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REVIEW

Vitamin D during pregnancy: why observational studies suggest deficiency and interventional studies show no improvement in clinical outcomes? A narrative review

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Abstract A considerable number of studies have examined vitamin D status during pregnancy. Although data from observational studies denote vitamin D hypovitaminosis (deficiency or insufficiency) during pregnancy is associated with a plethora of adverse maternal and neonatal outcomes, data from interventional (supplementation) trials fail to reveal a significant impact on maternal and offspring health. The aim of this narrative review was to critically appraise the methodology of the most representative published randomized controlled trials in an attempt to explain the difference between observational and supplementation results. We found that this difference could be attributed to a variety of factors, namely: (i) study design (lack of a specific outcome in conjunction with timing of supplementation, enrolment of participants with heterogeneous vitamin D status); (ii) pitfalls in the interpretation of vitamin D equilibrium (lack of determination of plasma half-life); (iii) supplementation regimen (administration of a wide range of regimens, in terms of dose, bolus and form); (iv) geographical characteristics (vitamin D needs could vary significantly within a country, particularly in areas with a wide range of latitude gradient); (v) adaptations of vitamin D metabolism during pregnancy (vitamin D and calcium equilibrium are changed during pregnancy compared with the non-pregnant state) and (vi) supplementation of populations with low baseline 25(OH)D values would likely manifest beneficial effects. All these parameters should be taken into consideration in the design of future vitamin D supplementation trials.

Keywords Vitamin D · Randomized controlled trial · Hypovitaminosis D · Pregnancy

Introduction

Pregnancy is a unique state in a woman's life, characterized by a continuum of biologic events that enables tissue maturation, aiming for foetal adaptation to future environmental and nutritional influences [1, 2]. On this critical time frame, several exogenous stimuli could affect maternal–neonatal syncytium and have an impact on pregnancy outcome, maternal health and future offspring development [1–3]. A wide range of nutritional deficiencies have been recognized as a preventable cause of adverse health events, rendering scientific communities and health organizations worldwide to establish specific nutritional recommendations aiming for optimal foetal development [4]. This suggested diet model, although very similar to a balanced healthy one suggested for most adults, incorporates recommendations, which apply to consumption of micronutrients and vitamins, which have been recognized to be of critical importance in protecting the developing foetus [4, 5].

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On that basis, data from observational studies during the last decade have suggested a potential adverse effect of maternal hypovitaminosis D during pregnancy on maternal and offspring health outcomes [6, 7]. Gradually, interventional studies have been conducted, focusing on the potential beneficial effects of vitamin D supplementation on these outcomes. However, the majority of these studies failed to show any benefit from vitamin D supplementation during pregnancy. The reasons for absence of an agreement between data from observational and supplementation studies remain obscure. The aim of this narrative review was to critically appraise the methodology of the published randomized controlled trials, in an attempt to explain why data from supplementation trials fail to reveal a significant impact on maternal and offspring health outcomes, as data from observational studies are suggesting. This narrative review included the most representative randomized controlled studies on the field according to study sample and methodology (Table 1).

Maternal outcomes

There have been a number of observational studies finding better pregnancy outcomes related to vitamin D levels. Vitamin D has been hypothesized [8] to play a significant role in the disparities of major pregnancy adverse outcomes between non-Hispanic black and non-Hispanic white women, as maternal hypovitaminosis D has been substantially more common in black women [9]. Previous results indicate that the higher the vitamin D levels at delivery, the lower the risk of having primary caesarean section [10]. Women with vitamin D levels above 15 ng/mL (37.5 nmol/L) were one-quarter as likely to require primary caesarean section as women with lower levels [10]. In addition, adjusted serum 25(OH)D concentrations in early pregnancy have been found to be lower in women who developed pre-eclampsia later in pregnancy compared with controls, whereas a 50 nmol/L decline in maternal vitamin D levels increased the risk of pre-eclampsia twice [11]. The same group demonstrated a U-shaped relation between maternal serum 25(OH) D and risk of small-for-gestational age birth in white mothers with the lowest risk from 60 to 80 nmol/L [12].

