Review Article

Vitamin D deficiency and pregnancy disorders

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Abstract: Vitamin D deficiency (VDD) is a global health care issue, with billion carrying deficiency or insufficiency around the world. This hormone plays a kaleidoscope of important roles throughout pregnancy, so that maintenance of an adequate vitamin D status is essential in this setting. Several studies, which have investigated vitamin D status in different populations of pregnant women, have convincingly reported high prevalence of vitamin D insufficiency, consistently higher than 50%. Although VDD developing during pregnancy has been associated with some adverse maternal and pregnancy outcomes, especially gestational diabetes mellitus (GDM) and preeclampsia (PE), the risk estimated are considerably heterogeneous, which makes it challenging to define operative recommendations. The mean differences in serum 25-hydroxy-cholecalciferol [25(OH)D] between GDM and non-GDM pregnant women varies between –4.93 and –7.36 nmol/L, whilst that between pre-eclamptic and non-pre-eclamptic pregnant women is even wider, being comprised between –3.86 and –14.53 nmol/L. It is hence still unclear whether assessment of vitamin D during pregnancy shall be recommended, as well as which dose of vitamin D shall be administered in pregnant women with VDD. Further studies shall be planned, which will need to be based on reliable quantification techniques [preferably liquid chromatography tandem mass spectrometry (LC-MS/MS)-based methods], but will also need to reduce the impact of many confounding factors such as ethnicity, sunlight exposure and sampling time.

Keywords: Vitamin D; pregnancy; preeclampsia (PE); gestational diabetes mellitus (GDM)

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Introduction

Vitamin D deficiency (VDD) is a global health problem with over a billion people worldwide being deficient or insufficient (1,2). Pregnant women are at especially high risk, since the prevalence of VDD has been estimated to be up to 50% in this population (3,4). A number of interventional and observational investigations studies have investigated the impact of VDD on maternal and foetal outcome so far, including studies in gestational diabetes mellitus (GDM), preeclampsia (PE), small for gestational age (SAG) and other tissue-specific conditions, which have ultimately generated inconsistent results (5). Heterogeneity in study findings could be attributable to differences in ethnicity, geographic setting, stage of gestation, endpoints, level and duration of vitamin D supplementation (5). Different methods and sensitivities of analytical techniques to measure Vitamin D metabolites are additional factors that may have contributed to complicating the whole scenario. This review is hence aimed at presenting an updated overview on the role of vitamin D in pregnancy and on the impact of VDD on pregnancy outcomes, with particular focus on the role that different laboratory methods have on the expected results (6).

Vitamin D synthesis and metabolism

Vitamin D belongs to a group of fat-soluble secosteroids, whose more important compounds are ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3) (3,7). The two molecules mainly differ for the presence of a methyl group on C24 and for a double bond between C22 and C23, which are present in the side chain of vitamin D2 but not in vitamin D3 (8,9). Vitamin D2 is principally generated through ultraviolet (UV) radiation (UVB radiation, in particular) of ergosterol, which is normally found in plants, fungi, and invertebrates (10). Vitamin D3, is characterized by a similar genesis, though the provitamin is 7-dehydrocholesterol, which is found in vertebrates. (11). Even In humans, vitamin D3 is synthesized in the skin, at the level of the Malpighian layer, a process triggered supported by sunlight exposure (5). The UVB range stimulating the effective generation of vitamin D in the skin is narrow (i.e., between 290–315 nm) (3,12).

