β (95% CI) for BW (g) per 1nmol/l increase in 25(OH)D** | **Adjusted regression co-efficient β (95% CI) for BW (g) per 1 nmol/l increase in 25(OH)D** | **Conclusion** |
| **Ardawi, 199787** | 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% |  | | | | 25(OH)D <20 nmol/l  (n=24) | | | | | | 25(OH)D >20 nmol/l (n=240 | | | Not given | Not given | No difference in offspring BW in mothers with 25(OH)D <20 nmol/l at delivery compared to those with 25(OH)D >20 nmol/l |
| BW | | | | 3323 (439) | | | | | | 3481 (410) | | |
| **Weiler, 200586** | 3 (med) | Winnipeg, Canada  Sample size for analysis=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 2 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) |  | | | | 25(OH)D <37.5 nmol/l  (n=18) | | | | | | 25(OH)D ≥37.5 nmol/l  (n=32) | | | Not given | Not given | Offspring BW in mothers with 25(OH)D ≥37.5 nmol/l significantly lower than in mothers with 25(OH)D <37.5 nmol/l  p=0.022 |
| BW | | | | 3698 (380) | | | | | | 3399 (451) | | |
| **Mannion, 200683** | 1 (med) | Calgary, Canada  n=279 women,  207 women restricted milk intake (≤250ml milk) which equates to ≤90 IU vitamin D and 72 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. 3 or 4 times during pregnancy (1 cup of milk = 90 IU vitamin D) | In those not restricting milk, Vitamin D intake= 524 (180) IU/day  In those restricting milk, <2.25mcg/day per day, vitamin D intake=316 (188) IU/day | In those not restricting milk, BW=3530 (466)  In those restricting milk, BW=3410 (475)  p (diff. between groups) =0.07 | | | | | | | | | | | | | Not given | Not given  β for each 40 IU/day increase in vitamin D intake = 10.97 (1.19, 20.75) p=0.029 | Vitamin D intake in pregnancy is positively associated with offspring BW |
| **Morley, 200691** | 8 (low) | Melbourne, Australia  n=374 women (232 recruited in winter, 127 in summer) | Cohort | Sex, maternal height, whether first child, smoking, season of blood sample | 11 weeks and 28-32 weeks | Winter recruitment, geometric mean at 11 wks= 49.2; 26-32 wks=48.3  Summer recruitment geometric mean at 11 weeks= 62.6; 26-32 wks=68.9 | 3540 (520) | | | | | | | | | | | | | At 28-32 wks β for every Log2 increase in 25(OH)D =  40 (-39-119) | At 28-32 wks β for every Log2 increase in 25(OH)D =  31 (-51, 112) | No significant association seen between Log 25(OH)D at 11 wks (data not given) or 28-32 wks and offspring birth weight |
|  | 25(OH)D  <28 nmol/) at 28-32 wk | | | | 25(OH)D >28 nmol/l at 28-32 wk | | | Diff | | | | Adj Diff |
| BW | 3397 (57) | | | | 3555 (52) | | | -157 | | | | -153 |
| **Sabour, 200688** | -2 (high) | Tehran, 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 | Overall group mean (SD) | | | | | 3190 (450) | | | | | | | | Not given | Not given | No significant association seen between vitamin D intake and birth weight  p=0.53 |
| Vit D intake <200 IU/day | | | | | 3150 (480) | | | | | | | |
| Vit D intake >200 IU/day | | | | | 3190 (440) | | | | | | | |
| **Magbooli, 200789** | 1 (med) | Tehran, Iran  n=552 women | Cross-sectional | None | Delivery\* | 27.82 (10.86)\* | 3190 (225) | | | | | | | | | | | | | Not given | Not given | No significant association seen between serum 25(OH)D3 and birth weight. p not given |
| **Clifton-Bligh, 200892** | 6 (low) | New South Wales, Australia  n=307 women (included 81 women with GDM) | Cohort | Gestational age | Mean (SD) 28.7 (3.3) weeks | 53.8 (23.9) | Not given | | | | | | | | | | | | | Not given | Not given | No association between maternal 25(OH)D and offspring birth weight  p>0.4 |
| **Harvey , 200864** |  | Southampton Women’s Survey  n=604 women | Cohort | Gestational age, maternal age, maternal BMI, parity | 34 weeks |  | 3506 (441) | | | | | | | | | | | | | β per Log 25(OH)D increase = 31.59 (-44.19, 107.36)  p=0.42 | β per Log 25(OH)D increase = 68.27 (-7.16, 143.71)  p=0.08 | No significant association seen between maternal serum Log 25(OH)D and offspring birth weight |
| **Gale, 200825** | 4 (med) | Princess Anne Cohort, Southampton, 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 | Divided into quartiles 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, 21.7)  p=0.247 | β per Log 25(OH)D increase =  52.9 (-14.4, 120.3)  p=0.123 | No significant association seen between maternal serum Log 25(OH)D and offspring birth weight |
| **Farrant, 200990** | 5 (low) | Mysore Parthenon Study, India  n=559 women (included 34 women with GDM) | Cohort | Maternal age, fat mass, diabetes 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 | Geometric mean (IQR) = 2900 (400) | | | | | | | | | | | | | β per Log 25(OH)D increase=  -26.82  (-79.28, 25.65)  p=0.32 | β per Log 25(OH)D increase=  -72.47  (-195.82, 50.88)  p=0.25 | No association seen between late pregnancy maternal Log serum 25(OH)D and offspring birth weight when data analysed both continuously or dividing the group into categories using 25(OH)D <50nmol/l as a threshold (p=0.8) |
| **Scholl, 200984** | 2  (med) | The Camden Study, New Jersey, 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 | 3196 (12.77) | | | | | | | | | | | | | Not given | Not given | Positive association seen between vitamin D intake and birthweight  p for trend = 0.043 (after adjustments)  When comparing birth weight in those with intake of <200 IU /day (inadequate intake) to those >200 IU/day (adequate intake , p=0.0270 (after adjustments) |
| Vitamin D intake  (IU/day) | | | | | | BW | | | | | | |
| <285 | | | | | | 3163(21) | | | | | | |
| 285-368 | | | | | | 3187(20) | | | | | | |
| 368-440 | | | | | | 3193(19) | | | | | | |
| 440-535 | | | | | | 3207(19) | | | | | | |
| >535 | | | | | | 3228(23) | | | | | | |
| **Amirlak, 200980** | 2 (med) | UAE  n=84 healthy Arab and South Asian women with uncomplicated term deliveries | Cross-sectional | Cord blood vitamin A, Maternal ferritin | Delivery | 18.5 (11.0, 25.4) | 3317 (510) | | | | | | | | | | | | | Unadjusted β not given  Unadjusted r= 0.23; p<0.05 | 11.6 (3.0-20.1)  P=0.009 | Positive correlation seen between maternal 25(OH)D at delivery and birthweight.  For every 1 unit increase in 25(OH)D, birth weight increased by 11.6 g |
| **Bowyer, 200981** | 4 (med) | Sydney, Australia  n=971 women | Cohort | Gestation, maternal age, overseas maternal birth place | 30-32 weeks | 52.0 (17, 174)  Median Vit D concentration according to group:  Vit D ≤25 nmol l (n=144)= 18 (17, 22)  Vit D 26-50 (n=317) = 39 (32, 45)  Vit D >50 (n=510) = 73 (60-91) | 25(OH)D nmol/l | | Unadjusted birth weight | | | | | | | Adjusted birth weight | | | | Not given | Not given | Offspring birth weight 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 (90-305) | | | | | | | 151 (50-250) | | | |
| **Prentice, 200995** | 5 (low) | Gambia, Africa  Subset of pregnant Gambian women participating in a calcium supplementation trial  n=125 women | Cohort | Season, mat height, weight, weight gain, infant sex and whether received calcium supplement | 20 weeks and 36 weeks | 20 weeks=103 (25)  36 weeks=111 (27) | | | 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 birth weight when analysed both continuously and categorically (25(OH)D >80 nmol/l vs <80 nmol/l) |
| **Sayers, 200942** | 3  (med) | Avon Longitudinal Study of Parents and Children (ALSPAC), UK  n=13904 women |  | Nil | Not directly measured  Ambient UVB measured during 98 days preceding birth |  | | | Boys (n=7192)=3429 (608)  Girls (n=6722) =3327 (550) | | | | | | | | | | | 1.46 (-8.14, 11.06)  p=0.77 |  | No association between UVB exposure in 3rd trimester and birth weight |
| **Leffelaar, 201082** | 4  (med) | Amsterdam Born Children and their development (ABCD) study  cohort=3730 women , all term offspring (37 wks) | 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 (median 40.4); <29.9 (median 19.9) | | | Overall=3515.6 (489.1) | | | | | | | | | | | 1.404  (0.893, 1.916) | 0.068  (-0.483, 0.619) | When analysed continuously, no significant relationship observed between maternal early pregnancy 25(OH)D and offspring birth weight.  When analysed according to categories of 25(OH)D status, deficient vitamin D status (<29.9 nmol/l) was significantly associated with a lower birth weight.  Adjusted β=-64 (-107.1, -20.9)  Insufficient vitamin D (30-49.9 nmol/l)was not significantly associated with birthweight  Adjusted β= 1 (-35.1, 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) | | | | | | |
| **Watson, 201085** | 3 (med) | 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 pre-schoolers, 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 | | | 3551 (544) | | | | | | | | | | | Not given | Not given | Vitamin D intake at 4 months is positively associated with Log (Vitamin D).  pP=0.015  No significant association seen at 7 months  p value not given |
| **Viljakainen, 201094** | 3 (med) | Helsinki, Finland  n=125 women recruited during last trimester (Oct-Dec). All Caucasian, non-smokers, primiparous | Cohort | Parental size, maternal wt gain in pregnancy, solar exposure, total intake of vitamin D and initial 25(OH)D conc. | First trimester (8-10 weeks) and 2 days post-partum. Mean of 2 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 | | |  | 25(OH)D below median (42.6 nmol/l) | | | | 25 (OH)D above median (42.6 nmol/l) | | | | P (diff. between means) | | Not given | Not given | No significant difference in offspring birth weight or z-score birth weight if maternal 25(OH)status below median compared to above (median=42.6 nmol/l)  A weak inverse correlation was observed with postpartum 25(OH)D and birth weight z-score (r= -0.193, p=0.068). This was further weakened after adjustment for confounders (p=0.07) |
| BW (g) | 3700 (400) | | | | 3520 (440) | | | | 0.052 | |
| BW z-score | 0.12 (0.81) | | | | -0.23 (1.09) | | | | 0.082 | |
| **Dror, 201293** | 7 (low) | Oakland California  n=120 women | Cross-sectional | Gestational age, maternal age, maternal BMI, maternal height, ethnicity, parity, GDM | Peri-natal | 75.5 (32.3) | | | 3420 (542) | | | | | | | | | | | -0.63  (-3.68-2.43)  p=0.69 | -1.79  (-4.57-0.98)  p=0.20 | No association seen between maternal serum 25(OH)D and offspring birth weight |

\* Measured 25(OH)D3

**Table 9: The effect of Vitamin D supplementation in gestation on offspring birth weight (BW) – 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)/ Mean (SE)\* or median (IQR) maternal**  **25(OH)D concentration (nmol/l)** | | | **Mean (SD) or Mean (SE)\* birth weight (g) in un-supplemented group** | **Mean (SD) or Mean (SE)\* birth weight (g) in supplemented group** | **Conclusion** |
| **Brooke, 19804** | -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) | 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)\* | | | 3034 (64) | 3157 (61) | No significant difference in BW between groups  p>0.05 |
| **Marya, 19815** | -6 (high) | Rohtak, India  n=120 women | 3 arms:  Randomised to either no supplement (n=75) or 1,200 IU vitamin D + 375 mg calcium/ day∆  throughout the 3rd trimester (n=25); or oral 600,000 IU vitamin D2; 2 doses in 7th and 8th months gestation (n=20) | Nil | Not measured | Not measured | | | 2730 (360) | 1200IU/+ ca=2890 (320)  600,000 IU=3140 (450) | BW significantly higher in those taking supplements and highest in the 600,000 IU group  p=0.05 for un-supplemented vs. 1200 IU group  p=0.001 for non-supplemented vs. 600,000 IU group |
| **Congdon, 198322** | -9 (high) | Leeds, UK  n=64, all Asian women | Either 1000 IU vitamin D plus calcium (calcium dose not given) daily in the 3rd trimester (n=19) or no supplement (n=45) | Nil, but groups similar in terms of maternal age, infant sex, gestation length, birth weight | Not measured | Not measured | | | 3056 (59)\* | 3173 (108)\* | No significant difference in BW between the two groups  (p value not given) |
| **Delvin, 19867** | -2 (high) | Lyon, France  n=40 women | Randomised to either no supplement (n=20) or 1000 IU vitamin D3/day during 3rd trimester (n=20) | Nil  Groups similar in terms of maternal age and parity. All deliveries occurred in the same month (June) | At recruitment and at delivery |  | Mean (SD) 25(OH)D in suppl. group | Mean (SD) 25(OH)D in un-sup group | Not given | Not given | No significant difference in BW between the 2 groups  (p value not given) |
| At recruit-ment | 54.9 (10.0) | 27.5  (10.0) |
| Delivery | 64.9 (17.5) | 32.4 (20.0) |
| **Mallet, 19868** | -3 (high) | Rouen, France  n=77, all white women | 3 arms:  Randomised to either no supplement (n=29) or 1,000 IU vitamin D/day∆ in last 3 months of pregnancy (n=21), or single oral dose of vitamin D∆ 200,000 IU in 7th month (n=27) | Nil, but groups of similar maternal age, parity, calcium intake and frequency of outdoors outings | During labour (February and March) | Overall mean not given  According to group:  Un-supplemented=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 BW between the 3 groups  p value not given |
| **Marya, 19886** | -2 (high) | Rohtak, India  n=200 women | Randomised to either no supplement (n=100) or oral 600,000 IU vitamin D3; 2 doses in 7th and 8th 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:  Un-supplemented=35.71 (6.17) IU/day  Supplemented group=35.01 (7.13) IU/day | | | 2800 (370) | 2990 (360) | BW significantly higher in the supplemented group  p<0.001 |
| **Kaur, 199198** | -7 (high) | Rohtak, India  n=50 women | Randomised to either no supplement (n=25) or oral 60,000 IU vitamin D3; 2 doses in 6th and 7th month gestation (n=25) | Nil, but groups had similar maternal age, maternal weight, length of gestation, parity and haemoglobin | Not measured | Not measured | | | 2756 (60)\* | 3092 (90)\* | BW significantly higher in the supplemented group  p<0.001 |
| **Yu, 200996** | 5 (low) | London, UK  n=179 women | 3 arms  Randomised to either no supplement (n=59) or oral vitamin D2 800 IU/day vitamin 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 4 ethnic groups (Caucasian, Black, Asian, Middle Eastern) | Nil  No significant difference in baseline characteristics across the 3 groups | Measured at 26-27 weeks and again at delivery |  | 27 wks | Delivery | Not given | Not given | No significant difference in BW across the 3 groups |
| No sup | 25  (21-38) | 27  (27-39) |
| 800 IU daily | 26  (20-37) | 42  (31-76) |
| single sup | 26  (30-46) | 34  (30-46) |
| **Hollis, 201197** | 10 (low) | Charleston, USA | 3 arms  Randomised to either oral vitamin D3 400 IU/day (n=111) or 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 |  | Mean of measurements between  20-36 weeks | delivery | No un-supplemented group. All groups received some form of vitamin D3 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 BW across the 3 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) |

