ENDOCRINOLOGY IN PREGNANCY

Influence of maternal vitamin D status on obstetric outcomes and the fetal skeleton

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Abstract

Vitamin D status has been increasingly associated with wide-ranging clinical outcomes. There is now a wealth of observational studies reporting on its associations with obstetric complications, including pre-eclampsia, gestational diabetes and the mode and timing of delivery. The findings are inconsistent, and currently there is a lack of data from high-quality intervention studies to confirm a causal role for vitamin D in these outcomes. This is similarly true with regards to fetal development, including measures of fetal size and skeletal mineralisation. Overall, there is an indication of possible benefits of vitamin D supplementation during pregnancy for offspring birthweight, calcium concentrations and bone mass as well as for reduced maternal pre-eclampsia. However, for none of these outcomes is the current evidence base conclusive, and the available data justify the instatement of high-quality randomised placebo controlled trials in a range of populations and health care settings to establish the potential efficacy and safety of vitamin D supplementation to improve particular outcomes.

Introduction

The classical role of vitamin D is in calcium and phosphate homoeostasis: it is without doubt that severe vitamin D deficiency (VDD) can result in rickets, osteomalacia and hypocalcaemia. However, there has been increasing evidence to suggest that VDD is associated with wide-ranging clinical outcomes, including pregnancy complications and adverse fetal development. As a result, a number of national guidelines recommend vitamin D

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supplementation during pregnancy (1, 2, 3), although this is not currently supported by the World Health Organisation (4). In the present review, we consider the evidence basis for antenatal vitamin D supplementation to prevent obstetric complications and the influence of vitamin D on fetal growth and skeletal development.

Literature search

The present review is based on literature identified through our recently published systematic review of vitamin D in pregnancy (in relation to both maternal and offspring outcomes), in which published and grey literature were comprehensively searched for maternal and offspring health outcomes across a wide range of databases from their inception until 2012 (5). A full systematic update was outside the scope of the present review, but we aimed to identify important additional studies using the US National Library of Medicine National Institutes of Health (www.pubmed.com) with the search terms 'vitamin D' AND 'pregnancy' up to August 2014.

Vitamin D physiology and epidemiology in pregnancy

Vitamin D can be derived from the diet as either ergocalciferol (vitamin D_2) from plant sources or as cholecalciferol (vitamin D_3) from animal sources. However, the majority is formed endogenously within the skin from the action of ultraviolet B (UVB; 290–315 nm wavelength) to convert 7-dehydrocholesterol to pre-vitamin D_3. Hydroxylation within the liver produces 25-hydroxyvitamin D (25(OH)D). This is the main circulating form of vitamin D, and it is found either bound to vitamin D binding protein (VDP), bound to albumin or in the free form. 25(OH)D acts as a reservoir for conversion to 1,25-dihydroxyvitamin D (1,25(OH)$_2$D), primarily in the renal proximal tubular cells, but also within bone, the parathyroid gland and placenta. Although 1,25(OH)$_2$D is the active metabolite, its production is regulated in response to serum calcium and its half life is short, at 4–6 h. Conversely, hepatic 25-hydroxylation is not physiologically regulated, and 25(OH)D has a half-life of ∼2–3 weeks (6). Therefore, serum 25(OH)D is currently considered the best marker of vitamin D status (7).

The primary function of 1,25(OH)$_2$D is calcium and phosphate homoeostasis, which occurs in conjunction with parathyroid hormone (PTH). Thus, low serum ionised Ca^{2+} stimulates PTH release, which simultaneously increases renal calcium reabsorption in the distal tubule of the kidney, decreases proximal tubule phosphate reabsorption, and increases 1,25(OH)$_2$D synthesis. The main action of 1,25(OH)$_2$D is to increase the uptake of dietary calcium through the intestinal enterocytes, but it also enables the PTH-induced mobilisation of calcium and phosphate from bone mineral (8).

