

Early-Life Effects of Vitamin D: A Focus on Pregnancy and Lactation

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Key Messages

- The active form of vitamin D – 1,25-dihydroxyvitamin D (1,25(OH)₂D) – increases during pregnancy, remains elevated throughout, and, unlike at other times during the lifecycle, is directly affected by circulating 25-hydroxyvitamin D (25(OH)D) concentration with the optimal point of conversion of 25(OH)D to 1,25(OH)₂D at 100 nmol/L (40 ng/mL).
- Lactation has increased demands on the mother regarding nutrient intake delivered through her breast milk to her recipient infant: when a mother is vitamin D deficient, her milk is deficient, which can be remedied by direct infant supplementation; however, this treats only the infant.
- A safe alternative during lactation to infant supplementation is direct maternal vitamin D supplementation at higher doses than usual (6,400 IU/day), improving the vitamin D status of the mother, the content of the milk, and, consequently, the vitamin D status of the infant, effectively treating both the mother and the infant.

Keywords

1,25-dihydroxyvitamin D · 25-hydroxyvitamin D · Cholecalciferol · Calcidiol · Clinical nutrition · Human nutrition · Infancy and childhood · Lactation · Pregnancy

Abstract

Vitamin D is an endocrine regulator of calcium and bone metabolism. Yet, its effects include other systems, such as innate and adaptive immunity. Unique to pregnancy, circulating

1,25-dihydroxyvitamin D (1,25(OH)₂D) increases early on to concentrations that are 2–3 times prepregnant values. At no other time during the lifecycle is the conversion of 25-hydroxyvitamin D (25(OH)D) to 1,25(OH)₂D directly related and optimized at ≥ 100 nmol/L. Vitamin D deficiency appears to affect pregnancy outcomes, yet randomized controlled trials of vitamin D supplementation achieve mixed results depending on when supplementation is initiated during pregnancy, the dose and dosing interval, and the degree of deficiency at the onset of pregnancy. Analysis of trials on an intention-to-treat basis as opposed to the use of 25(OH)D as the intermediary biomarker of vitamin D metabolism yields differing results, with treatment effects often noted only in the most deficient women. Immediately after delivery, maternal circulating 1,25(OH)₂D concentrations return to prepregnancy baseline, at a time when a breastfeeding woman has increased demands of calcium, beyond what was needed during the last trimester of pregnancy, making one question why 1,25(OH)₂D increases so significantly during pregnancy. Is it to serve as an immune modulator? The vitamin D content of mother's milk is directly related to maternal vitamin D status, and if a woman was deficient during pregnancy, her milk will be deficient unless she is taking higher doses of vitamin D. Because of this relative "deficiency," there is a recommendation that all breastfed infants receive 400 IU vitamin D₃/day starting a few days after birth. The alternative – maternal supplementation with 6,400 IU vitamin D₃/day, effective in safely raising maternal circulating vitamin D, that of her breast milk, and effective in achieving sufficiency in her recipient

breastfeeding infant – remains a viable option. Additional research is needed to understand vitamin D's influence on pregnancy health and the effect of maternal supplementation on breast milk's immune signaling.

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Conception Onward

From the moment of conception, there are tremendous changes that must occur for growth and shaping of a single-cell organism to billions of cells as the construct of diverse systems, which function in concert to form a living human being. It is in the context of this timing, this concert of matter and energy transfer across cells, that we can appreciate what is happening surrounding conception. Conception does not occur in a hostile or nonnurturing environment, yet the very invasion of extravillous cytotrophoblasts into the uterine wall is an invasive and inflammatory process [1–3]. Pregnancy is a state of change and flux that must balance between negentropy—organization of tissue—and cellular death and apoptosis – necessary for refinement of tissue and organ structure. The very event of conception is dependent upon a functional neuroendocrine system in both the mother and father, a functioning uterus with a rich lining to allow for invasion of the extravillous cytotrophoblasts into the uterine wall, and a dynamic synchrony of cell division and cell death. The initiation of human life at the moment of conception involves a myriad of ancient signaling hormones, which include vitamin D [4, 5].

Vitamin D as Prehormone

Long known as an endocrine facilitator in its role as a prehormone affecting calcium and bone metabolism and homeostasis, vitamin D is something more as well. Our understanding of vitamin D has expanded in the decades since its discovery in the early 20th century. There are provocative experimental models in animals that extend to observational and some clinical trials in humans, which suggest that vitamin D plays a role in both innate and adaptive immunity, affecting our ability to survive infectious insults as well as long-latency diseases, such as autoimmune diseases and cancers, all of which depend on a balanced and functional immune system [6].