∆ = not known whether supplementation was vitamin D2 or vitamin D3

**Table 10: The effect of maternal vitamin D status in gestation on offspring birth length– Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and 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)** | **Mean (SD) or median (IQR) birth length (cm)** | | | | | | | | | | | | **Unadjusted regression co-efficient β (95% CI) for birth length (cm) per 1nmol/l increase in 25(OH)D** | **Adjusted regression co-efficient β (95% CI) for birth length (cm) per 1 nmol/l increase in 25(OH)D** | **Conclusion** |
| **Ardawi, 199787** | 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% |  | | | | 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 in mothers with 25(OH)D <20 nmol/l at delivery compared to those with 25(OH)D >20nmol/l |
| Birth length (cm) | | | | 51.7 (2.9) | | | | | 51.0 (2.4) | | |
| **Sabour, 200688** | -2 (high) | Tehran, 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 | 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 compared to those with inadequate intake  p=0.03 |
| Vit D intake <200 IU/day | | | | | | | 49.5 (3.77) | | | | |
| Vit D intake >200 IU/day | | | | | | | 50.37 (2.73) | | | | |
| **Mannion, 200683** | 1 (med) | Calgary, Canada  n=279 women,  207 women restricted milk intake (≤250ml milk) which equates to ≤90 IU vitamin D and 72 not restricting milk intake | Cohort |  | Not measured directly  Repeat 24 hour dietary telephone recall. 3 or 4 times during pregnancy (1 cup of milk = 90 IU vitamin D) | | In those not restricting milk, Vitamin D intake= 524 (180) IU/day  In those restricting milk, <2.25mcg/day per day, vitamin D intake=316 (188) IU/day | In those not restricting milk, unadjusted birth length= 51.4 (3.6)  In those restricting milk, unadjusted birth length= 51.1 (3.5)  P (diff. between groups)=0.46 | | | | | | | | | | | | Not given | Not given | No difference in offspring birth length in mothers restricting milk intake in pregnancy compared to those with unrestricted intake |
| **Morley, 200691** | 8 (low) | Melbourne, Australia  n=374 women (232 recruited in winter, 127 in summer) | Cohort | Sex, maternal height, whether first child, smoking, season of blood sample | 11 weeks and 28-32 weeks | | Winter recruitment, geometric mean at 11 wks=49.2; 26-32 wks=48.3  Summer recruitment geometric mean at 11 weeks=62.6; 26-32 wks=68.9 |  | 25(OH)D<28 (nmol/l) at 28-32 wk | | | 25(OH)D >28 (nmol/l) at 28-32 wk | | | | Diff (95% CI) | | | Adj Diff (95% CI) | At 28-32 wks β for every Log2 increase in 25(OH)D =  -0.3 (-0.08-0.6) | At 28-32 wks β for every Log2 increase in 25(OH)D =  -0.3 (-0.1-0.6)) | No significant association seen between Log 25(OH)D at 11 wks (data not given) or 28-32 wks and offspring birth length |
| BL | 49.8 (2.7) | | | 50.4 (2.4) | | | | -0.6  (-1.5-0.3) | | | -0.6  (-1.5-0.3) |
| **Magbooli, 200789** | 1 (med) | Tehran, Iran  n=552 women | Cross-sectional | None | Delivery\* | | 27.82 (21.71)\* | 50.02 (1.58) | | | | | | | | | | | | Not given | Not given | No significant association seen between serum 25(OH)D3 and offspring birth length  p not given |
| **Clifton-Bligh, 200892** | 6 (low) | New South Wales, Australia  n=307 women (included 81 women with GDM) | Cohort | Gestational age | Mean (SD) 28.7 (3.3) weeks | | 53.8 (23.9) | Not given | | | | | | | | | | | | Not given | Not given | No association between maternal 25(OH)D and offspring birth length  p>0.4 |
| **Gale, 200825** | 4 (med) | Princess Anne Cohort, Southampton, 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 >50nmol/l  28.3% had levels 27.5-50 nmol/l  21.1% had levels <27.5 nmol/l | Not given | | | | | | | | | | | | β per Log 25(OH)D increase =  0.23 (-0.09, 0.54)  p=0.150 | β per Log 25(OH)D increase =  0.18 (-0.10, 0.46)  p=0.215 | No association seen between maternal serum 25(OH)D and offspring birth length |
| **Farrant, 200990** | 5 (low) | Mysore Parthenon Study, India  n=559 women (included 34 women with GDM) | Cohort | Maternal age, fat mass, diabetes status | 30 (+/- 2) weeks | | 37.8 (24.0-58.5)  60% of women ha d 25(OH)D <50 nmol/l, 31% below 28 nmol/l | Geometric mean =48.9 (2.2) | | | | | | | | | | | | β per Log 25(OH)D increase= -0.07 (-0.34, 0.20)  p=0.6 | β per Log 25(OH)D increase= -0.27 (-0.80, 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 <50nmol/l as a threshold (p=0.9) |
| **Prentice, 200995** | 5 (low) | Gambia, Africa  Subset of pregnant Gambian women participating in a calcium supplement trial  n=125 women | Cohort | Season, mat height, weight, weight gain, infant sex and whether received calcium supplement | 20 weeks and 36 weeks | | 20 weeks = 103 (25)  36 weeks = 111 (27) | 50.5 (1.9)\* | | | | | | | | | | | | 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) |
| **Sayers, 200942** | 3  (med) | ALSPAC, cohort, UK  n=10584 women | Cohort | Nil | | Not directly measured  Ambient UVB measured during 98 days preceding birth | Not measured | Boys (n=5447)=50.93 (2.61)  Girls (n=5140)=50.19 (2.44) | | | | | | | | | | | | β per 1 SD increase in UVB 0.10 (0.05-0.15)  p=0.00004 | No adjustments made | Maternal UVB exposure in late pregnancy is positively associated with offspring birth length |
| **Leffelaar, 201082\*\*** | 4  (med) | Amsterdam Born Children and their development (ABCD) study  cohort=3730 women, all term offspring (≥37 wks) | 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 (median 40.4)  Deficient ≤29.9 (median (19.9) |  | | All | | | 25(OH)D≤29.9 | | | 25(OH)D 30-49.9 | | | 259OH)D ≥50 | 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 |
| Unadj  Length  at 1  month | | 54.8  (0.05) | | | 54.2  (0.09) | | | 54.8  (0.10) | | | 55.1  (0.06) |
| **Viljakainen,201094** | 3 (med) | Helsinki, Finland  n=125 women recruited during last trimester (Oct-Dec). All Caucasian, non-smokers, primiparous | Cohort | Parental size, maternal wt gain in pregnancy, solar exposure, total intake of vitamin D and initial 25(OH)D conc. | First trimester (8-10 weeks) and 2 days post-partum. Mean of 2 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 |  | | | 25(OH)D below median (42.6 nmol/l) | | | 25 (OH)D above median (42.6 nmol/l) | | | | P (diff. 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 to above (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 |
| Unadj. Birth length (cm) | | | 51.0 (1.9) | | | 50.5 (1.8) | | | | 0.140 | |
| Unadj. z-score birth length | | | 0.14 (1.0) | | | -0.20 (0.96) | | | | 0.104 | |
| **Dror, 201293** | 7 (low) | Oakland California  n=120 women | Cross-sectional | Gestational age, maternal age, maternal BMI, maternal height, ethnicity, parity, GDM | | Peri-natal | 75.5 (32.3) | Not given | | | | | | | | | | | | -0.004  p=0.53 | -0.009 (-0.022-0.004)  p=0.18 | No association seen between maternal serum 25(OH)D and offspring birth length |

\*Measured 25(OH)D3

\*\*Measured when infant was 1 month old

**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)\* birth length (cm) in un-supplemented group** | **Mean (SD) or Mean (SE)\* birth length (cm) in supplemented group** | **Conclusion** |
| **Brooke, 19804** | -2 (high) | London, UK, n=126 women(all Asian) | Double-blinded  Randomised to either placebo (n=67) or 1000 IU/day of vitamin D2 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)\* | 49.7 (0.3)\* | No significant difference in birth length between groups  p>0.05 |
| **Marya, 19886** | -2 (high) | Rohtak, India | Randomised to either no supplement (n=100) or oral 600,000 IU vitamin D3; 2 doses in 7th and 8th 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  Un-supplemented = 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 |

**Table 12: The effect of maternal vitamin D status in gestation on offpring head circumference (HC) – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and 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)** | **Mean (SD) or median (IQR) HC (cm)** | | | | | | | | | | | **Unadjusted regression co-efficient β (95% CI) for HC (cm) per 1nmol/l increase in 25(OH)D** | **Adjusted regression co-efficient β (95% CI) for HC (cm) per 1 nmol/l increase in 25(OH)D** | **Conclusion** |
| **Ardawi, 199787** | 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% |  | | | 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 in mothers with 25(OH)D <20 nmol/l at delivery compared to those with 25(OH)D >20nmol/l |
| HC (cm) | | | 34.8 (1.3) | | | | | 34.11 (1.46) | | |
| **Mannion, 200683** | 1 (med) | Calgary, Canada  n=279women, 207 women restricted milk intake (≤250ml 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. 3 or 4 times during pregnancy (1 cup of milk = 90 IU vitamin D) | In those not restricting milk, Vitamin D intake= 524 (180) IU/day  In those restricting milk, <2.25mcg/day per day, vitamin D intake=316 (188) IU/day | In those not restricting milk, unadjusted HC= 34.6 (1.5)  In those restricting milk, unadjusted HC= 34.3 (1.5)  P (diff. between groups)=0.19 | | | | | | | | | | | Not given | Not given | No difference in offspring HC in mothers restricting milk intake in pregnancy compared to those with unrestricted intake |
| **Morley, 200691** | 8 (low) | Melbourne, Australia  n=374 women (232 recruited in winter, 127 in summer) | Cohort | Sex, maternal height, whether first child, smoking, season of blood sample | 11 weeks and 28-32 weeks | Winter recruitment, geometric mean at 11 wks= 49.2; 26-32 wks=48.3  Summer recruitment geometric mean at 11 weeks= 62.6; 26-32 wks=68.9 | HC | 25(OH)D  <28 (nmol/l) at 28-32 wk | | | 25(OH)D ≥28 (nmol/l) at 28-32 wk | | | Diff | | | Adj. Diff | At 28-32 wks β for every Log2 increase in 25(OH)D = -0.02 (-0.2, 0.2) | At 28-32 wks β for every Log2 increase in 25(OH)D = -0.05 (-0.3, 0.2) | No significant association seen between Log 25(OH)D at 11 wks (data not given) or 28-32 wks and offspring HC |
|  | 34.5 (1.5) | | | 34.7 (1.5) | | | -0.2 | | | -0.2 |
| **Sabour,200688** | -2 (high) | Tehran, 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 | Overall group mean (SD) | | | | | | 34.81 (6.55) | | | | | Not given | Not given | No significant association seen between maternal vitamin D intake and offspring HC  P=0.47 |
| Vit D intake <200 IU/day | | | | | | 34.51 (2.66)) | | | | |
| Vit D intake >200 IU/day | | | | | | 35.19 (10.38 | | | | |
| **Magbooli, 200789** | 1 (med) | Tehran, Iran  n=552 women | Cross-sectional | None | Delivery\* | 27.82 (21.71)\* | Not given | | | | | | | | | | | Not given | Not given | No significant association seen between serum 25(OH)D3 and offspring HC.  p not given |
| **Clifton-Bligh, 200892** | 6 (low) | New South Wales, Australia  N=307 women (included 81 women with GDM) | Prospective cohort | Gestational age | Mean (SD) 28.7 (3.3) weeks | 53.8 (23.9) | Not given | | | | | | | | | | | Not given | Not given | No association between maternal 25(OH)D and offspring HC  p>0.4 |
| **Gale, 200825** | 4 (med) | Princess Anne Cohort, Southampton, 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 >50nmol/k  28.3% had levels 27.5-50 nmol/l  21.1% had levels <27.5 nmol/l | Not given | | | | | | | | | | | β per Log 25(OH)D increase =  0.06 (-0.14, 0.26)  p=0.557 | β per Log 25(OH)D increase =  0.06 (-0.13, 0.25)  p=0.530 | No association seen between maternal serum 25(OH)D and offspring HC |
| **Farrant, 200990** | 5 (low) | Mysore Parthenon Study, India  n=559 women (included 34 women with GDM) | cohort | Maternal age, fat mass, diabetes status | 30 (+/- 2) weeks | 37.8 (24.0-58.5)  60% of women had 25(OH)D <50 nmol/l, 31% below 28 nmol/l | 53.40 (1.53) | | | | | | | | | | | β per Log 25(OH)D increase=  -0.002  (-0.19-0.19)  P=0.98 | β per Log 25(OH)D increase=  -0.01 (-0.41-0.39)  P=0.96 | No association seen between late pregnancy maternal Log serum 25(OH)D and offspring HC at birth |
| **Prentice, 200995** | 5 (low) | Gambia, Africa  Subset of pregnant Gambian women participating in a calcium supplementation trial  n=125 women | Cohort | Season, mat height, weight, weight gain, infant sex and whether received calcium supplement | 20 weeks and 36 weeks | 20 weeks =103 (25)  36 weeks =111 (27) | 35.5 (1.6)\* | | | | | | | | | | | -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 |
| **Viljakainen, 201094** | 3 (med) | Helsinki, Finland  n=125 women recruited during last trimester (Oct-Dec). All Caucasian, non-smokers, primiparous | Cohort | No adjustments made for HC | First trimester (8-10 weeks) and 2 days post-partum. Mean of 2 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 | HC (cm) | | 25(OH) below median (42.6 nmol/l) | | | 25 (OH)D above median (42.6 nmol/l) | | | | P (diff. between means) | | Not given | Not given | No significant difference in offspring HC if maternal 25(OH) below median compare to above (median=42.6 nmol/l) |
|  | | 35.7 (1.4) | | | 35.5  (1.6) | | | | 0.511 | |
| **Dror, 201293** | 7 (low) | Oakland California  n=120 women | Cross-sectional | Gestational age, maternal age, maternal BMI, maternal height, ethnicity, parity, GDM, infant age in days, infant feeding practice (breast, formula, mixed) | Peri-natal | 75.5 (32.3) | Not given\*\* | | | | | | | | | | | -0.003 (-0.012, 0.005)  p=0.46 | 0.005 (-0.013, 0.003)  p=0.23 | No association seen between maternal serum 25(OH)D and offspring HC |