During pregnancy, alterations to calcium and phosphate metabolism occur to allow the accretion of calcium within the fetal skeleton, particularly during the final trimester (9). This occurs through increased maternal intestinal calcium absorption (10, 11) and the mobilisation of calcium within the maternal skeletal (12) but without alteration to the maternal serum ionised calcium concentration. Maternal calcitropic hormones, including 1,25(OH)$_2$D, likely play an important role in these adaptations, seeing as total 1,25(OH)$_2$D increases during the second and third trimesters (10, 13). This could, however, also reflect the increase in VDP from early to late pregnancy (11, 14). The increase in 1,25(OH)$_2$D appears to be independent of PTH, which remains within the normal adult range throughout pregnancy (9). However, PTH-related protein (PTHrP) is elevated in the maternal circulation beginning in early pregnancy and might contribute to the rise in 1,25(OH)$_2$D (13). The effect of pregnancy on 25(OH)D is less well understood: Zhang et al. (14) observed a reduction in 25(OH)D in late pregnancy as compared to early pregnancy, but this might have been a reflection of seasonal variation, seeing as all of the subjects were recruited in summer months. In contrast, Ritchie et al. (11) reported no significant differences in 25(OH)D measured in 14 women before pregnancy, during each trimester and during lactation. Nonetheless, biochemically low levels of 25(OH)D are highly prevalent: in a cohort of predominantly Caucasian women in the UK, 31% had a serum 25(OH)D of <50 nmol/l, which is widely considered to be insufficient, and 18% had <25 nmol/l, which is often considered deficient (15). However, in an ethnically more diverse UK population, 36% of women had a 25(OH)D of <25 nmol/l at pregnancy booking (16). Indeed, dark skin pigmentation and extensive skin covering (e.g. for religious or cultural reasons) are the strongest risk factors for VDD. Obesity is also associated with biochemically low 25(OH)D levels, whereas in pregnancy, the use of vitamin D supplements may prevent deficiency (15). Maternal 25(OH)D in pregnancy is an important consideration, because the fetus is entirely dependent on the mother for 25(OH)D. 25(OH)D readily crosses the placenta, and maternal and umbilical cord venous blood 25(OH)D are moderately to highly correlated, with umbilical cord

concentrations typically being lower than those of the maternal blood, although the reported correlation coefficient does vary markedly between studies ($r = 0.44$–0.89 (17, 18, 19, 20)). Randomised controlled trials have clearly demonstrated that vitamin D supplementation during pregnancy can increase umbilical cord venous and neonatal serum 25(OH)D as compared to a placebo (21, 22, 23, 24, 25, 26, 27, 28).

Obstetric complications

Observational studies

Numerous observational studies have reported associations between either vitamin D intake in pregnancy or serum measurement of 25(OH)D and pregnancy complications, including gestational hypertension (GHT) and pre-eclampsia (PET), gestational diabetes (GDM), timing of delivery and mode of delivery. The interpretation and comparison of these studies is limited by the timing of 25(OH)D measurements, which range from first trimester to delivery, the definitions used for both VDD and the outcome, the covariates that are included and the study design (e.g. prospective cohort or case–control).

Gestational hypertension and pre-eclampsia

Although the aetiology of PET is poorly understood and likely multifactorial, there is some evidence that maternal calcium status might be important and that calcium supplementation can reduce PET risk, particularly in women with low calcium intake (29). Thus, exploring a role for calcitropic hormones, including vitamin D, is a sensible approach. Several case–control and prospective cohort studies have demonstrated that women who developed PET had lower serum 25(OH)D as compared to controls in early (30, 31, 32), mid- (33, 34) or late (30, 35, 36) pregnancy and that VDD increased the risk of PET (30, 35, 37). One case–control study suggested that women with serum 25(OH)D of <37.5 nmol/l at <22 weeks gestation had a fivefold higher risk of PET than did women with a 25(OH)D of >37.5 nmol/l, independent of ethnicity, season, gestational age at sampling, pre-pregnancy BMI and educational achievement (30). Similarly, in a cohort of 23 425 pregnant women in Norway, lower vitamin D intake that was estimated from a food frequency questionnaire at 22 weeks gestation was associated with a significantly increased risk of PET (38). The lower vitamin D intake in women who developed PET was mostly the result of a difference in vitamin D obtained from supplements, which

suggests that supplementation might prevent PET. However, these findings are not supported by all studies (32, 39, 40, 41, 42, 43, 44, 45, 46). Indeed, in a prospective cohort of 1591 women, for each additional 25 nmol/l increment in 25(OH)D in early pregnancy, the risk of gestational hypertension (GHT) (without PET) increased by 30%, but no effect on PET risk was observed (43), which highlights possible detrimental effects of higher vitamin D status.

In recent years, there have been several published meta-analyses of the relationship between maternal vitamin D status and PET risk, as shown in Table 1 (5, 47, 48, 49, 50, 51). As with the observational studies, the conclusions of these meta-analyses are inconsistent. In our own meta-analysis, we found no significant reduction in the risk of PET with higher vitamin D status (Fig. 1) (5). In contrast, Aghajafari et al. (49) found that the increased risk of PET in VDD was only observed in studies in which blood sampling occurred after 16 weeks gestation and when VDD was defined as 25(OH)D of <75 nmol/l and not <50 nmol/l. However, Tabesh et al. (50), who included a larger number of studies that defined VDD as <50 nmol/l, demonstrated an increased risk of PET, which was not found when deficiency was defined as <38 nmol/l. Importantly, the total number of women included in these meta-analyses varied from 610 to 2485 (excluding those based on intake only and the most recent meta-analyses, which included novel data (47)). However, between January 2013 and July 2014, at least a further 14 case–control or prospective cohort studies that measured serum 25(OH)D and assessed PET risk have been published (32, 36, 37, 44, 45, 46, 47, 52, 53, 54, 55, 56, 57, 58, 59). These newer studies include data for an additional 21 000 women, considerably more than were included in the published meta-analyses.