There have been several previous reports suggesting an association between vitamin D insufficiency in pregnancy and gestational diabetes mellitus (GDM) [13–15]. In a recent meta-analysis of 10 studies, low 25(OH)D was associated with increased risk for GDM [16]. Although observational studies demonstrate a strong association among maternal hypovitaminosis D and adverse birth outcomes, results from vitamin D supplementation trials for pre-eclampsia, GDM and other maternal outcomes, fail to converge to a meaningful outcome, revealing the potential for systematic failures within the field. At this context, most representative randomized controlled trials (RCT) will be discussed below, aiming to identify potential research gaps in the existing literature.

Pre-eclampsia

A series of supplementation studies have examined potential adverse maternal and pregnancy outcomes, mainly pre-eclampsia. Studies with pre-eclampsia risk reduction as their primary outcome can be divided into observational and randomized. A large, prospective observational trial from Norway [17] studied 23,423 nulliparous women, categorized into two groups, according to the use of vitamin D supplements before or during pregnancy. Vitamin D supplementation during pregnancy with 400–600 IU daily resulted in 27 % risk reduction of pre-eclampsia [odds ratio (OR) 0.73, 95 % confidence interval (CI) 0.58–0.92]. However, the beneficial effect of vitamin D intake should not be attributed to supplementation per se, due to the high intake of vitamin D-rich long n-3 fatty acids in the local diet. In another large, Hungarian study [18], routine vitamin D supplementation, either as a mono-therapy or contained in a multi-vitamin regimen, with a dose of 3000 IU/week (average 400 IU/day) starting from the 20th gestational week, resulted in a reduced risk of pre-eclampsia in a dose–response manner.

In the field of RCTs, Marya et al. [19] in a placebo-controlled, non-blinded trial studied two groups of 200 pregnant women (supplementation group, 375 mg calcium and 1200 IU of vitamin D daily from the 20–24th gestational week; non-supplementation group, normal diet) and found no statistical difference in the incidence of pre-eclampsia between them. The only blinded, supplementation study available so far [20] administered 400 IU (control group), 2000 IU or 4000 IU of vitamin D_3 daily during pregnancy. Cord blood 25-hydroxy-vitamin D [25(OH)D] concentrations were 45.5 ± 25.3 nmol/L in the 2000 IU group and 66.3 ± 25.8 nmol/L in the 4000 IU group. Higher 25(OH)D concentrations did not alter cord blood calcium or phosphorus. The study suffered from a high dropout rate, while powered only for a biochemical endpoint. However, by combining all available randomized data so far, a reduced OR of pre-eclampsia is evident in supplemented women with a pooled OR of 0.66 (95 % CI 0.52–0.83, $p = 0.001$) [16].

Gestational diabetes mellitus

Although some observational studies support an association between maternal hypovitaminosis D during pregnancy and the development of GDM [13–16], data from

Table 1 Summary of vitamin D supplementation studies during pregnancy regarding maternal and foetal outcomes

