

Vitamin D administration during pregnancy as prevention for pregnancy, neonatal and postnatal complications

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Abstract Pregnancy represents a time of rapid bodily change, which includes physical proportions, physiology and responsibility. At this context, maternal vitamin D stores have been the objective of extensive scientific research during the last decades, focusing on their potential effects on maternal and neonatal health. 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 including asthma, multiple sclerosis and other neurological disorders. On that basis, this review aims to provide to the reader new insights into the vitamin D requirements and function during pregnancy supported by recent data and will not discuss the classical roles of vitamin D and skeletal function during pregnancy. In addition, we will focus on recent results that demonstrate that maternal vitamin D supplementation could reduce neonatal respiratory and neurological complications, suggesting that available guidelines should be updated, since it remains unclear why these recommendations are not updated according to recent results. Also, with regard to randomized controlled trials (RCT's) for vitamin D, we consider that they are largely doomed to fail. The reasons for

this are many and specific cases of this failure will be presented in this text.

Keywords Vitamin D · Cholecalciferol · Pregnancy · Lactation · Health outcomes · Preeclampsia · Asthma · Complications of pregnancy

1 Introduction

Pregnancy is a time of rapid change for both the mother and fetus, which includes changes in physical proportions, physiology and responsibility [1]. A hormone that is significantly affected during pregnancy is 1,25-dihydroxy-vitamin D, increasing more than 2–3 fold in the first weeks of pregnancy, the significance of which remains unclear. Maternal vitamin D status and its effect on pregnancy outcomes have been the focus of extensive scientific research during the last decades [2, 3]. A growing body of observational studies suggests that maternal hypovitaminosis D (as defined by circulating maternal 25-hydroxyvitamin D [25(OH)D] concentrations <20 ng/ml or <50 nmol/l [2] is a significant risk factor for adverse neonatal outcomes [2, 3] including asthma, multiple sclerosis and other neurological disorders [3–13]. In addition, available physiological studies indicate that during pregnancy significant changes in the requirement and metabolism of vitamin D are evident [2, 3, 15].

Supported by recent data, this comprehensive review aims to provide the reader with new insights into vitamin D requirements and functions during pregnancy that go beyond the classical roles of vitamin D and skeletal function during this unique time in the lifecycle (for more information about the classical roles, the following are helpful [14–17]). By reviewing the similarities and differences in vitamin D metabolism during the pregnant and nonpregnant state, a better

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appreciation of the complexity of the vitamin D metabolic pathways is achieved. By focusing on recent results that demonstrate that maternal vitamin D supplementation could reduce neonatal respiratory and neurological complications, the impetus to change available guidelines and conduct additional research is more apparent.

To aid the reader, the salient points of this review are summarized in Table 1.

1.1 Vitamin D metabolism during normal pregnancy as compared to the non-pregnant state

A striking difference exists in vitamin D metabolism during pregnancy and fetal development compared with non-pregnancy and non-fetal states, a point that has been known for at least the past three decades but which has received little attention until recently [18–22]. The conversion of vitamin D to its metabolite 25(OH) D appears unchanged during pregnancy, following first-and-zero-order enzyme kinetics [23]. By contrast, the conversion of 25(OH) D to 1,25(OH)₂D during pregnancy is unique and unparalleled during life. At no other time during lifecycle is 25(OH) D so closely linked with 1,25(OH)₂D production. By 12 weeks of gestation, 1,25(OH)₂D serum concentrations are more than twice that of a non-pregnant adult and continue to rise two- to threefold from the non-pregnant baseline rising to over 700 pmol/L within weeks of conception, attaining circulating concentrations that would be toxic to the non-pregnant individual due to hypercalcemia, but which are seemingly essential during pregnancy [24]. Similarly, circulating 1,25(OH)₂D concentrations in cord blood are even more closely tied to fetal circulating concentrations of its precursor 25(OH) D [25, 26]. In neither the mother nor fetus does this conversion seem to be controlled by the classic calcium homeostatic mechanisms during the pregnant state [16, 24].

The rise in circulating 1,25(OH)₂D concentrations in the mother/fetus is a remarkable observation. Early-on, the thought was that this increase was to ensure adequate delivery of calcium to the maternal skeleton for preservation and fetal skeletal development. Calcium homeostasis, however, is not linked with this increase in 1,25(OH)₂D because at 12 weeks of gestation there is no increase in calcium demand by either the mother or fetus. In contrast, this increased concentration of 1,25(OH)₂D sustained during pregnancy is not sustained

during lactation when maternal calcium demand is at least as high as during pregnancy [27]. Thus, in the mother and fetus during pregnancy, the rise in 1,25(OH)₂D is dependent on substrate availability—in this case—25(OH) D, and is largely independent of calcium homeostasis [24]. It remains unclear what the exact mechanism (s) is/are that are driving this profound change noted early in pregnancy.

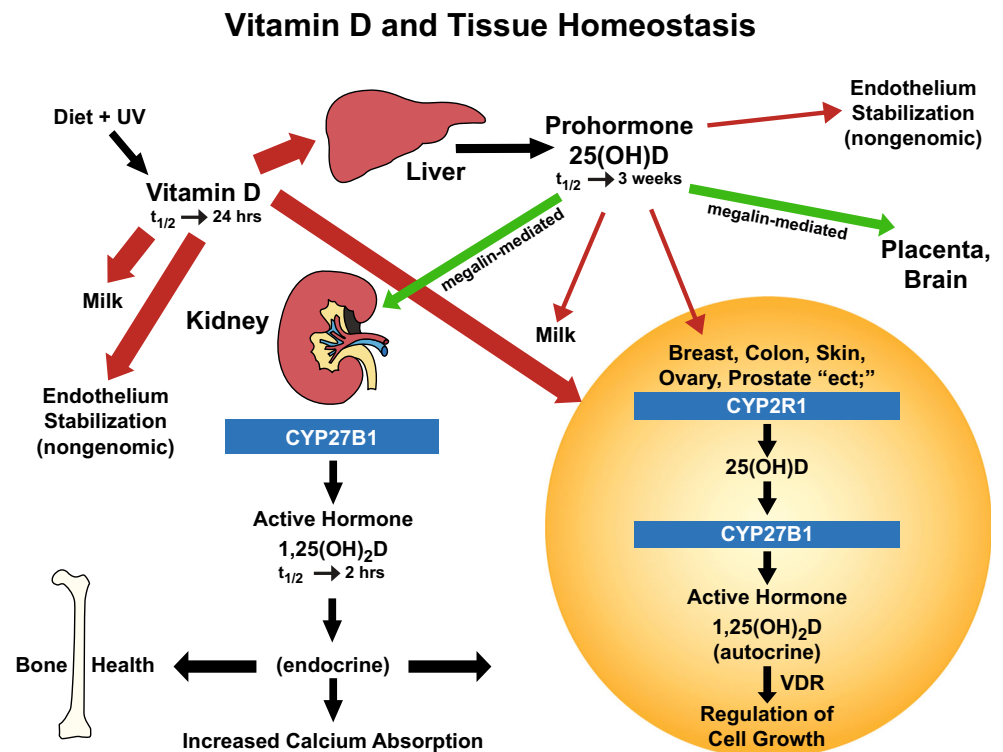
In humans, vitamin D₃ is naturally obtained when sunlight in the UVB range strikes the skin and causes 7-dehydrocholesterol to be converted, following a membrane-enhanced thermal-dependent isomerization reaction, into vitamin D₃, which then diffuses into the circulation through the capillary bed [28]. Vitamin D also is obtained orally through the diet as either vitamin D₂ or D₃. As far as can be determined from the literature, this absorption process is primarily diffusion-based, is dependent on bile acid solubilization, and is not saturable [29–31]. When vitamin D₃ enters the circulation after UV exposure, it is primarily associated with vitamin D binding protein (VDBP). In contrast, after intestinal absorption, it is coupled with both VDBP and lipoproteins [32]. Vitamin D from either route is delivered primarily to the liver, where 25(OH) D is produced, becomes associated with VDBP, and is discharged into the circulation [33]. Not only circulated to the liver, vitamin D also is circulated to all tissues in the body; many of which are now known to contain both the activating hydroxylase and the vitamin D 25-hydroxylase that converts vitamin D into 25(OH) D, thus achieving autocrine production of 25(OH) D in those tissues [34–38] (see Fig. 1).