Gestational diabetes

Similarly to PET, conflicting findings have been reported for 25(OH)D status in case–control and prospective cohort studies of GDM risk: both lower (52, 60, 61, 62, 63, 64, 65) and similar serum 25(OH)D (66, 67) during pregnancy in women with and without GDM have been reported. One study of women who were referred for GDM screening did not find a difference in the prevalence of GDM in women with 25(OH)D of more than and <50 nmol/l, but the women with 25(OH)D of <50 nmol/l did have higher fasting blood glucose, HBA1c and insulin resistance. However these women also had higher BMI, lower physical activity and were less likely to be Caucasian, which might have confounded the findings (68). Three separate

Table 1 Meta-analyses of maternal vitamin D status (intake and serum 25-hydroxyvitamin D level) and risk of pre-eclampsia.
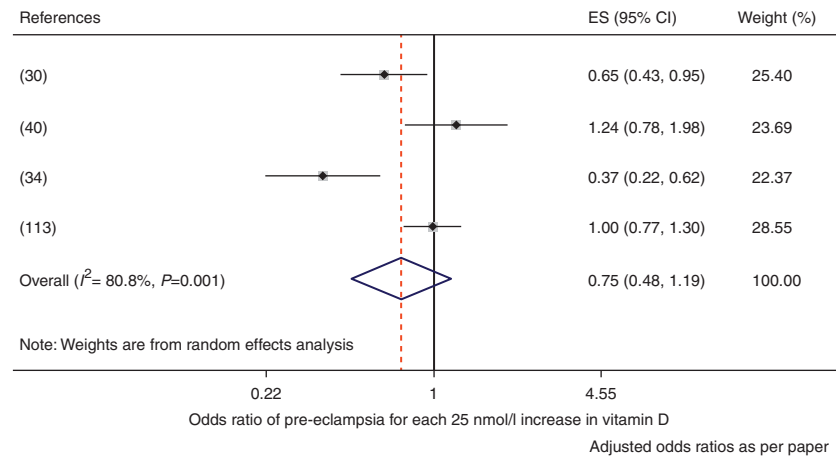
Author	Publication cut-off	Number of studies included	Number of women included	Comparison	Risk of pre-eclampsia with low vitamin D status	
					Direction of effect	Reported odds ratio (95% CI)
Vitamin D intake						
Thorne-Lyman (2012) (48)	June 2011	2	25 141	Highest vs lowest category of vitamin D intake	↔	0.95 (0.86, 1.06)
Hypponen (2013) (47)	March 2013 + inclusion of novel data	2	77 165	Self-supplementation vs unsupplemented	↑	1.23 (1.15, 1.33)
Serum 25(OH)D						
Aghajafari (2013) (49)	August 2012	2	697	Serum 25(OH)D ≥50 nmol/l vs <50 nmol/l	↔	1.27 (0.67, 2.42)
		5	1165	Serum 25(OH)D ≥75 nmol/l vs <75 nmol/l	↑	2.11 (1.36, 3.27)
		7	1862	Higher serum 25(OH)D as defined by each study vs lower serum 25(OH)D	↑	1.79 (1.25, 2.58)
		7	1862	Higher serum 25(OH)D as defined by each study vs lower serum 25(OH)D, adjusted for 'critical confounders'	↔	1.51 (0.89, 2.57)
Hypponen (2013) (47)	March 2013 + inclusion of novel data	6	6864	Higher serum 25(OH)D as defined by each study vs lower serum 25(OH)D	↑	1.92 (1.12, 3.33)
Tabesh (2013) (50)	December 2012	4	931	Serum 25(OH)D ≥38 nmol/l vs <38 nmol/l	↔	Actual odds ratios not reported
		5	1775	Serum 25(OH)D ≥50 nmol/l vs <50 nmol/l	↑	
		8	2485	Higher serum 25(OH)D as defined by each study vs lower serum 25(OH)D	↑	
Wei (2013) (51)	October 2012	6	610	Serum 25(OH)D ≥50 nmol/l vs <50 nmol/l	↑	2.09 (1.50, 2.90)
		5	802	Serum 25(OH)D ≥75 nmol/l vs <75 nmol/l	↑	1.78 (1.23, 2.56)
Harvey (2014) (5)	June 2012	4	628	Each 25 nmol/l increase in serum 25(OH)D	↔	0.78 (0.59, 1.05)

meta-analyses of published studies all concluded that women with GDM had significantly lower mean 25(OH)D than normoglycaemic women did (49, 51, 69), with the mean difference in 25(OH)D ranging from 3.9 to 7.4 nmol/l. Furthermore, these meta-analyses suggested that the risk of GDM was increased by 40–60% in women with VDD (49, 51, 69), as shown in Fig. 2 (49). However, as was the case with the studies that assessed PET risk, there is now substantially more data available than there was for these meta-analyses (44, 52, 62, 63, 64, 65, 67, 70). Although many of the smaller studies would have supported the previous conclusions, a large prospective cohort of women in Australia, including 5109 women, of whom 7.4% developed GDM, first trimester VDD (defined either as <25 nmol/l or <37.5 nmol/l) was not associated with an increased risk of GDM as compared to 50–75 nmol/l 25(OH)D after adjustment for age, parity, smoking during pregnancy, maternal weight, previously diagnosed hypertension, diabetes, season at sampling, country of birth or socio-economic disadvantage (52). Furthermore, in 1953 women in southern China, vitamin D sufficiency (25(OH)D of >75 nmol/l) at 16–20 weeks gestation was associated with a small, but statistically significant, increased risk of GDM (OR 1.02, 95% CI 1.00, 1.04) (44).