First author Year Country	Study design	Maternal 25(OH)D: time of measurement	Maternal 25(OH)D: assay	Maternal 25(OH)D: mean (SD)	Conclusion
Maternal					
Marya RK 1987 India [19]	RCT Group A: no supplement ($n = 200$) Group B: 375 mg/day Ca^{2+} +1200 IU vit D from GW 20–24 until term ($n = 200$)	Not measured	Not measured	Not measured	No difference in rates of pre-eclampsia Reduced SBP and DBP in Group B at GW 32 and 36 ($p < 0.001$) *Comment: High risk of bias [lack of 25(OH)D estimation]*
Hossain N 2014 Pakistan [22]	Group A: 200 mg ferrous sulphate and 600 mg Ca^{2+} daily) Group B: 4000 IU vit D_3 od, from GW 20 until term	At recruitment and at delivery	Chemiluminescence immunoassay based on a direct competitive immunoassay technique	Group A: from 6.9 ± 7.0 to 6.3 ± 4.0 ng/dL ($p = 0.06$) Group B: from 18.3 ± 11.0 to 8.8 ± 11.8 ng/dL ($p = 0.001$) Neonatal 25(OH)D: Group A: 19.2 ± 12.2 ng/dL Group B: 6.3 ± 5.2 ng/dL	No difference in obstetrical outcomes Inefficacy of vit D regimen to achieve normalization of maternal vitamin D status (only 15 % of pregnant women achieved concentrations > 30 ng/ml) *Comment: inadequate in severely deficient populations. Low baseline 25(OH)D in severely deficient populations require higher doses. These populations are likely to manifest beneficial effect compared to population with higher baseline 25(OH)D values*
Yap C [23]	RCT Group A: 5000 IU vit D od ($n = 89$) Group B: 400 IU vit D od ($n = 90$) Primary end point: fasting plasma insulin during 75-g OGTT, performed between GW 26 and 28	At baseline, at GW 34–36 and within 2 days after delivery	DiaSorin LIAISON chemiluminescent immunoassay		No difference in glucose levels during OGTT Group A: vit D supplementation commencing at a mean of GW 14 does not improve glucose levels in pregnancy Group B: inverse relationship between pre-treatment 25(OH)D and fasting ($p < 0.001$) and 2-h ($p < 0.001$) glucose level during OGTT *Comment: The study was not powered to detect a difference in risk of GDM. Supplementation commenced late (at GW 15)*
Offspring					
Marya RK 1988 India [26]	RCT Group A ($n = 100$): no supplement Group B ($n = 100$): oral 600,000 IU vit D_3 (2 doses in 7th and 8th gestational months)	Not measured	Not measured	Not reported	Higher mid-arm circumference, triceps skinfold and infrascapular skinfold in Group B ($p < 0.01$ for all) *Comment: High risk of study bias Maternal 25(OH)D not reported*
Brooke OG 1980 UK [27]	RCT, Asian women Group A (n = 67): placebo Group B (n = 59): vit D_2 1000 IU/d during last trimester	GW 28–32 and at delivery	Competitive protein-binding assay after chromatographic purification of lipid extracts of serum	At allocation: 20.1 ng/dL (1.9 nmol/L) At term: Group A: 16.2 ng/dL (2.7 nmol/L) Group B: 168.0 ng/dL (12.5 nmol/L)	Greater fontanelle area in Group B ($p < 0.05$). No difference in forearm length or triceps skinfold thickness. *Comment: High risk of bias* *Assay flaw likely*

Table 1 continued

First author Year Country	Study design	Maternal 25(OH)D: time of measurement	Maternal 25(OH)D: assay	Maternal 25(OH)D: mean (SD)	Conclusion
Delvin E 1986 France [28]	RCT Group A ($n = 20$): no supplement Group B ($n = 20$): 1000 IU vit D_3/d during 3rd trimester	At recruitment and at delivery	Radioligand assay	At recruitment: Group A: 27.5 ng/dL (10.0 nmol/L) Group B: 54.9 ng/dL (10.0 nmol/L) At term: Group A: 32.4 ng/dL (20.0 nmol/L) Group B: 64.9 ng/dL (17.5 nmol/L)	No difference in BW between the two groups (p value not given) *Comment: Supplementation inadequate in severely deficient populations*
Mallet E 1986 France [29]	RCT Group A ($n = 29$): no supplement Group B ($n = 21$): 1000 IU vit D/d during 3rd trimester Group C ($n = 27$): single oral dose of vit D 200,000 IU during 7th month	At delivery	Radioligand assay	Group A: 9.4 ng/dL (4.9 nmol/L) Group B: 25.3 ng/dL (7.7 nmol/L) Group C: 26.0 ng/dL (6.4 nmol/L)	No significant difference in BW between the three groups (p value not given) *Comment: Unknown effect of pharmacodynamics of bolus supplementation. Daily dosing inadequate?*
Yu 2009 UK [25]	RCT Group A ($n = 59$): no supplement Group B ($n = 60$): oral vit D_2 800 IU/d from GW 27 Group C ($n = 60$): single 200,000 IU vit D_2 at GW 27	At GW 26–27 and at delivery	Not reported	Group A: from 25 to 29 ng/dL Group B: from 26 to 42 ng/dL Group C: from 26 to 34 ng/dL	No difference in BW and rate of SGA among groups ($p = 0.7$) *Comment: Supplementation inadequate in severely deficient populations* *Highest 25(OH)D levels attained in Group B*
Goldring S 2012 UK [32]	RCT Group A: no vit D Group B: 800 IU D_2 od Group C: single oral bolus 200,000 IU D_3 at GW 27	At recruitment and at delivery	Diasorin Radioimmunoassay	Neonatal 25(OH)D [mean (IQR)]: Group A: 17 (14–22) nmol/L Group B: 26 (17–45) nmol/L ($p = 0.001$ Group C: 25 (18–34) nmol/L ($p = 0.001$)	No effect of maternal supplementation in decreasing wheezing in offspring at age of 3 years *Comment: Inadequate supplementation: all neonatal groups vit D deficient*
Hollis B 2011 USA [20]	RCT Group A (n = 111): oral vit D_3 400 IU/d Group B (n = 122): oral vit D_3 2000 IU/d Group C (n = 117): oral vit D_3 4000 IU/d from GW 12-16 until term	At recruitment, then monthly and at delivery	Diasorin Radioimmunoassay	Group 1: 78.9 ± 36.5 nmol/L Group 2: 98.3 ± 34.2 nmol/L Group 3: 111.0 ± 40.4 nmol/L ($p < 0.0001$)	No significant difference in BW among the ($p = 0.23$) *Comment: High dropout rate, adequately powered*
Roth Bangladesh 2013 [30]	RCT ($n = 160$) Group A: placebo Group B: vit D_3 35,000 IU/w from GW 26–30 until delivery	Maternal 25(OH)D: At recruitment and at delivery Neonatal 25(OH)D: At delivery	High-performance liquid chromatography tandem mass spectroscopy	Maternal 25(OH)D: Group A: from 45.3 ± 19.4 to 38.3 ± 18.1 nmol/L Group B: from 38.5 ± 21.4 to 134.9 ± 30.6 nmol/L Neonatal 25(OH)D: Group A: 39.0 ± 18.7 nmol/L Group B: 102.8 ± 28.6 nmol/L ($p < 0.001$)	Mean length-for-age z-score (LAZ) similar between groups at delivery, but 0.44 (95 % CI 0.06–0.82) higher in Group B. vs. Group A at 1 year, corresponding in a sex-adjusted increase of 1.1 cm (95 % CI 0.06–2.0). Average LAZ during infancy was 0.41 higher in vitamin D vs placebo (95 % CI, 0.11–0.71, $p = .01$) *Comment: Small study sample, not adequately powered for given outcomes*