\*HC measured in infant at 2 weeks

\*\* HC measured in infant between 8-21 days old

**Table 13: The effect of vitamin D supplementation in gestation on offspring head circumference (HC) – 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)\* HC (cm) in un-supplemented group** | **Mean (SD) or Mean (SE)\* HC (cm) in supplemented group** | **Conclusion** |
| **Brooke, 19804** | -2 (high) | London, UK, n=126 women (all Asian) | Double-blinded  Randomised to either placebo (n=67) or 1000 IU/day of vitamin D2 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)\* | 34.5 (0.1)\* | No significant difference in HC between groups  p>0.05 |
| **Marya, 19886** | -2 (high) | Rohtak, India  n=200 women | Randomised to either no supplement (n=100) or oral 600,000 IU vitamin D3; 2 doses in 7th and 8th 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  Un-supplemented = 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 |

**Table 14: The effect of maternal vitamin D status in gestation on offspring bone mass – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First author and 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)D3 was measured** | **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 co-efficient (r) or regression co-efficient (β) (95% CI)** | | | | | | **Adjusted correlation co-efficient (r) or regression co-efficient (B) (95% CI)** | | | | **conclusion** |
| **Weiler, 200586** | 3 (med) | Cross-sectional | Winnipeg, Canada  Overall cohort= 342 women  Sample size for analysis=50  Neonates delivered at term and assessed within 15 days of birth by DXA | Lumbar spine (LS) BMC (g)  LS BMC/body weight (wt) (g/kg)  Femur BMC  Femur BMC/wt  Whole Body (WB) BMC  WB BMC/ wt | 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 | 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 lumbar spine BMC or lumbar spine BMC/body weight, femur BMC or whole body 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/wt (g/kg) | 0.59  (.14) | | 0.66  (.125) | | 0.08 |
| Femur BMC (g) | 2.8  (0.7) | | 2.9  (0.6) | | 0.60 |
| Femur BMC/wt (g/kg) | 0.71  (.17) | | 0.81  (.15) | | 0.027 |
| WB BMC (g) | 76.4  (12.9) | | 75.7  (13.7) | | 0.86 |
| WB BMC/wt (g/kg) | 19.49  (3.05) | | 21.33  (2.03) | | 0.017 |
| **Javaid, 20062** | 5 (low) | Cohort | Princess Anne Cohort, Southampton, UK  n=198 women  Children assessed at mean 8.9 years by DXA | WB BMC (g)  BA (cm2) BMD (g/cm2)  Lumbar spine (LS) BMC(g)  BA (cm2) BMD (g/cm2) | Gestational age , offspring age at DXA | 34 weeks | 25(OH)D conc (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 |
| **Prentice, 200995** | 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 DXA for whole body measurements | Radial midshaft BMC (g) and bone width  WB BMC (g/cm)  WB BA (cm2) | Season, mat height, weight, weight gain, infant sex and whether received calcium supplement | 20 weeks and 36 weeks | 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 |
| **Sayers, 200942** | 3  (med) | Cohort | ALSPAC, cohort, UK  n=6955 women  Children assessed at mean age 9.9 years by DXA | WB less head BMC, (g), BA (cm2) BMD (g/cm2), aBMC (g) | BMC adjusted for area  BA adjusted for height | Not directly measured  Ambient UVB measured during 98 days preceding birth | 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.  No relationship was observed with maternal UV exposure and aBMC |
| BMC (g) | | 9.6  (5.3, 13.8) | | <0.0001 | |
| BA (cm2) | | 8.1  (4.3, 11.9) | | <0.0001 | |
| BMD (g/cm2) | | 0.003  (0.001, 0.004 | | <0.0001 | |
| aBMC (g) | | 0.69  (0.22, 1.60) | | 0.14 | |
| **Akcakus 2009100** | 4  (med) | Cross-sectional | Turkey  Cohort=100 women  3 groups, 30 SGA, 40 AGA, 30 LGA infants  Most women veiled  Children assessed within 24hour of birth by DXA | WB BMC(g)  WB BMD (g/cm2) | Nil | Delivery | 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 |
| **Viljakainen, 201094** | 3 (med) | Cohort | Helsinki, Finland  n=125 women recruited during last trimester (Oct-Dec). All Caucasian, non-smokers, primiparous  Children assessed when newborn by pQCT of tibia | Tibial BMC (g/cm), tibial CSA (mm2) and tibial BMD (mg/cm3) | 3 models:  1: adjusted for z score birth weight  2: as above + maternal height  3: as above +log(age of newborn at pQCT) | First trimester (8-10 weeks) and 2 days post-partum. Mean of 2 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 | | Bone outcome | | | | r for log 25(OH)D  p value | | r after adjust 1 | | r after adjust 2 | r after adjust 3 | A positive significant association seen between maternal 25(OH)D status and offspring tibial BMC and tibial CSA.  Tibial BMC and CSA significantly higher in those with maternal 25(OH)D above median than those below even after adjustments  No association seen with tibial BMD |
| Tibial BMC | | | | 0.149, p=0.163 | | 0.232  P=0.034 | | 0.230  p=0.036 | 0.192  P=0.085 |
| Log (tibial CSA) | | | | 0.197, p=0.05 | | 0.214  p=0.05 | | 0.218  P=0.048 | 0.226  P=0.042 |
| **Viljakainen 201199** | 4 (med) | 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). 55 children had bone data at both time-points | Tibial BMC (g/cm), tibial CSA (mm2) and tibial BMD (mg/cm3) | Sex, birth weight z score, walking age, exclusive breast feeding and offspring 25(OH)D at 14 months | First trimester (8-10 weeks) and 2 days post-partum. Mean of 2 values used to calculate “vitamin D status” | Not given  Overall median “vitamin D status=” 42.6 | | Not given | | | | | | Not given | | | | No difference in tibial BMC or BMD in offspring with maternal 25(OH)D above median than those below  CSA higher at 14 months in offspring with maternal 25(OH)D above median than those below.  This suggest that postnatal vitamin D supplementation only partly improved the differences in bone variables induced by maternal vitamin D status during pregnancy |
| **Dror, 201293** | 7 (low) | Cross-sectional | Oakland California, USA  n=120 women  Children assessed between 8-21 days old by DXA | WB BMC  WB aBMC | Maternal height, GDM, infant age at DXA, feeding practice (breast, formula, mixed), infant weight-for-height z score, infant height-for- age z score, bone area and size for gestational age | Per-natal | 75.5 (32.3) | | WB BMC β= -0.02 (p=0.52) | | | | | | WB aBMC: β= 0.0007 (-0.031, 0.032)  P=0.97 | | | | No association seen between maternal 25(OH)D and offspring WB BMC or WB aBMC either analysed continuously or categorically |

SGA = small for gestational age, AGA = appropriate for gestational age, LGA = large for gestational age

WB BMC= whole body bone mineral content, WB BMD = whole body bone mineral density, WB BA= whole body bone area, aBMC= bone mineral content adjusted for bone area)

DXA= Dual energy X-ray absorptiometry

SPA= Single photon absorptiometry

pQCT= peripheral quantitative computed tomography

**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 un-supplemented group** | **Mean (SE) bone outcome(units) in supplemented group** | **Conclusion** |
| **Congdon, 198322** | -9 (high) | Leeds, UK  n=64, all Asian women | Either 1000 IU vitamin D plus calcium (calcium dose not given) daily in the 3rd trimester (n=19) or no supplement (n=45)  Offspring assessed within 5 days of birth. Method of bone measurement not given | Forearm BMC (units not given) | Nil, but groups similar in terms of maternal age, infant sex, gestation length, birth weight | | Not measured | Not measured | 3.10 (0.10) \* | 3.19 (0.12)\* | No difference in forearm BMC between groups  p value not given |

\* Results expressed in arbitrary units proportional to the mineral mass per unit length of the radius and ulna combined

**Table 16: The effect of maternal vitamin D status in gestation on offspring anthropometry and body composition – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First author and 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)D3 was measured** | | **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 co-efficient (r) or regression co-efficient (B) (95% CI)** | | | | | | | | | **Adjusted correlation co-efficient (r) or regression co-efficient (B) (95% CI)** | | | | | | **Conclusion** | |
| **Weiler, 200586** | | 3 (med) | | Cross-sectional | | Winnipeg, Canada  Sample size for analysis=50 women  Neonates delivered at term and assessed within 15 days of birth by DXA | | | Whole body fat (%) | | | 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 2 groups (o=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) | | |  | | Maternal 215(OH)D <37.5 nmol | | | Maternal 25(OH)D >37.5 nmol | | | | Not given | | | | | | No significant difference in offspring whole body fat in those with maternal 25(OH)<37.5 nmol/l compared to those with maternal 25(OH)D >37.5 nmol/l | |
| Mean (SD) neonatal whole body fat (%) | | 12.7 (4.1) | | | 10.6 (4.1) | | | |
| **Morley, 200691** | | 8 (low) | | Cohort | | Melbourne, Australia  n=374 women (232 recruited in winter, 127 in summer)  Neonates assessed between 12-72 h 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 first child, smoking, season of blood sample | | | 11 weeks and 28-32 weeks | | | Winter recruitment, geometric mean at 11 wks=49.2; 26-32 wks=48.3  Summer recruitment geometric mean at 11 weeks=62.6; 26-32 wks=68.9 | | |  | | | | β (95% CI) for every Log2 increase in maternal 25(OH)D (i.e. doubling of 25(OH)D at 28-32 wks | | | | | Adjusted β (95% CI) for every Log2 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, -0.02) | | | | | -0.2 (-0.4, -0.06) | | | | | |
| Triceps skinfold (mm) | | | | -0.3 (-0.5, -0.02) | | | | | -0.1 (-0.4, 0.1) | | | | | |
| Suprailiac skin fold (mm) | | | | -0.06 (-0.4, 0.1) | | | | | -0.06 (-0.4, 0.2) | | | | | |
| Mid upper arm circumference (cm) | | | | 0.08 (-0.07, 0.2) | | | | | 0.1 (-0.06, 0.3) | | | | | |
| Calf circumference (cm) | | | | 0.05 (-0.1, 0.2) | | | | | 0 (-0.2, 0.2) | | | | | |
| **Gale, 200825** | | 4 (med) | | Cohort | | Princess Anne Cohort, Southampton, UK  Children assessed at birth n=466), 9 months (n=440) and 9 years (n=178) using measuring tape with DXA at 9 years only | | Mid-upper arm circumference (cm) at birth and 9 months  Fat mass (kg) Lean mass (kg) at 9 years | | | Adjusted for age of child at scan | | | Late pregnancy  (median (IQR) 32.6 (32-33.4) weeks | | | 50 (30-75.3)  50.4% had 25(OH)D >50nmol/k  28.3% had levels 27.5-50 nmol/l  21.1% had levels <27.5 nmol/l | | | P value for difference in offspring outcome according to quartile 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 am circumference at birth | | | | | 0.080 | | | |
| Mid-upper arm circumference at 9 months | | | | | 0.581 | | | |
| Fat mass at 9 years | | | | | 0.090 | | | |
| Lean mass t 9 years | | | | | 0.090 | | | |
| **Sayers, 200942** | | 3  (med) | | Cohort | | ALSPAC, cohort, UK  n=6955 women  Children assessed at mean age 9.9 years by DXA | | Lean mass (kg)  fat mass (kg) | | | Nil | | | Not directly measured  Ambient UVB measured during 98 days preceding birth | | | | 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, 237) | | | | | 0.00002 | |
| Fat mass (kg) | | 73.9  (-44.2, 191.9) | | | | | 0.22 | |
| **Krishnaveni, 2011102** | | 4 (med) | | 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 | | Arm muscle area (AMA; cm2)  Subscapular skinfold, thickness (mm),  Triceps skinfold thickness (mm),  Waist circumference,  Fat mass (kg),  Percent body fat (%),  Fat-free mass (kg),  Percent fat-free mass (%) | | | Offspring sex and age, maternal BMI, gestational diabetes, 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) | | 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 arm-muscle area in comparison to those children born to mothers without deficient.  No significant difference seen in any of the other anthropometric or body composition measurements | | |
|  | | β | | P value | |
| **5 yr** | | | | | |
| AMA | 0.4 | | | 0.01 | |
| Subscap | .004 | | | 0.86 | |
| Triceps | 0.01 | | | 0.55 | |
| Waist | 0.07 | | | 0.81 | |
| Fat mass | -0.01 | | | 0.92 | |
| %Fat | -0.4 | | | 0.48 | |
| Fat-free mass | 0.1 | | | 0.33 | |
| % fat free mass | 0.3 | | | 0.51 | |
| **9.5 yr** | | | | | |
| AMA | 0.7 | | | 0.02 | |
| Subscap | -.009 | | | 0.80 | |
| Triceps | .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 | |
| **Crozier, 2012103** | | 8 (low) | | Cohort | | Southampton Women’s Survey, UK  Children assessed at birth (574), 4 years (565) and 6 years (447) using DXA | | 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 | | | | 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.  Negative association late pregnancy maternal 25(OH)D and fat mass at 6 years after adjusting for confounders.    No significant association seen at 4 years after adjustments for confounders. | | |
| Birth fat mass (SD) | 0.06  (-0.01, 0.12) | | | | | 0.09 | | | 0.08  (0.02, 0.15) | | | 0.02 | | |
| Birth fat-free mass (SD) | 0.02  (-0.03, 0.07) | | | | | 0.44 | | | 0.04  (-0.02, 0.09) | | | 0.17 | | |
| 4-y fat mass (SD) | -0.09  (-0.16,  -0.02) | | | | | 0.02 | | | -0.01  (-0.08, 0.07) | | | 0.81 | | |
| 4-y fat-free mass (SD) | 0.03  (-0.02, 0.08) | | | | | 0.21 | | | 0.03  (-0.02, 0.08) | | | 0.30 | | |
| 6-y fat mass (SD) | -0.16  (-0.23,  -0.08) | | | | | <0.001 | | | -0.10  (-0.17,  -0.02) | | | 0.01 | | |
| 6-y fat-free mass (SD) | 0.01  (-0.04, 0.06) | | | | | 0.65 | | | 0.02  (-0.03, 0.07) | | | 0.43 | | |

DXA = Dual energy X-ray absorptiometry

**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** | **Mean (SE) maternal**  **25(OH)D concentration (nmol/l)** | **Mean (SD)/ Mean (SE)\* offspring outcome (units) in un-supplemented group** | | **Mean (SD)/ Mean (SE)\* offspring outcome(units) in supplemented group** | | | **Conclusion** |
| **Brooke 19804** | -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 (cm2) | 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)\* | Triceps skinfold  (cm) | 3.6 (0.1)\* | Triceps skinfold (cm) | | 3.8 (0.1)\* | Significantly greater fontanelle area in the supplemented group (p<0.05).  No significant difference in forearm length or triceps skinfold thickness |
| Forearm length (cm) | 8.1 (0.1)\* | Forearm length  (cm) | | 8.1 (0.1)\* |
| Fontanelle area | 6.1 (0.7)\* | Fontanelle area | | 4.1 (0.4)\* |
| **Marya, 19886** | -2 (high) | Rohtak, India  N=200 women | | Randomised to either no supplement (n=100) or oral 600,000 IU vitamin D3; 2 doses in 7th and 8th 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 | Not measured directly, but mean daily vitamin D intake given as follows:  Un-supplemented = 35.71 (6.17) IU/day  Supplemented group = 35.01 (7.13) IU/day | Mid-arm circum (cm) | 9.44 (0.85) | Mid-arm circum (cm) | 9.82 (0.72) | | Significantly higher mid-arm circumference, triceps skinfold and infrascapular skinfold in the supplemented group (all p<0.01) |
| Triceps skinfold (mm) | 7.30 (0.83) | Triceps skinfold (mm) | 7.72 (0.67) | |
| Infrascap skinfold (mm) | 7.49 (0.89) | Infrascap skinfold (mm) | 7.82 (0.67) | |