Caesarean delivery

Unsurprisingly, in recent years, there has also been an increase in studies that have reported maternal vitamin D status in relation to the mode and timing of delivery.

References	ES (95% CI)	Weight (%)
(30)	0.65 (0.43, 0.95)	25.40
(40)	1.24 (0.78, 1.98)	23.69
(34)	0.37 (0.22, 0.62)	22.37
(113)	1.00 (0.77, 1.30)	28.55
Overall (I^2= 80.8%, P=0.001)	0.75 (0.48, 1.19)	100.00

Note: Weights are from random effects analysis

0.22   1   4.55

Odds ratio of pre-eclampsia for each 25 nmol/l increase in vitamin D

Adjusted odds ratios as per paper

Figure 1

Forest plot of the association between maternal vitamin D status and risk of pre-eclampsia (observational studies). Reproduced from Harvey N, Holroyd C, Ntani G, Javaid M, Cooper P, Moon R, Cole Z, Tinati T, Godfrey K, Dennison E *et al*. Vitamin D supplementation in pregnancy: a systematic review. *Health Technology Assessment* 2014 **18** 1–190.

Again, these are inconsistent. After adjusting for potential confounding factors, three studies, which assessed 25(OH)D in early pregnancy, when attending for GDM screening and at delivery, reported an increased risk of Caesarean delivery (68, 71, 72). Conversely, two studies which measured 25(OH)D in the first trimester demonstrated no increased risk (42, 44). Assessment of the influence of VDD on the mode of delivery is further complicated by the underlying cause for intervention. Savvidou *et al.* (73) additionally categorised women requiring emergency caesarean delivery as a result of a failure to progress and for fetal distress. Neither group had significantly different serum 25(OH)D levels in early pregnancy from those of women who delivered vaginally (73).

Preterm delivery

More studies have concluded that maternal 25(OH)D status is not related to preterm birth (39, 42, 52, 74, 75, 76, 77, 78) than have shown that VDD increases this risk (68, 79, 80). Furthermore, Zhou *et al.* (44) reported that women with higher vitamin D status at 16–20 weeks gestation had a higher odds of preterm delivery, and Hossain *et al.* (81) similarly found that cord blood 25(OH)D was higher in preterm (<37 weeks gestation) deliveries (mean 55 nmol/l) as compared to term pregnancies (mean 40 nmol/l, P=0.009) in women in Pakistan. Interestingly, two of the studies which suggested that VDD increased the risk of preterm delivery used a definition of <35 weeks gestation for preterm (79, 80), whereas all but one (78) of the studies

which reported either no relationship or that VDD reduced the risk of preterm delivery considered preterm delivery to be at <37 weeks gestation. Although this might suggest that VDD is particularly associated with an increased risk of very preterm birth, Schneuer *et al.* (52), who prospectively studied first trimester 25(OH)D status in more than 5000 women, found that VDD did not increase the risk of either all or spontaneous preterm birth at <34 weeks gestation before or after adjustment for potential confounding factors. However, differences in the timing of 25(OH)D assessment, and the inclusion of only twin pregnancies in one study that showed increased risk (79), could account for these different findings. Furthermore, Bodnar *et al.* (80) observed that only non-white mothers had an increased risk of preterm birth with low 25(OH)D at 26 weeks gestation, which suggests that the stratification of women by ethnicity in future intervention studies might be necessary.

Intervention studies of vitamin D supplementation to reduce obstetric complications

Observational data cannot confirm a causal effect of vitamin D or justify population-wide supplementation, particularly seeing as some studies have suggested possible detrimental effects of higher 25(OH)D (43, 44, 81). Because 25(OH)D status is primarily determined by environmental factors, confounding and reverse causality need to be considered, and differences in the covariates included in multivariate models might explain the inconsistent findings. For example, obese individuals