BW birth weight; *CI* confidence interval; *DBP* diastolic blood pressure; *GDM* gestational diabetes mellitus; *GW* gestational week; *od* once daily; *OGTT* oral glucose tolerance test; *RCT* randomized controlled trial; *SBP* systolic blood pressure; *SD* standard deviation; *SGA* small for gestational age

supplementation studies are limited. A double-blinded RCT in 54 women diagnosed with GDM reported an improvement in fasting glucose and Homeostasis Model Assessment-Insulin Resistance (HOMA-IR) index after two doses of oral 50,000 IU vitamin D, given 21 days apart, compared with placebo [21]. However, significant differences in these parameters were noted at baseline, making the results difficult to interpret. In an open-label RCT of vitamin D supplementation in two groups of Pakistani women, 4000 IU of vitamin D were administered compared to calcium and ferrous sulphate supplementation [22]. The obstetrical outcomes were identical in both groups. This effect was attributed to the inefficacy of the given vitamin D regimen to achieve normalization of maternal vitamin D status (only 15 % of pregnant women achieved concentrations above 30 ng/mL), leaving the majority in the deficiency range.

In an Australian double-blind controlled supplementation study [23], a cohort of 209 pregnant women before 20th gestational week were randomized in either 5000 IU ($n = 89$) or 400 IU ($n = 90$) of oral vitamin D_3 daily, until delivery. Endpoints were maternal glucose concentrations in Oral Glucose Tolerance Test (OGTT, 26th–28th gestational week), neonatal 25(OH)D concentrations, obstetric and neonatal outcomes and assessment of maternal insulin resistance. Although an inverse association between baseline 25(OH)D and fasting and 2-h glucose concentrations was found in post hoc analysis, the 5000 IU group failed to demonstrate significant differences in mean fasting, 2-h blood glucose concentrations and HOMA-IR, compared to the 400 IU group. Overall, all women in the 5000 IU group which developed GDM ($n = 7$) manifested adequate vitamin D status at the time of diagnosis. However, this study was not adequately powered to detect a difference between groups in the incidence of GDM.

Recent elegant results [24] indicated parathyroid hormone levels (PTH), as a significant underlying factor that could improve the interpretation of the conflicted literature on GDM and maternal vitamin D status. Prevalence of GDM progressively increased from the first to third tertile of PTH and was independently associated with GDM. These findings indicate that vitamin D supplementation during pregnancy might be beneficial in women whose 25(OH) D concentrations are unable to suppress PTH. Given the fact that 25(OH)D induced suppression of PTH varies widely among patients, future research agenda on this field, could focus on this specific patient population.