On reaching the circulation, the primary determinant of how long a vitamin D metabolite will stay in circulation is its affinity for VDBP [39]. Vitamin D, 25(OH) D, and 1,25(OH)₂D have vastly different dissociation constants with regard to VDBP: for 25(OH) D, it is approximately 10⁻⁹ M; for vitamin D and 1,25(OH)₂D, it is approximately 10⁻⁷ M [40]. When taking into account vitamin D's relative insolubility, its dissociation constant probably is reduced to approximately 10⁻⁸ M when measured *in vitro* [41]. The dissociation constants are more meaningful when translated into the circulating half-lives of these compounds; thus, where the half-life of 25(OH) D is weeks; for vitamin D, it is 1 day; and for 1,25(OH)₂D, a few hours [42–44]. These dissociation constants also dictate the “free” concentration of compound that is available to diffuse across a cell membrane into cells to be metabolized or to modulate cell activity (see Fig. 1). In the case of these three compounds, the

Table 1 Key Points Regarding Vitamin D Metabolism and Its Extra-skeletal effects during pregnancy

- Vitamin D metabolism during pregnancy is unique differing from the non-pregnant state.
- Circulating levels of 1,25(OH)₂D manifest a “dissociation” from calcium homeostasis during pregnancy, that would result in severe hypercalcemia during non-pregnant state.
- Maternal hypovitaminosis D during pregnancy alters maternal gene expression.
- Vitamin D deficiency during pregnancy contributes to increased complications of pregnancy with later complications noted in the offspring, including asthma.
- Comorbidities of pregnancy are decreased in women whose circulating 25(OH)D concentrations are at least 40 ng/mL (100 nmol/L).

Fig. 1 Diagram of the metabolic processes providing vitamin D and its metabolites to various tissues in the body. Tissue distribution of vitamin D and 25(OH)D based on simple diffusion (red arrows) or endocytosis (green arrows). Endocytosis requires the tissue-specific megalin-cubilin system, whereas simple diffusion is primarily controlled by the dissociation constant of the vitamin D compound for the VDBP. Bolder red lines indicate greater diffusion rates due to higher dissociation constant. $t_{1/2}$, half-life. Modified from reference [97] and used with permission



“free” circulating concentrations are greater for 1,25(OH)₂D than for intact vitamin D, which in turn is larger than that for 25(OH)D, matching their relative circulating half-lives.

Besides simple cellular diffusion of free compound, there exists an important transport system that affects vitamin D metabolism—the megalin-cubilin endocytotic system [45]. This system is key in the delivery of 25(OH)D to the 25-hydroxyvitamin D-1- α -hydroxylase in the kidney [46], but also in the parathyroid glands, regulating calcium homeostasis [47]. The megalin-cubilin system also functions in the placenta [48] and brain [49]. Where tissues lack this endocytotic system, however, diffusion of vitamin D compounds in relation to free circulating concentrations becomes inherently important. Interestingly, VDBP-knockout animal models show normal survival when given dietary vitamin D on a daily basis [50, 51]; because vitamin D metabolite cellular access could only be by diffusion in these animals, this shows that the parent compound vitamin D is normally transferred in wild-type animals through simple membrane diffusion.

Why is calcium metabolism uncoupled from 1,25(OH)₂D generation during pregnancy and not lactation? One of the leading theories is that 1,25(OH)₂D is an important immune modulator involved in maternal tolerance to the foreign fetus whose DNA is only half that of the mother’s. Support for this premise comes from careful analysis of preeclamptic mothers (preeclampsia is a complication in pregnancy characterized by new-onset high blood pressure and signs of damage to another organ system, including systemic vasculitis, often involving the kidneys as well as the placenta that usually begins after

20 weeks of gestation). In early epidemiological studies involving pregnant women with preeclampsia, vitamin D deficiency has been implicated [52, 53]. Experimental animal models have also strongly suggested vitamin D deficiency as a potential mechanism of placental dysfunction [54, 55].

Vitamin D is a known modulator of inflammation [56]. Until recently, native dietary vitamin D₃ was thought to be bio-inactive, and the beneficial effects of vitamin D were thought to be largely mediated only by 1,25(OH)₂D [57]. In many disease states, low circulating 25(OH)D concentrations are associated with multiple inflammatory diseases such as cardiovascular, arthritis, multiple sclerosis, cancer, schizophrenia and sepsis [3–10, 12, 13, 58]. Common to all of these diseases is the disruption of endothelial stability and an enhancement of vascular leak. Experimental animal models of preeclampsia clearly demonstrate this endothelial instability leads to placental ischemia [59]. To that end, Gibson et al. [60, 61] have identified vitamin D₃ as an effective stabilizer of endothelium and endothelium “leak” through non-genomic mechanisms. This membrane stabilization process is dependent on the highly structurally-specific open b-ring, the cis-triene structure of vitamin D not present in its precursor 7-dehydrocholesterol (which has a closed ring) [60]. This is an important new observation: vitamin D₃, 25(OH)D₃ and 1,25(OH)₂D₃ all have the ability to control “endothelial leak,” and that on an equimolar basis, vitamin D₃ is more potent in this function than are 25(OH)D₃ or 1,25(OH)₂D [60]. Because these previously considered ‘inactive’ sterols can promote stabilizing activity at doses lower than necessary for an

interaction with VDR, the stabilizing phenomena appears to occur in a VDR-independent manner. The authors point out that this study does not call into question a role for $1,25(\text{OH})_2\text{D}_3$ or VDR in immunomodulatory mechanisms, but rather, that these results suggest the existence of an alternative pathway of vitamin D activity, and that the inverse correlations between circulating $25(\text{OH})\text{D}_3$ concentrations and certain diseases could be due to the effects of D_3 and $25(\text{OH})\text{D}_3$ directly on endothelial stability [60]. Such findings support what has been observed in certain disease states, including preeclampsia.

As depicted in Fig. 1, besides being the most potent stabilizer, vitamin D_3 would also be the metabolite most accessible to the cell membrane to impart its function. As discussed earlier, circulating $25(\text{OH})\text{D}_3$ is almost totally bound to the VDBP and its “free” concentration so miniscule that there simply is not enough to matter. $1,25(\text{OH})_2\text{D}_3$, while existing in a high circulating “free” form, simply circulates at a level of insignificance for this function [18]. Vitamin D_3 , however, if given at physiological levels of 4000 IU/d or greater, would circulate in the “free” form at significant concentration and would be available for membrane insertion and subsequent endothelial stabilization that is likely to have profound effects on several disease processes [60]. This represents a new frontier in our understanding of vitamin D’s mode of action.