Since vitamins are organic residues required as nutrients for the body (because they cannot be independently synthesized), this term is applied exclusively to ergocalciferol, whilst cholecalciferol is considered a real hormone, because the organism is capable to independently carry out its synthesis (13). Approximately 80–90% of vitamin D human pools originate from endogenous synthesis of cholecalciferol by sunlight action in the skin, whilst the residual 10–20% is present in form of ergocalciferol and cholecalciferol taken with food. Vitamin D is produced in summer in higher quantity than during the rest of the year both during pregnancy and after birth (14,15), so that the excess is accumulated in the adipose tissue, making it available during periods of reduced light exposure, such as during the cooler seasons. However, the current Western lifestyle, with most activities taking place indoors, often prevents sufficient sunlight expose even during the summer. Moreover, latitude, clothing, sunscreen, and skin pigmentation may also contribute to lowering the vitamin D synthesis (16,17), so that dietary intake is becoming increasingly important. Exogenous vitamin D2 and D3 are both absorbed in the duodenal and at the level of the first jejunal loops thanks to the presence of bile salts and lipids in the intestinal lumen. They are then conveyed with chylomicrons within the lymphatic vessels. In the blood, the adsorbed vitamin D2 and D3 along with the endogenous vitamin D3 are transported by a specific α1 globulin (vitamin D binding protein, DBP), produced by the liver. DBP, whose half-life is approximately 2.5–3 days, binds vitamin D and its metabolites with high affinity, and is filtered and partially reabsorbed by the kidney (3). The exogenous vitamin D is then stored in the adipose tissue (and in small quantities in muscles, skin and bone tissue) or, after binding to chylomicrons, can be uptaken by the liver (13). Vitamins D2 and D3 are then converted in the liver into the pro-hormone 25-hydroxy-cholecalciferol [25(OH)D, also known as calcidiol or calcifediol, by cytochrome p450 and subsequently released into the bloodstream associated with the DBP. The 25(OH)D3 (half-life of about 10–20 days and metabolically inactive] is the major circulating metabolite of vitamin D, and its concentration in blood is used as a surrogate index of vitamin D status. In the proximal renal tube, 25(OH)D3 may undergo two further processes of hydroxylation. The former process is catalyzed by 1α-hydroxylase, which generates 1,25-dihydroxy-cholecalciferol [or $1,25(OH)_2D3$ or calcitriol (plasma half-life of 10–15 hours, plasma concentration 20–60 pg/mL], the active component and specific natural ligand of vitamin D receptors (VDR). The latter reaction involves the 24-hydroxylase, which generates the inactive form 24,25-dihydroxy-cholecalciferol [$24,25(OH)_2D3$] (3,18). The final products are excreted in the bile. The activity of 1α-hydroxylase is regulated by both availability of 25(OH) D substrate and co-factors, as well as by modulation of *CYP27B1* and *CYP24* genes (9,19). In addition to parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF-23), 1α-hydroxylase is regulated by blood concentration of calcium and phosphorus. Hypocalcemia and hypophosphatemia contribute to its stimulation, whilst hypercalcemia and hyperphosphoremia produce an inhibitory effect.

Vitamin D activity

The main activity of Vitamin D occurs at the level of three target tissues: kidney, bone and intestine, in cooperation with two other peptide hormones, PTH and FGF23 (20). PTH is the major trigger of $1,25(OH)_2D3$ renal production. Calcitriol directly inhibits synthesis and secretion of PTH and proliferation of parathyroid cells and, indirectly, contribute to enhance serum calcium level. The latter suppresses the release of PTH through the calcium sensitive receptors (CaSR) present on parathyroid glands. Furthermore, 1,25(OH)2D3 acts by increasing levels of VDR and promotes the transcription of *CaSR*, thus sensitizing the parathyroid glands to the inhibition of PTH secretion by plasmatic calcium. On the other hand,