In summary, as far as maternal outcomes are concerned, although supplementation studies homogeneously indicate a beneficial effect on the reduction of pre-eclampsia risk, the absence of pre-conception vitamin D values, the limited power and the heterogeneity in thresholds used, do not allow for definitive conclusions to be drawn. In the case of GDM, risk reduction data do not indicate a beneficial effect, so far.

Foetal outcomes

Anthropometry at birth or during early life is the most studied extra-skeletal clinical outcome of maternal vitamin D supplementation. So far, only two [20, 25] out of six RCTs [20, 25–29] defined 25(OH) D concentrations as their primary outcome and had adequate sample size. The only study, which demonstrated effects on neonatal clinical parameters [26], was conducted in India, an area with profound vitamin D deficiency. Administration of two doses of 600,000 IU vitamin D in the third trimester of pregnancy resulted in a significant increase in birth weight of the offspring, compared to the non-supplemented group. This study did not define a primary outcome parameter, used no placebo and reported no data on 25(OH) D concentrations.

Anthropometry

In an effort to assess post-partum beneficial effects of optimization of maternal vitamin D status in offspring linear growth, a randomized, double-blind, supplementation trial [30] (35,000 IU/week vs. placebo) during the first trimester of pregnancy evaluated longitudinally neonatal anthropometry from birth to 12 months of age. The primary analysis included evaluation of mean length-for-age Z-score based on international standards. At birth, no differences among the two groups were evident, whereas at 1 year of age, mean length-for-age was higher in the supplemented group, in conjunction with an increase in longitudinal growth during early infancy, interpreted in an increase of 1.1 cm throughout the first year of life, after controlling for sex. A large-scale, supplementation study [20] treated 257 pregnant women with 400, 2000 or 4000 IU of vitamin D_3 daily during pregnancy. Achieved cord blood 25(OH)D concentrations were 45.5 ± 25.3 nmol/L in the low-dose group and 66.3 ± 25.8 nmol/L in the high-dose group. A positive association between vitamin D dose and neonatal weight percentile, as well as a negative association between 25(OH)D concentrations and premature labour and infection was evident. These results might have been biased by the small study sample ($n = 162$, supplemented until term) and the high percentage of maternal vitamin D deficiency (35 %) in the group supplemented with 4000 IU daily.

Immune system

The effect of maternal vitamin D supplementation on offspring immune profile remains controversial. In a large

cohort of more than 5000 young adults [31], supplementation with more than 2000 IU vitamin D daily in the first year of life was found to be associated with an increased prevalence of allergic rhinitis. Although this trial centred on supplementation during infancy and not during pregnancy, it could comprise a spectrum of long-term effects of vitamin D equilibrium on immune regulation. This research question was the main objective in an RCT where 180 pregnant women were allocated randomly from 27th gestational week to either, single bolus of 200,000 IU per os or 800 IU d of ergocalciferol daily [32]. No significant effect of maternal supplementation was evident on the risk of offspring wheeze, atopy (assessed by skin test) or lung function at 3 years of age. It has to be noted, however, that the supplemented groups demonstrated inadequate vitamin D status at both dosing regimens (daily dose 26 nmol/L, bolus dose 25 nmol/L), an effect that might influence potential beneficial effects of vitamin D supplementation.

In summary, as far as foetal outcomes are concerned, supplementation studies indicate a potential beneficial effect on offspring anthropometry. However, several parameters regarding timing, duration and dose of supplementation regimen remain to be elucidated before incorporating this evidence into daily clinical practice.

Why data from supplementation studies fail to reveal favourable clinical outcomes?

Previous observations [11–16] suggest that the beneficial effects of vitamin D supplementation during pregnancy are attained with maternal 25(OH)D values of at least 100 nmol/L [20]. Desired levels, however, should be tailored to each according to a plethora of parameters, including the desired health outcome and population characteristics. Based on the data by the supplementation studies discussed above, a series of reasons can be implicated. These reasons are discussed in the following paragraphs and illustrated in Table 2.

Study design

It could be argued that the link between maternal hypovitaminosis D and adverse outcomes is not causative and that decreased vitamin D concentrations are a consequence or a confounder, rather than a disease per se. However, recent data on cardiovascular disease (CVD) risk [33], suggest a causal association between serum 25(OH)D concentrations and CVD risk, as do most well-designed RCTs. The term "well-designed RCT" is of outmost importance in understanding the potential beneficial effects of vitamin D supplementation during pregnancy. As described recently by Heaney [34], a vitamin D RCT should focus on a specific outcome, enrol only participants with low 25(OH)D concentrations and supplement with appropriate doses and regimens of vitamin D_3.