25(OH)D concentration insufficiency and GDM by cut-off levels
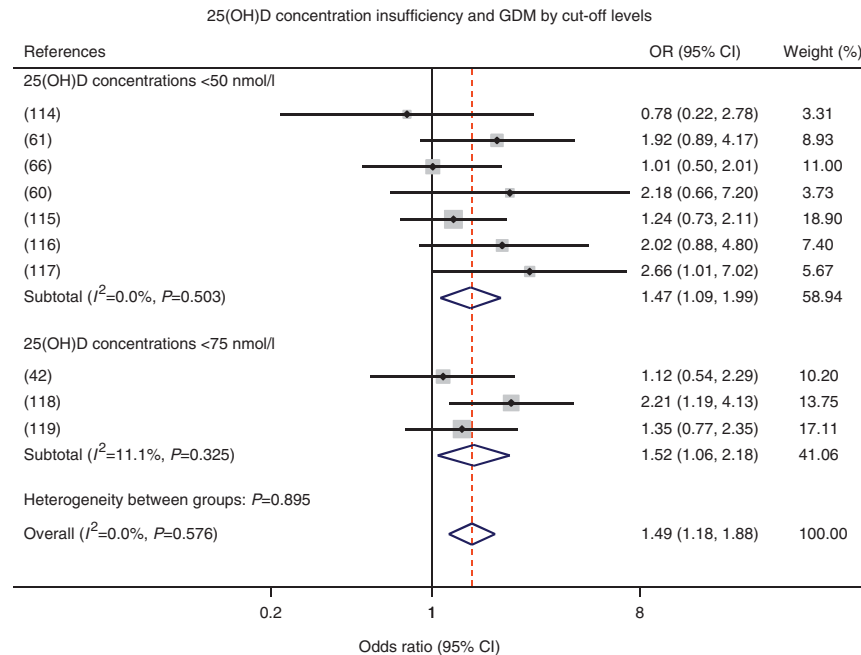
Figure 2

Meta-analysis of maternal serum 25(OH)D in pregnancy and gestational diabetes. Reproduced from Aghajafari F, Nagulesapillai T, Ronksley PE, Tough SC, O'Beirne M & Rabi DM. Association between maternal serum 25-hydroxyvitamin D level and pregnancy and neonatal outcomes: systematic review and meta-analysis of observational studies. *BMJ* 2013 **346** f1169. doi: 10.1136/bmj.f1169 with permission from BMJ Publishing Group Ltd.

have lower 25(OH)D status and a higher incidence of GDM, GHT, PET, caesarean section and preterm delivery (82, 83). Similarly African-American women are more likely to require delivery by Caesarean section and to experience pre-eclampsia and preterm labour (84). Whether these outcomes can truly be attributed to lower 25(OH)D as compared to Caucasian women and can therefore be prevented with vitamin D supplementation must be established through intervention studies.

Despite the expansiveness of the observational data, there are currently few trials of antenatal vitamin D supplementation that report on maternal outcomes other than maternal/neonatal vitamin D and calcium status (85). In three of the five studies, the interventional product contained only vitamin D (26, 86, 87), whereas the other two assessed the effects of combined vitamin D and calcium supplementation (88, 89) (Table 2). The interpretation of these two studies with regards to GHT and PET is limited, because calcium supplementation is known to reduce the risk of PET (29). Nonetheless, high-dose vitamin D supplementation, with or without calcium supplementation, did not improve the incidence of GHT, PET, GDM, or preterm delivery as compared to either usual care

or low-dose supplementation (26, 86, 87, 88, 89). However, these studies were most likely underpowered to detect a difference in these outcomes. GDM complicates ∼4.5% of pregnancies in the UK (90). Thus, to detect a 50% reduction in this incidence with 80% power at the 5% significance level, 1010 women would have been needed in each study arm. Because PET occurs in 2–3% of pregnancies, even larger study numbers would be needed to detect it.

Although trials of vitamin D supplementation have not yet demonstrated a reduction in the incidence of PET or GDM, there is some evidence to support its effects on blood pressure and glucose metabolism when they are considered as continuous outcomes. For example, Marya *et al.* (89) demonstrated a reduction in both systolic and diastolic BP in women randomised to vitamin D and calcium supplementation as compared to those who received usual care. Confirmation of this finding using vitamin D alone is now needed. Three studies have assessed the effects of vitamin D supplementation on insulin resistance. In an unblinded study of 113 Iranian women randomised to one of three treatment groups (200 IU/day, 50 000 IU/month, 50 000 IU/fortnight) from 12 weeks gestation until delivery, insulin resistance, as

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Table 2 Intervention studies of vitamin D supplementation (alone and in combination with calcium supplementation) in pregnancy to reduce obstetric complications.

Study	Population	Gestation at randomisation (weeks)	Interventional medicinal product (IMP)	Control	Effect of IMP vs control on incidence of obstetric events					
					Hypertensive disorders		GDM	Preterm delivery	Caesarean section	Intrauterine death/stillbirth
					GHT	PET				
Vitamin D supplementation										
Hossain (2014) (86) (Karachi, Pakistan)	n=178	20	4000 IU/day oral cholecalciferol	Usual care	↕	↕		↕	↕	↓ (0 vs 1 case, P=0.05)
Wagner (2013) (26) (South Carolina, USA)[1]	n=504	12–16	2000 IU/day oral cholecalciferol (n=201); 4000 IU/day oral cholecalciferol (n=193)	400 IU/day oral cholecalciferol (n=111)	↕		↕	↕		
Yap (2014) (87) (Sydney, Australia)	n=179 25(OH)D <80 nmol/l at baseline	<20	5000 IU/day oral cholecalciferol	400 IU/day oral cholecalciferol		↕	↕	↕	↕	
Vitamin D+calcium supplementation										
Kalra (2011) (88) (Lucknow, India)	n=140	12–24	Group 1: 60 000 IU single-dose oral cholecalciferol at recruitment + 1 g elemental Ca/day until delivery (n=48) Group 2: 120 000 IU oral cholecalciferol at recruitment and 28 weeks gestation + 1 g elemental Ca/day until delivery (n=49)	Usual care (n=43)	↕				↕	↕
Marya (1987) (89) (Rothak, India)	n=400	20–24	1200 IU/day vitamin D+375 mg calcium	Usual care		↕				