FGF23 inhibits the renal production of $1,25(OH)_2D3$ and increases the expression of *CYP24* gene, whilst $1,25(OH)_2D3$ stimulates the production of FGF23. FGF23 is mainly expressed by osteocytes but also by osteoblasts and lining cells (21). On the bone and in the intestine, the vitamin D metabolite $1,25(OH)_2D3$ acts increasing serum calcium and phosphorus, with the aim of maintaining normal concentrations of these ions in serum and thus ensuring normal bone mineralization. Kidney is the third major target organ of $1,25(OH)_2D3$. Here calcitriol seems to strength the effects of PTH, stimulating calcium resorption from distal tube. Regarding its effects on phosphatemia, vitamin D stimulates reabsorption from proximal tube (more or less based on the plasmatic concentration of PTH) (13). VDRs are also present in bone marrow, cartilage, hair follicle, adipose tissue, adrenal gland, brain, stomach, small intestine, distal kidney tubule, colon, pancreas (B cells), liver, lung, muscle, activated Band T lymphocytes, heart cells, vascular smooth muscle cells, gonads (22). Some tissues also express the *CYP27B1* gene and may produce $1,25(OH)_2D3$ independently. The biological significance of these observations is confirmed by evidence of many extra-skeletal effects of vitamin D, such as regulation of cell proliferation and differentiation, as well as hormonal secretion and immune-modulation (23). The maintenance of vitamin D homeostasis during pregnancy is essential for stimulating calcium absorption for adequate intrauterine bone mineral accumulation of fetus, as well as for improving maternal resistance to fetal and paternal alloantigens (21,24,25).

25(OH)D measurement

The most abundant vitamin D metabolite, 25(OH)D, is currently regarded as the reference analyte for assessment of vitamin D status in clinical practice, and this is also due to its relatively long half-life. The methods for 25(OH)D measurement can be essentially divided into immunochemical (based on radioactive, enzymatic or chemiluminescence detection) and chromatographic [liquid chromatography, high performance liquid chromatography (HPLC) and liquid chromatography tandem mass spectrometry (LC-MS/MS)]. According to the vitamin D External Quality Assessment Scheme (DEQAS), overseeing method comparability across 56 countries, ~79% of the 861 participating laboratories in the year 2017 used automated immunoassays for measuring 25(OH)D, ~3% used manual immunoassays, whilst ~18% used LC-MS/MS and ~2% used HPLC (26). Despite increasing efforts have been made in the attempt of assuring high-quality methods for vitamin D assessment, differences in analytical assays still lead to remarkable biases. Controversial results have also been observed for serum 25(OH)D in response to vitamin D supplementation in different populations, including pregnant women. In earlier years, automated immunoassays had largely replaced manual assays because of their easier operation, better standardization and higher throughput. However, the major limitation of these techniques, entailing intrinsic cross-reactivity of antibodies, has paved the way to strong renaissance of LC-MS/MS systems. LC-MS/MS has also replaced HPLC methods due to its higher sensitivity and specificity. The major advantages and disadvantages of LC-MS/MS methods compared to HPLC and immunoassay for 25(OH)D measurement are summarized in *Table 1*. Thus, LC-MS/MS currently represents the preferred technique for 25(OH)D measurement in patients and this is especially true for pregnant women in whom the immunoassay assessment has been proven vulnerable to varying values of DBP (27,28).

Definition of VDD and insufficiency

VDD has been defined as a 25(OH)D level <50 nmol/L, whilst vitamin D insufficiency is defined as a 25(OH)D level between 52–72 nmol/L. It is commonly known, however, that VDD varies by age group, so that controversies remain regarding the standardized levels for identifying deficiencies. Despite these disagreements, most of the studies performed in pregnancy are aligned in defining VDD as a serum 25(OH)D level <25 nmol/L (i.e., <10 ng/mL) and vitamin D insufficiency as a serum level between 25–50 nmol/L (i.e., between 10–20 ng/mL) (29,30).

Vitamin D and calcium metabolism in pregnancy

During pregnancy, important changes in vitamin D and calcium metabolism occur, for providing the calcium needed for foetal bone mineralization. In the first trimester, the foetus accumulates 2–3 mg/day of calcium in the skeleton, an amount that nearly doubles in the last trimester (3,9). This actually happens as a result of increased intestinal absorption and decreased urinary excretion of calcium (in the mother) due to the considerable increase in serum vitamin D levels. In fact, plasma levels of $1,25(OH)_2D$ increase in early pregnancy, reaching a peak in the third trimester and returning to normal levels during

Table 1 Advantages and disadvantages of current LC-MS/MS, HPLC methods and immunoassays for 25(OH)D measurements