Vitamin D economy

Theoretically, serum vitamin D concentrations are the result of endogenous production and dietary intake [35, 36]. As a consequence, the potential beneficial effects of vitamin D supplementation should be interpreted in the context of attained concentrations of serum 25(OH)D and not just the dose administered [37]. Vitamin D supplementation markedly differs from other interventions, where a pharmaceutical compound is given. In the former case, serum 25(OH)D concentrations are under the confounding contribution of newly synthesized 25(OH)D, whereas in the latter, serum/plasma concentrations depend on the administered dose [36]. This phenomenon is of particular importance in defining the vitamin D dose–response relationship in supplementation trials. Moreover, 25(OH)D half-life depends on serum 25 (OH) D concentrations as higher concentration lead to faster destruction rates in order to maintain equilibrium [37]. Thus, incorporation of vitamin D equilibrium parameters, such as 25(OH)D plasma half-life, could provide an additional insight to supplementation studies, by identifying vitamin D kinetics (e.g. storage and release from fat and muscle) [38].

Supplementation regimen

An ideal vitamin D supplementation trial in pregnancy would use a reference population, with different baseline vitamin D status, aiming at attaining sufficient serum 25(OH)D concentrations, in order to establish a "supplementation and result" relationship [39]. Nevertheless, this outcome could be significantly affected by the regimen and dose of vitamin D used in each study. Since even a large bolus of 50,000 or 100,000 IU of vitamin D would rapidly (in a few days) be absorbed and undetectable from the serum [34]. It has to be noted that several supplementation trials used this type of bolus administration, with a potential effect on their outcomes [25, 29]. In this context, the duration of supplementation could also play a role in maintaining adequate vitamin D concentrations. Although the optimal dosing and duration for specific outcomes remains to be defined, by supplying constant doses of vitamin D for 3–4 months, a steady state will be attained [40]. This is not the case in bolus regimens with monthly or weekly patterns of supplementation. The short duration of vitamin D supplementation during pregnancy (i.e. weeks or months) may also not be adequate to alter the pathogenetic pathways of

Table 2 Parameters that may interpret the inability of vitamin D supplementation trials during pregnancy to reveal a significant impact on maternal and offspring health

Parameter	Study flaws	Implications	Perspective
Vitamin D expenditure	Inaccurate estimation of vitamin D production	Interpretation of serum vitamin D concentrations, including storage and release from fat and muscle	Measurement of 25(OH)D half-life
Supplementation regimen	Bolus regimen rapidly metabolized Supplementation inadequate in severely deficient populations Supplementation for 6 months or less	Inadequate vitamin D concentrations attained for manifesting biological actions Not adequate for attaining biological actions	Chronic, stable doses of oral Supplementing for more than 6 months or increasing dosage
Geographical parameters	Severely deficient populations UVB exposure Dietary, sartorial habits	Inadequate supplementation Lack of surrogate markers of the primary source of vitamin D Reduced dietary vitamin D intake, use of sunscreens or body coverage for religious reasons	Incorporation of all local climatic parameters into design of a supplementation regimen
Metabolism during pregnancy	Increase in VDBP concentrations	Decrease in bioavailable VDBP concentrations	Estimation of bioavailable vitamin D Estimation of VDBP changes
Assay methodology Baseline vitamin D values	Failure to measure different vitamin D forms, such as epimers Interpretation of findings in meta-analyses by not taking into account baseline 25(OH)D values	Inaccurate estimate of bioactive forms Supplementation of pregnant women with baseline 25(OH)D < 50 nmol/L would likely show beneficial effects compared to those with higher baseline levels	Administration of accurate vitamin D measurement (e.g. LC–MS/MS) Administration of appropriate doses of vitamin D at baseline in severely deficient populations, in order to achieve sufficiency

LC–MS/MS liquid chromatography–mass spectrometry; *UVB* ultra-violet B radiation; *VDBP* vitamin D-binding protein

diseases in which vitamin D is speculated to be involved, such as in pre-eclampsia and GDM. This hypothesis is supported by previous results by Heaney et al., which demonstrated that the calculated oral input of cholecalciferol required to maintain 25(OH)D concentrations in healthy men, particularly in winter was 3000–5000 IU [41].

