Maternal vitamin D levels during pregnancy and neonatal health: evidence to date and clinical implications

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Ther Adv Musculoskel Dis

2016, Vol. 8(4) 124–135

DOI: 10.1177/1759720X16656810

***Abstract*:** Low maternal vitamin D levels during pregnancy have been associated with a plethora of adverse neonatal outcomes, including small for gestational age and preterm births, detrimental effect on offspring bone and teeth development, and risk of infectious diseases. Although most observational studies indicate a significant linear relationship between maternal 25-hydroxyvitamin D and the above outcomes, some randomized controlled trials to date are inconclusive, mostly due to differences in study design and supplementation regimen. The currently available results indicate that vitamin D supplementation during pregnancy reduces the risk of preterm birth, low birth weight, dental caries of infancy, and neonatal infectious diseases such as respiratory infections and sepsis. This narrative review aims to summarize available trial results regarding the effect of low maternal vitamin D levels during pregnancy, in conjunction with neonatal outcomes on the field, with a discourse on the appropriate clinical approach of this important issue.

Keywords: hypovitaminosis D, infectious diseases, pregnancy, preterm birth, small for gestational age

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Introduction

Low maternal vitamin D levels during pregnancy have been associated with a number of adverse neonatal outcomes, including small for gestational age (SGA), preterm birth, detrimental effect on offspring bone and teeth development, and risk of infectious diseases. A growing body of observational studies indicated that maternal hypovitaminosis D (as defined by maternal 25 -hydroxyvitamin D [25(OH)D] levels $<$20 ng/ml or $<$50 nmol/l) is a significant risk factor for adverse neonatal outcomes. Hence, some interventional studies have been conducted focusing on the potential beneficial effects of vitamin D supplementation. However, many of these studies failed to show any benefit from vitamin D supplementation during pregnancy. The main reasons for this phenomenon are mainly differences in study design and a wide heterogeneity in the pregnant populations studied. This narrative review aims to summarize available clinical randomized and observational data with a discourse on the appropriate clinical approach for daily clinical practice.

Metabolism of calcium and vitamin D in pregnancy

Vitamin D plays an important role during pregnancy and this has been demonstrated by findings on nuclear vitamin D receptors (VDRs) and vitamin-D-activating 1α-hydroxylase in pregnancy-specific tissues, i.e. the decidua and placenta [Evans *et al.* 2004]. This phenomenon occurs in order to allow the accretion of calcium within the fetal skeleton, in particular during the third trimester of pregnancy. An increase in levels of 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] has been reported during the early stages of pregnancy, up to twofold increase at the end of pregnancy, in order to meet the increased fetal calcium demands for adequate bone mineral accrual. Moreover, this process has been suggested to be connected to normal immunological adaptations

for successful maintenance of pregnancy [Karras *et al.* 2014b].

The increase in $1,25(OH)_2D$ is mostly due to an increased production rather than a decreased clearance as demonstrated in pregnant rats and rabbits [Delvin *et al.* 1988; Paulson *et al.* 1990]. This increase in $1,25(OH)_2D$ seems to be independent of PTH, which remains within the normal adult range throughout pregnancy [Kovacs, 2001]. However, the increase of PTH-related peptide (PTH-rP) during early pregnancy may contribute to the rise in $1,25(OH)_2D$ [Ardawi *et al.* 1997]. In pregnancy, vitamin D renal 1α-hydroxylase may be upregulated up to two- to fivefold as demonstrated by *in vitro* measurements of homogenates of rabbits and guinea pigs maternal kidney [Fenton and Britton, 1980; Kubota *et al.* 1982]. Furthermore, parathyroidectomy in pregnant sheep does reduce, but not eliminate, the pregnancy related increase in $1,25(OH)_2D$. However, other hormones could contribute to the bioregulation of 1α-hydroxylase such as PTH-rP, estradiol, prolactin, and placental lactogen [Spanos *et al.* 1976, 1981; Baksi and Kenny, 1978].