**Table 18: The effect of maternal vitamin D status in gestation on offspring asthma and atopy– Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and year** | **Bias score** | **Cohort details** | **Study type** | **Adjustments** | **When was maternal serum 25 OH D measured** | **Mean (SD) or median (IQR) 25OHD3 concentration (nmol/l- unless other stated)** | **Risk of Asthma/Wheeze/ Eczema** | | | | | **Conclusion** |
| **Camargo, 2007106** | 2 (med) | Massachusetts, USA  Cohort = 2128 women  1194 (56%) studied for outcome | Cohort | Sex, birth weight, 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 food frequency questionnaire at initial prenatal visit and 26-28 weeks gestation. | Not measured  Mean vitamin D intake (mean of early pregnancy and 26-28 week for each participant) was 548 (167) IU/day. | In comparison to the lowest quartile, mothers in the highest quartile of daily vitamin D intake had a lower risk of having a child with recurrent wheeze at 3years (OR 0.38; 95%CI 0.22-0.65). | | | | | 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 |
| **Devereux, 200727** | -1 (high) | Aberdeen, Scotland  Cohort = 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, birth weight, birth order, season of last menstrual period, maternal intakes of vitamin E, zinc and calcium. | Not measured  Estimated from food frequency questionnaire at 32 weeks gestation. | 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 to the lowest quintile, the highest quintile of maternal vitamin D intake displayed lower risk of “ever wheeze” (OR: 0.48; 95%CI: 0.25-0.91), and “wheeze in the previous year” (OR: 0.35; 95%CI 0.15-0.83) at 5years determined by parental questionnaire.  No differences in atopic sensitization or spirometry. | | | | | Low maternal vitamin D intakes during pregnancy are associated with increased wheezing symptoms in children at 5 years. |
| **Gale, 200825** | 4 (med) | Princess Ann Cohort, Southampton, UK  n= 440 at 9 months  n=178 at 9 years | Cohort | Nil | Late pregnancy  Median (IQR)= 32.6 (33-33.4) weeks | 50 (30-75.3)  50.4% had 25(OH)D >50nmol/k  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 | 30-50 | 50-75 | >75 |
| Visible eczema on examination at 9 months | 1.0 | 0.59  (0.14-2.50) | 0.79 (0.21-3.00) | 3.26 (1.15-9.29) |
| Atopic eczema at 9 months (UK working party criteria) | 1.0 | 1.11 (0.43-2.84) | 1.75 (0.73-4.17) | 1.62 (0.67-3.89) |
| Reported eczema at 9 years | 1.0 | 0.71 (0.15-3.39) | 0.49 (0.08-2.68) | 1.89 (0.51-6.99) |
| Reported asthma at 9 years | 1.0 | 2.05 (0.36-11.80) | 2.05 (0.36-11.80) | 5.40 (1.09-26.65) |
| **Erkkola, 2009104** | -1 (high) | Finland – 3 university hospitals  Cohort = 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 years, maternal intake of vitamin C, vitamin E, selenium and zinc | Not measured  Estimated from food frequency questionnaire. Completed retrospectively after delivery for 8th month of pregnancy. | 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 0.59-0.99) and allergic rhinitis (HR 0.84; 95% CI 0.72-0.98) but not atopic eczema (OR 0.94; 95% CI 0.83-1.07) at 5 years | | | | | Maternal vitamin D intake during pregnancy inversely associated with the development of asthma and allergic rhinitis |
| **Miyake, 2010105** | -1 (high) | Osaka, Japan  Cohort = 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, birth weight, age at child assessment. | Not measured  Self administered validated questionnaire of dietary intake. Measured between 5 and 39 weeks of pregnancy. | 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 0.43-0.97) and eczema (adjusted OR 0.41-0.98) 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. |
| **Nwaru, 2010111** | 3 (med) | Finland  Cohort = 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 food frequency questionnaire. Completed retrospectively after delivery for 8th month of pregnancy. | 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 sensitization (specific IgE≥0.35KU/l) to food allergens (adjusted OR 0.56 (95%CI 0.35-0.91, p<0.026) but not inhaled allergens (adjusted OR 0.76 (95%CI 0.50-1.17) at 5 years of age. | | | | | Increasing maternal intake of vitamin D was inversely associated with sensitization to food allergens. |
| **Camargo, 2011107** | 3 (med) | 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, birth weight, child’s gender and ethnicity, smoking, number of children in household, during of exclusive breastfeeding. | Not measured  Cord blood 25(OH)D were measured | Not measured  Median cord blood 25(OH)D= 44nmol/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: 1.17–2.26] for 25–74 nmol/L, and 2.15 [95% CI: 1.39–3.33] 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. |
| **Cremers, 2011110** | 3 (med) | Netherlands  Cohort = 2834 women (2343 women with a conventional lifestyle; 491 women with an alternative lifestyle with regards to child rearing practices, diet an vaccination programmes)  415 (14.6%) 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, birth weight, exposure to tobacco smoke, season of blood sampling, physical activity | 36 weeks gestation | 46.0 (18.2) nmol/l | No association between maternal plasma 25(OH)D at 36weeks gestation and offspring FEV1 (p=0.99) nor FVC p=0.59) at 6-7 years | | | | | No association between maternal late pregnancy 25-hydroxyvitamin D levels and lung function in children aged 6-7 years. |
| **Rothers, 2011108** | 2 (med) | Tucson, Arizona, USA  Cohort = 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 | 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<50nmol/l and >100nmol/l.  Greater risk of skin-prick testing positivity to aeroallergens at 5 years in children with cord 25(OH)D ≥100nmol/l compared with reference group (25(OH)D 50-74.9nmol/l): OR3.4; 95%CI 1.0-11.4, p=0.046) | | | | | Non-linear relationship between vitamin D status at both and markers of atopy at 5 years |
| **Morales 2012109** | 3 (med) | Spain,  Cohort=2860 women enrolled in the INMA project (Infancia y Medio Ambiente)  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, and area of study | Between 12-23 weeks gestation  Mean (SD) =12.6 (2.5) weeks | Median= 73.6 (56.2–92.6) nmol/l | No significant association seen between maternal 25(OH)-vitamin 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)-vitamin D and offspring wheeze at 1 year and 4 years, or offspring asthma at 4-6 years |

**Table 19: The effect of maternal vitamin D status in gestation on risk of offspring being born small for gestational age – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and year** | **Bias score** | **Study details** | **Study type** | **Confounders / adjustments** | **Number of weeks gestation when 25(OH)D was measured** | **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 appropriate for GA (AGA)** | **Odds ratio (95% CI) of offspring being SGA from univariate analysis** | | | | **Odds ratio (95% CI) of offspring being SGA from multivariate analysis** | | **Conclusion** |
| **Akcakus, 2006100** | 4 (med) | Turkey  Cohort=100 women  Cases of SGA\*=30  Most women veiled | Cross-sectional | NIl | Delivery | 21.75 (7.5) | 21.5 (7.5) | Not given | | | | Not given | | No difference in maternal 25(OH)D at delivery in SGA infants compared to AGA infants |
| **Mehta, 2009118** | 3 (med) | Tanzania  Overall Cohort=1078. Women all HIV infected taking part in a clinical trial of vitamin use  Cases of SGA\*=74  Cohort for analysis= 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) | 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, 1.91)  p=0.31 | | | | 1.25 (0.82, 1.90)  p=0.31 | | No relationship between SGA risk and maternal 25(OH)D amongst women with HIV |
| **Leffelaar, 201082** | 5 (low) | Amsterdam Born Children and their development (ABCD) study, Netherlands  Cohort=3730 women  Cases of SGA\*=9.2% (approx. 343) | Prospective cohort | 2 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 and vitamin D status | Early pregnancy (mean 13 weeks) | 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  (1.0-3.2) | | 1.8  1.3-2.5 | 1.9  (1.4-2.7) |
| 30-49.9 | | 1.5  (1.1-2.0) | | 1.2  0.9-1.7 | 1.2  (0.9-1.3) |
| 50+ | | 1.0  (Ref) | | 1.0  (Ref) | 1.0  (Ref) |
| **Bodnar, 2010112** | 7 (low) | Pittsburg, USA  Overall cohort size=1198 women  Cases of SGA\*=111  Controls=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 | Geometric mean (95% CI) according to race  White= 73.2 (69.7, 76.8)  Black=39.8 (36.7, 43.2) | Geometric mean (95% CI) according to race  White= 71.5 (64.0, 79.9)  Black= 39.8 (33.6, 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 amongst black mothers  No sig. 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 amongst white mothers with the lowest risk between 60-80 nmol/l |
| 25(OH)D  Nmol/l | White | | Black | White | Black |
| <37.5 | 10.6  (2.6, 42.5) | | 1.4  (0.5, 3.1) | 7.5  (1.8, 31.9) | 1.5  (0.6, 3.5) |
| 37.5-75 | 1.0  (ref) | | 1.0 (Ref) | 1.0  (ref) | 1.0  (ref) |
| >75 | 1.9  (1.1, 3.4) | | 1.9  (1.1, 3.4) | 2.1  (1.2, 6.8) | 2.2  (0.5, 5.5 |
| **Shand,2010114** | 6 (low) | Vancouver, Canada  All women had either clinical or biochemical risk factors for preeclampsia∆  Cohort=221 women  Cases of SGA\*\*=13 | Cohort | Maternal age, ethnicity, parity, BMI, season, multivitamin use, smoking | Between 10 and 20 weeks 6 days (mean 18.7 (1.88) weeks) | Not given | Not given | Unadjusted values not given | | | | 25(OH)D conc | OR  (95% CI) | No significant relationship seen between maternal 25(OH)D and risk of infant being SGA |
| <37.5 | 1.78  (0.52, 6.03) |
| <50 | 2.34  (0.65, 8.49) |
| <75 | 2.16  (0.26, 18.2) |
| **Robinson**  **2011113** | 1 (med) | South Carolina, USA  All women has early onset preeclampsia (EOSPE)∆∆  Cases=33  Controls=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 birth weights converted to percentile growth for gestational age | Not given | 41.9 (22.2- 57.4) | 63.1 (39.9-82.4) | Not given | | | | Not given | | Serum 25(OH)D significantly lower in women with EOSPE and SGA offspring compared to EOSPE controls with normal sized offspring  p=0.02 |
| **Fernandez-Alonso, 2012115** | 3 (med) | Almeria, Spain  Cohort=466 women  Cases of SGA\* =46 | Cohort | Nil | Between 11-14 weeks | 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 |

\* SGA defined as infants born <10th percentile of birth weight according to nomograms based on gender and gestational age

\*\*SGA defined as infants born <3rd percentile of birth weight according to nomograms based on gender and gestational age

∆ Defined as past obstetric history of early-onset or severe preeclampsia, unexplained elevated α-fetoprotein ≥ 2.5 multiples of the median (MoM), unexplained elevated human chorionic gonadatrophin, or low pregnancy-associated plasma protein A ≤ 0.6 MoM

∆∆ Defined as meeting the American College of Obstetrics and Gynecology criteria for severe preeclampsia and having this diagnosis at <34 weeks gestation

**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** | **Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l)** | | | **Percentage of infants SGA\* in un-supplemented group** | **Percentage of infants SGA\* in supplemented group** | **Conclusion** |
| **Brooke, 19804** | -2 (high) | London UK, n=126 women (all Asian) | Double-blinded  Randomised to either placebo (n=67) or 1000 IU/day of vitamin D2 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 25OHD3 = 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; X2 = 3.1 |
| **Yu, 200996** | 5 (low) | London, UK  n=119 women | 3 arms  Randomised to either no supplement (n=59) or oral vitamin D2 800 IU/day vitamin from 27 weeks onwards (n=60), or a single 200,000 IU D2l at 27 weeks gestation (n=60)  Each group contained equal numbers of 4 ethnic groups (Caucasian, Black, Asian, Middle Eastern) | Nil  No significant difference in baseline characteristics across the 3 groups | Measured at 26-27 weeks and again at delivery |  | 27 wks | Delivery | 17% | 15% in daily dose group  13% in stat dose group | No significant difference in rate of SGA across the 3 groups  p=0.7 |
| No sup | 25  (21-38 | 27  (27-39) |
| Daily sup | 26  (20-37) | 42  (31-76) |
| Single sup | 26  (30-46) | 34  (30-46) |

\* SGA defined as infants born <10th percentile of birth weight

**Table 21: The effect of maternal vitamin D status in gestation on preterm birth of the offspring – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and year** | **Bias score** | **Study details** | **Study type** | **Confounders / adjustments** | **Number of weeks gestation when 25(OH)D was measured** | **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** | | **Odds ratio (95% CI) of offspring being preterm from univariate analysis** | | **Odds ratio (95% CI) of offspring being preterm from multivariate analysis** | | **Conclusion** |
| **Delmas, 1987117** | -4 (high) | Lyon, France.  n=9 women (controls)  n=10 women (cases of preterm)\*  None of the women were taking supplemental vitamin D | Case-control | None | Delivery | 44.9 (17.5) | | 47.4 (7.5) | | Not given | | Not given | | No difference in maternal 25(OH)D at delivery in preterm compared to full-term births  p value not given |
| **Mehta, 2009118** | 2 (med) | Tanzania  Overall Cohort=1078 women  Women all HIV infected taking part in a clinical trial of vitamin use  Cases of preterm\*\* birth=204  Cases of severe preterm\*\*\* birth=70  Cohort for analysis=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) | Mean not given  34% of preterm, 37% of severe preterm had 25(OH)D <80 nmol/l  66% of preterm, 63% of severe preterm had 25(OH) D>80 nmol/l | | Not given | | RR if maternal 25(OH)D <80 nmol/l compared to >80 nmol/l  Preterm= 0.83 (0.65, 1.07) p=0.14  Severe preterm= 0.77 (0.49, 1.19) p=0.24 | | Adjusted RR if maternal 25(OH)D <80 nmol/l compared to >80 nmol/  Preterm= 0.84 (0.65, 1.07). p=0.15  Severe preterm= 0.77 (0.50, 1.18). p=0.23 | | No increased risk of preterm or severe preterm birth if maternal 25(OH)D <80nmol/l compared with > 80nmol/l |
| **Baker, 2011119** | 5 (low) | North Carolina, USA  Overall cohort size= 4225 women  Cases of preterm birth #=40  Controls=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 | 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) | Adj 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, 4.26)  p=0.61 | <50 | 0.82 (0.19, 3.57)  p=0.79 |
| 50-74.9 | 8 (20) | 50-74.9 | 24 (20) | 50-74.9 | 1.01  (0.42, 2.46)  p=0.99 | 50-74.9 | 0.87 (0.34, 2.25)  p=0.77 |
| ≥75 | 29 (72.5) | ≥75 | 88 (73.3) | ≥75 | 1 (Ref) | ≥75 | 1 (Ref) |
| **Shand,2010114** | 6 (low) | Vancouver, Canada  All women had either clinical or biochemical risk factors for preeclampsia∆  Cohort=221 women  Cases of preterm birth\*\*=18 | Cohort | Maternal age, ethnicity, parity, BMI, season, multivitamin use, smoking | Between 10 and 20 weeks 6 days (mean 18.7 (1.88) weeks) | Not given | | Not given | | Unadjusted values not given | | 25(OH)D conc (nmol/l) | OR  (95% CI) | No significant relationship seen between maternal 25(OH)D and risk of preterm birth using 3 different maternal 25(OH)D cut-offs |
| <37.5 | 0.97  (0.43, 2.21) |
| <50 | 1.02  (0.48, 2.17) |
| <75 | 0.79 (0.31, 2.06) |
| **Hossain, 2011120** | 4 (med) | Karachi, Pakistan  Cohort=75 women  Cases of preterm birth\*\* = not given  26% of women covered their arms, hands and head; 76% also covered their face | Cross-sectional | None | At delivery | 42.2 (19.5)+ | | 32.9 (16.8)+ | | Not given | | Not given | | Maternal 25(OH)D tended to be higher in those who delivered preterm but did not achieve statistical significance (p=0.057) |
| **Shibata, 2011116** | 4 (med) | Toyoake, Japan  Cohort size=93 women. Deliveries spread equally across seasons)  Cases of threatened premature delivery ∆∆= 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 wks) | 30.0 (8.0) | | 37.9 (12.7) | | Not given | | β=-0.019 (p=0.023) | | Significantly lower maternal 25(OH)D in women with threatened premature delivery compared to normal deliveries.  p for difference in means=0.002 |
| **Fernandez-Alonso, 2012115** | 3 (med) | Almeria, Spain  Cohort= 466 women  Cases of preterm birth\*\*= 33 | Cohort | Nil | Between 11-14 weeks | 25(OH)D conc  (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) |