Methods	Advantages	Disadvantages
LC-MS/MS	Minimal consumable cost	High capital cost for instrumentation
	High sample throughput	High level of expertise required
	Sample preparation relatively simple and adaptable	Difficult to separate epi-25(OH)D3 from 25(OH)D3 (same mass and chromatographic behavior)*
	High sensitivity and specificity	
	Able to distinguish 25(OH)D2 (from Vitamin D supplementation) from 25(OH)D3 metabolites due to the different molecular mass	
	Multiple metabolite analysis in a single experimental run	
HPLC-UV	Minimal consumable cost	Low sample throughput
	Chromatographic interferences can be identified	Prone to analytical interference
Immunoassay	Minimal expense of instrumentation	High cost of kit consumable
	Minimal expertise required	High sample to sample variation in bias between different methods
	Kit methods simple to set up	Under- or over-estimation of total 25(OH)D due to the inability to distinguish 25(OH)D2 from the 25(OH)D3 form
		Cross-reactivity of other vitamin D metabolites recognised by the antibody, such as 24,25$(OH)_2$D

*, the presence of epimers has proven to be relevant in pediatric specimens but C3 epimer may represent a possible interference in adults as well. LC-MS/MS, liquid chromatography tandem mass spectrometry; HPLC, high performance liquid chromatography; 25(OH)D, 25-hydroxyvitamin D; UV, ultraviolet; 24,25(OH)2D, 24,25-dihydroxyvitamin D.

lactation (5). Conversely, maternal serum calcium levels fall during pregnancy, due to reduction of serum albumin, while ionized calcium levels remain unchanged (3). Many other physiological adaptations occur during pregnancy, including increase in DBP, placental VDR and renal and placental CYP27B1 activity to maintain normal serum levels of 25(OH)D and calcium (31). The stimulus for increased synthesis of 1,25$(OH)_2$D is unclear, considering that PTH levels do not change during pregnancy. An effective trigger of placental transfer of calcium and placental synthesis of vitamin D is PTH-related peptide (PTHrP), produced in the foetal parathyroid and placental tissues, which increases the synthesis of vitamin D (9). PTHrP in maternal circulation acts through the PTH/PTHrP receptor in the kidney and bones, thus increasing the synthesis of 1,25$(OH)_2$D and helping the regulation of calcium and PTH level in pregnancy (9,21). Although placenta may synthesize 1,25$(OH)_2$D, most of this metabolite in maternal circulation is indeed produced by maternal kidney (9). Other signals involved in this regulatory process include prolactin and placental lactogen hormone, which increase intestinal calcium absorption, reduce urinary calcium excretion and stimulate the production of PTHrP and 1,25(OH)2D. In addition, the increase in maternal blood levels of calcitonin and osteoprotegerin protects the mother's skeleton from excessive calcium resorption (9,21). The most important contribution of vitamin D during pregnancy is the stimulation of calcium absorption and placental calcium transport (32). Vitamin D may also regulate the immune system and inhibit inflammation processes by restraining inflammatory cytokines including TNF-α, IFN-γ, IL-6, and promoting the release of antimicrobial peptide cathelicidin in the placenta (33). Calcitriol also plays an important role in placental physiology, since it stimulates endometrial decidualization, synthesis of estradiol and progesterone, along with regulating the expression of human chorionic gonadotrophin (hCG) and human placental lactogen (hPL) (33).

Prevalence of VDD in pregnant women

The studies that investigated the status of vitamin D in

different populations of pregnant women have consistently found a high prevalence of vitamin D insufficiency so far. The main finding, originated from 86 studies published up to the year 2015, have been summarized by Saraf and coauthors in a systematic review and meta-analysis (34). Briefly, the average maternal 25(OH)D concentrations were found to be highly variable among different regions and also across studies performed within the same region. In particular, the mean 25(OH)D concentrations ranged between 47 and 65 nmol/L in American populations, between 15 and 72 nmol/L in Europe, between 13 and 60 nmol/L in Eastern Mediterranean, between 20 and 52 nmol/L in South-East Asia, and between 42 and 72 nmol/L in Western Pacific. The only study investigating vitamin D status in African women found a mean concentration of 92 nmol/L. Therefore, the prevalence of vitamin D insufficiency or deficiency, defined as a 25(OH)D concentration of <50 and <25 nmol/L in pregnant women were as follows: 64% and 9% in the Americas, 57% and 23% in Europe, 46% and 79% in Eastern Mediterranean, 83% and 13% in Western Pacific. In South East Asia the only available data was that concerning values <50 nmol/L, which were found to have a prevalence of 87% (34).