On the other hand, available evidence regarding maternal 25(OH)D levels in pregnancy provided controversial results. For example, some researchers have reported a decrease in 25(OH)D in late pregnancy [Zhang *et al.* 2014], while others [Ritchie *et al.* 1998] did not detect any significant differences in 25(OH)D measured before or during pregnancy, and during lactation. However, maternal 25(OH)D level remains an important parameter that should be assessed during pregnancy because the fetus is totally dependent on the mother for 25(OH)D [Markestad *et al.* 1984; Karras *et al.* 2013]. Interestingly, randomized controlled trials (RCTs) have demonstrated that vitamin D supplementation during pregnancy contribute to increased umbilical cord and neonatal serum 25(OH)D [Grant *et al.* 2014; Sablok *et al.* 2015].

In rats, maternal increase of intestinal absorption of calcium mainly occurs at mid-pregnancy, just before the skeletal mineralization of the fetus [Quan-Sheng and Miller, 1989]. It was speculated that prolactin might have an effect on the increased intestinal absorption of calcium, as demonstrated in pregnant vitamin-D-deficient rats treated with prolactin [Pahuja and Deluca, 1981]. This increase in duodenal calcium absorption has been found to occur before the rise in $1,25(OH)_2D$ level, suggesting that the intestinal calcium absorption is mediated via additional factors besides vitamin D [Halloran and Deluca, 1980].

Interestingly, there is a decrease of total serum calcium despite the increase in maternal intestinal absorption of calcium in pregnancy. The decrease of serum calcium could be considered as a consequence of several changes related to pregnancy such as the fall in serum albumin and the increase of intravascular fluid volume leading to hemodilution [Kovacs, 2008]. However, serum ionized calcium, which represents the physiologically important fraction, is kept at the nonpregnant levels throughout pregnancy [Dahlman *et al.* 1994].

Maternal hypovitaminosis D during pregnancy: the extent of the problem

Vitamin D deficiency during pregnancy has been documented in many populations across the globe and is often associated with adverse maternal and birth outcomes [Karras *et al.* 2014a]. In a large multiethnic cohort of pregnant women and their infants in Netherlands [Vinkhuyzen *et al.* 2015], 25(OH)D was measured in the sera of 7256 pregnant women at around 20 weeks of gestation and 5023 neonatal cord blood samples. The authors found that 26% of mothers and 46% of neonates had 25(OH)D levels below 25 nmol/l. In a systematic review from the sunny Mediterranean region [Karras *et al.* 2014a], the authors concluded that vitamin D deficiency (as defined by maternal 25(OH)D levels <20 ng/ml or <50 nmol/l) in pregnancy is quite common, with the prevalence of vitamin D insufficiency (defined by 25(OH)D levels between 50 and 75 nmol/l) ranging from 9% to 41%, whereas that of vitamin D deficiency ranged from 23% to 90%. Therefore, it becomes evident that even in countries with abundant sunshine, the absence of preventive strategies combined with sociocultural factors can negate the benefits of sun exposure.

On the other hand, seasonal variation has been shown to influence maternal levels of circulating 25(OH)D [Haggarty *et al.* 2013]. Similarly, season of birth has been demonstrated to have a great impact on new born 25(OH)D levels, especially in high-latitude countries. Indeed, a study from Denmark described that babies born in the summer had almost 100% increase in 25(OH)D levels compared with those born in winter [Mcgrath *et al.* 2010]. In this regard, a recent

study was conducted on a dataset of 450,000 participants from the UK Biobank [Day *et al.* 2015]. It demonstrated that birth weight and adult height were significantly associated with season of birth, with individuals born during the summer having significantly higher mean birth weight and attained a significantly taller adult height compared with those born during the rest of the year [Day *et al.* 2015]. By taking into account that offspring's vitamin D concentration is affected by maternal ones, it becomes evident that maintaining maternal vitamin D status through supplementation during winter, could be beneficial on neonatal outcomes, in particular in countries with high latitude [Cooper *et al.* 2016].