Supplementation before pregnancy and achievement of vitamin D sufficiency may be more effective than supplementation during pregnancy for both maternal and foetal outcomes. In addition, recent results from a post hoc exploratory analysis from two large supplementation trials, demonstrated that maternal vitamin D status closest to delivery date was more significantly associated with preterm birth, suggesting that late intervention may be beneficial for reducing the risk of preterm delivery [42]. It becomes evident that the most appropriate timing for supplementing future mothers, depends on what disease team is aimed to be prevented. On that basis, aiming for prevention of pregnancy diseases where their initial pathophysiological manifestations are initiated from the very early stages of pregnancy (e.g. GDM, pre-eclampsia), giving vitamin D in third trimester may be too late. On the other hand, previous results indicate that supplementation during the third trimester of pregnancy for prevention of respiratory infections in early infancy or preterm birth might be beneficial [42].

Ideally, vitamin D supplementation for preventing adverse outcomes during pregnancy should aim to attain 25(OH)D levels of at least 100 nmol/L (40 ng/mL) [20]. In a previous landmark supplementation study [20] a biphasic relationship between circulating 25(OH)D and $1,25(OH)_2D_3$ was demonstrated, with circulating levels of at least 100 nmol/L (40 ng/mL) required to support maximum $1,25(OH)_2D_3$ output in the pregnant women. Normalization of 1, 25(OH) $_2D_3$ concentrations in this setting would enhance $1,25(OH)_2D_3$ control on gene expression, which is very important for the developing foetus. Most available RCTs did not make enough of a move along the regression fit in order to attain physiological levels of 1, 25(OH)D which could partially explain the lack of a significant differences in outcomes [19, 34, 37].

Currently, there seems to be a growing consensus that, for equimolar quantities, orally administered D3 raises serum 25(OH)D by about twice as much as D2 [43–45]. This has been shown for bolus doses, short-term continuous administration (12 weeks), and long-term continuous administration (12 months) [46–48]. Consequently, in some of the previous supplementation trials during pregnancy, related mostly to offspring outcomes, the lack of a beneficial effect, could at least partially be attributed to the use of vitamin D2 [25, 27, 32] instead of vitamin D3.

Geographical characteristics

As vitamin D is generated by an environmental factor (sunshine exposure), it can be affected by geographical factors [49, 50]. Reports from Europe, USA and Africa indicate that populations from different countries share more common vitamin D-related characteristics than cohorts from the same country [51, 52]. This concept is further supported by Kimlin et al. [53], who assessed vitamin D data from seven US locations. During 8 months (March to October), no latitude gradient (from 18° to 44°N) of vitamin D was observed. In contrast, during cooler months (November to February) vitamin D was strongly determined by latitude. These observations indicate that vitamin D could vary significantly within a country, particularly in areas with a wide range of latitude gradient. Moreover, vitamin D status of immigrant populations in Europe was poor compared with that of the indigenous European populations [54], indicating that social and cultural habits are different as well. Indeed, the approach to vitamin D status taking into account specific geographical characteristics, such as latitude, ultra-violet B (UVB) radiation and microclimate, as well as the specific social and dietary habits could improve the interpretation of differences reported on vitamin D status in different geographical regions. Baseline 25(OH)D concentrations in conjunction with ethnicity and individual response to solar UVB, according to skin phototype and racial variances in alleles of vitamin D-binding protein (VDBP) could minimize heterogeneity among studies [55].

Metabolism during pregnancy

Vitamin D equilibrium during pregnancy manifests unique adaptations [7, 56]. Previous results demonstrated that circulating 1,25(OH)2D3 levels at 12 weeks gestation are approximately triple that of normal, non-pregnant female and normal male subjects [20, 57].

Pregnant women manifest extremely high concentrations of $1,25(OH)_2D$ without evidence of hypercalcemia, whereas VDBP concentrations increase in response to high oestrogen concentrations [58]. VDBP is increasingly recognized as a vital parameter in the interpretation of vitamin D status [37, 59]. Recently, results from a large study in community-dwelling black Americans, demonstrated low concentrations of total 25(OH)D and VDBP compared to white population, resulting in similar concentrations of estimated bioavailable 25(OH)D [60]. Although the assay methodology used in this was prone to overestimation of bioavailable 25(OH)D by 2–2.5 times owing to underestimation of vitamin D–binding protein in black people [61], racial differences could theoretically explain these findings. Conversely, the increase in VDBP during pregnancy could

decrease bioavailable vitamin D, albeit concentrations considered as normal according to current vitamin D criteria.