\*No threshold for preterm birth given. Gestational age determined by the scoring system of Dubowitz (based on examination of the neonate and scored on neurological and physical examination features

\*\*Preterm birth defined as delivery at <37 weeks gestation

\*\*\* Severe preterm birth defined as delivery at <34 weeks gestation

# Preterm birth defined as delivery at >23 weeks and <35 weeks gestation

+ 25(OH)D3 measured

∆ Defined as past obstetric history of early-onset or severe preeclampsia, unexplained elevated α-fetoprotein ≥ 2.5 multiples of the median (MoM), unexplained elevated human chorionic gonadatrophin, or low pregnancy-associated plasma protein A ≤ 0.6 MoM

∆∆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)

**Table 22: The effect of maternal vitamin D status in gestation on risk of Type 1 Diabetes Mellitus (DM) in the offspring – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and year** | **Bias score** | **Study details** | **Study type** | **Confounders / adjustments** | **Number of weeks gestation when 25(OH)D was measured** | **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** | **Odds ratio (95% CI) of offspring developing Type 1 Diabetes from univariate analysis** | | | **Odds ratio (95% CI) of offspring developing Type 1 Diabetes from multivariate analysis** | | | | **Conclusion** |
| **Stene, 2003122** | 2 (med) | Norway  Cases of offspring Type 1 DM=545  (mean age 10.9 (3.4) years)  Controls=1668 | Case-control | Controls matched for period of birth (between 1/1/1985 – 31/12/1999)  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 amongst 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, 1-4 times per week” or “yes, 5+ times per week” | Not measured | Not measured | Vit D suppl. in pregnancy | OR (95% CI) | | Vit D suppl 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, 1-4 times per week | 0.86  (0.63, 1.18) | | Yes, 1-4 times per week | | 1.09  (0.77, 1.56) | |
| Yes, 5+ times per week | 0.89  (0.69, 1.13) | | Yes, 5+ times per week | | 0.98  (0.73, 1.31) | |
| p for trend | 0.28 | | p for trend | | 0.94 | |
| **Marjamaki, 2010123** | 6 (low) | Diabetes Prediction and Prevention study (DIPP), Finland  Cohort size=3723 women and their children with increased genetic risk of diabetes\*  Cases of offspring Type 1 DM=74 (children observed for mean 4.3 (range 0.2-8.9) years | Prospective cohort | 2 models:  HR1 adjusted for genetic risk and familial type 1 DM  HR2 adjusted for genetic risk, familial Type 1 diabetes, 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 8th month of pregnancy and the use of supplements | Not given | Not given | Not given | | | HR given  HR1=1.18 (0.74, 1.87); p=0.49  HR2=1.08 (0.65, 1.79); p=0.77 | | | | Maternal intake of vitamin D, either from food or supplements is not associated with type 1 DM or advanced B cell autoimmunity in the offspring |
| **Sorensen, 2012121** | 8 (low) | Norway  Overall cohort=29072 women  Cases of offspring type 1 DM= 109 (mean age at diagnosis 9.0 (3.6) years  Controls=219 | Nested case-control | No significant difference between cases and controls in terms of maternal age, parity, gestational week of blood sample, frequency of C-section or maternal diabetes pre-pregnancy. Significantly more female offspring in cases than controls.  Adjustments:  2 models:  OR1 adjusted for sex of child and season of blood sample  OR2 adjusted for age of child at diagnosis, offspring sex, mothers age at delivery, parity, gestational week of blood sample, pre-gestational diabetes, season of blood sample, region of residence, percentage undergoing C-section | Median (IQR) cases=37 (22-38) wks  Median (IQR) controls=37(24-38) wks | 65.8 (26.5)) | 73.1 (27.2) | 25(OH)D conc | | OR | 25(OH)D conc | OR1 | | OR2 | Trend towards higher risk of type 1 diabetes in the offspring with lower levels of maternal 25(OH)D in late pregnancy, especially in those with 25(OH)D under 54 nmol/l |
| >89 | | 1.0  (ref) | >89 | 1.0  (ref) | | 1.0  (ref) |
| >69-89 | | 1.32  (0.63, 2.76) | >69-89 | 1.35  (0.63, 2.89) | | Not given |
| >54-69 | | 1.73  (0.86, 3.48) | >54-69 | 1.78  (0.85, 3.74) | | Not given |
| ≤54 | | 2.25  (1.14, 4.46) | ≤54 | 2.38  (1.12, 5.07) | | 2.39  (1.07-5.11 |
| Test for trend  Cont. | | P=0.022 | Test for trend  Cont. | 0.031 | | 0.032 |

\* 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

**Table 23: The effect of maternal vitamin D status in gestation on risk of low birth weight (LBW)\* in the offspring – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and year** | **Bias score** | **Study details** | **Study type** | **Confounders / adjustments** | **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** | **Odds ratio (95% CI) of offspring having LBW from univariate analysis** | **Odds ratio (95% CI) of offspring having LBW from multivariate analysis** | **Conclusion** |
| **Sabour, 200688** | -2 (high) | Tehran, Iran  n=449 women  Cases of LBW\*- not given | Cross-sectional | Nil | 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 (1000mg ca, 200 IU vitamin D)  p=0.007 |
| **Maghbooli, 200789** | 1 (med) | Tehran, Iran  n=552 women  Cases of LBW\*=5.4% (30) | Cross-sectional | None | Delivery\*\* | Not given | Not given | Not given | Not given | No significant association seen between serum 25(OH)D3 and LBW  p not given |
| **Mehta, 2009118** | 3 (med) | Tanzania  Overall Cohort=1078. Women all HIV infected taking part in a clinical trial of vitamin use  Cases of LBW\*=80  Cohort for analysis=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) | Mean not given  35% of LBW had 25(OH)D <80 nmol/l  65% had 25(OH) D>80 nmol/l | Not given | 0.85 (0.55, 1.32) | 0.84 (0.55, 1.28) | No relationship between LBW risk and maternal 25(OH)D amongst women with HIV  p=0.42 |

\* LBW defined as infants born <2500g

\*\*Measured 25(OH)D3

**Table 24: The effect of maternal vitamin D status in gestation and offspring serum calcium (Ca) concentration – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and 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)** | **Mean (SD) offspring serum Ca (mmol/l)** | | **Unadjusted regression co-efficient β (95% CI) or correlation coefficient r (95% CI) for offspring serum Ca (mmol/) per 1nmol/l increase in 25(OH)D** | **Adjusted regression co-efficient β (95% CI) or correlation coefficient r (95% CI) for offspring serum Ca (mmol/) per 1nmol/l increase in 25(OH)D** | **conclusion** |
| **Ardawi, 199787** | 5 (low) | Jeddah, Saudi Arabia  Cohort size=264 women | Cross-sectional | nil | Delivery | 47.71 (15.77)  25(OH)D <20 nmol/l (inadequate) in 23%  25(OH)D>20 nmol/l (adequate) in 77% | Mean cord Ca =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 Ca    No difference in cord Ca if group divided according to maternal 25(OH)D using 20 nmol/l as a threshold (p>0.05) |
| Maternal 25(OH)D | Mean (SD) cord calcium concentration (mmol/l) |
| <20  (n=24) | 2.48 (0.18) |
| >20  (n=240) | 2.40 (0.22) |

**Table 25: The effect of Vitamin D supplementation in gestation on offspring serum calcium (Ca) 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** | **Mean (SD)/ Mean (SE)\* or median (IQR)**  **25(OH)D concentration (nmol/l)** | | | | | **Mean (SD) or Mean (SE)\* offspring serum calcium conc (mmol/l) in un-supplemented group** | | | **Mean (SD) or \*Mean (SE) serum calcium conc (mmol/l) in supplemented group** | | | **Conclusion** |
| **Brooke, 19804** | -2 (high) | London, UK,  n=126 women (all Asian) | | Double-blinded  Randomised to either placebo (n=67) or 1000 IU/day of vitamin D2 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 | At allocation 25(OH)D = 20.1 (1.9)\*  At term, placebo group= 25(OH)D= 16.2 (2.7\*)  At term, supplemented group 25(OH)D = 168.0 (12.5)\* | | | | | cord | 2.65 (0.02)\* | | Cord | 2.71 (0.02)\* | | No significant difference in cord Ca between groups at birth , but significantly higher levels in the treatment group at day 3 and 6, but higher rates of breast feeding in the treatment group, which in itself was positively associated with offspring calcium conc. Compared to bottle feeding)  When groups considered separately, a weak correlation see between maternal 25(OH)D and cord Ca in the treatment group. r= 0.31, p<0.05  5 cases of symptomatic hypocalcaemia in control group, 0 in treatment group (X2 = 4.6, p<0.01) |
| Day 3 | 2.18 (0.04)\* | | Day 3 | 2.30 (0.04)\* | |
| Day 6 | 2.29 (0.02\* | | Day 6 | 2.49 (0.04) | |
| **Cockburn, 198021** | -1 (high) | Edinburgh, UK  n=1139 women | | Either given placebo (n=633) or 400 IU vitamin D2 (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 to May.  Maternal age, parity, type of delivery, offspring Apgar score at birth, social class, maternal preeclampsia, birth weight and gestational age were not associated with offspring 6 day Ca concentration | 24, 34 weeks and delivery |  | | 25(OH)D in  placebo | | 25(OH)D in supp | Cord | 2.69 (0.26)  (n=452) | | Cord | 2.66 (0.27)  (n=262) | | No significant difference in cord blood serum Ca at delivery.  Significantly higher serum Ca 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 (Ca<1.85 mmol/l) compared with 13% in the placebo group. |
| 24 wks | | 32.5  (n=82) | | 39.0  (n=82) |
| 34 wks | | 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) |
| **Marya, 19815** | -6 (high) | Rohtak, India  n= 120 women | | 3 arms:  Randomised to either no supplement (n=75) or 1,200 IU vitamin D + 375mg calcium/ day∆  throughout the 3rd trimester (n=25); or oral 600,000i IU vitamin D2; 2 doses in 7th and 8th months gestation (n=20) | Nil | Not measured | Not measured | | | | | 2.52 (0.23)  (value represents cord blood at delivery) | | | 1200IU/+ ca= 2.55 (0.17)  600,000 IU = 2.67 (0.12)  (values represents cord blood at delivery) | | | No difference in cord calcium between un-supplemented and 1200 IU+ 375 mg Ca/ day supplementation  Cord Ca significantly higher in those taking 600,000iu supplement compared to un-supplemented (p=0.001) |
| **Congdon, 198322** | -9 (high) | Leeds, UK  n=64, all Asian women | | Either 1000 IU vitamin D plus calcium (calcium dose not given) daily in the 3rd trimester (n=19) or no supplement (n=45) | Nil, but groups similar in terms of maternal age, infant sex, gestation length, birth weight | Not measured | Not measured | | | | | 2.50 (0.03) | | | 2.64 (0.05) | | | Cord Ca significantly higher in the supplemented group  P<0.025 |
| **Mallet, 19868** | -3 (high) | Rouen, France  n=77 women | 3 arms:  Randomised to either no supplement (n=29) or 1,000 IU vitamin D/day∆ in last 3 months of pregnancy (n=21), or single oral dose of vitamin D∆ 200,000iu in 7th month (n=27) | | Nil, but groups of similar maternal age, parity, calcium intake and frequency of outdoors outings | During labour (February and March) | Overall mean not given  According to group:  Un-supplemented = 9.4 (4.9)  1000IU/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)  (values represents cord blood at delivery) | | | No significant difference in serum Ca between the 3 groups  1 case of neonatal hypocalcaemia observed in the un-supplemented group (serum Ca 1.69 mmol/l) |
| **Delvin, 19867** | -2 (high) | Lyon, France  n=40 women | Randomised to either no supplement (n=20) or 1000 IU vitamin D3/day during 3rd 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 breast fed from the 6th hour of life | At recruitment (n=50) and at delivery |  | 25(OH)D in suppl. group | | 25(OH)D in unsuppl group | | When measured | | Mean infant serum Ca (SE) (mmol/l) | When measured | | Mean infant serum Ca (SE) (mmol/l) | Significant correlation between maternal 25(H)D and cord blood total Ca concentration (p<0.005)  No significant difference in cord blood total Ca concentration at delivery between groups.  At day 4, infant Ca levels were significantly higher in those in the supplemented group (p<0.025)  Infant Ca fell significantly more from delivery to day 4 in the un-supplemented group compared to the supplemented group (p<0.05) |
| At recruit-ment (185 days gest) | 54.9 (10.0)\* | | 27.5  (10.0)\* | | Cord at delivery  n=15 | | 2.63 (0.025)\* | Cord at delivery  n=15 | | 2.55 (0.5)\* |
| Delivery | 64.9 (17.5)\* | | 32.4 (20.0)\* | | Infant day 6  n=12 | | 2.1 (0.05)\* | Infant day 6  n=13 | | 2.28 (0.5)\* |
| **Marya, 19886** | -2 (high) | Rohtak, India  n=200 women | Randomised to either no supplement (n=100) or oral 600,000 IU vitamin D3; 2 doses in 7th and 8th 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  Un-supplemented = 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 Ca concentration significantly higher in the supplemented group (P<0.001) |

Table includes any studies that measured maternal vitamin D status in pregnancy and either cord calcium concentration of offspring serum calcium concentration.