There are 2 forms of vitamin D: ergocalciferol (or vitamin D₂, which is synthesized by plants and fungi) and cholecalciferol (or vitamin D₃, which is synthesized in the skin of humans and animals). Humans can metabolize both forms of vitamin

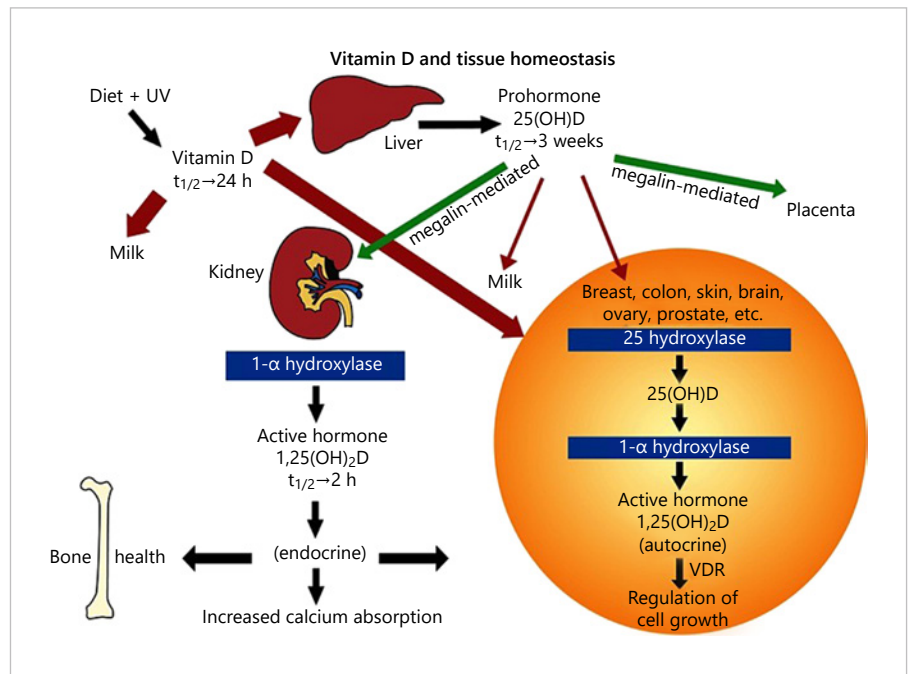
D. Pre-vitamin D₃ is synthesized in the epidermal layer of the skin by keratinocytes mainly in the stratum basale and stratum spinosum when 7-dehydrocholesterol is exposed to ultraviolet B light in the wavelength of 290–320 nm [7]. Through this photolytic energy transfer, pre-vitamin D is formed, and with further thermally induced isomerization in the skin, the parent compound vitamin D₃ is produced. Vitamin D₃ is carried into the bloodstream bound to vitamin D-binding protein (VDBP) or, less frequently, to albumin. Once vitamin D (either form D₂ or D₃) enters the circulation, either through epidermal transfer or intestinal absorption, it associates with VDBP, a 58-kD globular protein that binds vitamin D and its metabolites with various affinities based on the number and position of polar functional groups and/or methyl groups [8]. The initial step in the metabolic activation of vitamin D is the enzyme-catalyzed insertion of an OH group at carbon 25; this oxidation process is primarily a hepatic microsomal function mostly by CYP2R1, a 25-hydroxylase [9], producing 25-hydroxyvitamin D (25(OH)D), the most abundant circulating form of vitamin D [10].

Vitamin D plays a role in both innate and adaptive immunity, affecting our ability to survive infectious insults as well as long-latency diseases

As shown in Figure 1 (from Hollis and Wagner [11], with permission), following formation in the liver, 25(OH)D appears in the circulation – bound primarily to VDBP. The half-life of the parent compound is 12–24 h, while that of its first metabolite 25(OH)D is 2–3 weeks. The conversion of 25(OH)D to the active hormone 1,25-dihydroxyvitamin D (1,25(OH)₂D) through the CYP27B1 enzyme mainly occurs in the proximal tubules of the kidney, and then it is carried throughout the body also bound to VDBP.

Unlike 25(OH)D, 1,25(OH)₂D has a much shorter half-life of 4–8 h. VDBP preferentially binds 25(OH)D with higher affinity than 1,25(OH)₂D or the parent compound [12]. The high affinity of VDBP for the vitamin D and its metabolites, coupled with the excessive binding capacity, keeps “free” or unbound concentrations of vitamin D and its metabolites at quite low relative concentrations [13, 14]. This is important because only the “free” concentrations of the vitamin and its metabolites have transmembrane diffusion capabilities, thus exerting their

Fig. 1. Diagram of the metabolic processes providing vitamin D and its metabolites to various tissues of 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 VDBP. Bolder red lines indicate greater diffusion rates due to a higher dissociation constant. $t_{1/2}$, half-life. (Hollis and Wagner [11], 2013, with permission.)



biologic function. What influences vitamin D status throughout the lifecycle is parathyroid hormone (PTH). When circulating $1,25(\text{OH})_2\text{D}$ concentrations decrease, PTH increases, affecting intestinal absorption of vitamin D and conversion of vitamin D from its precursor in the skin. The measurement of intact PTH (iPTH) has long been considered an indicator of vitamin D deficiency and is used as a marker [15].