1.2 Observational studies suggesting the function of vitamin D extends beyond calcium homeostasis during pregnancy

While not the focus of this chapter, it is important to mention that the role of vitamin D on skeletal function during pregnancy requires minimal supplementary vitamin D to meet these needs [64]. Beyond skeletal issues, what would these other issues be with respect to vitamin D in pregnancy? To discover what these might be, we rely on associative or observational studies, and in the past 15 years or so many of these studies have been performed.

Early observational/correlation studies uncovered strong relationships between maternal circulating levels of $25(\text{OH})\text{D}$ and preeclampsia [52, 53, 62, 63], altered placental vascular pathology [64], cesarean section [65], glucose tolerance [66], adverse birth outcomes due to race [67], and altered brain [11–13] and respiratory function in children [4]. More recent observational studies have pointed to maternal vitamin D deficiency (<20 ng/mL or 50 nmol/L) and insufficiency (<80 nmol/L or 32 ng/mL) as risk factors for abnormal fetal growth patterns, adverse birth outcomes, and reproductive failure [71–73]. A recent meta-analysis of observational studies supports the premise that maternal vitamin D deficiency ($25\text{OHD} <20$ ng/mL) increases the risk of preterm birth [68]. Maternal vitamin D status is associated with maternal health and complications during pregnancy, and one would expect

this to influence preterm birth rates. Preeclampsia certainly is linked with vitamin D deficiency early-on [53, 63, 67, 69–71]. Spontaneous preterm births where there was no antecedent risk factor other than vitamin D deficiency is associated with lower circulating $25(\text{OH})\text{D}$ [72].

Vitamin D deficiency during pregnancy has been documented in many populations across the globe and is often associated with adverse maternal and birth outcomes [75]. In a large multiethnic cohort of pregnant women and their infants in Netherlands [76], $25(\text{OH})\text{D}$ 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 $25(\text{OH})\text{D}$ levels below 25 nmol/L (10 ng/mL). In a comprehensive narrative review from the sunny Mediterranean region [77], the authors concluded that vitamin D deficiency (as defined by maternal $25(\text{OH})\text{D}$ levels <20 ng/ml or <50 nmol/L) in pregnancy is quite common, 23% to 90% depending on the study, with the prevalence of vitamin D insufficiency (defined by $25(\text{OH})\text{D}$ levels between 50 and 75 nmol/L) ranging from 9% to 41%. 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.

Complicating maternal vitamin D status during pregnancy, seasonal variation has been shown to influence maternal circulating $25(\text{OH})\text{D}$ concentrations [78]. Similarly, season of birth has been demonstrated to have significant impact on newborn $25(\text{OH})\text{D}$ levels, especially in high latitude countries. In this regard, a recent study was conducted on a dataset of 450,000 participants from the UK Biobank [79]. It demonstrated that birth weight and adult height were significantly associated with season of birth, with individuals born during the summer had significantly higher mean birth weight and a significantly taller adult height compared to those born during the rest of the year. While height may be a marker of skeletal integrity influenced by vitamin D in a classical endocrine manner, it could also be a marker of the general health status of child that could be influenced by vitamin D in a non-endocrine manner. By taking into account that offspring vitamin D concentration is affected by maternal status, it becomes evident that maintaining maternal vitamin D status through supplementation during winter could be beneficial for neonatal outcomes, in particular in countries at higher latitudes [80].

A number of elegant observational studies indicated a linear association between maternal hypovitaminosis D and adverse neonatal outcomes. In a large U.S. multicenter study, maternal $25(\text{OH})\text{D}$ was positively associated with birth weight and head circumference (81). In addition, maternal $25(\text{OH})\text{D}$ level ≥ 37.5 nmol/L in the first trimester was associated with a 50% risk reduction of small for gestational age (SGA) births. Similarly, a multi-ethnic study from the Netherlands reported that mothers with adequate vitamin D had infants with higher birth weight and lower risk of SGA [82]. In an Australian

study conducted by Bowyer et al. [73], 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, some of which may have reflected pre-term births. Similarly, in study by Burris et al., comprised of women living in Massachusetts who were from Caucasian and African ethnic groups, lower circulating maternal vitamin D levels were associated with increased risk of small-for-gestational age (SGA) status [74]. In a study involving 500 Iranian pregnant women with 25(OH) D₃ < 75 nmol/L (<30 ng/mL) beginning at weeks 12–16 of pregnancy, those women randomized to the 50,000 IU vitamin D₃ every 2 weeks until delivery group had a lower incidence of gestational diabetes, but the intervention had no effect on other outcomes including birth weight [75].

Not all intervention vitamin D pregnancy studies have demonstrated significant results. In a Spanish study, lack of association was reported between maternal vitamin D and birth outcomes, including SGA and newborn anthropometry [85]. The findings were based on a single measurement of circulating 25(OH) D obtained during the first prenatal visit. Overall, prevalence rates of maternal hypovitaminosis D were rather decreased with only 19.7% of meeting the definition of vitamin D deficiency [25(OH) D < 50 nmol/L]. In a study from the UK [86], a serum sample taken from mothers during late pregnancy (28- to 42-weeks), and stored at -40 °C for 5 years prior to measurement of total 25(OH) D by radioimmunoassay showed 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.

It becomes evident that, while public policy cannot be set for supplementation practices based on observational studies, this information is invaluable at pointing research in the direction that could yield public policy changes in vitamin D consumption. Such studies were important in designing and in the conduct of randomized clinical trials (RCTs). Even with RCTs, however, whose results can be problematic when analyzed on an intent-to-treat basis and when there is high non-adherence to protocol (as is often the case), the potential good or harm of a given treatment at higher doses can be reduced or minimized. As such, a biomarker of a drug or in this case “vitamin” or pre-prohormone is better served as the biomarker of effect. For these reasons, analyses of effect of vitamin D therapies using 25(OH) D concentrations is a far better indicator of true “effect.”

1.3 Randomized controlled trials investigating vitamin D supplementation during pregnancy

Enthusiasm for evidence-based medicine (EBM) has resulted in the extension of its methods to the evaluation of nutrient

effects. Heaney [76] pointed out that EBM, as applied in the evaluation of drugs, is poorly suited to the study of nutrients. In a drug trial, the placebo group will be totally devoid of the compound in question; not so for a nutrient like vitamin D. To perform a true RCT for vitamin D, one would have to make sure all subjects were vitamin D-deficient at the study onset. For the duration of the study, all subjects would have to remain indoors to avoid any sun exposure. Then and only then could a true RCT be performed for any given function of vitamin D. Dr. Heaney [18] went on to propose five rules for individual clinical studies of nutrient effects: 1) basal nutrient status must be measured, used as an inclusion criterion for entry into the study, and recorded in the report of the trial; 2) the intervention must be large enough to change nutrient status and must be quantified by suitable analysis; 3) the change in nutrient status produced in those enrolled in the report of the trial must be measured and reported; 4) the hypothesis to be tested must be that a change in nutrient status produces the sought-after-effect; and 5) co-nutrient status must be optimized in order to ensure that the nutrient is the only nutrition-related, limiting factor in the response. We have added one additional rule to this group: 6) the nutrient in question has to follow an appropriate dosing schedule matching the physiologic system being investigated [57]. Needless to say, while almost all vitamin D RCT's to this point would fail based on these criteria, evaluation of existing evidence with respect to pregnancy becomes the basis for optimizing dietary and clinical recommendations.

Vitamin D supplementation trials involving pregnant women have been performed since 1980 [1]. These early studies were small, did not look at meaningful endpoints, and supplemented with minimal doses of vitamin D [1]. As a result, no meaningful information or public policy changes occurred because of them.