Maternal hypovitaminosis D and fetal bone growth

In a systematic review of the effect of maternal 25(OH)D on fetal bone growth [Galthen-Sorensen *et al.* 2014], five observational studies [Mahon *et al.* 2010; Fernández-Alonso *et al.* 2011; Ioannou *et al.* 2012; Young *et al.* 2012; Walsh *et al.* 2013] were analyzed. Since no RCTs were available on the relation between maternal vitamin D levels and future bone mechanical behavior in the offspring, scientific evidence was derived from observational studies. Among those five, only three were declared to be of fair quality by the reviewers. The findings from those remaining three studies were contradictory, with only one study [Young *et al.* 2012] finding positive association between maternal 25(OH)D levels and fetal bone development. The other two studies failed to detect any association [Mahon *et al.* 2010; Ioannou *et al.* 2012]. The authors of the systematic review concluded that the current evidence is not adequate in supporting an association between maternal 25(OH) D level and fetal bone formation, and that more studies are needed [Galthen-Sorensen *et al.* 2014].

Moreover, vitamin D deficiency has been linked to craniotabes in one observational study [Reif *et al.* 1988], but not in another earlier study [Kokkonen *et al.* 1983]. A large-scale Japanese study [Yorifuji *et al.* 2008] demonstrated that 37.3% of infants with craniotabes had 25(OH)D levels < 25 nmol/l, with these findings persisting until 1 month of age in the majority of breast-fed infants. It was concluded that *in utero* vitamin D deficiency is associated with craniotabes in normal infants.

In this regard, current data indicate that severe maternal vitamin D deficiency might predispose to offspring skeletal deformities in early infancy and childhood. Concerning neonatal and childhood rickets prevention, there is some evidence of benefit from vitamin D supplementation of pregnant women at high risk of vitamin D deficiency, such as dark skinned women or those with limited Sun exposure due to social or sartorial habits. Less evidence exists in the case of pregnant women currently regarded as being at low risk of deficiency, such as white European populations. For instance, a longitudinal study [Javaid *et al.* 2006] demonstrated that inadequate maternal vitamin D levels during pregnancy were associated with reduced whole body and lumbar spine bone mineral content (BMC) in 9-year-old offspring. Indeed, maternal serum 25(OH)D levels in the third trimester of pregnancy demonstrated a weak linear dose–response positive association with whole-body BMC and lumbar spinal BMC ($r = 0.17$, $p = 0.03$). However, Lawlor and coauthors studied 3960 mother–child pairs enrolled in the Avon Longitudinal Study of Parents and Children (ALSPAC) [Lawlor *et al.* 2013]. They found no association between maternal 25(OH)D levels in any trimester and offspring BMC or other bone outcomes. In clinical terms, available evidence does not justify vitamin D supplementation in pregnant women for optimization of bone mass in their offspring. The Maternal Vitamin D Osteoporosis Study (MAVIDOS) [Cooper *et al.* 2016] was the first multicenter, double-blind, randomized, placebo-controlled trial that supplemented pregnant women from 14 weeks gestation, to either cholecalciferol 1000 ($n = 565$) IU/day or matched placebo ($n = 569$), with neonatal whole-body BMC, assessed within 2 weeks of birth by dual-energy X-ray absorptiometry (DXA), as the primary outcome. Neonatal whole-body BMC of infants born to mothers assigned to cholecalciferol 1000 IU/day did not significantly differ from that of infants born to mothers assigned to placebo [61.6 g (95% confidence interval [CI] 60.3–62.8) *versus* 60.5 g (59.3–61.7), respectively; $p = 0.21$]. Of major interest, prespecified secondary analyses, revealed an interaction between treatment and season for births in the winter, since neonatal BMC and bone mineral density, were greater in the offspring of mothers who had received cholecalciferol, suggesting a benefit for maternal vitamin D supplementation during winter, for future preventive health strategies. No adverse events were deemed to be treatment related. With the exclusion of pregnant populations at high risk of

maternal vitamin D deficiency, where supplementation for rickets prevention seems indicated, appropriate approach for other ethnic cohorts is still under investigation. Well-designed RCTs of vitamin D supplementation during pregnancy are needed to assess the potential benefits of maternal vitamin D levels on infant and child bone outcomes.