Vitamin D and pregnancy outcomes

VDD during pregnancy has consistently been associated with adverse maternal and pregnancy outcomes, mostly encompassing PE and GDM. A number of systematic reviews of the literature have efficiently summarized the results of single studies, often reporting controversial, which were essentially attributed to wide heterogeneity of the populations and the methods used for vitamin D assessment. We describe here, in *Tables 2,3*, a summary of the current published meta-analyses which quantitatively evaluated the association between vitamin D status and risk of GDM (35-40) and PE (36,37,41,42). Although data are almost convergent in demonstrating an overall significant association between VDD or vitamin D insufficiency and increased risk of both pregnancy disorders, most meta-analyses have important limitations. Not all studies shown in *Tables 2,3* provided disease prevalence or frequency of VDD. In some studies, the definition of GDM and PE was not specified. No sufficient information was then provided regarding the sampling timing. Pooled estimates have been calculated with random effect model in some meta-analyses and with fixed effect model in others. Heterogeneity is often high. Finally, the risk estimates differ widely between different meta-analyses, although the natural overlap of studies included. Accordingly, although the VDD seems to be convincingly associated with risk of developing both GDM and pre-eclampsia, an effective evaluation of the strength of this association is far from being established.

The underlying mechanism justifying the association between vitamin D and GDM has not been well understood so far, although some hypotheses have been made (43). The first implies a direct action of vitamin D on pancreatic β-cell function, which occurs through expression of VDR and 25-hydroxyvitamin D-1-α-hydroxylase in the pancreatic β-cells. The second mechanism may involve an influence of vitamin D on insulin resistance, through regulation of intracellular calcium, which influences glucose transport in target tissues. The third putative explanation concerns the effect of vitamin D on systemic inflammation, which is associated with insulin resistance in diabetes mellitus.

The mechanisms through which low serum vitamin D levels can affect the risk of pre-eclampsia are also still unclear, although this association remains biologically plausible. In particular, it has been proposed that vitamin D may help preventing the development of hypertension in gestational women by acting as endocrine suppressor of renin biosynthesis, thus behaving as effective regulator of the renin-angiotensin system which plays a critical role in regulation of blood pressure and electrolyte and plasma volume homeostasis (44). In addition, vitamin D may influence blood pressure through suppression of vascular smooth muscle cell proliferation and improve endothelial cell-dependent vasodilatation, also inhibiting anticoagulant activity (45). In vitro studies have demonstrated that vitamin D leads to an up-regulation of regulatory T cell responses, whilst proinflammatory responses are typically down-regulated (46), thus representing an adaptation to maternal tolerance which would finally contribute to lower the risk of developing pre-eclampsia.

Conclusions

Although maternal vitamin D status seems to be associated with incidence of GDM and PE, the risk estimated varied widely between different meta-analyses, thus making results of difficult interpretation. The mean differences in serum 25(OH)D between GDM and non-GDM pregnant women ranges between –4.93 and –7.36 nmol/L, whilst that between pre-eclamptic and non-pre-eclamptic pregnant women displays a wider variation, being comprised between –3.86 and –14.53 nmol/L. It is hence still unclear

Table 2 Meta-analyses evaluating the association between vitamin D status and risk of GDM