**Table 26: The effect of maternal vitamin D status in gestation on offspring blood pressure – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First author and 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)D3 was measured** | **Mean (SD) or median (IQR) maternal 25(OH)D concentration (nmol/l)** | **Mean (SD) offspring blood pressure according to maternal 25(OH)D category/ Unadjusted correlation co-efficient (r) or regression co-efficient (B) (95% CI)** | | | | | | | | | **Adjusted correlation co-efficient (r) or regression co-efficient (B) (95% CI)** | **Conclusion** |
| **Gale, 200825** | 4 (med) | Cohort | Princess Anne Cohort, Southampton, UK  n=178 women, and  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 >50nmol/k  28.3% had levels 27.5-50 nmol/l  21.1% had levels <27.5 nmol/l |  | 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 |
|  | <30 | | -50 | | -75 | >75 | |  |
| Systolic BP (mm Hg) | 103.4  (7.94) | | 102.2  (7.26) | | 101.9  (8.18) | 102.9  (8.10) | | 0.47 |
| Diastolic BP (mm Hg) | 59.8 (5.25) | | 60.1 (5.49) | | 60.2 (5.7) | 59.9 (6.2) | | 0.75 |
| **Krishnaveni 2011102** | 4 (med) | 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, 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) | Maternal 25(OH)D | | | | | | | | | Comparing offspring of mothers with and without 25(OH)D deficiency (deficient=0, non-deficient=1)  5 yr systolic BP β=0.3 (-1.32, 1.89; p=0.72)  5 yr diastolic BP β= -0.3 (-1.67, 0.98; p=0.61)  9.5 yr systolic BP β=-1.2 (-2.87, 0.42;p=0.15)  9.5 yr diastolic BP β=0.4 (-0.90, 1.74; p=0.53) | No significant difference in offspring BP at 5 and 9.5 years in those born to mothers with 25(OH)D deficiency in late pregnancy compared to those born to mothers without vitamin D deficiency |
|  | | < 50 nmol/l (deficient) | | >50 nmol/l (non-deficient) | | | p value | |
| Systolic BP at 5 yr (mm Hg) | | 96.7 (8.4) | | 97.0 (8.1) | | | 0.67 | |
| Diastolic BP at 5 yr (mm Hg) | | 58.3 (6.8) | | 57.9 (6.6) | | | 0.54 | |
| Systolic BP at 9.5 yr (mm Hg) | | 101.6 (8.7) | | 100.5 (8.3) | | | 0.2 | |
| Diastolic BP at 9.5 yr (mm Hg) | | 58.3 (6.5) | | 58.7 (7.2) | | | 0.5 | |

**Table 27: The effect of maternal vitamin D status in gestation on maternal preeclampsia – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and year** | **Bias score** | **Study details** | **Study type** | **Confounders / adjustments** | **Number of weeks gestation when 25(OH)D was measured** | **Mean (SD) or Mean (SEM)\* or median (IQR) 25(OH)D concentration (nmol/l) in cases** | | **Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in controls** | | **Odds ratio/ Relative risk of preeclampsia from univariate analysis** | | | | | **Odds ratio/ relative risk of pre-eclampsia from multivariate analysis** | | | | | **Conclusion** |
| **Seely, 1992128** | 2 (med) | Boston, USA  12 cases  24 controls | Case-control | No adjustments, but cases and controls similar for age, gestation, number Caucasian, height, weight, no. primiparous | Mean 35.5 (0.6) weeks for cases and 36 (0.4) wks for controls | 73.9 (7.5)\* | | 89.3 (11.7)\* | | Unadjusted OR not given | | | | | OR not given | | | | | No statistically significant relationship seen |
| **Bodnar, 2007124** | 8 (low) | Pittsburgh, USA  Cohort size=1198 women  55 cases  220 controls  All women nulliparous | Nested case-control | Controls randomly selected and un-matched  Adjusted for:  Maternal race/ethnicity, pre-pregnant BMI, education, season, gestational age at collection | 2 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) | | 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, 14.1)  50 nmol/l reduction in 25(OH)D increased risk of pre-eclampsia OR 2.4; (95% CI 1.1-5.4)  **At delivery :**  25(OH)D significantly lower in cases (15% reduction; p<0.05) | | | | | At <22 weeks a strong inverse relationship between preeclampsia and 25(OH)D was observed (p=0.02) |
| **Oken, 2007131** | 5 (low) | Project Viva, Eastern Massachusetts, USA  n=1718 women  Cases= 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) | | 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 preeclampsia = 0.99 (0.87, 1.13) | | | | | No significant relationship seen |
| **Azar, 2011130** | 5 (low) | Oklahoma, USA  All white women with Type 1 diabetes  Cohort = 151 women  23 cases  24 controls | Nested case-control | Cases and controls matched for age, diabetes duration, HbA1c and parity.  Higher BMI and lower HDL cholesterol in the cases  Adjusted for parameters that differed between groups (BMI and HDL cholesterol) | 3 visits  Mean 12.2 (1.9) wks  Mean 21.6 (1.5) wks  Mean 31.5 (1.7 weeks) | Visit 1 | 44.4 (32.9-51.4) | Visit 1 | 47.2 (37.4-58.2) | Visit 1  (early pregnancy) | | | 0.91 (0.88-0.95) | | Visit 1 | | | 0.99 (0.77-1.30) | | No statistically significant relationship seen at any time point (after adjusting for confounders) |
| Visit 2 | 44.2 (35.7-58.2) | Visit 2 | 43.4 (30.0-61.4) | Visit 2  (mid-pregnancy) | | | 1.02 (0.98-1.06) | | Visit 2 | | | 1.02 (0.78-1.33) | |
| Visit 3 | 47.2 (23.5-55.4) | Visit 3 | 44.9 (33.2-65.9) | Visit 3  (late pregnancy) | | | 0.90 (0.73-1.11) | | Visit 3 | | | 0.92 (0.75-1.14) | |
| **Baker, 2010126 \*\*** | 9 (low) | Boston, USA, cohort size =3992 women  44 cases  201 controls | 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) | | 98 (680- 114) | | OR for severe pre-eclampsia | | | | | Adjusted OR for severe pre-eclampsia | | | | | Lower 25(OH)D3 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) | | | - | >75 | 1 (Ref) | | | - |
| 50-74.9 | 1.53  (0.67,3.49) | | | 0.31 | 50-74.9 | 2.16  (0.86,5.40) | | | 0.10 |
| <50 | 3.63  (1.52,8.65) | | | 0.004 | <50 | 5.41  (2.02,14.52) | | | 0.001 |
| **Haugen, 2009125** | 2 (med) | Norwegian mother and child cohort, Norway  n=23,425 women  Cases= 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) | | 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 Vit D intake (IU/day) | | OR | | | Total Vit D intake (IU/day) | | OR | | |
| <200 | | 1 | | | <200 | | 1 | | |
| 200-399 | | 0.93 (0.81,1.07) | | | 200-399 | | 0.99 (0.85,1.14) | | |
| 400-599 | | 0.81 (0.67,0.97) | | | 400-599 | | 0.87 (0.73,1.05) | | |
| 600-799 | | 0.69 (0.55,0.87) | | | 600-799 | | 0.77 (0.61,0.96) | | |
| >800 | | 0.78 (0.65,0.92) | | | >800 | | 0.89 (0.89,1.06) | | |
| **Powe, 2010129** | 4 (med) | Massachusetts General Hospital Obstetric maternal Study, Massachusetts, USA  Cohort size=9930 women  Cases=39  Controls=131 | Nested case control | Controls unmatched  Adjusted for:  BMI, non-white race and summer blood collection | first trimester | 68.5 (0.48)\* nmol/l | | 72.0 (2.0)\* nmol/l | | OR per 25 nmol/l increase in 25(OH)D =  0.86 (0.60,1.25)  If Vit D <37.5 nmol/l OR=2.49 (0.89,6.90) | | | | | OR per 25 nmol/l increase in 25(OH)D = 1.24 (0.78,1.98)  If Vit D <37.5 nmol/l OR=1.35 (0.4,4.5) | | | | | No significant relationship seen  (p=0.435) |
| **Robinson, 2010127\*\*** | 5 (low) | South Carolina, USA  Cases=50  Controls=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) | | 80 (50-110) | | OR per 25 nmol/l increase in 25(OHD = 0.58 (0.43,0.77) | | | | | OR per 25 nmol/l increase in 25(OH)D =0.37 (0.22,0.62) | | | | | Lower 25(OH)D associated with increased risk of severe early preeclampsia  p<0.001 |
| **Shand,2010114** | 6 (low) | Vancouver, Canada  All women had either clinical or biochemical risk factors for preeclampsia∆  Cohort=221 women  Cases=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) | | 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 (0.31,2.62) | | | |
| <50 | 1.39 (0.54,3.53) | | | |
| <75 | 0.57 (0.19,1.66) | | | |
| **Hossain, 2011120** | 4 (med) | Karachi, Pakistan  Cohort=75 women  Cases= 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)+ | | 36.2 (18.4)+ | | Not given | | | | | 25(OH)D3 tertile | | | Adjusted OR(95% CI) for preeclampsia (systolic BP≥140, and/or diastolic BP≥90mmHg | | Women in the lowest and middle tertile for 25(OH)D3 more likely to meet criteria for Preeclampsia compared to those in the highest tertile.  25(OH)D3 of 50nmol/l maximum identified as the threshold relating to increased risk for preeclampsia |
| Highest tertile | | | 1.0 (Ref) | |
| Middle tertile | | | 11.05 (1.15,106.04) | |
| Lowest tertile | | | 3.38 (0.40,28.37) | |
| **Fernandez-Alonso, 2012115** | 3 (med) | Almeria, Spain  Cohort=466 women  Cases=7 | Cohort | Nil | Between 11-14 weeks | Overall mean not given | | Not given | | Not given | | | | | Not given | | | | | No significant association between development preeclampsia as a function of first trimester 25(OH)D status (p=0.51) |
| 25(OH)D conc | n |
| <50 | 2 |
| 50-75 | 3 |
| >75 | 2 |

\*Mean (SEM)

\*\*Severe preeclampsia

+ 25(OH)D3 measured

∆ Defined as past obstetric history of early-onset or severe preeclampsia, unexplained elevated α-fetoprotein ≥ 2.5 multiples of the median (MoM), unexplained elevated human chorionic gonadatrophin, or low pregnancy-associated plasma protein A ≤ 0.6 MoM

**Table 28: The effect of Vitamin D supplementation in gestation on preeclampsia – Intervention studies**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First author, year** | **Risk of bias** | **Setting** | **Randomisation** | **Adjustments/ confounders accounted for** | **Number of weeks gestation when 25(OH)D3 measured** | **Mean (SD)**  **25(OH)D concentration (nmol/l- unless other stated)** | **No. of cases in un-supplemented group** | **No. of cases in supplemented group** | **Conclusion** |
| **Marya, 1987132** | -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 2 groups (p>0.05)  Significantly reduced diastolic and systolic BP 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 effect of maternal vitamin D status in gestation on risk of gestational diabetes (GDM) – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and 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** | | **Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in unaffected controls** | | **Odds ratio (95% CI) of GDM from univariate analysis** | | **Odds ratio of GDM from multivariate analysis** | | | **Conclusion** |
| **Maghbooli, 2008133** | 3 (med) | Tehran, Iran  Overall cohort size=741 women  Cases of GDM=52  Controls=527 | Cross-sectional | Nil.  Cases significantly older, higher parity and higher BMI. | 24-28 weeks\*\* | 16.49 (10.44)\*\* | | 22.97 (18.25)\*\* | | Not given | | Not given | | | 25(OH)D3 significantly lower in individuals with GDM  p=0.009 |
| **Clifton-Bligh, 200892** | 6 (low) | New South Wales, Australia  Cases of GDM= 81 women  Normal pregnancies=183 women | Prospective cohort | Age, BMI, ethnicity, season | Mean (SD) 28.7 (3.3) weeks | 48.6 (24.9) | | 55.3 (23.3) | | Not given | | OR if 25(OH)D <50 nmol/l= 1.92 (0.89,4.17) | | | 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, -0.01) |
| **Zhang, 2008135** | 8 (low) | Omega Study, Seattle and Washington, USA  Overall cohort size=953 women  Cases of GDM=57 women (70% white)  Controls=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 DM  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) | | 30.1 (9.7) | | 25(OH)D conc | Unadjusted OR (95% CI) | 25(OH)D conc | OR1 (95% CI) | OR2 (95% CI) | 25(OH)D is early pregnancy is significantly associated with an elevated risk of GDM |
| 75+ | 1 (referent) | 75+ | 1 (ref) | 1 (ref) |
| 50-74 | 1.86  (0.86,4.01) | 50-74 | 1.86  (0.84,4.09) | 1.56  (0.69,3.52) |
| <50 | 4.33  (1.78,10.5) | <50 | 3.74  (1.47,9.50) | 2.66  (1.01,7.02) |
| P for trend | 0.001 | P for trend | 0.006 | 0.05 |
| Per 12.5 nmol/l reduction | 1.44  (1.16,1.69) | Per 12.5 nmol/l reduction | 1.36  (1.11,1.69) | 1.29  (1.05,1.60) |
| **Farrant, 200990** | 5 (low) | Mysore Parthenon Study, India  Cases of GDM=34 women  Normal pregnancies=525 women | Prospective cohort | Maternal age, fat mass, diabetes status | 30 weeks | 38.8 | | 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 GTT and 25(OH)D in those with 25(OH)D <50 nmol/l |
| **Soheilykhah, 2010134** | 3 (med) | Iran  Cases of GDM=54 women  Controls=111 women | Case-control | Nil  Controls matched for gestational age, maternal age, maternal BMI | 24-28 weeks | 24.05 (20.65)\*\* | | 32.25 (35.8)\*\* | | 25(OH)D3 conc | OR (95% CI) of GDM | No multivariate analysis performed | | | Significantly increased risk of GDM if 25(OH)D3 <37.5 nmol/l. |
| <50 | 2.02 (0.88,4.6) |
| <37.5 | 2.66 (1.26,5.6) |
| **Makgoba, 2011136** | 7 (low) | London, UK  Overall cohort size=1200 women  Cases of GDM=90 women  Controls=158 women | Nested case-control | Unclear how cases and controls were matched  Cases had higher BMI, prior history of Type 2 DM and a family history of Type 2 DM, 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+6 weeks | 47.2 (26.7) | | 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; 0.782 in multivariate analysis  25(OH)D negatively associated with fasting glucose (p=0.0009), 2 hour glucose following GTT (p=0.002) and HbA1c (0.002) at 28 weeks in univariate analysis. After adjustments however the only significant relationship remaining was with 2 hour glucose (p=0.048) |
| **Baker, 2012137** | 7 (low) | North-Carolina, USA  Overall cohort=4225 women  Cases of GDM=60 women  Controls=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 | | Mean not given | | 1.25 (0.39,4.05) if 25(OH)D <50 compared with those with 25(OH)D >75 | | 0.78 (0.22,2.78) 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 conc | N (%) | 25 (OH)D conc | N (%) |
| <50 | 5 (8.3) | <50 | 8 (6.7) |
| 50-74.9 | 11 (18.3) | 50-74.9 | 24 (20) |
| 75+ | 44 (73.3) | 75+ | 88 (73.3) |
| **Fernandez-Alonso, 2012115** | 3 (med) | Almeria, Spain  Cohort=466 women  Cases of GDM=36 | Prospective cohort | Nil | 11-14 weeks | Overall mean not given | | 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 |
| 25(OH)D conc | N |
| <50 | 109 |
| 50-75 | 191 |
| >75 | 166 |