Maternal hypovitaminosis D and newborn anthropometry, SGA and preterm birth

Observational studies indicating a linear association

A number of elegant observational studies indicated a linear association between maternal hypovitaminosis D and adverse neonatal outcomes. In a combined *post hoc* analysis of two studies, maternal 25(OH)D below 50 nmol/l was associated with increased risk of preterm birth [Wagner *et al.* 2015]. Interestingly, this association was stronger near the delivery date, indicating that optimization of maternal vitamin D levels in the third trimester of pregnancy might be beneficial. In a large US multicenter study, maternal 25(OH)D was positively associated with birth weight and head circumference [Gernand *et al.* 2013]. In addition, maternal 25(OH)D level $\geqslant$37.5 nmol/l in the first trimester was associated with a 50% risk reduction of SGA. Similarly, a multiethnic study from the Netherlands reported that mothers with adequate vitamin D, had infants with higher birth weight and lower risk of SGA [Leffelaar *et al.* 2010].

In an Australian study [Bowyer *et al.* 2009], immigrant, dark skinned, and veiled women were at greater risk of developing vitamin D deficiency. Moreover, lower vitamin D levels in those pregnant women were associated with increased risk of low birth weight. Similarly, lower maternal vitamin D levels were associated with increased risk of SGA in another study comprising women from Caucasian and African ethnic groups [Burris *et al.* 2012]. However, race was speculated to influence the association between maternal vitamin D levels and birth outcome. Indeed, in a study of pregnant American women [Bodnar *et al.* 2010], a U-shaped association between vitamin D levels and risk of SGA was described in Caucasian but not African women. The majority of blood sampling was conducted between 0 and 13th gestational week (GW). Maternal vitamin D levels at $<$22th GW had a U-shaped relation with SGA risk in white women. White mothers with SGA infants were more likely than white controls to have serum 25(OH)D $<$37.5 nmol/l (defined as deficiency) and $>$75 nmol/l (defined as sufficiency) ($p < 0.00001$) with an adjusted OR of 2.4 (1.2–4.7). In the quartile analysis, women in the upper and bottom quartile were approximately 2.7 and 3.9 times more likely to have a SGA infant after adjustment for confounders including body mass index (BMI), maternal age, smoking, and multivitamins use. No similar association was found in black women. Although interesting, the above findings might have resulted from confounding factors such as prior use of vitamin D supplements or intake of fatty fish. White women had higher levels of 25(OH)D as expected, but it could be argued that a U-shaped phenomenon, at least at bottom quartiles of serum 25(OH)D, should also be evident for black women, where maternal hypovitaminosis D was more prevalent. Another confounder could be the time of blood collection since the majority of blood samples were collected between 0 and 13th GW. Similarly, a study from the UK found that lower maternal vitamin D levels was associated with greater risk of SGA in Caucasian but not in African mothers [Ertl *et al.* 2012].

Observational studies indicating lack of association

In a Spanish study, lack of association was reported between maternal vitamin D and birth outcomes including SGA and newborn anthropometry [Rodriguez *et al.* 2014]. The finding was based on a single measurement of 25(OH)D obtained during the first prenatal visit. Overall, prevalence rates of maternal hypovitaminosis D were rather decreased with only 19.7% of women reported with deficiency [25(OH)D $<$50 nmol/l]. In the same perspective, a group from Canada measured maternal 25(OH)D level during the first trimester in mothers with high risk for preeclampsia [Shand *et al.* 2010]. They failed to find an association between maternal vitamin D levels and birth weight.

In a study from the UK [Gale *et al.* 2008], a serum sample was taken from mothers during late pregnancy (28 to 42 weeks), and samples were stored at -40°C for 5 years prior to measurement of total 25(OH)D by radioimmunoassay. The group claimed there was no association between maternal 25(OH)D levels and infant anthropometry at birth or at age of 9 months. Several factors including childhood nutrition, as well as data

from a large part of the original cohort were not reported in this study.