In 2001, Hollis and colleagues conceived a large RCT investigating the supplementation of vitamin D to a pregnant population that involved supplementing pregnant women less than 16 weeks of gestation in a double-blind fashion with up to 4000 IU/d vitamin D₃ until delivery [25]. The goal of the study was to see how much vitamin D was required to raise circulating maternal 25(OH) D concentrations to at least 80 nmol/L or 32 ng/mL by the end of gestation, the circulating concentration shown to suppress secondary hyperparathyroidism [77]. Using mathematical calculations from previous studies, it was calculated how much vitamin D₃ would be needed to achieve this endpoint [78, 79]. At the initiation of these RCTs, endpoints were safety of the dosing, attained circulating concentrations of maternal 25(OH) D, growth parameters of the infant, and bone-mineral-density of the mother and infant.

The results of RCT's of vitamin D supplementation during pregnancy published by Hollis et al. and others within the past 5 years are summarized in Table 2 [24, 75, 80–86]. Earlier studies are not included here as they had small sample sizes

Table 2 Recently Completed Randomized Controlled Trials Providing Vitamin D during Pregnancy

Study	Country	Subjects	Trial Type	Intervention	Baseline 25(OH)D (ng/mL)	Endpoint 25(OH)D (ng/mL)	Findings	Adverse Events
Hollis et al. [15, 52] 2011, 2013	USA	350	RCT	400, 2000, or 4000 IU/d vitamin D ₃ from 14 wks ^a gestation until delivery	400 IU/d: 24.6 2000 IU/d: 23.3 4000 IU/d: 23.2	400 IU/d: 31.6 2000 IU/d: 39.3 4000 IU/d: 44.4	Vitamin D decreased cesarean section and comorbidities of pregnancy	none
Wagner et al. [53, 54] 2013, 2016	USA	2013: 160 2016: 504	2013: RCT 2016: post-hoc combined RCT	2013: 2000 or 4000 IU/d vitamin D ₃ from 14 wks ^a gestation 2016: 400, 2000 or 4000 IU/d vitamin D ₃ from 14 wks ^a gestation	400 IU/d: 24.6 2000 IU/d: 23.2 4000 IU/d: 22.8	400 IU/d: 30.7 2000 IU/d: 37.1 4000 IU/d: 41.9	Vitamin D decreased preterm births and complications of pregnancy	none
Sablok et al. [56] 2015	India	165	RCT	0 IU vitamin D ₃ ; 60,000 IU/bolus vitamin D ₃ at 20 wks ^a gestation; 120,000 IU/bolus vitamin D ₃ at 20 and 24 wks ^a gestation or 120,000 IU/bolus vitamin D ₃ at 20, 24, 28 and 32 wks ^a gestation	All patients <10.0	0 IU: <10.0 120,000 IU/bolus: 15.2 120,000 IU/4 doses: 26.0	Vitamin D greatly decreased complications of pregnancy	none
Mojibian et al. [57] 2015	Iran	500	RCT	400 IU/d vitamin D ₃ or 50,000 IU/bolus every 2 wks vitamin D ₃ starting at 14 wks ^a gestation	400 IU/d: 15.3 50,000 IU/bolus every 2 wks: 14.5	400 IU/d: 27.2 50,000 IU/bolus every 2 wks: 37.9	Vitamin D significantly decreased incidence of gestational diabetes	none
Chawes et al. [58] 2016	Denmark	623	RCT	800 or 2800 IU/d vitamin D ₃ from 24 wks ^a gestation	800 IU/d: 13.0 2800 IU/d: 31.0	800 IU/d: 29.0 2800 IU/d: 43.0	Vitamin D tended to decrease infant wheezing but was not statistically significant	none
Litonjua et al. [59] 2016	USA	881	RCT	400 or 4400 IU/d vitamin D ₃ from 16 wks ^a gestation	400 IU/d: 23.0 4400 IU/d: 23.0	400 IU/d: 27.0 4400 IU/d: 39.0	Trend where higher treatment group had decreased asthma and wheezing in infants up to 3 years follow-up ($p < 0.05$); post hoc analysis by circulating 25(OH)D was highly significant	none

and were not powered to detect differences in clinical outcome measures. A review of those earlier clinical trials was previously published [1]. As for the other factors mentioned in the previous section on vitamin D relationships based on observational studies, those associations had not been made at study initiation. As such, these endpoints were analyzed as post hoc analyses.

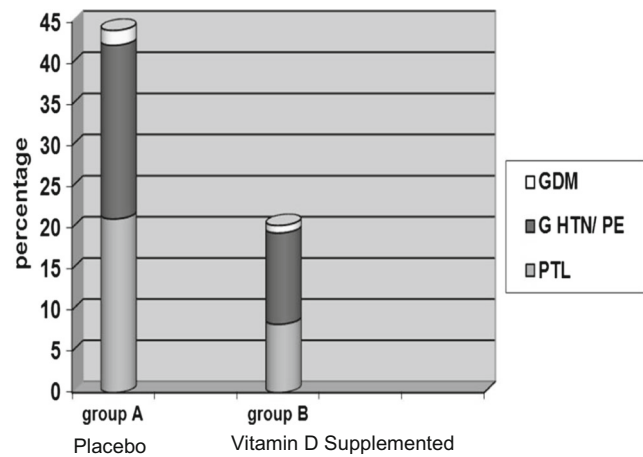
The main finding of these more recent studies was that a 4000 IU/d dose of vitamin D₃ safely elevates circulating 25(OH) D to a concentration that, regardless of race, fully normalizes vitamin D metabolism and calcium homeostasis in the pregnant women. Further, in these trials, which cumulatively involved more than 2000 pregnant women, were without a single adverse event observed attributable to vitamin D supplementation (Table 2). Of major interest, data from Hollis et al. studies [25, 92, 93] when analyzed on an intent-to-treat basis, clearly demonstrated increased vitamin D supplementation decreased complications of pregnancy and C-section births [24, 80]. Further, RCT data have clearly demonstrated that higher doses of vitamin D during pregnancy improve birth outcome data [80, 82].

Hence, it becomes evident that the majority of available observational data favor maternal hypovitaminosis D (as defined by circulating 25(OH) D concentrations <20 ng/mL or 100 nmol/L) as a significant risk factor for adverse neonatal outcomes, including low birth weight, SGA and preterm birth. Available findings from RCTs analyzed by intent-to-treat or treatment group, however, are inconclusive, mostly due to issues regarding study design, regimen of vitamin D supplementation, and the impact of nonadherence on circulating vitamin D and its metabolites. Further studies are urgently needed in this field, primarily in pregnant populations with profound maternal hypovitaminosis D.

RCT studies have recently demonstrated vitamin D to decrease complications of birth and gestational diabetes [75, 84]. The most informative of these RCT studies was performed by Sablok et al. [84]. These investigators took a vitamin D deficient population of pregnant women, with circulating 25(OH) D concentrations of <10 ng/ml, and supplemented the treatment arm with substantial amounts of vitamin D starting at 20 weeks of gestation. The control group received placebo and thus remained profoundly vitamin D deficient throughout pregnancy (see Fig. 2). Vitamin D treatment in these women resulted in a substantial decline in the complications of pregnancy. Further, the compliance rate of the women was 100% because the physicians administered the vitamin D to each patient.

1.4 Supplementing vitamin D during pregnancy to prevent childhood diseases

In this section, we review the data that links pregnancy health with later outcomes during childhood, which include childhood asthma and wheezing, as well as neurodevelopment and autoimmune consequences. In grappling with the potential



Adapted from: SablokA, et al. Clinical Endocrinology 2015; 83: 536.