All vitamin D moieties are capable of binding to the vitamin D receptor (VDR). As shown in Figure 1, the conversion of vitamin D to $25(\text{OH})\text{D}$ and of $25(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}$ in the nuclear membrane of the cell is not limited to the liver and kidneys, respectively; keratinocytes and many cells throughout the body, including monocytes, macrophages, and prostate and breast cells, can convert vitamin D_3 to $25(\text{OH})\text{D}$ and then to the active form $1,25(\text{OH})_2\text{D}$ [16, 17]. $1,25(\text{OH})_2\text{D}$'s endocrine effects include the following classic triad of action: (1) increase in intestinal calcium (as Ca_2+ ions) absorption through the actions of calbindin; (2) increase in urinary calcium reabsorption; and (3) regulation of PTH in a negative feedback loop that allows calcium to be absorbed from the gastrointestinal tract, reabsorbed from urine, and metabolized from bone in order to maintain calcium homeostasis within the body. Because calcium is essential to all tissues and organs, particularly the heart, skeletal muscle, and brain, the body will claim calcium, if necessary, from the skeleton. In individuals with vitamin D deficiency, only trace amounts of vitamin D will be found in the body because whatever comes into the circulation is quickly converted to $25(\text{OH})\text{D}$ and then

to $1,25(\text{OH})_2\text{D}$ to maintain calcium homeostasis [18]. For this reason, $1,25(\text{OH})_2\text{D}$ is not the indicator of vitamin D status and why $25(\text{OH})\text{D}$ with its longer half-life should be used.

Another important factor influencing the conversion rate of $25(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}$ is the tissue transport mechanism for these secosteroids referred to as the megalin-cubilin system. The megalin-cubilin endocytic system [19] serves as an essential delivery system of $25(\text{OH})\text{D}$ to the $25(\text{OH})\text{D}$ - $1-\alpha$ -hydroxylase in the kidney, necessary in the conversion of $25(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}$ [19]. This system also exists in the parathyroid glands and, therefore, plays an important role in the endocrine function of vitamin D to maintain calcium homeostasis. Interestingly, the megalin-cubilin system also functions in the placenta and likely orchestrates maternal-fetal calcium homeostasis [20]. For those tissues that lack this endocytic transport system, free circulating concentrations of vitamin D moieties reach target cells through passive diffusion. For additional information, there are excellent reviews available that detail vitamin D metabolism in the nonpregnant individual [17, 21–23].

Also of importance is that $1,25(\text{OH})_2\text{D}$ itself is responsible for reducing $1,25(\text{OH})_2\text{D}$ concentrations in cells primarily by stimulating its catabolism through the induction of 24-hydroxylase, 24CYP24A1. This enzyme hydroxylates both $25(\text{OH})\text{D}$ and $1,25(\text{OH})_2\text{D}$ in the 24 position to form $24,25(\text{OH})_2\text{D}$ and $1,24,25(\text{OH})_3\text{D}$ [24]. As is discussed next, during pregnancy, there is increased $1,25(\text{OH})_2\text{D}$ concentration presumed to be due to decreased catabolism.

Differences in Vitamin D Metabolism during Pregnancy

From early on in pregnancy, circulating $1,25(\text{OH})_2\text{D}$ concentrations increase without the predicted surge in PTH that causes a rise in calcitriol in nonpregnant individuals. While calcitriol is synthesized by the placenta, during pregnancy it is mainly synthesized by the kidneys [25]. There appears to be a slower rate of catabolism of $1,25(\text{OH})_2\text{D}$ to $24,25(\text{OH})_2\text{D}$ [26]. What purpose does this early and sustained rise in $1,25(\text{OH})_2\text{D}$ serve? There has been much speculation about this. It has been theorized for decades that this increase during pregnancy was due to increased fetal calcium requirements, most notable during the last trimester [27]. Elevated circulating $1,25(\text{OH})_2\text{D}$ was also thought to continue during lactation [28], but later, with more sensitive assay methodology surrounding the measurement of $1,25(\text{OH})_2\text{D}$, this was shown not to be the case [29, 30]. The return to prepregnancy circulating concentrations of $1,25(\text{OH})_2\text{D}$ during lactation is poorly understood and suggests that the role of $1,25(\text{OH})_2\text{D}$ during pregnancy may be for reasons that extend beyond calcium metabolism and which surround vitamin D's role in immune function [25]. The above occurs in the presence of a continued high calcium requirement of the breastfeeding infant of at least 200–350 mg/day for growth that is comparable to fetal requirements during the last trimester of pregnancy.