RCTs indicating beneficial effects of vitamin D supplementation

A trial from the US [Hollis *et al.* 2011] showed that women who received 400, 2000 or 4000 IU vitamin D_3/day from 12–16 weeks until birth, had significantly fewer pregnancy complications including SGA birth [Hollis and Wagner, 2013]. A previous study from India demonstrated that mothers who received vitamin D supplementation during pregnancy gave birth to infants with greater birth weight, height, and head circumference [Kalra *et al.* 2012]. Their finding was based on supplementation with one oral dose of 1 500 μg D_3 during second trimester or two doses of 3000 μg D_3 in the second and third trimesters. Another study in Bangladesh [Roth *et al.* 2013] used a supplementation regimen consisting of 35,000 IU vitamin D_3/week over the median period of 10 weeks beginning from week 26–30 of pregnancy. Despite the fact that they found no difference between the infants of the supplemented group and placebo group at birth, infants of supplemented mother had significantly higher length-for-age *z*-score at 1 year of age. In summary, these two studies used different supplementation regimen of vitamin D, and they focused on profoundly deficient populations.

More interestingly, in a recent RCT on 180 pregnant women from Delhi, Sablok and coauthors [Sablok *et al.* 2015] supplemented the intervention group with different vitamin D regimen based on their 25(OH)D level at the initial visit. More specifically, women with 25(OH)D >50 nmol/l received one dose of 60,000 IU at 20 weeks, women with 25(OH)D between 25 and 50 nmol/l received two doses of 120,000 IU at 20 and 24 weeks, while women with 25(OH)D <25 nmol/l received four doses of 120,000 IU at 20, 24, 28, and 32 weeks. The intervention group gave birth to newborns with higher birthweight 2.6 kg ± 0.33 compared with 2.4 kg ± 0.38 in the nonintervention group. In general, one could speculate that positive findings might not be applicable to European populations with higher baseline 25(OH)D levels and with a potential risk of a U-shaped phenomenon, although such data with the exception of one study [Bodnar *et al.* 2010] have not been reported previously.

RCTs indicating neutral effects of vitamin D supplementation

Some neutral effects on pregnancy outcome have been also reported in the literature. A recent study from Iran [Mojibian *et al.* 2015] included 500 women with 25(OH)D_3 < 75 nmol/l at weeks 12–16 of pregnancy. It demonstrated that supplementation with 50,000 IU vitamin D_3 every 2 weeks until delivery reduced the risk of gestational diabetes, but had no effect on other outcomes including birth weight. Similarly, in an interventional study from Pakistan [Hossain *et al.* 2014], pregnant mothers were supplemented with 4000 IU vitamin D_3/day from 20 weeks until delivery. Supplementation did not have an effect on neonatal anthropometry despite the fact that neonatal 25(OH)D levels were significantly higher in the supplemented group. However, the majority of supplemented women, failed to achieve optimal levels of 25(OH)D. In another trial from the UK [Yu *et al.* 2009], a multiethnic group of women were recruited at 27 weeks of gestation and randomized into three treatment groups: a single oral dose of 200,000 IU vitamin D_3, a supplement of 800 IU vitamin D_2/day from 27 weeks until delivery, and a no-treatment group. They found that vitamin D supplementation had no influence on birth weight [Yu *et al.* 2009]. Similarly, an older French study [Mallet *et al.* 1986] failed to find an effect on birth weight when supplementing pregnant women with 1000 IU vitamin D_3/day during the last trimester. In both latter studies, the authors reported only baseline level of 25(OH)D.

However, it is now being hypothesized that baseline 25(OH)D level is a major determinant of study outcome. Indeed, Cannell and coauthors suggested that RCTs with baseline 25(OH)D <50 nmol/l are twice as likely to show benefits compared with those with baseline >50 nmol/l [Cannell *et al.* 2014]. This might also explain the reason why Cooper and colleagues [Cooper *et al.* 2016] failed to detect a beneficial effect of vitamin D supplementation in their recently published RCT. Indeed, the study included pregnant mothers from different UK centers with 25(OH) D ranging between 25 and 100 mmol/l. The supplementation regimen consisted of 1000 IU/day administered from approximately 14 weeks of gestation. The outcome measure was whole-body BMC at birth. Although no measurable benefit on neonatal bone health was identified, the study reinforced the safety of vitamin D supplementation by measuring different safety outcomes

Table 1. Available meta-analyses on neonatal outcomes (preterm birth, low birth weight, and SGA).