Fig. 2 Effect of vitamin D supplementation starting at 20 weeks of pregnancy with respect to the development of complications of pregnancy. Pregnancy complication in the form of preterm labor (PTL), gestational hypertension (GHTN)/preeclampsia (PE) or gestational diabetes mellitus (GDM) were observed in 25/57 (44%) women taking placebo compared to 22/108 (20.4%) women being supplemented with vitamin D. Significance between groups was $p < 0.02$. From reference [84] and used with permission

effect of maternal vitamin D status during pregnancy and later childhood health, one has to take into account not only the genetic and epigenetic makeup of that child, but also the environmental exposures that may confound the picture. While it is not a simple association that exists, understanding this complex, dynamic interplay allows us to assess the role that vitamin D plays.

1.5 Supplementing vitamin D during pregnancy to prevent childhood asthma

Previous beneficial outcomes in childhood asthma rates after vitamin D supplementation during pregnancy [87, 88] comprised the initial theoretical basis for conducting a RCT using vitamin D supplementation during pregnancy to prevent the development of childhood asthma. The Vitamin D Antenatal Asthma Reduction Trial (VDAART) was a double-blind RCT performed at three clinical centers: Boston, St. Louis and San Diego; and involved giving supplemental vitamin D₃ (400 or 4400 IU/d) to pregnant women across the three major racial/ethnic groups in the US from 16 weeks of gestation until delivery. The primary endpoint was prevention of asthma/wheeze in the infant/child at 1, 2 and 3 years post birth. Nearly 900 high-risk subjects were enrolled and completed the study, which recently was published [86]. When analyzed by treatment group, asthma at 3-years approached significance with lower rates of asthma in the higher dose groups ($p = 0.051$). Further analysis of the VDAART study [86] revealed some additional findings. The more dramatic findings were noted when the biomarker 25(OH) D concentration, independent of protocol adherence, was used as the outcome

measure. Presented as supplementary data, Weiss et al. analyzed maternal 3rd trimester circulating 25(OH) D concentration (103) and its impact on asthma. Adherence or compliance was a significant problem that could not be dealt with in the intent-to-treat study analysis and when a participant did not follow the protocol or was “non-adherent to protocol,” that affected that participant’s circulating 25(OH) D, particularly affecting the higher dose treatment group [86]. Most affected was the African American subcohort who comprised 43% of the total study subjects and adhered to the prescribed supplementation regimen—as assessed by pill counts and electronic medical cap monitoring—50% of the time. What was the result of this? This non-adherence could have biased the study toward null results. In fact, when this bias is factored into the results, the strength of the findings become more significant [86, 89]. If one uses circulating 25(OH) D concentration instead of treatment with its inherent bias (affected by nonadherence), the effect of vitamin D in the prevention of childhood asthma becomes more apparent ($p < 0.02$; Fig. 3) [89, 90]. This effect is especially true for the African American pregnant women in VDAART study [91]. Based on the post hoc analysis, vitamin D supplementation during pregnancy will decrease asthma or recurrent wheezing rates in children (Fig. 4).

A nearly identical RCT study performed in Denmark also recently was published [85]. The authors of these two RCTs performed a meta-analysis [92]. The results from these RCTs

and meta-analysis studies showed that vitamin D₃ given to a pregnant woman reduced the risk of asthma/wheeze in her child. The mechanisms by which this occurs remain unknown but it is likely that epigenetic in utero changes triggered by the vitamin D administered to the pregnant women impart functional changes in the fetus [93, 94].

If one looks at our original pregnancy study from an intent-to-treat fashion, the results are muddled most likely due to non-adherence [24]; however, taking adherence into account by using circulating 25(OH) D levels as the independent variable, the true effect on vitamin D supplementation on preterm birth is exposed (106) (Figs. 5 and 6). The same associations from our VDAART trial also hold true for the prevention of preeclampsia [70].

Vitamin D supplementation and the resulting vitamin D status of the mother and fetus during pregnancy appear to affect gene expression of multiple cell types, which in turn, affect several highly functional modules related to systemic inflammation and immune responses and implicates the emergence of a distinctive immune response in women destined to develop preeclampsia [94, 95]. As an important example, Mirzakhani et al. [69], in their gene expression sub-study identified a set of vitamin D-associated genes related to preeclampsia. The study demonstrated genomic connectivity to known vitamin D-signaling pathways indicating the functional cohesiveness of vitamin D to the preeclampsia disease

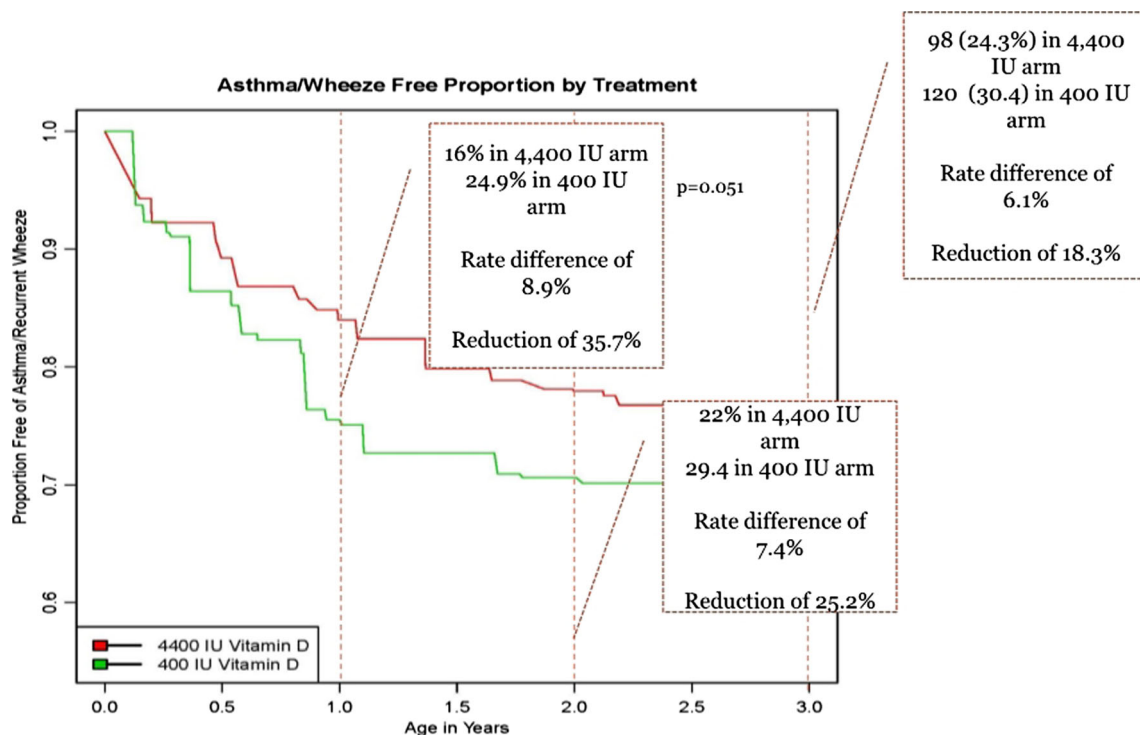
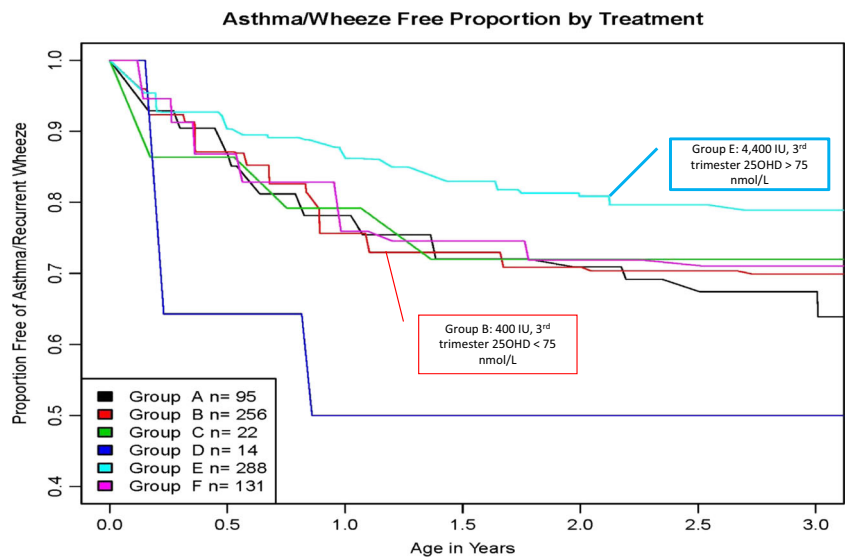