First author, year of publication	No. studies	No. total patients	Vitamin D assay method (No. studies for method)	GDM criteria* (No. patients for criteria)	Prevalence (mean) and definition of VDD	Association between VDD and GDM	Differences in serum 25(OH)D in GDM women with respect to non-GDM women
Poel *et al.* 2012 (35)	7 studies (3 cross-sectional, 2 case-control, 2 nested case-control)	2,146 women—433 with GDM	Not specified	145 Carpenter & Coustan; 57 ADA; 244 ADPS; 60 NDDG; 90 WHO	55.64% in 6 studies [defining VDD as serum 25(OH)D levels <50 nmo/L] 70.6% in 1 study [defining VDD as serum 25(OH)D levels <25nmo/L]	Unadjusted OR =1.609 (95% CI: 1.19 to 2.17). I^2 =5.8% (P=0.38); adjusted for BMI and maternal age (6 studies) OR =1.57 (95% CI: 1.11 to 2.22). I^2 =17% (P=0.011); adjusted for BMI, maternal age and ethnicity (3 studies) OR =1.84 (95% CI: 1.07 to 3.15). I^2 =47% (P=0.03)	−5.33 nmol/L (95% CI: −9.7 to −0.9; P=0.018)
Wei *et al.* 2013 (36)	12 studies (4 nested case-control, 4 prospective cohort, 2 cross-sectional, 2 case-control)	4,126 women—623 with GDM; 3,840 women—542 with GDM	5 LC-MS; 2 CLIA; 2 RIA; 2 ELISA; 1 HPLC	Not specified	Not reported; 10 studies defined VDD as serum levels of 25(OH)D <50 nmol/L, 8 studies defined VDD as serum levels of 25(OH)D <75 nmol/L	OR =1.38 (95% CI: 1.12 to 1.70) for 25(OH)D <50 nmol/L. I^2 =0%; OR =1.55 (95% CI: 1.21 to 1.98) for 25(OH)D <75 nmol/L. I^2 =7%	−5.98 nmol/L (95% CI: −9.14 to −2.81; I^2 =45%)
Aghajafari *et al.* 2013 (37)	10 studies (3 case-control, 3 prospective, 3 nested case-control, 1 cross-sectional)	4,112 women—687 with GDM	4 HPLC and LC-MS; 2 RIA; 2 ECLIA; 1 ELISA; 1 EIA	Not specified	Not reported; 7 studies defined VDD as serum levels of 25(OH)D <50 nmol/L, 3 studies used the cut-off of <75 nmol/L	OR =1.49 (95% CI: 1.18 to 1.89). I^2 =0%, P=0.58; adjustment for country of origin, 25(OH)D cut-off concentration gestational age at sampling, study design and 25(OH)D quantification methods did not change the pooled estimated; OR adjusted for critical confounders[§] 1.98 (95% CI: 1.23 to 3.23)	−7.36 nmol/L, (95% CI: −10.16 to −4.56) The weighted mean difference did not change significantly when stratified by country of origin and study design
Zhang *et al.* 2015 (38)	20 studies (8 cross-sectional, 5 case-control, 5 nested case-control, 2 prospective cohort)	9,209 women—1,737 with GDM	4 RIA; 3 ECLIA; 4 CLIA; 4 ELISA; 5 LC-MS	168 Carpenter & Coustan; 477 ADA; 81 ADPS; 178 NDDG; 397 WHO; 332 IADPSG; 36 CDA	50% (VDD defined as serum levels of 25(OH)D <50 nmol/L)	OR =1.53 (95% CI: 1.33 to 1.75). I^2 =16.20%, P=0.252; OR for severe deficiency 1.59 (95% CI: 1.11 to 2.27); OR for insufficiency 1.39 (95% CI: 1.07 to 1.82)	−4.93 nmol/L (95% CI: −6.73 to −3.14; I^2 =61.40%, P=0.001)

Table 2 (*continued*)