\*\* Measured 25(OH)D3

**Table 30: The effect of maternal vitamin D status in gestation on Caesarean section (C-section) – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and 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 C-section** | | **Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in vaginal deliveries** | **Odds ratio/ Relative risk of C-section from univariate analysis** | **Odds ratio of C-section from multivariate analysis** | | | | **Conclusion** |
| **Ardawi, 199787** | 5 (low) | Jeddah, Saudi Arabia  Cohort size=264 women | Cohort | nil | Delivery | Not given  C-section incidence of 12.5% (n=3) if 25(OH)D <20 nmol/l  c-section rate of 9.59% (n=23) if 25(OH)D >20 nmol/l | | Not given | Not given | Not given | | | | 25(OH) <20 nmol/l was associated with an increased rate of C-section but results not significant (p>0.05). |
| **Brunvand, 1998140** | 1 (med) | Pakistan  Cases=37 women  Controls=80 women  All nulliparous Pakistani women of low social class  Cases all had emergency C-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 birth weight.  Maternal height and birth weight included in logistic regression model | Just before delivery\*\* | 26 (15-37)\*\* | | 19 (11-27)\*\* | Not given | 1.03 (0.99,1.06) | | | | No significant association seen between maternal 25(OH)D concentration and risk of emergency C-section due to obstructed labour |
| **Merewood, 2009139** | 6 (low) | Boston, USA  Cohort=277 women  Cases=67 women  All cases were women having primary C-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 | 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 (1.20,4.92) | If 25(OH)D <37.5 nmol/l, adjusted OR= 3.84 (1.71,8.62) | | | | 25(OH)D <37.5 nmoll/l is significantly associated with an increased risk of primary C-section |
| **Scholl, 2012138** | 5 (low) | Camden cohort, New Jersey, USA  Cohort=1153 women  Cases=290 women (173 primary C-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 | Not given | | Overall mean not given | Not given | 25(OH)D conc. | OR1 (95% CI) | | OR2 (95% CI) | Serum 25(OH)D <30 was associated with a significantly increased risk of overall C-section in both regression models .  Regarding primary C-section, if BMI is not included in the model (OR1), serum 25(OH)D <30 was associated with a significantly increased risk of primary C-section,  When maternal BMI is included (OR2) in the model the trend remains but the relationship no longer remains significant (p=0.054)  Risk of overall C-section and primary C-section due to prolonged labour was significantly higher iif 25(OH)D <30 nmol/l even after adjusting for maternal BMI (OR2 = 2.24 (1.17,3.98) for primary C-section) |
| <30 | 1.70  (1.12,2.58) | | 1.66  (1.09,2.52) |
| 30-49.9 | 0.89  (0.63,1.25) | | 0.83  (0.59,1.17) |
| 50-125 | Ref | | Ref |
| >125 | 0.59  (0.17,2.08) | | 0.90  (0.49,1.66) |
| **Savvidou, 2012141** | 7 (low) | London, UK  Cohort=1000 women  Cases=199 women (111 emergency) | Cohort | Maternal age, racial origin, smoking, method of conception, season of blood sampling | Between 11-13 weeks | 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 multiples of the median after adjustments | | | | No significant association seen between maternal 25(OH)D concentration and risk of either elective or emergency C-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) | |
| **Fernandez-Alonso, 2012115** | 3 (med) | Almeria, Spain  Cohort=466 women  Cases=105 women (61 emergency) | Cohort | Nil | Between 11-14 weeks\*\* | Overall mean not given | | Not given | Not given | Not given | | | | No significant association between C-section rates as a function of first trimester 25(OH)D status  Overall C-section, p=0.65  Emergency C-section p=0.47  Elective C=section p=0.06 |
| 25(OH)D conc | N |
| <50 | 23 |
| 50-75 | 41 |
| >75 | 41 |

\*\* Measured 25(OH)D3

**Table 31: The effect of maternal vitamin D status in gestation on risk of bacterial vaginosis – Observational studies**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **First Author and 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 bacterial vaginosis** | **Mean (SD) or median (IQR) 25(OH)D concentration (nmol/l) in unaffected controls** | **Odds ratio of bacterial vaginosis from univariate analysis** | | **Odds ratio of bacterial vaginosis from multivariate analysis** | | **Conclusion** |
| **Bodnar, 2009142** | 5 (low) | Pittsburgh USA  Cohort=469 women all non-Hispanic white or non-Hispanic black)  Cases=192 (approx.) | 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 | Unadjusted geometric mean = 29.5 (27.1-32.0) | Unadjusted geometric mean = 40.1 (37.0-43.5) | Not given | | Prevalence ratio (PR) given | | 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 this this, no significant relationship was observed |
| 25(OH) conc nmol/l | Adjusted PR (95% CI) |
| 20 (25th centile) | 1.65  (1.01,2.69) |
| 50 (75th centile) | 1.26  (1.10,1.57) |
| 75 (90th centile | Referent |
| 90 (97th centile) | 1.32 (0.84,2.09) |
| **Hensel, 2011143** | 4 (med) | National Health and Nutrition Examination Survey (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 | Not given | Not given | Not given | | Adjusted odd ratio (95% CI) if Vitamin D deficient (<75 nmol/l) = 2.87 (1.13,7.28). p=0.03 | | Serum 25(OH)D <75 nmol/l is significantly associated with an increased risk of bacterial vaginosis |
| **Dunlop, 2011144** | 2 (med) | Sample of the Nashville Birth Cohort  Total cohort size=1547 women  Sample size=160 women (all non-Hispanic white or non-Hispanic black)  Cases=14 | Cross-sectional | Race, age, smoking, BMI, gestational age at delivery, payer source | At delivery | 45.0 (20.35) | 60.85 (29.93) | 25(OHD conc (nmol/l) | OR (95% CI | 25 (OH)D con (nmol/l) | Adjusted OR  (95% CI) | 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 |
| <30 | 7.58  (2.13,27.03) | <30 | 5.11  (1.19,21.97) |
| <50 | 1.4  (0.79,14.93) | <50 | 1.2  (0.39,3.85) |

* 1. Appendix 7: Forest plots

Figure 2: Forest plot of the effect of maternal vitamin-D on offspring birth weight – observational studies using log-transformed 25(OH)-D (unadjusted)

bwt_lreg_log_unadjusted.wmf

Figure 3: Forest plot of the effect of maternal vitamin-D on offspring birth weight – observational studies using log-transformed 25(OH)-D (adjusted)

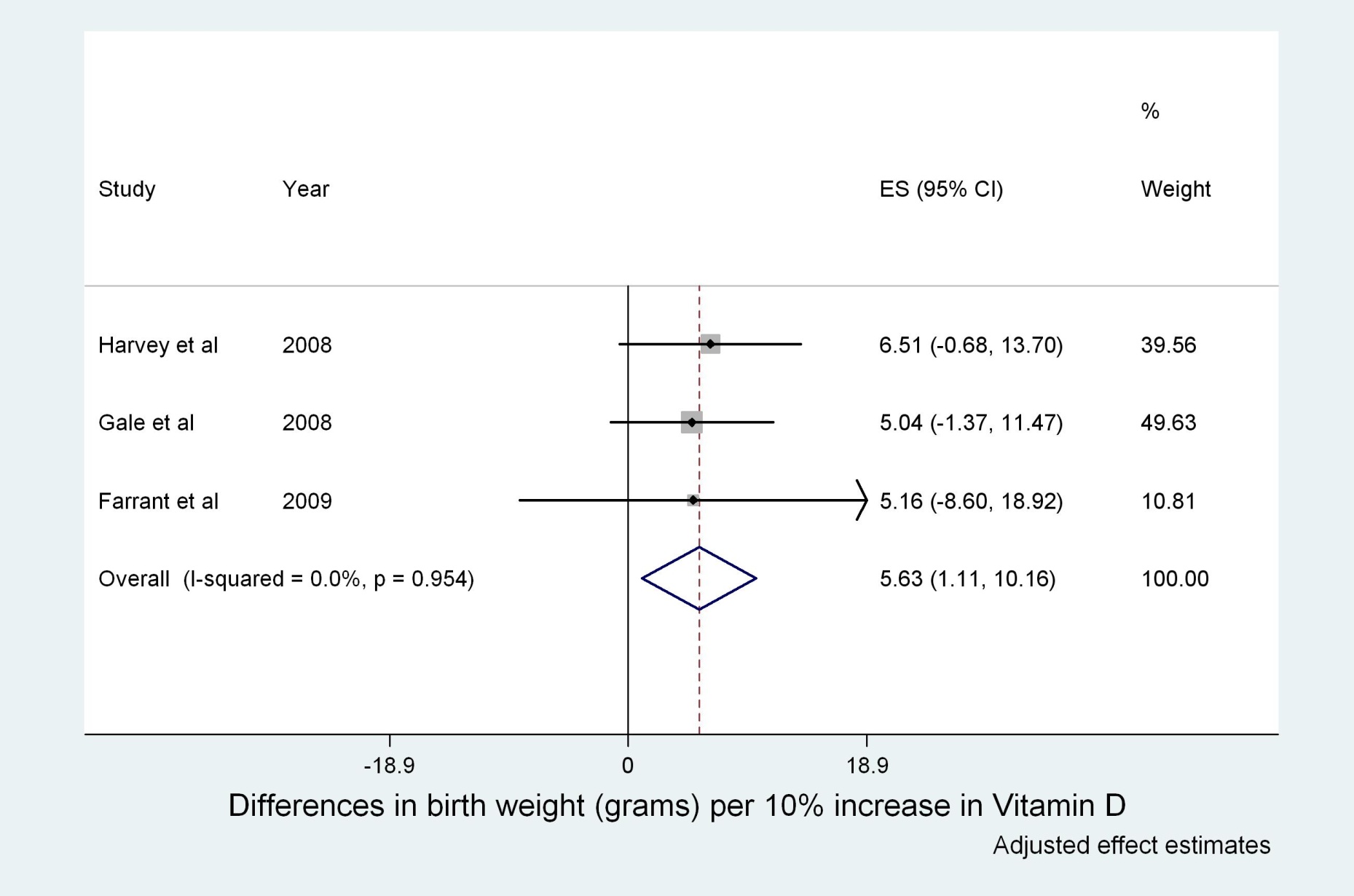


Figure 4: Forest plot 3 of the effect of maternal vitamin-D on offspring birth weight – observational studies (unadjusted)

bwt_reg_orig_unadj.wmf

Figure 5: Forest plot of the effect of maternal vitamin-D on offspring birth weight – observational studies (adjusted)

bwt_reg_orig_adj.wmf

Figure 6: Forest plot of the effect of maternal vitamin-D supplementation on offspring birth weight – intervention studies (low dose)

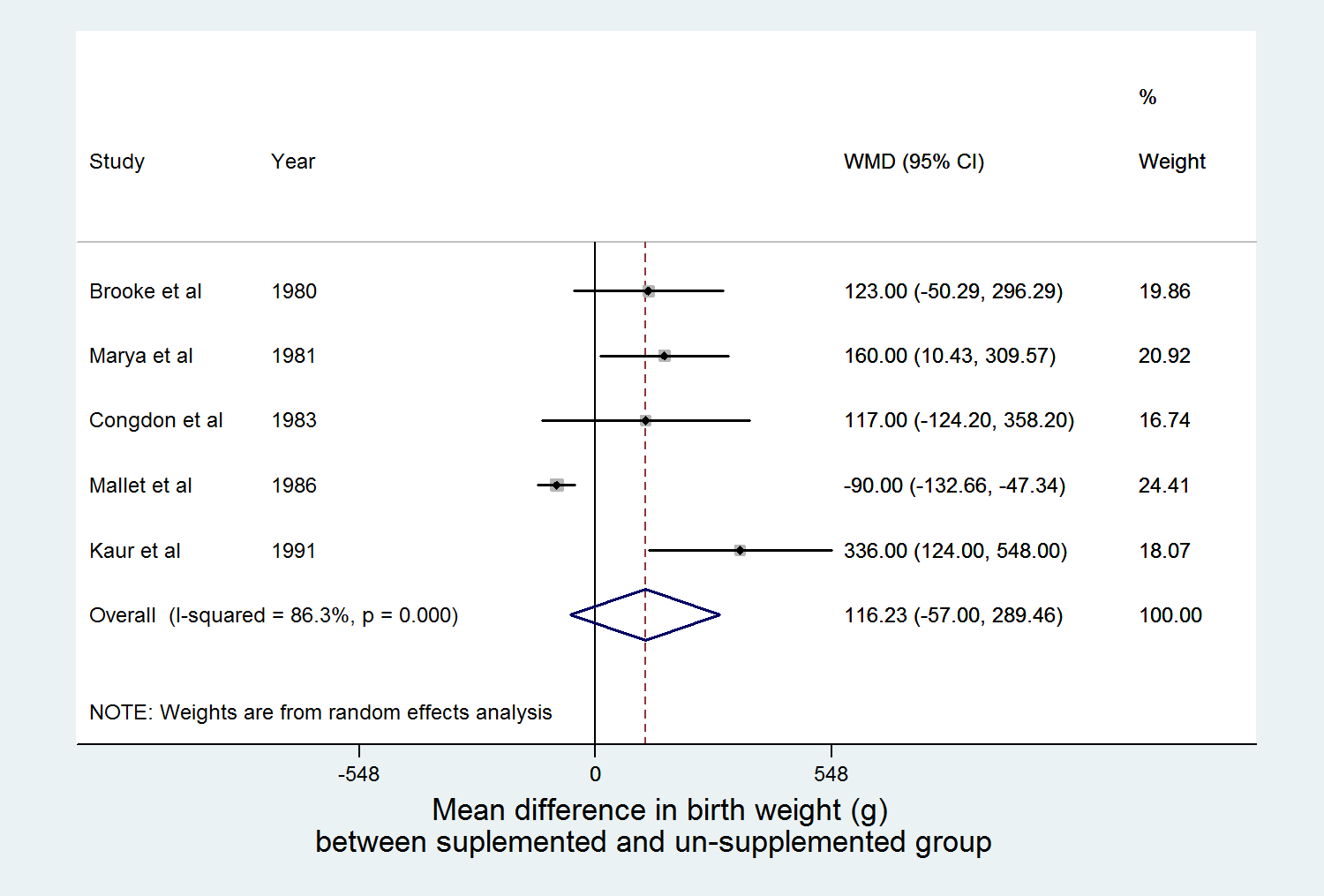


Figure 7: Forest plot of the effect of maternal vitamin-D supplementation on offspring birth weight – intervention studies (high dose)