Fig. 3 Kaplan-Meier survival estimates for the effect of vitamin D treatment during pregnancy on the development of asthma/recurrent wheeze by age 3 year analyzed in an intent-to-treat format. The hazard

ratio for the time to first event of asthma or recurrent wheeze was 0.8 at three years, $p = 0.051$. From reference [86] and used with permission

Fig. 4 Kaplan-Meier survival estimates for the effect of vitamin D treatment during pregnancy on the development of asthma/recurrent wheeze by age 3 year analyzed stratified by 3rd trimester maternal level of circulating 25(OH)D as an estimate of study compliance. The hazard ratio for the time to first event of asthma or recurrent wheeze now becomes 0.73 at three years, $P < 0.02$. From reference [86] and used with permission

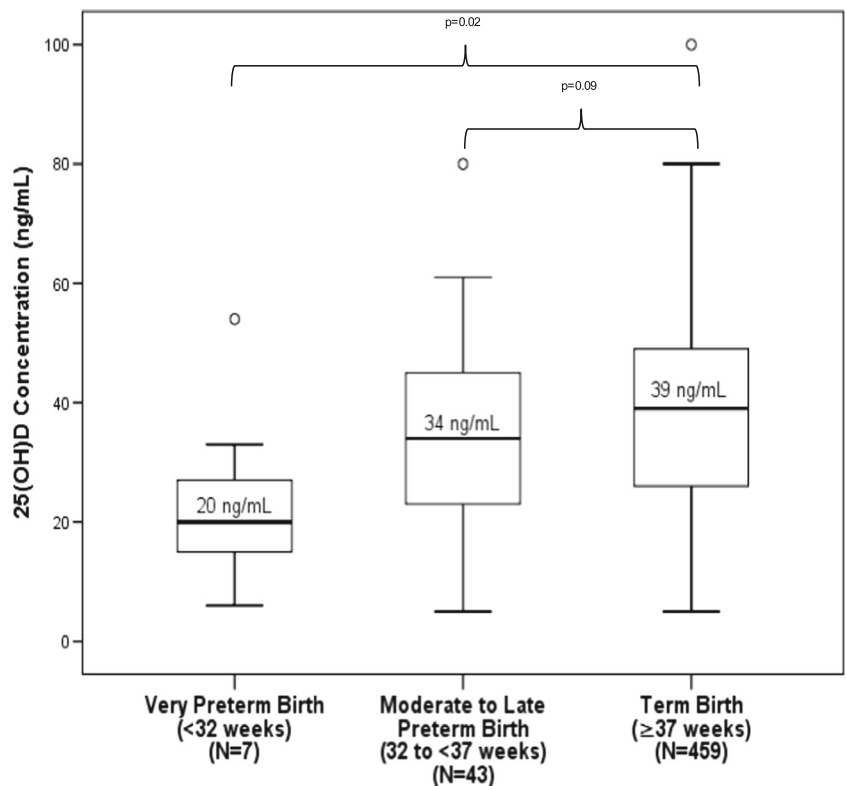


model (Figs. 7 and 8). Most of the genes in this replication model were associated with maternal systemic changes in immune—innate and humoral—and inflammatory responses (Figs. 7 and 8). A more detailed explanation of this interaction can be viewed within the publication [69]. This landmark study is the first of its kind to explain how supplemental vitamin D can reduce the incidence of a serious condition at both the clinical and genomic levels.

Maternal vitamin D status during pregnancy also appears to affect epigenetic regulation—DNA methylation—in the

offspring. DNA methylation data were obtained from a subset of mothers and their neonates ($n = 23$) participating in a recent vitamin D supplementation trial [93]. Linear regression analysis was performed with either cord blood 25(OH) D or maternal last trimester 25(OH) D as a continuous independent variable and trait of interest with mother’s age, baby’s sex, baby’s race included as covariates. Not unexpected, cord blood 25(OH) D was highly correlated with mother’s 25(OH) D ($r = 0.97$, $p = <0.001$). A total of 2427 CpG sites had raw p -value <0.01 , and 1375 CpG sites had raw p -value

Fig. 5 Circulating levels of maternal 25(OH)D with respect to birth staging. From reference [82] and used with permission



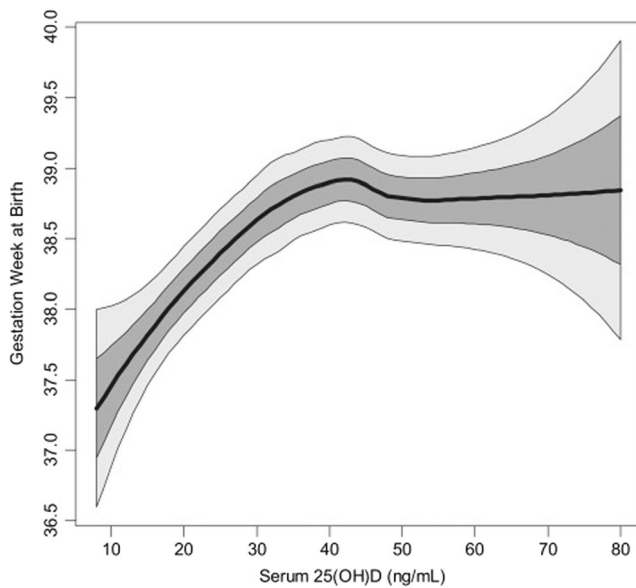


Fig. 6 LOESS curve of 25(OH)D concentration and gestational age (weeks) at birth to show the change in average behavior with 1 and 2 SD windows superimposed (NICHD and TRF, $n = 509$). Black line represents fitted LOESS curve; dark gray area represents 1 SD; and light gray area represents 2 SD. Multivariable log-binomial regression found that 25(OH)D concentrations >40 ng/ml reduces the risk of preterm birth by 59% compared to <20.0 ng/ml, adjusted for covariates. From reference [82] and used with permission

<0.01 . The pathway analysis for the top 200 genes showed that these genes were enriched in the regulation of metabolic processes, antigen processing and presentation, inflammation, regulation of cell death, cell proliferation, transmission of nerve impulse, neurogenesis, neuron differentiation and sensory organ development [93]. Maternal vitamin D supplementation and resultant cord blood 25(OH) D concentration were found to be associated with DNA methylation changes. While further analyses warranted to validate these findings, these preliminary data support the premise that maternal vitamin D status during pregnancy affects offspring through epigenetic mechanisms.