↔, no effect shown; ↓, vitamin D supplementation reduced the incidence of the outcome; GHT, gestational hypertension; PET, pre-eclampsia; GDM, gestational diabetes mellitus.
[1]Reported a combined analysis of data collected in two previous studies (22, 109).

assessed by HOMA-IR, increased significantly from baseline to delivery in all three groups, but the rise was significantly less in women randomised to 50 000 IU/fortnight than it was in women who received 200 IU/day (91). In contrast, Yap *et al.* (87) found no difference in either fasting blood glucose or that measured 2 h after glucose load in women randomised to either 400 IU/day or 5000 IU/day cholecalciferol, with similar results for HOMA-IR. Finally, in a small study of 54 women with a diagnosis of GDM, two doses of 50 000 IU cholecalciferol 3 weeks apart improved fasting blood glucose and insulin resistance as compared to a placebo. However, the women randomised to vitamin D supplementation had significantly higher insulin resistance at baseline, which makes these results difficult to interpret (92). Nonetheless, these findings support the need for further high-quality, large randomised controlled trials and the need to concurrently determine if any effects on maternal physiology might also have beneficial effects on maternal and/or fetal morbidity, for example, macrosomia or neonatal hypoglycaemia.

Fetal development

Early rickets and symptomatic neonatal hypocalcaemia have been reported in infants born to mothers with VDD (93, 94, 95). However, these outcomes are rarely reported in infants of white mothers, and they most commonly occur in those born to mothers with dark skin pigmentation, extensive skin covering and profound VDD. The fetus is dependent on the mother for the accretion of ∼30 g of calcium to enable skeletal development. As such, a subclinical role for vitamin D and/or calcium in fetal growth and bone development has been considered, yet maternal supplementation with calcium alone does not appear to have beneficial effects on fetal bone mineral accrual (85).

Size at birth

There are now a number of intervention studies that have assessed the effect of vitamin D supplementation on birth anthropometry, although the dose and timing of introduction of vitamin D have varied widely (Table 3). Most of the studies trialled supplementation with vitamin D alone and did not find a significant effect on birth weight, length or head circumference (Table 1). However, interestingly, vitamin D in combination with calcium did increase birth weight in three studies despite women in the control group also receiving calcium supplementation in two of these studies (88, 96, 97). Indeed, the prevalence of VDD at

baseline and the mean 25(OH)D achieved were similar in a study of women in Bangladesh who received 35 000 IU/day cholecalciferol from 26 to 30 weeks gestation (24) to those in women who participated in a study of 50 000 IU cholecalciferol per week in addition to 200 mg elemental calcium supplementation in Iran (97). Both studies included a similar number of women. However, in the former study, birth weight was similar in both the intervention and control groups, whereas in the latter study, mean birth weight in the intervention group was 170 g greater than that in the control group. These differing findings might suggest that the effect of vitamin D is dependent on the availability of calcium, or they could result from genetic/racial variation in response to vitamin D supplementation, but they nonetheless highlight the importance of using data obtained from an appropriate population in the development of antenatal supplementation policies.

Skeletal development

Currently, the data relating maternal 25(OH)D status to offspring bone development is largely observational in nature, but they do span antenatal measurements to peak bone mass. Indeed, using gestational ultrasound, smaller femoral volumes (98) and widening of the distal femoral metaphysis relative to femur length have been demonstrated in fetuses of mothers with low levels of serum 25(OH)D (99).

A number of studies have demonstrated associations between maternal 25(OH)D status in pregnancy and offspring bone mineralisation during the neonatal period. In 71 Korean neonates, those born in the summer (July–September) had a whole-body bone mineral content (BMC) that was 6% higher than that of infants born in the winter (January–March), and neonatal 25(OH)D at delivery was correlated with whole-body BMC in all children ($r=0.24$, $P=0.05$) (100). However, in three similar studies by the same authors in North America, a reversed pattern was observed, with whole-body BMC being 8–12% lower in infants born in the summer (101). The authors suggest that this difference reflects a low uptake of vitamin D supplementation throughout pregnancy in Korea but only during the first trimester in North America, which therefore indicates that early pregnancy during winter might impact skeletal development (101). However, Weiler *et al.* (102) studied 50 Canadian infants born between August and April, with the majority of mothers taking vitamin D supplementation during pregnancy. Infants with a cord blood 25(OH)D of <37.5 nmol/l ($n=18$) were heavier and longer than

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Table 3 Intervention studies of the effect of vitamin D supplementation during pregnancy on offspring anthropometry at birth.