These adaptive changes of vitamin D metabolism during pregnancy could interpret attained vitamin D concentrations, as optimal, although not. Interpreting bioavailable vitamin D concentrations in future trials in conjunction with VDBP concentrations could offer a new insight in the field. In addition, maternal vitamin D receptor (VDR) polymorphisms have been associated with an increase risk of GDM in Iranian population, as well as increased birth weight [62].

Assay methodology

Although the existence of various vitamin D forms, such as epimers, has been established, their clinical significance remains obscure. Most studies report on a minority of vitamin D metabolites, which are usually the circulating ones. The latter are convenient to be measured, but they are essentially inactive. Furthermore, recent data show that at least one epimer form has activity in vitro [63]. Indeed, a recent study has revealed that higher concentrations of the active form exist in diseases, such rheumatoid arthritis, and diabetes mellitus type 1 [64].

In recent years, there have been considerable advances in techniques for vitamin D measurement [65, 66]. High-quality assays for multiple vitamin D forms include liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) [67, 68]. With the development of more advanced assays, a thorough understanding of the interplay among the various vitamin D forms can be achieved. The accurate assay highlights a considerable proportion of vitamin D exists as epimers and there is a lack of correlation between the circulating and active forms. While previous data indicated that neonatal and infant vitamin D stores are reliant from maternal ones [63], routine accurate measurement of epimers could overcome the technical flaws provoked by the presence of a significant percentage of epimers as a fraction of total vitamin D levels [69]. These results underscore the need for accurate measurements to appraise vitamin D status. The results, based on specific and accurate measurement, revealed that maternal characteristics and active forms of vitamin D, along with their epimers explain 56 % of neonatal vitamin D concentration [63].

Baseline levels of maternal 25(OH)D

Robert Heaney recently [34] outlined the significance of different basal status values in interpreting the effect of clinical studies of nutrient effects. In the case of vitamin D supplementation studies, it has been hypothesized that supplementing populations with lower baseline concentrations give better results. This parameter could affect results of studies where vitamin D supplementation was used, in pregnant women with profound hypovitaminosis D. Hossain et al. [22] reported identical obstetrical outcomes in supplemented (highest dose used was 4000 IU) vs non-supplemented Pakistani women, since the majority of supplemented women, failed to achieve optimal concentrations of 25(OH)D. This phenomenon is of atmost importance since previous results, in the field of vitamin D effects on inflammation reported that RCTs with baseline 25OHD < 50 nmol/L were twice as likely to show benefits as those with baseline >50 nmol/L [70].These findings suggest that is that RCTs conducted in countries with lower 25OHD concentrations in general, such as Middle Eastern countries and Pakistan where women cover most of their body, are more likely to show beneficial effects than studies done in countries with higher 25OHD. This approach, however, should be combined with appropriate supplementation regimens, in order to achieve sufficient 25(OH)D levels in these populations.

Conclusions

Data from supplementation trials with vitamin D during pregnancy fail to reveal a significant impact on maternal and offspring health, at least in consistent way. Possible reasons for this fact may include:

1. *Study design* Methodology flaws, such as lack of a specific outcome in conjunction with timing of supplementation,
2. *Vitamin D equilibrium* Lack of parameters that describe vitamin D economy, such as plasma half-life.
3. *Supplementation regimen* Administration of a wide range of regimens, in terms of dose, bolus and form that prevent safe interpretation of study results.
4. *Geographical characteristics* Vitamin D needs could vary significantly within a country, particularly in areas with a wide range of latitude gradient.
5. *Metabolism during pregnancy* Vitamin D and calcium equilibrium are altered during pregnancy compared with the non-pregnant state.
6. *Populations with lower baseline 25(OH)D* would likely to manifest beneficial effects after supplementation.

Supplementation with a biomolecule that also derives from endogenous production and undergoes significant transformation in order to accomplish its skeletal and extra-skeletal actions is a challenging task. Considering the whole spectrum of parameters that could affect vitamin D homeostasis during pregnancy, it can be concluded that supplementation regimens should be specific, according

population's baseline vitamin D values and taking into account parameters such as the ones suggested by this study.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent For this type of study formal consent is not required.

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