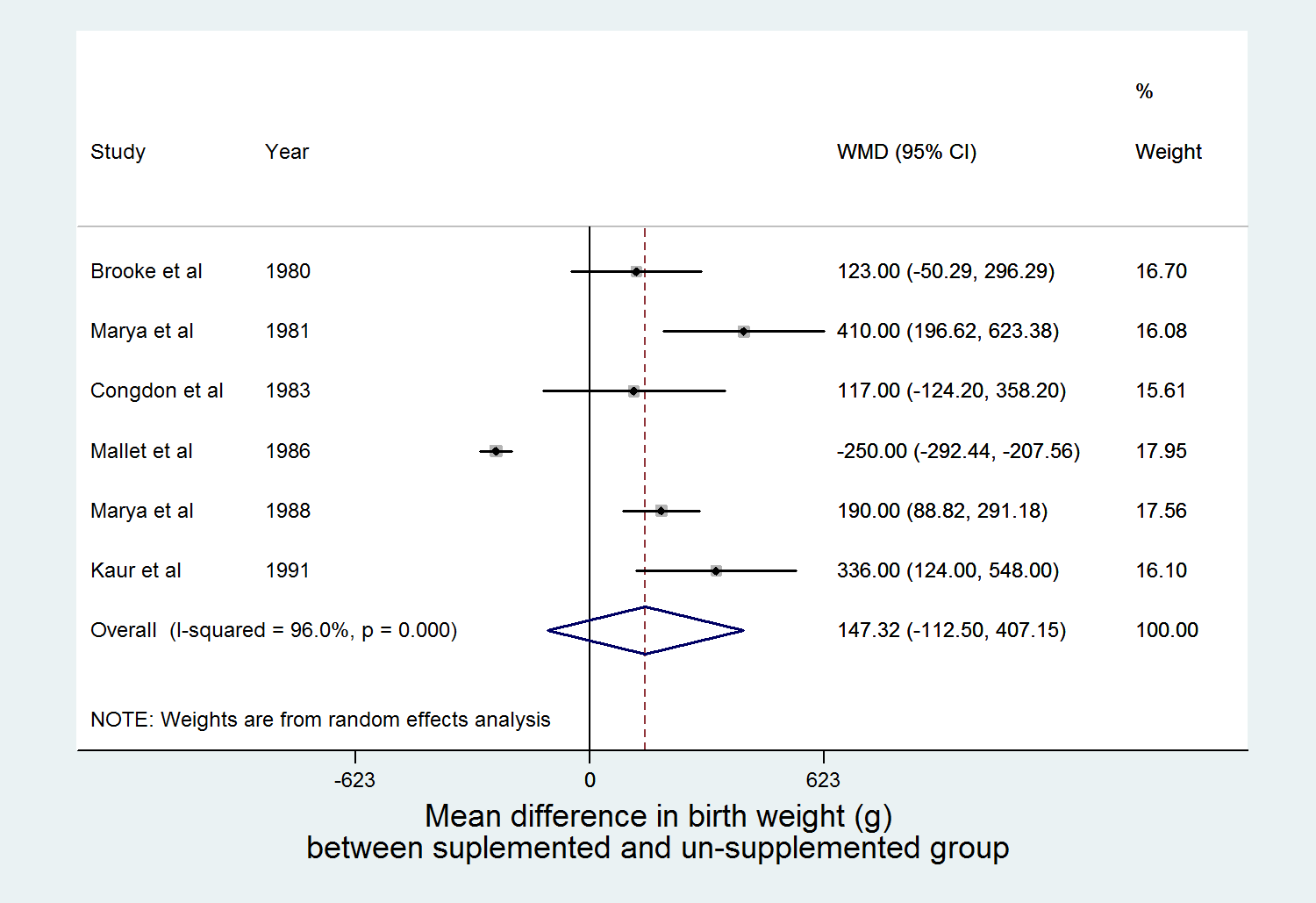


Figure 8: Forest plot of the effect of maternal vitamin-D supplementation on offspring calcium concentration – intervention studies (low dose)

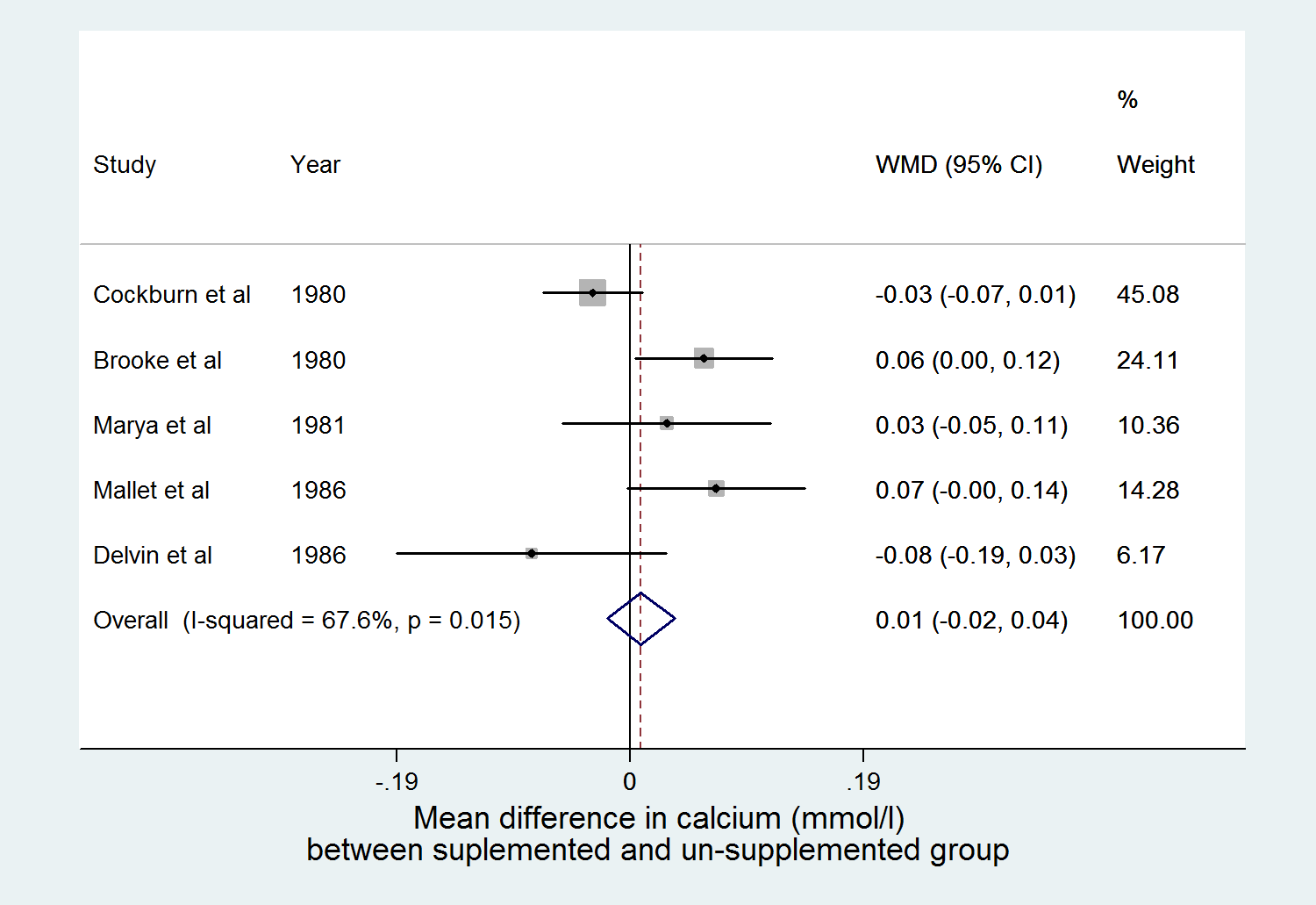


Figure 9: Forest plot of the effect of maternal vitamin-D supplementation on offspring calcium concentration – intervention studies (high dose)

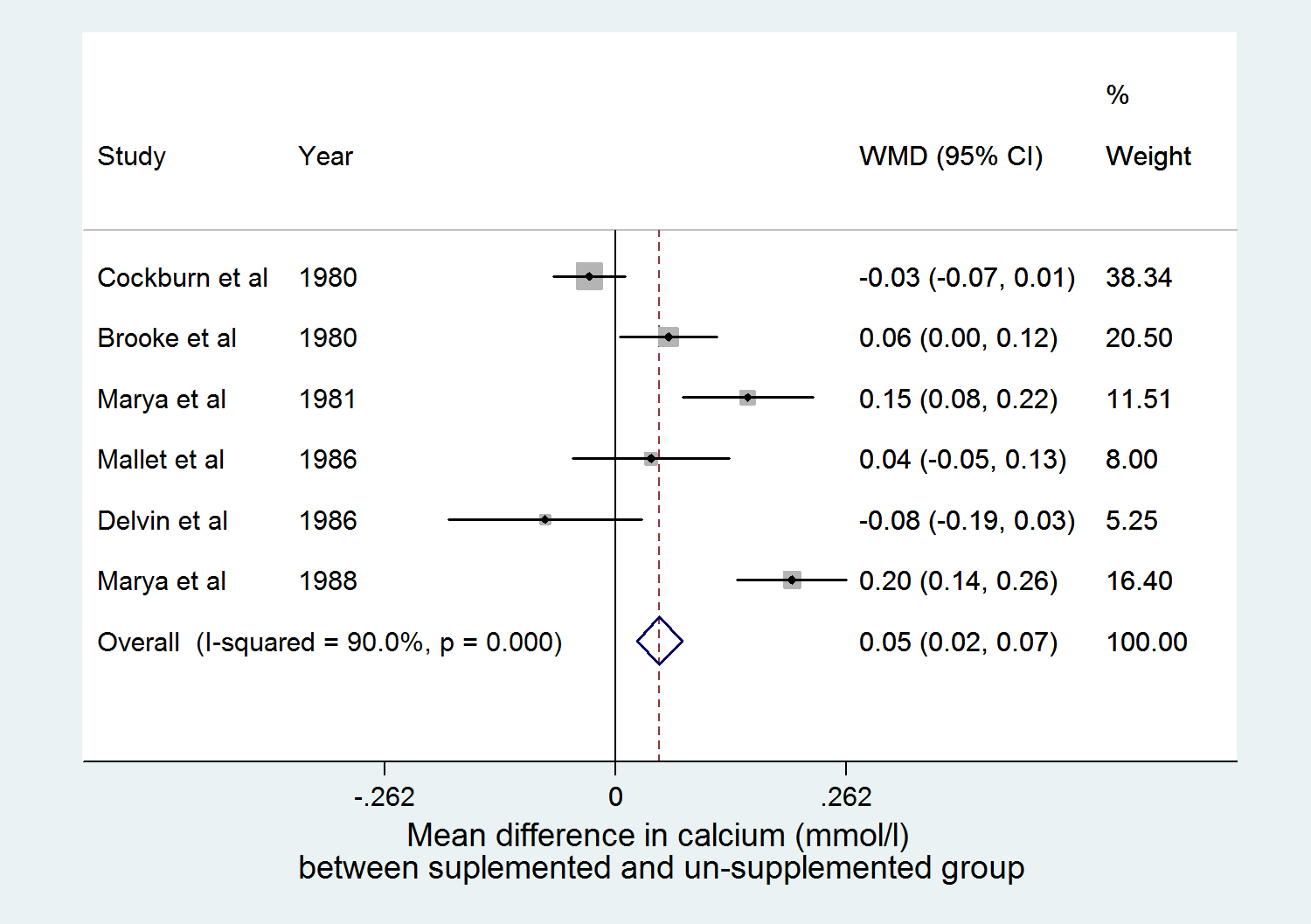


Figure 10: Forest plot of the effect of maternal vitamin-D on risk of preeclampsia – observational studies (unadjusted)

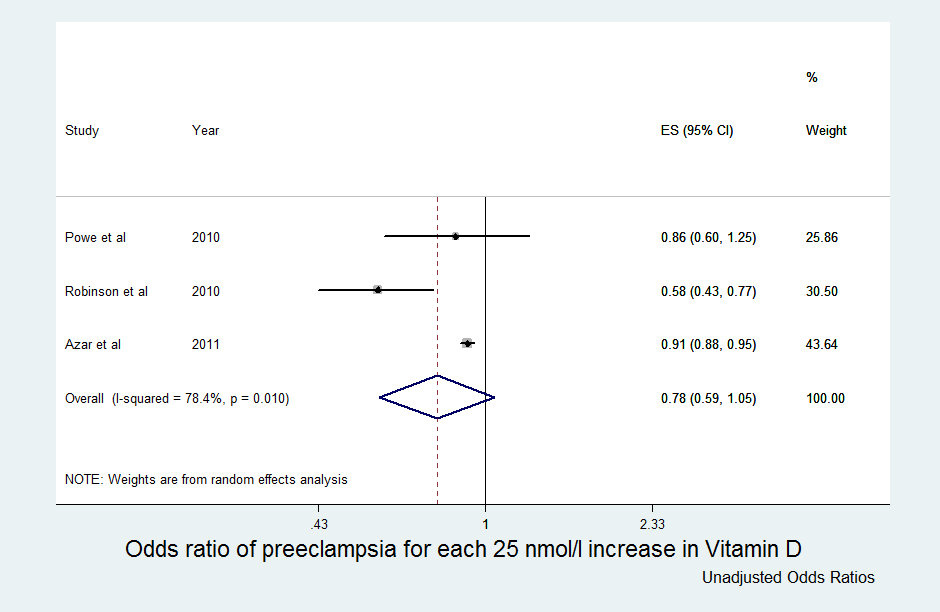
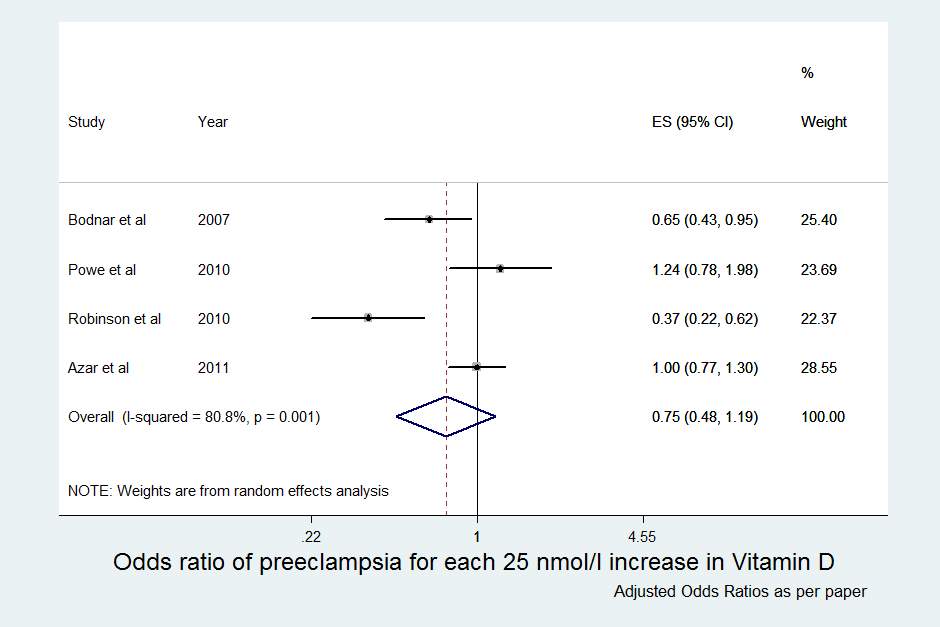


Figure 11: Forest plot of the effect of maternal vitamin-D on risk of preeclampsia – observational studies (adjusted)



* 1. Appendix 8: Original and final protocol (separate document)