Study	Population	Gestation at allocation/ randomisation	Interventional medicinal product (IMP)	Control	Effect of vitamin D supplementation		
					Birth weight	Birth length	Head circumference
Vitamin D only							
Brooke (1980) (21) (London, UK)	126 Asian women	28–32 weeks	1000 IU/day oral vitamin D	Placebo	↔	↔	↔
Mallet (1986) (23) (France)	68 women	Final trimester	Group A: 1000 IU/day oral vitamin D Group B: 200 000 IU single dose in month 7 of pregnancy	Usual care	↔	↔	
Marya (1988) (120) (Rohtak, India)	200 Indian women	7 months	Single dose of 600 000 IU cholecalciferol in months 7 and 8 of pregnancy	Usual care	↑	↑	↑
Yu (2009) (25) (London, UK)	180 women	27 weeks	Group A: 800 IU/day oral cholecalciferol Group B: 200 000 IU oral cholecalciferol in a single dose at 27 weeks gestation	Usual care	↔		
Dawodu (2013) (28) (Al Ain, UAE)	192 Arab women	12–16 weeks	Group A: 4000 IU/day oral cholecalciferol Group B: 2000 IU/day oral cholecalciferol	400 IU/day oral cholecalciferol	↔	↔	↔
Grant (2013) (22) (Auckland, New Zealand)	260 women	26–30 weeks	Group A: 1000 IU/day oral cholecalciferol Group B: 2000 IU/day oral cholecalciferol	Placebo	↔		
Wagner (2013) (26) (USA)	Combined analysis of two trials including a total of 513 women	12–16 weeks	Group A: 2000 IU/day oral cholecalciferol Group B: 4000 IU/day oral cholecalciferol	400 IU/day oral cholecalciferol	↔		
Roth (2013) (24) (Dhaka, Bangladesh)	148	26–30 weeks	35 000 IU/week oral cholecalciferol	Placebo	↔	↔	↔
Vitamin D + calcium							
Marya (1981) (96) (Rohtak, India)	120 Hindu women	Final trimester	Group A: 1200 IU/day vitamin D + 375 mg calcium during third trimester Group B: 600 000 IU vitamin D orally in the 7th and 8th months of pregnancy (n=20)	Usual care	↑		
Kalra (2011) (88) (Lucknow, India)	140 women	12–24 weeks	Group A: 60 000 IU oral cholecalciferol in a single dose at randomisation + 1 g/day calcium carbonate Group B: 120 000 IU oral cholecalciferol at randomisation and at 28 weeks gestation + 1 g/day calcium carbonate	1 g calcium carbonate/day	↑	↑	↑
Hashemipour (2014) (97) (Qazin, Iran)	109 women, 25(OH)D <75 nmol/l	24–26 weeks	50 000 IU/week cholecalciferol for 8 weeks in addition to the supplement received by control group	400 IU/day oral cholecalciferol; 200 mg elemental calcium	↑	↑	↑
Hossain (2014) (86) (Karachi, Pakistan)	198	20 weeks	4000 IU/day oral cholecalciferol, 600 mg calcium lactate and 200 mg ferrous sulphate	600 mg calcium lactate and 200 mg ferrous sulphate	↔	↔	↔

↔, no effect shown; ↑, vitamin D supplementation increased the outcome; ↓, vitamin D supplementation reduced the incidence of the outcome.

those with a cord blood 25(OH)D above this cut-point were, but skeletal size was not relatively increased, such that whole-body and femur BMC relative to body weight were significantly lower (102). In a Finnish study, peripheral quantitative computed tomography (pQCT) was used to assess both BMC and bone geometry of the tibia in 98 neonates. In that analysis, the mean of two maternal 25(OH)D measurements in early pregnancy and 2 days *postpartum* was used to define maternal vitamin D status, and the median for the cohort was used to establish two groups. BMC and bone cross-sectional area (CSA) were 13.9 and 16.3% higher respectively in infants of mothers with higher 25(OH)D (103). When these children were reassessed at 14 months of age, the difference in tibial BMC was no longer present, but the greater CSA had persisted (104). Conversely, in 125 Gambian mother–offspring pairs, no significant relationships were observed between maternal 25(OH)D at either 20 or 36 weeks gestation and offspring whole-body BMC or bone area at 2, 13 or 52 weeks of age (105). However, in contrast to the other studies, none of the mothers had a 25(OH)D of <50 nmol/l, which is consistent with the notion that poorer skeletal mineralisation might only occur in fetuses of mothers with the lowest vitamin D levels.