There is historical information as early as the 1940s with halibut liver oil – rich in both vitamins A and D and other vitamins – given as a supplement to pregnant women that showed benefit

Specific to pregnancy, there are changes in states of inflammation: early in pregnancy, there is inflammation – to allow the conceptus to invade the uterine milieu and for a network of channels between maternal and embryo to develop that give rise to the placenta, following a time of relative quiescence of those inflammatory processes that facilitate fetal growth beginning in the middle of the first trimester toward the end of pregnancy, with a return to a relatively in-

flammatory state with the onset of labor and the expulsion of the placenta [3]. Pregnancy represents tremendous change in numerous systems with most notable increases in estrogen, progesterone, human placental growth factor, the interleukins, as well as $1,25(\text{OH})_2\text{D}$. Each has its purpose, but with any system, various growth factors and cytokines do not operate in isolation, but there is much interaction.

There is evidence that maternal vitamin D deficiency – however this is defined – affects maternal and fetal outcomes. Although scientific inquiry on the topic with published observational and clinical vitamin D supplementation trials did not consistently appear in the literature until the late 1970s/early 1980s [31], there is historical information as early as the 1940s with halibut liver oil – rich in both vitamins A and D and other vitamins – given as a supplement to pregnant women that showed benefit [32]. Specifically, a study conducted by the People's League of Health in 1938–1939 involving over 5,000 pregnant women who were randomized to receive a cocktail of vitamins and halibut liver oil (a source of both vitamins A and D) or placebo was rediscovered by Olsen and Secher [32] and the results published in 1990. This nutritional supplement was superior compared to control in achieving reductions in preterm birth and preeclampsia. Since that time, studies that have focused on one nutrient instead of a combined nutritional supplement, with the exception of higher-dose vitamin D studies in the most deficient women, and more recently in systematic reviews and meta-analyses, have failed to demonstrate this effect. Much research has occurred with far more studies published each year on the topic. With those trials, there have been mixed results, with some studies showing a positive effect and others showing a minimal or no effect. There are, however, indisputable findings surrounding gene expression on the basis of maternal vitamin D status.

Focusing on vitamin D, the metabolism of this important prohormone during pregnancy is vastly different when compared to the nonpregnant state. As noted earlier, $1,25(\text{OH})_2\text{D}$ increases 2- to 3-fold within days of conception, while $25(\text{OH})\text{D}$ remains relatively stable within a certain range [33–35]. It is $25(\text{OH})\text{D}$ which crosses the placenta to the fetus and, thus, is the main pool of vitamin D in the fetus, not $1,25(\text{OH})_2\text{D}$; the fetus must synthesize $1,25(\text{OH})_2\text{D}$ from that pool. While the main source of the increased $1,25(\text{OH})_2\text{D}$ during pregnancy comes from the kidney, its other source is the placenta, with VDR and regulatory metabolic enzymes synthesized in the placenta and decidua. This is considered a potential critical point in the immunomodulation at the maternal-fetal interface and raises the question if maternal hypovitaminosis D during pregnancy leads to pregnancy-related disorders [36, 37].

Genetic Studies and Vitamin D Status

There is an increasing number of genetic studies to evaluate vitamin D's effect on gene expression. One of the first was a study by Al-Garawi et al. [38] who, in their post hoc analysis of a randomized clinical trial of maternal vitamin D supplementation in women who themselves or of whom a first-degree relative had allergy or asthma, sought to explore vitamin D's effect on genomic changes during pregnancy, which is one of the first reports of its kind. Women were randomized at 10–18 weeks of gestation to 400 and 4,400 IU vitamin D₃/day [39] with the primary outcome wheezing in the offspring at 3 and 6 years. An analysis of a subset of blood samples for RNA gene expression changes between the first and third trimesters was conducted. Using significance of analysis of microarrays (SAM) and clustered weighted gene co-expression network analysis (WGCNA) to identify major biological transcriptional profiles between those time points, 5,839 significantly differentially expressed genes were studied. Transcripts from these genes clustered into 14 co-expression modules, of which 2 (associated with immune defense pathways, extracellular matrix reorganization, and Notch signaling and transcription factor networks) showed significant correlation with maternal 25(OH)D concentrations. The findings show that maternal gene expression changes during pregnancy are affected by maternal vitamin D status, which, in turn, is a direct reflection of maternal vitamin D supplementation.