1.6 Neurodevelopment and autoimmune consequences

The development of multiple sclerosis (MS) is a result of a complex interaction between genes and environment with an important environmental factor being vitamin D deficiency [9]. While it is not yet understood how and when vitamin D acts to modulate MS risk, there is increasing evidence that this occurs through genetic alterations [8]. As described above, vitamin D supplementation during pregnancy alters transcriptome and epigenetic alterations through DNA methylation in genes that regulate metabolic processes, antigen processing, inflammation, regulation of cell death, cell proliferation, transmission of nerve impulse, neurogenesis, neuron differentiation and sensory organ development [93]. Observational studies strongly suggest vitamin D deficiency

during pregnancy is a strong causative agent in the development of MS in later life [5, 7].

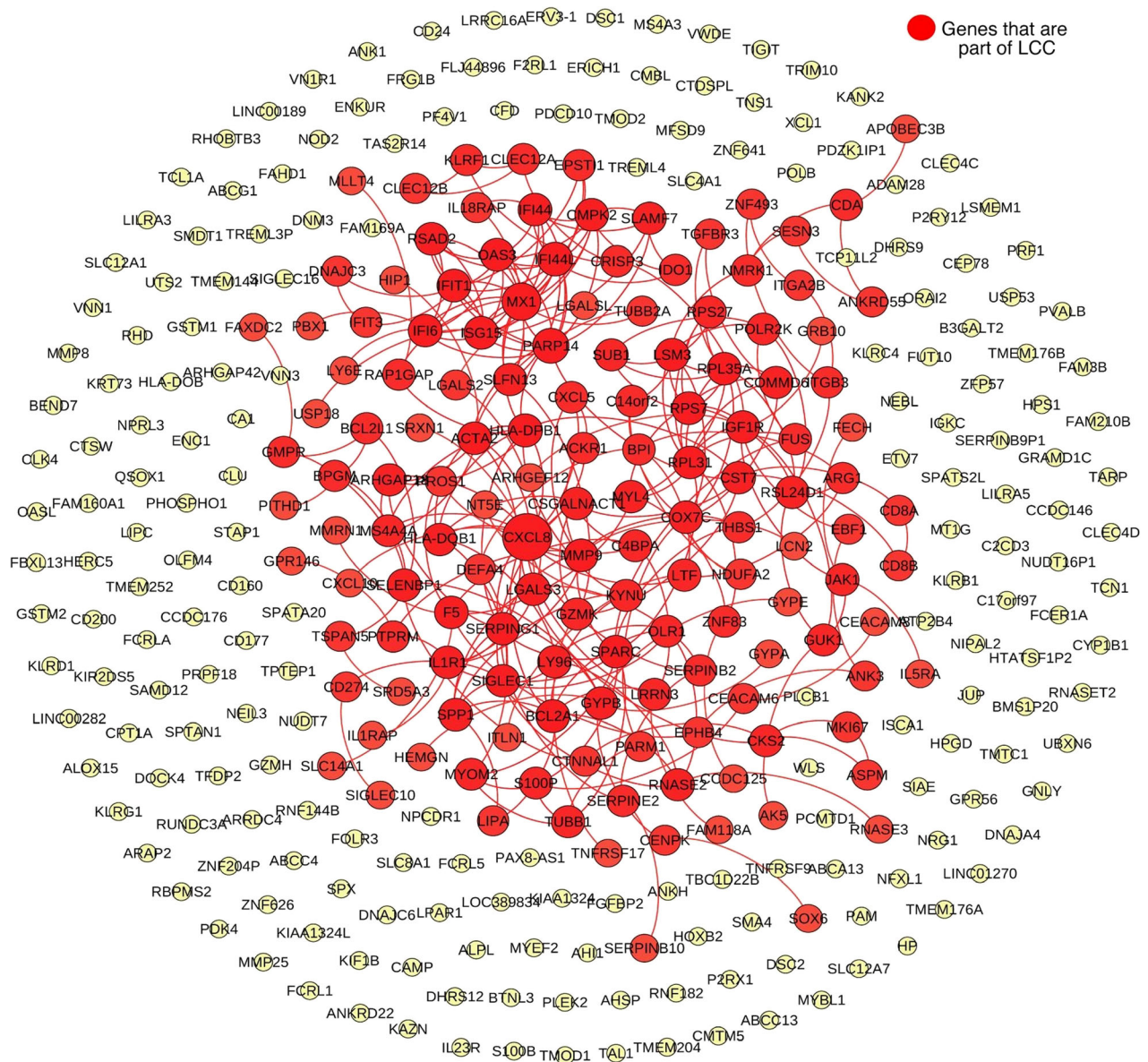
With regard to vitamin D deficiency during pregnancy and neurologic disease and altered development [10–13, 96], there is emerging evidence in animal models that suggests adverse neurological consequences may occur if vitamin D is restricted during pregnancy [13]. Observational data in humans supports the findings from the animal models [10–13]. A recent prospective, interventional vitamin D trial during pregnancy for the prevention of autism in the newborn [10] provides important supportive data of the link between vitamin D deficiency and neurodevelopment. From these data, the authors suggest that even performing an RCT would be unethical [10].

1.7 Current suggestions for vitamin D supplementation during pregnancy

At this time, based on RCT data as well as substantial observational and interventional data, we suggest that all pregnant women maintain a circulating 25(OH) D concentration of at least 40 ng/mL during the earliest time points of pregnancy [82]. This will insure maximum protection from pregnancy complications, including preeclampsia in the mother and asthma formation in the infant. To achieve this, intakes of at least 4000 IU/d vitamin D₃ will be required because of variable individual abilities to convert vitamin D to 25(OH) D [79]. These supplements have proven to be safe in thousands of patients over the past 15 years, as not a single adverse event has been observed related to vitamin D supplementation of around 4000 IU/day. Further, this level of supplementation lies within the safe intake level as defined by The Endocrine Society [2]. Clearly, large RCTs designed using criteria or “rules” as proposed by Heaney [76, 97–100] will not be easily available as a result of the huge economical burden that they would carry. Until then, clinicians could incorporate new results from available relevant RCTs in their daily practice and orientation, with the understanding that the lack of adequate vitamin D substrate during pregnancy imparts genetic and epigenetic alterations in both mother and fetus, with long-term consequences.

This brings up the question of when should vitamin D supplementation begin. Based on the earlier discussion

Fig. 7 Dose-response association between maternal serum 25(OH)D at study entry, 10–18 weeks of gestation, and the corresponding predicted probability of preeclampsia derived from a logistic regression model adjusted for confounders. The gray zone indicates 0.95 confidence bands. The minimum predicted risk for the population under study relative to the distribution of pregnant women falls at the concentrations of 40 to 50 ng/mL circulating 25(OH)D. Note on the upper X-axis not a single case of preeclampsia was observed in this population when circulating 25(OH)D exceeded 40 ng/mL. In other words, perfect protection from preeclampsia in this study population. From reference [69] and used with permission



Term	No. of involved genes	Corrected P value
Immune system process	109	1.31×10^{-16}
Immune response	83	1.12×10^{-15}
Defense response	82	4.07×10^{-13}
Response to stress	125	1.28×10^{-9}
Response to biotic stimulus	50	5.27×10^{-9}
Regulation of immune system process	63	1.17×10^{-6}
Innate immune response	50	0.000001
Inflammatory response	35	0.0000042
Platelet degranulation	12	0.0000606
Immune effector process	36	0.000174
Cellular response to cytokine stimulus	34	0.000536
Leukocyte migration	22	0.000583
Negative regulation of immune system process	22	0.00102
Cytokine-mediated signaling pathway	29	0.00139
Cell-surface receptor signaling pathway	81	0.00143
IL-10 production	8	0.00157
Cellular response to type I IFN	10	0.00294
Type I IFN signaling pathway	10	0.00294
Response to type I IFN	10	0.00411
Regulation of cell migration	29	0.00733
Cytokine production	28	0.0127
Lymphocyte-mediated immunity	15	0.0364
Adaptive immune response	19	0.043
Regulation of cytokine production	25	0.0498