There is evidence to support the persistence of these relationships outside of the neonatal period, although the data are less consistent. In the first study to report on the relationship between maternal 25(OH)D status and off-spring bone mineralisation in childhood, Javaid *et al.* (15) demonstrated positive associations between late pregnancy 25(OH)D and offspring whole-body and lumbar spine BMC, bone area and areal bone mineral density (aBMD) measured at 9 years (Fig. 3). Positive relationships with umbilical venous calcium concentration were also observed, which suggests that the effect of vitamin D on skeletal development might be mediated through placental calcium transport (15). This was initially supported by data from the Avon Longitudinal Study of Parents and Children (ALSPAC), in which maternal estimated u.v. B exposure in late pregnancy, which was used as a proxy measure of vitamin D status, was positively associated with offspring whole-body less head (WBLH) BMC and bone area at 9–10 years of age in 6955 children (106). However, subsequent reanalysis in a more limited subset of the ALSPAC cohort using serum 25(OH)D measured in pregnancy demonstrated no association with WBLH BMC or bone area (107). Interestingly, there was strong collinearly between maternal gestational u.v. B exposure and offspring age at bone assessment, which limits the interpretation of these studies (108). Finally, data from the Raine cohort in Western Australia provide support for
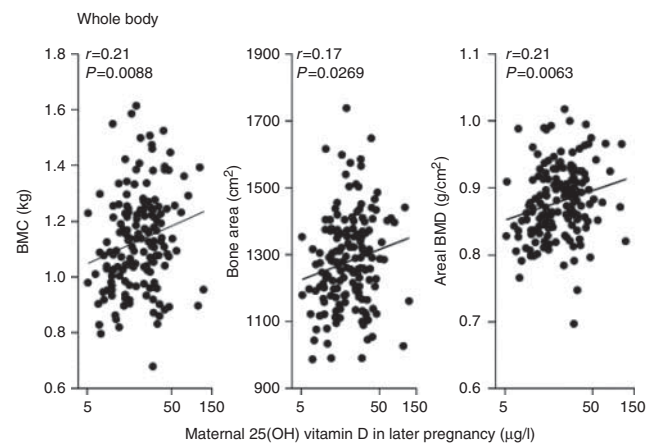
Figure 3

Maternal 25(OH)D concentration in late pregnancy and child-hood bone mass at age 9 years. Reprinted from Javaid MK, Crozier SR, Harvey NC, Gale CR, Dennison EM, Boucher BJ, Arden NK, Godfrey KM & Cooper C. Maternal vitamin D status during pregnancy and childhood bone mass at age 9 years: a longitudinal study. *Lancet* 2006 **367** 36–43, Copyright (2014), with permission from Elsevier.

a positive relationship between maternal gestational vita-min D status and offspring bone development to peak bone mass (109). In that study, whole-body BMC and aBMD were 2.7 and 1.7% lower respectively at 20 years of age in the offspring of mothers with 25(OH)D of <50 nmol/l (as compared to the offspring of mothers with >50 nmol/l) at 18 weeks gestation after adjustment for sex, age, height and body composition at 20 years, maternal height and pre-pregnancy weight, age at delivery, parity, education, ethnicity, smoking during pregnancy and season of maternal blood sampling.

Currently there is only one intervention study of the effects of vitamin D supplementation during pregnancy on offspring bone mineralisation. Congdon *et al.* (110) assessed forearm BMC using single-photon absorptio-metry in 64 infants of Asian mothers living in the UK who participated in a non-randomised study of vitamin D and calcium supplementation during pregnancy. Nine-teen women received 1000 IU vitamin D and a calcium supplement (of unknown strength) during the final trimester and were compared to 45 women who did not receive any supplementation. No significant differences were identified between these two groups, but interpre-tation of the study findings is limited by the small study size, the lack of randomisation and the technique used to assess BMC. The ongoing Maternal Vitamin D

Osteoporosis Study (MAVIDOS), in which more than 1000 women were randomised to 1000 IU cholecalciferol or placebo daily from 14 weeks gestation until delivery and in which offspring bone mineralisation were assessed at birth and at 4 years of age by dual energy X-ray absorptiometry (DXA) (111), will provide much-needed high-quality evidence on the role of vitamin D supplementation during pregnancy in fetal skeletal development (112).

Conclusion

There is now a wealth of observational data relating vitamin D status in pregnancy to obstetric complications, fetal growth and offspring bone development. The findings of these studies are inconsistent, and although they justify the need for assessing vitamin D supplementation in high-quality randomised controlled trials, observational data alone should not be used as a basis for population-wide vitamin D supplementation during pregnancy. Indeed, it is possible that the variability in findings of both observational and a few intervention studies reflects the wide heterogeneity in the populations studied (including the prevalence of VDD, calcium status and ethnic diversity), the dose of vitamin D, the timing of initiation or the assessment of 25(OH)D status and the definition used for the outcomes considered. Thus, any public health recommendations need to be based on an appropriate population. Furthermore, although the currently available data do not suggest any short-term detrimental effects on the mother or fetus, the long-term safety of vitamin D supplementation, particularly at supra-physiological doses, remains to be established.

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