Additional evidence of vitamin D's effect on gene expression comes from Baca et al. [40] and another from Barchitta et al. [41] in their focus on vitamin D-related genes. Baca et al. [40] conducted a meta-analysis of 2 large cohorts – the Epidemiology of Vitamin D Study (EVITA) and the Collaborative Perinatal Project (CPP) – where the combined analysis of more than 4,000 randomly selected samples showed that the maternal genotypes of 7 SNPs in VDR, 3 SNPs in GC (VDBP), and 1 SNP in the flanking regions of Cyp27B1 were associated with maternal vitamin D status as expressed as the log₂(OH)D concentration. Adjusting for multiple comparisons, 1 SNP in VDR and 2 SNPs in GC remained significant. The investigators theorized that SNPs in VDR may influence circulating 25(OH)D by changing the rate at which 25(OH)D is hydroxylated either directly or indirectly through a negative feedback loop. The 2 SNPs in GC are likely related to the response of an individual to vitamin D supplementation, with certain GC polymorphisms associated with an attenuated or refractory response to supplementation compared to other genotypes, such as 1S or 2 [42].

Barchitta et al. [41] conducted a study to examine the association of VDR polymorphisms and preterm birth and neo-

natal anthropometric measures. Utilizing the Italian “Mamma and Bambino” cohort ($n = 187$), they studied the most common polymorphisms – BsmI, ApaI, FokI and TaqI. The investigators found that for the FokI polymorphism, gestational duration (age) and birth weight (that are clearly linked) were statistically significantly lower with increasing number of the A allele. In addition, when compared to mothers with the GG or GA genotype, those mothers who carried the AA genotype had a higher risk of preterm birth (OR 12.049, 95% CI 2.606–55.709, $p = 0.001$). Further, the BsmI polymorphism appeared to be protective against preterm birth, both allelic (A vs. G: OR 0.74, 95% CI 0.59–0.93) and recessive (AA vs. GG + AG: OR 0.62, 95% CI 0.43–0.89, $p = 0.0001$). Mothers with the AA genotype exhibited a 12-fold increased risk of preterm birth that was independent of sociodemographic characteristics, lifestyle, vitamin D intake/use of supplements, type of delivery, and parity. The results of this study were combined with earlier reported studies, which strengthened the robustness of these findings.

Maternal gene expression changes during pregnancy are affected by maternal vitamin D status, which, in turn, is a direct reflection of maternal vitamin D supplementation

These genetic studies collectively suggest that genotyping of common allelic variants and polymorphisms may play an important role in vitamin D metabolism during pregnancy. The findings further suggest that certain functional genetic variants may contribute to vulnerability or risk of vitamin D deficiency. The findings suggest that there may be subgroups of women based on their genotype profile for relevant vitamin D-related genes who would benefit from certain dosing regimens while others would not. The changes in gene expression from the first trimester compared to the third may also suggest that the prescription of one vitamin D supplement dose throughout pregnancy does not meet the physiological needs of the pregnant woman and might be based more on convenience than what is needed for optimal vitamin D status.

Table 1. Systematic Reviews and Meta-Analyses of Various Clinical Studies Reporting the Effect of Maternal Vitamin D Status on Pregnancy Health Outcomes, 2018–2019