Study	Neonatal outcome	Number of trials included	Risk reduction	Inclusion/ exclusion criteria	Publication bias (size /negative)	Conclusion/ effect
Thorne-Lyman and Fawzi [2012]	Low birth weight	3	RR 0.40; 95% CI 0.23–0.71	Defined	Not assessed	Protective
	SGA	2	RR 0.67; 95% CI 0.40–1.11	Defined	Not assessed	Protective
De-Regil *et al.* [2016]	Low birth weight	3	RR 0.40; 95% CI 0.24–0.67	Defined	Included	Protective
	Preterm birth	3	RR 0.36; 95% CI 0.14–0.93	Defined	Included	Protective
Perez-Lopez *et al.* [2015]	Low birth weight	4	RR 0.72; 95% CI 0.44–1.16	Defined	Included	No effect
	Preterm birth	3	RR 1.26; 95% CI 0.60–2.63	Defined	Included	No effect
	SGA	3	RR 0.78; 95% CI 0.50–1.21	Defined	Included	No effect

CI, confidence interval; RR, Relative risk; SGA, small for gestational age.

[Cooper *et al.* 2016]. Hence, it becomes evident that, the majority of available observational data favor maternal hypovitaminosis D as a significant risk factor for adverse neonatal outcomes, including low birth weight, SGA, and preterm birth. However, available findings from RCTs are inconclusive, mostly due to issues regarding study design and regimen of vitamin D supplementation. Further studies are urgently needed in this field, primarily in pregnant populations with profound maternal hypovitaminosis D.

Meta-analyses of observational studies

In an attempt to synthesize the contradictory evidence in the field, researchers have turned to systematic reviews and meta-analyses, regarding the effect of maternal vitamin D levels and neonatal birth weight or risk for a birth of SGA neonate (Table 1). In this respect, three meta-analyses of observational studies have been published in recent years [Thorne-Lyman and Fawzi, 2012; Aghajafari *et al.* 2013; Wei *et al.* 2013]. The first study did not come to a clear conclusion in terms of vitamin D levels during pregnancy and birth weight [Thorne-Lyman and Fawzi, 2012]; perhaps due to different thresholds used for defining vitamin D deficiency or insufficiency; but the risk of SGA seemed to be higher in vitamin D deficient women. The second study showed that women with 25(OH)D levels <50 nmol/l were at increased risk of SGA [odds ratio (OR) 1.52, 95% CI 1.08–2.15] compared with those with >50 nmol/l [Aghajafari *et al.* 2013]. This risk was confirmed by the third meta-analysis [Wei *et al.* 2013], where maternal 25(OH)D below 50 nmol/l was associated with an increased risk of preterm birth and SGA (OR 1.85, 95% CI 1.52–2.26 for vitamin D insufficiency).

Maternal hypovitaminosis D impact on neonatal infections and respiratory health

Another adverse effect of low 25 (OH) D levels during pregnancy is increased risk of respiratory tract infections (RTIs) for the neonates. It was speculated that vitamin D reduces risk of RTIs from both bacteria and viruses through immunomodulation including decreased chemokine production, inhibition of dendritic cell activation, and alteration of T-cell activation [Hansdottir and Monick, 2011]. Clinical trials have provided good support for the role of vitamin D supplementation in reducing risk of RTIs [Bergman *et al.* 2013]. Countries where high proportion of the population is vitamin D deficient reported several studies linking low 25(OH)D level to risk of respiratory and other infectious diseases. For instance, a study in Turkey involving 30 term neonates with acute RTIs and 30 healthy controls described that the median 25(OH)D level of those with acute RTIs was 29 nmol/l (20–31 nmol/l) compared with 43 nmol/l (34–52 nmol/l) for the controls [Dinlen *et al.* 2016]. In a larger study in Turkey, 132 preterm infants (<32 weeks of gestation) diagnosed with respiratory distress syndrome were studied. A total of 31 of them developed bronchopulmonary dysplasia (BPD)