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First author, year of publication	No. studies	No. total patients	Vitamin D assay method (No. studies for method)	GDM criteria* (No. patients for criteria)	Prevalence (mean) and definition of VDD	Association between VDD and GDM	Differences in serum 25(OH)D in GDM women with respect to non-GDM women
Lu *et al.* 2016 (39)	20 studies (7 nested case-control, 7 prospective cohort, 4 case-control, 2 cross-sectional)	16,515 women. Prevalence of GDM not reported	15 immunoassay; 4 HPLC/MS; 1 HPLC	Not specified	Not reported; 15 studies defined VDD as serum levels of 25(OH)D <50 nmol/L, 5 studies used the cut-off of <75 nmol/L	Summary RR =1.45; 95% CI: 1.15 to 1.83; P<0.001. I^2 =66,6% (P=0.00) Adjustments: the association remain significant if the study was conducted in developed countries, did not use cohort design, used HPLC-MS assay to assess vitamin D levels, had a sample size of <1,000, a mean age of the patient was >30 years, did not adjust for potential confounding factors, and had a high quality	Not reported
Amraei *et al.* 2018 (40)	26 studies (8 cross-sectional, 6 prospective, nested case-control, 7 retrospective case-control and 5 prospective cohort)	29,533 women—5,464 with GDM	6 CL-MS; 4 ELISA; 4 RIA; 5 ECLIA; 4 CLIA; 1 AIAS; 1 HPLC; 1 Liebermann-Burchard method	Not specified	Not reported	VDD is correlated with an increased risk of gestational diabetes if the study did not use a cross-sectional and cohort design	−2.26 (95% CI: −0.39 to −0.14; I^2 =68.8%)

*, diagnostic criteria of GDM; §, include, depending on the study, one or more of the following: age, insurance status, body mass index, gestational, age at serum collection, season, diabetes status, smoking status, method of conception, race, family history of type 2 diabetes. C&C, Carpenter and Coustan; ADPS, Australasian Diabetes in Pregnancy Society; ADA, American Diabetes Association; NDDG, National Diabetes Data Group; WHO, World Health Organization; IADPSG, International Association of the Diabetes and Pregnancy Study Groups; CDA, Canadian Diabetes Association; RIA, radioimmunoassay; LC-MS, liquid chromatography-mass spectrometry; ECLIA, electrochemiluminescence immunoassay; ELISA, enzyme-linked immunosorbent assay; CLIA, chemiluminescence immunoassay; AIAS, automated immunoassay system; GDM, gestational diabetes mellitus; VDD, vitamin D deficiency.

Table 3 Meta-analyses evaluating the association between vitamin D status and risk of pre-eclampsia

First author, year of publication	No. studies	No. total patients	Vitamin D assay method (No. studies for method)	Pre-eclampsia criteria	Prevalence (mean) and definition of VDD	Association between VDD and Pre-eclampsia	Differences in serum 25(OH)D in pre-eclamptic women with respect to non-pre-eclamptic women
Hyppönen *et al.* 2013 (41)	6 studies (3 nested case-control, 2 prospective cohort, 1 case-control)	6,578 without PE—286 with PE	Not specified	Systolic BP ≥140 mmHg and/or diastolic BP was ≥90 mmHg and proteinuria was at least 300 mg/24 h	Not reported; 3 studies defined VDD as serum levels of 25(OH)D <50 nmol/L, 2 studies defined VDD as serum levels of 25(OH)D <37.5 nmol/L, 1 study defined VDD as serum levels of 25(OH)D <75 nmol/L	OR =0.52 (0.30–0.89), P=0.02. I^2 =60,9%, P=0.03 Estimated effect was similar both for studies using 37.5 and 50 nmol/L as the cut-off for low concentrations. There were no systematic differences between studies supporting a stronger versus a weaker association in relation to study size, assay type or trimester	Not reported
Tabesh *et al.* 2013 (42)	8 studies (4 nested case-control, 2 prospective cohort, 1 cross-sectional, 1 case-control)	2,485 women enrolled. Non-specified number of women with PE	Not specified	BP of >140/90 mmHg after 20 weeks gestation, accompanied by proteinuria of >0.3 g in a 24-hour urine collection or a urine dipstick result of +2 or greater. in 2 study dipstick result of 1+, 1 study used 2 definition of PE*	Not reported; 4 studies defined VDD as serum levels of 25(OH)D <50 nmol/L, 3 studies defined VDD as serum levels of 25(OH)D <38 nmol/L, 1 study used both cut-offs	Pooled RR not reported but significant. I^2 =52,7%; P=0.03 RR =2.78; (95% CI: 1.45 to 5.33), P=0.002 for both cross sectional and case control studies RR for studies considering VDD as serum levels of 25(OH)D <50 nmol/L comprises between 1.24 and 5.41. Association significant RR for studies considering VDD as serum levels of 25(OH)D <38 nmol/L comprises between 0.9 and 5. Association not significant	Not reported