First author [ref.], year	Topic: Effect of Vitamin D on ...	No. of studies included in analysis	No. of pooled participants	Findings
Amraei [65], 2018	Risk of gestational diabetes	26: 8 cross-sectional 6 prospective, nested case-control 7 retrospective case-control 5 prospective cohort studies	$n = 5,464$ GDM $n = 15,039$ without GDM	Risk of GDM, OR 1.18, 95% CI 1.01–1.35, $p < 0.0001$; 25(OH)D concentration lower in those with GDM, OR -0.26 , 95% CI -0.39 to -0.14 , $p < 0.0001$ No world regional differences Stated limitations: differences between studies in definition of low 25(OH)D and criteria for GDM; potential impact of other confounders, such as pregnancy wt gain, SES, and skin color could not be explored
Baca [40], 2018	Expression of VDR SNPs and association with log25(OH)D concentration during pregnancy	2 cohorts: Epidemiology of Vitamin D Study (EVITA) and Collaborative Perinatal Project (CPP)	EVITA: $n = 1,958$ randomly selected/analyzed CPP: $n = 4,285$ randomly selected/analyzed	Higher rates of vitD deficiency in black mothers Maternal genotypes of 7 SNPs in VDR, 3 SNPs in GC/VDBP, and 1 SNP in flanking regions of CYP27B1 were associated with difference in log25(OH)D concentration during pregnancy Adjusting for multiple comparisons, 1 SNP in VDR and 2 SNPs in GC/VDBP remained significant
Barchitta [41], 2018	Evaluation of VDR polymorphisms and their association with neonatal anthropometric measures and PTB	“Mamma and Bambino” cohort 11 observational clinical studies 3 case-control plus Mamma and Bambino included in meta-analysis	$n = 187$ Unclear total N $n = 763$	For FokI polymorphism, gestational duration and BW were decreased with an increase in the No. of A allele(s) Compared with GG and GA genotypes, mothers who carried the AA genotype exhibited higher PTB risk (OR 12.049, 95% CI 2.606 to 55.709, $p = 0.001$) Protective effect of BsmI polymorphism against PTB under the allelic (A vs G: OR 0.74, 95% CI 0.59–0.93) and recessive (AA vs GG+AG: OR 0.62, 95% CI 0.43–0.89) models
Bi [66], 2018	Infant/offspring growth, morbidity and mortality	24 RCTs	$n = 5,405$	VitD supplemented during pregnancy associated with lower risk of SGA (RR 0.72, 95% CI 0.52 to 0.99; RD -5.6% , 95% CI -0.86 to -10.34%), without risk of fetal or neonatal mortality or congenital anomaly Neonates of supplemented mothers: at birth, higher circulating 25(OH)D, serum calcium levels, and wt, carried through to 3, 6, 9, and 12 months after delivery Lower rates of fetal/neonatal mortality of mothers receiving 2,000 IU vitD/day and risk not reduced above that dose (RR 0.35, 95% CI 0.59 to 0.80)
Fang [67], 2019	Association of maternal vitD deficiency (<20 ng/mL/<50 nmol/L) during pregnancy with LBW	16 cohort or case-control	$n = 8,403$ from 8 studies analyzed for LBW risk $n = 11,867$ from 10 studies analyzed for BW differences	Maternal vitD deficiency associated with LBW (OR 2.39, 95% CI 1.25 to 4.57, $p = 0.008$) Total mean BW decreased by 0.08 kg or 80 g (OR -0.08 kg, 95% CI -0.10 to -0.06 , $p < 0.001$)
Fogacci [68], 2019	Effect of maternal vitD-S on risk of preeclampsia	27 RCTs Low degree of heterogeneity 3 studies excluded that included multivitamins with vitD	$n = 4,777$ total VitD treatment group $n = 2,487$ Control group $n = 2,290$	Decreased risk of preeclampsia with higher maternal 25(OH)D (OR 0.37, 95% CI 0.26 to 0.52) If vitD-S initiated before 20 wks’ gestation, lower risk of maternal preeclampsia (OR 0.35, 95% CI 0.26 to 0.52, $p < 0.001$) Increased vitD dosage inversely associated with preeclampsia risk (slope of log OR -1.1 , 95% CI -1.73 to -0.46 , $p < 0.001$ corresponding to OR 0.33, 95% CI 0.18 to 0.63, $p < 0.001$) Risk not associated with maternal age
Gallo [69], 2019	Risk of vitD deficiency in mother and neonate Risk of abnormal maternal homeostatic model assessment of insulin resistance Effect on BW Risk of preeclampsia, cesarean section, gestational age and neonatal length	20 RCTs qualitative analysis 17 RCTs quantitative analysis Significant heterogeneity between studies	$n = 2,844$	Good evidence to support maternal vitD-S, increased both maternal (13 studies, MD 14.1 ng/mL [35.2 nmol/L]; 95% CI 9.6 to 18.6 ng/mL [24.0 to 46.4 nmol/L]) and neonatal (cord blood) 25(OH)D (9 studies, MD 9.7, 5.2, 14.2 ng/mL [24.2, 12.9, 35.5 nmol/L]) Fair evidence that vitD-S was associated with decreased maternal HOMA-IR and increased BW in offspring Null effect seen for preeclampsia, mode of delivery, infant gestational age, or birth length

Table 1 (continued)