[Cetinkaya *et al.* 2015b]. All of them had 25(OH)D level <25 nmol/l. The univariate regression analysis OR was 0.76 for maternal 25(OH)D level. Furthermore, in a study involving preterm infants (<32 weeks gestation) in Ireland, 25(OH)D level <30 nmol/l in preterm infants at birth was associated with increased oxygen requirement ($p = 0.008$), increased duration of intermittent positive-pressure ventilation during resuscitation at delivery ($p = 0.03$), and greater need for assisted ventilation ($p = 0.01$) [Onwuneme *et al.* 2015]. A recent RCT from Denmark studied 623 pregnant women [Chawes *et al.* 2016]. Focusing mainly on offspring respiratory health, the group reported that daily supplementation with 2400 IU vitamin D_3 from week 24 until week 1 postpartum had a positive impact. Indeed, babies of the intervention group had significantly less episodes of troublesome lung symptoms during the first 3 years of life [Chawes *et al.* 2016]. Concurrently, results from the Vitamin D Antenatal Asthma reduction Trial (VDAART) conducted in the US on 806 pregnant women with high risk of having children with asthma were recently published [Litonjua *et al.* 2016]. The results demonstrated that daily supplementation of pregnant mothers with 4000 IU vitamin D_3 reduced the incidence of asthma and wheezing in their offspring at the age of 3 by 6.1%. This finding, however, did not reach statistical significance [Litonjua *et al.* 2016].

On the other hand, vitamin D deficiency is an important risk factor for sepsis. A systematic review and meta-analysis found that vitamin D deficiency was associated with a pooled OR of 1.78 (95% CI 1.55–2.03) [Upala *et al.* 2015]. It is thought that vitamin D can reduce the risk of sepsis by increasing levels of cathelicidin LL-37, which exhibits some antimicrobial activity under physiological conditions [Mookherjee *et al.* 2007]. In this respect, a study in Turkey investigated the correlations between maternal and neonatal 25(OH)D levels and risk of neonatal sepsis. For the entire group of 50 infants with early-onset neonatal sepsis (EONS), the postpartum maternal and neonatal 25(OH)D levels were 56 nmol/l (SD = 17 nmol/l), compared with 91 nmol/l (SD = 5 nmol/l) and 45 nmol/l (SD = 12 nmol/l) respectively for the controls [Cetinkaya *et al.* 2015a]. A second study in Turkey involving 40 neonates with EONS and 43 controls found that cord blood 25(OH)D levels were 32 nmol/l (8–197 nmol/l) for those with EONS and 53 nmol/l (13–295 nmol/l) for controls, $p = 0.04$ [Cizmeci *et al.* 2015]. Moreover, solar UVB exposure and vitamin D supplementation have been found to reduce the risk of dental caries [Grant, 2011; Hujoel, 2013]. Thus, it would be expected that low 25(OH)D in infancy is associated with increased risk of dental caries. Indeed, a study of 207 women and infants in Canada found that mothers of children assessed at 1 year of age who had dental caries had 25(OH)D levels of 41 ± 20 nmol/l *versus* 52 ± 27 nmol/l for mothers of children without dental caries [Schroth *et al.* 2014]. There was a monotonic reduction in decayed teeth score with increasing 25(OH)D level, going from 2.1 at 5 nmol/l to 0.3 at 145 nmol/l. The primary mechanism appears to be induction of cathelicidin LL-37, which reduces the amount of cariogenic bacteria in the mouth [Grant, 2011]. However, in this study, it appears that the primary effect was on primary dentition involving calcium.