Table 3 (*continued*)

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First author, year of publication	No. studies	No. total patients	Vitamin D assay method (No. studies for method)	Pre-eclampsia criteria	Prevalence (mean) and definition of VDD	Association between VDD and Pre-eclampsia	Differences in serum 25(OH)D in pre-eclamptic women with respect to non-pre-eclamptic women
Wei *et al.* 2013 (36)	6 studies (3 prospective, 2 nested case-control, 1 case-control)	6 studies considering as cut-off 25(OH)D <50 (1,799 without PE, 209 with PE); 5 studies considering as cut-off 25(OH)D <75 nmol/L (1,134 without PE, 177 with PE)	2 LC-MS, 1 ELISA, 2 ECLIA, 2 RIA	Not specified	Not reported; 6 studies defined VDD as serum levels of 25(OH)D <50 nmol/L, 5 studies defined VDD serum levels of 25(OH)D <75 nmol/L	OR =2.09 (95% CI: 1.50 to 2.90) for 25(OH)D <50 nmol/L. I^2=0%; OR =1.78 (95%CI 1.23 to 2.56) for 25(OH)D <75 nmol/L. I^2 =28%	−3.86 nmol/L (95% CI: −5.13 to −2.59) For studies with blood sampling at before 20 weeks: −3.60 nmol/L (95% CI: −5.06 to −2.15) For studies with blood sampling at after 20 weeks: −4.68 nmol/L (95% CI: −7.28 to −2.07)
Aghajafari *et al.* 2013 (37)	9 studies (4 case-control, 3 nested case-control, 2 prospective)	1,789 without PE, 286 with PE	5 HPLC or LC-MS, 1 RIA, 1 ELISA, 2 ECLIA	PE was defined by new onset hypertension after 20 weeks of gestation (systolic BP >140 mmHg or diastolic BP >90 mmHg) and proteinuria 0.3 g or more per day or 2 or more + on dipstick testing	Not reported; 2 studies defined VDD as serum levels of 25(OH)D <50 nmol/L, 5 studies defined VDD as serum levels of 25(OH)D <75 nmol/L	OR =1.79 (95% CI: 1.25 to 2.58). I^2 =0%, P=0.81 (from 7 studies) The estimate of association varied by indicators of quality gestational age at sampling, definition of insufficiency, and method for quantification OR adjusted for critical confounders[§] 1.51 (0.89 to 2.57). No more significant	−14.53 nmol/L, (95% CI: −22.57 to −6.49) (from 5 studies)

*, blood pressure >160/110 mmHg accompanied by proteinuria or systolic blood pressure of at least 90 mmHg plus 5 g proteinuria in a 24-hour urine sample; [§], include, depending on the study, one or more of the following: age, body mass index, gestational age at serum sampling, season, parity, education, race. VDD, vitamin D deficiency; BP, blood pressure; PE, preeclampsia; LC-MS, liquid chromatography-mass spectrometry; ELISA, enzyme-linked immunosorbent assay; ECLIA, electrochemiluminescence immunoassay; RIA, radioimmunoassay; HPLC, high performance liquid chromatography.

whether assessment of vitamin D during pregnancy shall be recommended, as well as which dose of vitamin D shall be administered in pregnant women with VDD. Further studies should be designed, considering the significance of the method used for vitamin D quantification (and preferentially use LC-MS/MS based methods), the influence of confounding factors, the choice of the sampling time.

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