First author [ref.], year	Topic: Effect of Vitamin D on ...	No. of studies included in analysis	No. of pooled participants	Findings
Li [70], 2019	VitD-S during pregnancy and the risk of wheezing in offspring	4 prospective cohorts 3 RCTs	$n = 6,068$ mother/child pairs	Inverse relationship between maternal vitD intake during pregnancy and occurrence of wheezing in offspring (pooled OR 0.68, 95% CI 0.55 to 0.83, $p < 0.01$) Inverse relationship between maternal vitD intake during pregnancy and eczema but not significant (pooled OR 0.95, 95% CI 0.75 to 1.21, $p = 0.66$) Reported U-shaped dose curve between maternal vitD intake and risk of wheezing in offspring, with lowest risk in 800-IU group but were not able to control for timing of dose, maternal asthma, parental smoking, and other potential confounders
Maugeri [71], 2019	Effects of vitD-S on birth size	13 RCTs 17 comparison groups		Maternal vitD-S associated with BW (12 RCTs; MD 103.17 g, 95% CI 62.29 to 144.04), length (6 RCTs; MD 0.22 cm, 95% CI 0.11 to 0.33), and head circumference (6 RCTs; MD 0.19 cm, 95% CI 0.13 to 0.24) Also associated with reduced risk of LBW (3 RCTs; RR 0.40, 95% CI 0.22 to 0.74) and SGA (5 RCTs; RR 0.69, 95% CI 0.51 to 0.92)
Ojo [72], 2019	Effect of vitD-S on glycemic control in women with GDM	5 RCTs	$n = 173$	Compared to controls, vitD-S associated with decrease in fasting blood glucose (mean 0.46 mmol/L, 95% CI -0.68, -0.25, $p < 0.001$), glycated hemoglobin (mean 0.37%, 95% CI -0.65, -0.08, $p < 0.01$), and serum insulin concentration (mean 4.10 μ IU/mL, 95% -5.50, -2.71, $p < 0.001$)
Pacheco-González [73], 2018	Prenatal vitD status and later offspring respiratory and allergy outcomes	34 observational 26 separate study populations 25 longitudinal and 1 case-control 16 countries represented	n not listed	Risk of RTIs: comparing highest with lowest 25(OH)D category, pooled OR 0.64 (95% CI 0.47, 0.87) Positive borderline association with lung function at school age (FEV ₁ z-score coefficient 0.07, 95% CI -0.01, 0.15) No associations found for wheeze, asthma, atopic eczema, allergic rhinitis, and allergic sensitization
Santamaria [74], 2018	Prenatal vitD status and offspring growth, adiposity, and metabolic health	30 observational	$n = 35,032$ mother/offspring pairs	Low prenatal vitD associated with lower BW (g) (MD -100.69, 95% CI -162.25, -39.13), increased risk of SGA (OR 1.55, 95% CI 1.16, 2.07), and an elevated wt (g) in infants at the age of 9 months (MD 119.75, 95% CI 32.97, 206.52) No associations between prenatal vitD status and other growth parameters at birth, age 1 year, 4-6 yrs, or 9 yrs, or with diabetes type 1
Shen [75], 2018	Effect of maternal or neonatal (cord blood) vitD status on later risk of wheezing 5 yrs of age and >5 yrs	3 RCTs 33 cohort studies	$n = 1,619$	No statistically significant association between maternal or cord blood 25(OH)D or intake early in life and asthma either at 5 or >5 yrs
Shi [76], 2019	Maternal vitD intake during pregnancy and later risk of asthma and wheeze in offspring	10 observational, with 14 independent reports	2,073 incident cases of asthma 1,875 cases of wheeze Total 23,030 mother/child pairs	Compared to offspring of nonsupplemented mothers, offspring of vitD-S mothers with reduced risk of asthma or wheeze in infants Combined OR infant wheeze 0.65 (95% CI 0.54 to 0.79) and asthma 0.78 (95% CI 0.69 to 0.89)
Tous [77], 2019	Association of low prenatal 25(OH)D (using 3 different threshold levels), PTB, and anthropometric and neurodevelopmental outcomes in offspring	54 observational	$n = 67,484$	Mothers with 25(OH)D threshold value of <30 nmol/L, at greater risk of offspring with: - lower BW (MD -87.82 g, 95% CI -119.73 to -55.919) - lower head circumference (MD -0.19 cm, 95% CI -0.32 to -0.06) - increased risk of SGA and PTB (OR 1.59, 95% CI 1.24 to 2.03) With threshold of <50 nmol/L, offspring with: - increased risk for SGA (OR 1.43, 95% CI 1.08 to 1.91) - increased risk for PTB (OR 1.38, 95% CI 1.08 to 1.52) When maternal 25(OH)D 75 nmol/L, not associated with BW, SGA status, or PTB Offspring of vitD insufficient/deficient mothers had lower scores on mental index (OR -1.12, 95% CI -1.82 to 0.42) and language (OR -0.35, 95% CI -1.00 to 0.31, but not statistically significant)

Table 1 (continued)

First author [ref.], year	Topic: Effect of Vitamin D on ...	No. of studies included in analysis	No. of pooled participants	Findings
Yuan [78], 2019	Association of maternal 25(OH)D and risk of preeclampsia	1 nested case-control 20 additional clinical studies	<i>n</i> = 122 women with preeclampsia <i>n</i> = 480 controls <i>n</i> = 39,031 participants: 3,305 with preeclampsia, various ethnicities	65.6% with preeclampsia had 25(OH)D <50.0 nmol/L vs. 55.3% of controls 25(OH)D was significantly lower in women with preeclampsia than in controls (median [IQR] 43.3 [35.5, 55.2] vs. 47.5 [37.6, 60.4] nmol/L, <i>p</i> = 0.014) Women with 25(OH)D <50.0 nmol/L with 65% increase in preeclampsia risk (95% CI 1.02 to 2.69) compared with women with 25(OH)D 50.0 to 74.9 nmol/L Meta-analysis showed that low 25(OH)D concentrations were associated with significantly increased risk of preeclampsia by 62% (pooled OR 1.62, 95% CI 1.36 to 1.94), and risk effect of low 25(OH)D concentrations existed in most subgroups
Zhang [79], 2018	Effect of maternal vitD status on risk of GDM	87 observational and 25 RCTs	<i>n</i> = 55,859 in observational studies <i>n</i> = 2,445 in RCTs	Low 25(OH)D associated with increased GDM (OR 1.850, 95% CI 1.471 to 2.328) 25(OH)D associated with fasting glucose and HOMA-IR index