Achieving adequate maternal vitamin D levels

In a previous landmark randomized clinical trial of safety and effectiveness of vitamin D supplementation during pregnancy, pregnant women received 400, 2000, or 4000 IU vitamin D from about 12 weeks of gestation until delivery. Maternal vitamin D level of ⩾80 nmol/l was achieved, in 50%, 71%, and 82% of the three interventional groups, respectively. These levels were significantly correlated with neonatal levels [Hollis *et al.* 2011]. Interestingly, achieving maternal circulating levels of 100 nmol/l was required to stabilize $1,25(OH)_2D$. The authors suggested that vitamin D RDA in pregnant women of all race should be raised to 4000 IU. Although supplementation approach must be tailored to each specific population depending on local climate, it is clear that doses up to 4000 IU might be appropriate in profound hypovitaminosis D [Hossain *et al.* 2014]. On the other hand, clinicians must be cautious in supplementing Caucasian populations with high doses, since allergic reactions in the offspring have been also reported [Hyppönen *et al.* 2004, 2009; Niruban *et al.* 2014]. On the other hand, accurate prevalence data on vitamin D levels could be essential for optimizing design and analysis of future clinical trials. As described recently [Heaney *et al.* 2014], a vitamin D RCT should focus on a specific outcome, enroll only participants with low 25(OH)D concentrations and supplement with appropriate doses and regimens of vitamin D_3 in order to change the nutrient status. In

addition, conutrient status must be optimized in order to ensure that the test nutrient is the only nutrition-related limiting factor in the response. In the case of vitamin D studies in pregnancy, dietary calcium intake and sunshine exposure are essential parameters, which could permit the evaluation of a potential cause and effect relationship in pregnant populations sharing similar dietary habits and climate conditions.

Measurement of 25(OH)D

Serum 25(OH)D levels are measured either by immunochemical or physical (e.g. liquid chromatography–tandem mass spectrometry (LC-MS/MS)) detection methods. It is noteworthy that 25(OH)D is a difficult analyte to measure, and this is exemplified by the substantial variation among 25(OH)D assays. Numerous factors have been identified to have an impact on assay performance [Farrell *et al.* 2014; Glendenning, 2015]. One of these is the ability of the assay to fully recognize 25(OH)D_3 as well as 25(OH)D_2, as both metabolites are metabolically active. Furthermore, 25(OH)D needs to be liberated from the vitamin D binding protein (VDBP) for proper 25(OH)D measurement. Hence, any alteration in VDBP levels, such as the elevation of VDBP levels during pregnancy, would cause some immunoassays to generate markedly different 25(OH)D results when compared with LC-MS/MS [Heijboer *et al.* 2012; Cavalier *et al.* 2014]. Finally, the presence of many other circulating metabolites of vitamin D may interfere with either immunoassays or LC-MS/MS methods. Of particular relevance is the presence of a C3-epimeric form of 25(OH) D, a vitamin D metabolite of unknown biological significance. It has been shown that C3-epimer can contribute up to 60% of total 25(OH)D in infants up to 1 year of age. This epimer has been shown also to be elevated, though to a lesser extent, in pregnant women and cord blood (6–10%) [Bailey *et al.* 2013; Karras *et al.* 2013; Ooms *et al.* 2016]. In conclusion, it is important to realize that substantial variations in 25(OH)D assays exist, which impedes direct comparison of 25(OH)D results from different studies and when pooling 25(OH)D results from different studies in systematic reviews.

Conclusion

Maternal vitamin D has been shown to have its main impact on offspring birth weight and bone mass as well as immunity. However, it is difficult to draw final conclusions on the need for vitamin D supplementation during pregnancy due to the heterogeneous design of the studies in terms of the length of hypovitaminosis D, regimen of vitamin D supplementation, and other potential confounding factors. Current available data indicate that vitamin D supplementation during pregnancy reduces the risk of preterm birth, low birth weight, dental caries of infancy, and neonatal infectious diseases such as respiratory infections and sepsis. However, additional well-designed large RCTs are needed. Concerning safety, low doses of vitamin D during pregnancy have been reported to be safe, at least in the short term. No results are yet available regarding the potential long-term side effects of vitamin D supplementation on neonatal health, thus further high-quality research is needed in order to follow up the health of offspring of mothers supplemented with vitamin D during pregnancy.

Funding

WBG receives funding from Bio-Tech Pharmacal, Inc. (Fayetteville, AR) and the Vitamin D Society (Woodstock, ON, Canada) and has received funding recently from the Vitamin D Council (San Luis Obispo, CA).

Conflict of interest statement

The authors declare that there is no conflict of interest.

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