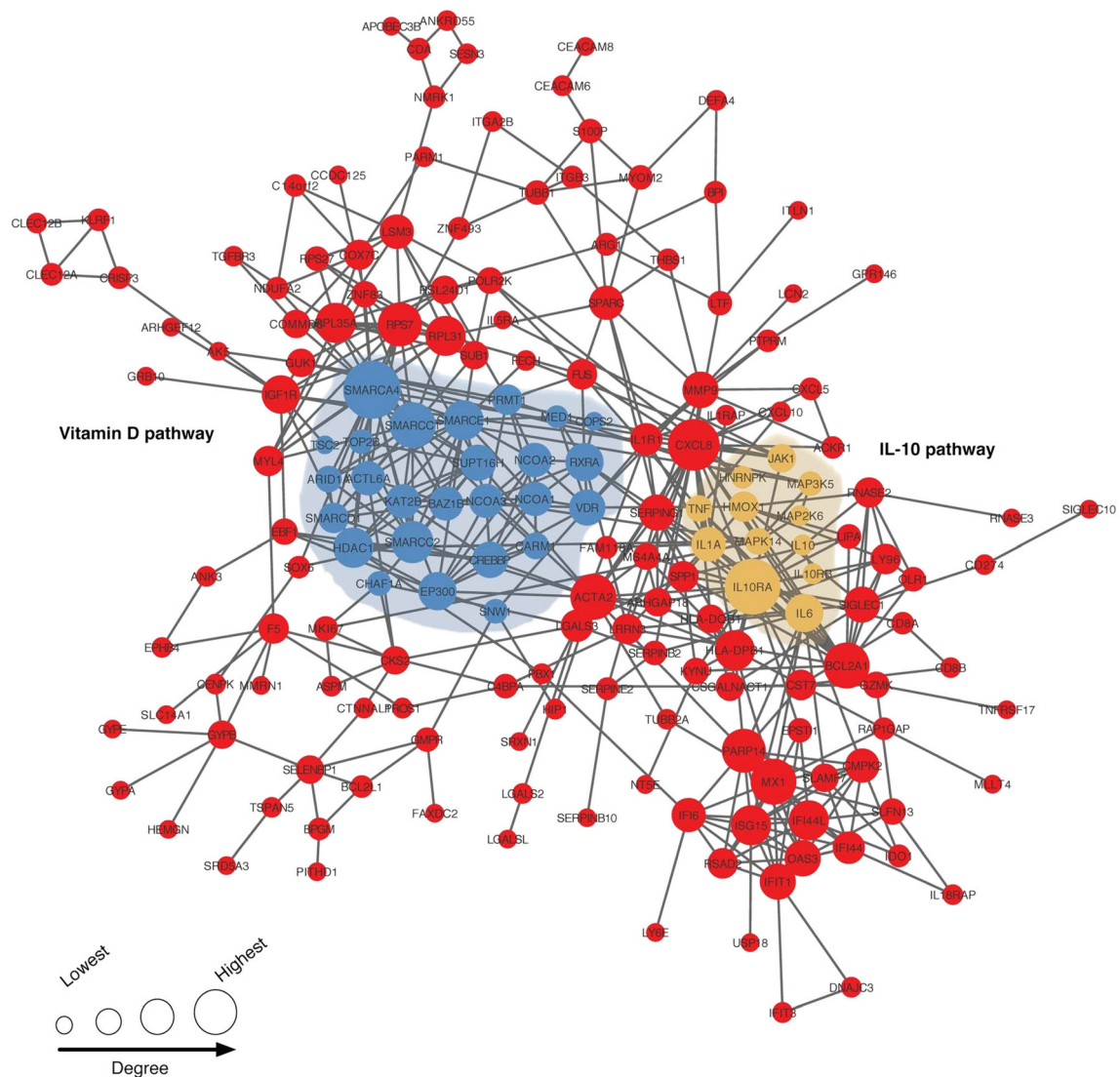


Fig. 8 LCC genes (143 genes, highlighted in red) corresponding to the “observable preeclampsia module” among the replicated differentially expressed genes ($N = 348$) and their biological processes. From reference [69] and used with permission

regarding placental gene expression and epigenetic factors that are associated with vitamin D status, the association of placental inflammation and disease states that manifest as a function of maternal vitamin D status early in pregnancy (e.g., preeclampsia and gestational diabetes), it appears prudent that vitamin D supplementation begin before placentation (and trophoblast invasion). Further work is needed to assess the impact of vitamin D supplementation initiated preconceptionally, and the impact it would have on pregnancy outcomes.

1.8 Conclusions and future perspectives

Pregnancy represents a dynamic period that involves a multitude of physiologic as well as physical changes for both the mother and her developing fetus. One aspect of this period of intense change involves vitamin D metabolism, where

1,25(OH)₂D increases 2–3 fold during the early weeks of pregnancy despite minimal increased calcium demands during that time of gestation. The reason for this marked increase in 1,25(OH)₂D is not entirely clear but it is speculated that it involves the role of vitamin D as an immune mediator in preventing fetal rejection by the mother. Why the 1- α -hydroxylase is more active in the pregnant than non-pregnant state that allows 1,25(OH)₂D concentrations to soar also is unknown. The question of whether or not less activation of the 1- α -hydroxylase leads to more inflammatory changes in certain women who go on to develop preeclampsia is an open question and warrants further study.

While there have been numerous observational studies that suggest vitamin D’s role in maintaining maternal and fetal well-being, until recently, there have been few randomized clinical trials with vitamin D supplementation. One has to exhibit caution, however, even with RCTs, whose results can

be problematic when analyzed on an intent-to-treat basis and when there is high non-adherence to protocol (as if often the case), thereby diluting the potential good or harm of a given treatment at higher doses. As such, a biomarker of a drug or in this case “vitamin” or pre-prohormone is better served. For these reasons, analyses of effect of vitamin D therapies using 25(OH) D concentrations is a far better indicator of true “effect.” When pregnancy outcomes are analyzed using the biomarker 25(OH) D instead of treatment dose, there are notable differences in maternal and fetal outcomes, with improved health in those women who attain a circulating 25(OH) D concentration of at least 100 nmol/L (40 ng/mL). Because an important issue is the timing or initiation of vitamin D treatment/supplementation, and given the potential effect of vitamin D on placental gene expression and its effects on inflammation within the placenta, it appears crucial to start vitamin D treatment before placentation (and trophoblast invasion).

Future work is needed to decipher the vitamin D requirements of pregnant women, taking into account a variety of lifestyles, body types and baseline vitamin D status and choosing a biomarker concentration that is linked with pregnancy outcome data. In addition, determining the role of vitamin D in the nonclassical, immune pathways continues to be a challenge that once answered will substantiate recommendations and public health policies.

Compliance with ethical standards

Conflict of interest Authors declare no conflict of interest.

Ethical approval Two of the authors (BWH and CLW) were involved in the conduct of several of the trials summarized in this review. The studies were approved by the local Human Subjects’ Institutional Review Board and were conducted according to federal and institutional guidelines in place for the conduct of human investigation in the United States.

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