BW, birth weight; GDM, gestational diabetes mellitus; HOMA-IR, homeostatic model assessment of insulin resistance; LBW, low BW; MD, mean difference; OR, odds ratio; PTB, preterm birth; RCTs, randomized clinical trials; SNPs, single nucleotide polymorphisms; SGA, small for gestational age; SES, socioeconomic status; vitD, vitamin D; vitD-S; vitamin D supplementation; VDR, vitamin D receptor; wt, weight; wks, weeks; yrs, years.

Vitamin D Clinical Trials during Pregnancy

The issue with nutrient studies is that they often are designed more like a drug study, where the starting concentration of the “drug” is zero, compared with a nutrient study, such as vitamin D, where there is some vitamin D concentration in everyone, and, thus, baseline 25(OH)D concentration is variable and not zero. Heaney [43] described the qualities that should define a nutrient study:

- 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 the status of other nutrients must be optimized to guarantee that the nutrient being studied is the only nutrition-related limiting factor in the response.

One might add another stipulation to the list – that the nutrient being investigated has to follow an appropriate dosing schedule to match what happens naturally. In the case of vitamin D, there is a plethora of data that show substantial physiological differences between daily, weekly, and monthly vitamin D dosing regimens [11].

In reviewing past clinical trials of vitamin D, most lack all 5 of the Heaney criteria and often the 6th dosing criterion. While

systematic reviews and meta-analyses provide larger combined sample sizes, the analyses of several limited studies only compound the problem. More recently, the rigor of clinical trials with increased sample sizes has improved the consistency of pooled/aggregate data, and some compelling evidence from these clinical trials suggests that vitamin D sufficiency during pregnancy enhances maternal and fetal health. The “translation” of the laboratory data to the clinic and bedside supports this emerging concept that vitamin D plays a role not only in calcium homeostasis and bone function but also in immune function. Beyond the scope of this review for an exhaustive summary, below are some of the highlights of those systematic reviews and meta-analyses to date, with an emphasis on the salient findings, and their strengths and weaknesses.

As mentioned in the attributes of a well-designed nutrient study as outlined by Heaney [43], part of the issue is that these supplementation trials have varied by the onset of supplementation during pregnancy, the dosing and timing of that dose, the degree of vitamin D deficiency at the onset of the trial, and different methodology used in measuring 25(OH)D. There have been numerous systematic reviews and meta-analyses on the topic, also with mixed findings. Restricting systematic reviews and meta-analyses to the 2 recent years 2018–2019, there are over 30 publications on the topic of vitamin D and pregnancy outcomes. The analyses cover such topics as gestational age, infant birth weight, gestational diabetes and insulin resistance, small-for-gestational age, preeclampsia, and maternal and neonatal vitamin D status at de-

livery. Compared to analyses performed in earlier years when there were few published randomized controlled trials that were often plagued with small sample sizes, the more recent reviews consistently have shown benefit of maternal vitamin D supplementation during pregnancy. The highlights of some of the larger systemic reviews and meta-analyses published in the past 2 years (2018–2019) are summarized in Table 1. With each review, there is evidence that there are still limitations to the clinical studies and there is a need for continued research, especially with genetic and epigenetic considerations in place and design of nutrient studies that take into account the Heaney criteria [43].

Association during Pregnancy: Linkage of 25(OH)D to 1,25(OH)₂D and a Unique Evolutionary Advantage?

Taken together, there is evidence to suggest that vitamin D deficiency increases the risk of adverse pregnancy outcomes in both the mother and her developing fetus. The question at the heart of the matter is what 25(OH)D concentration should be the target for pregnant women? What this target might be is suggested by this kinetic reaction saturation graph (Fig. 2) of 25(OH)D and 1,25(OH)₂D, which shows that 25(OH)D has direct influence on 1,25(OH)₂D concentrations throughout pregnancy, an event which does not occur during any other time during the human lifecycle. As is noted in our study reporting these results, as lower concentrations of 1,25(OH)₂D increase, first-order kinetics becomes zero-order kinetics, with a plateauing of the graph and an inflection point at 40 ng/mL (100 nmol/L) 25(OH)D – the level required to optimize 1,25(OH)₂D production during pregnancy [34].

If this inflection point of 40 ng/mL (100 nmol/L) represents where there is optimal conversion of 25(OH)D to 1,25(OH)₂D, one would predict that attaining this level during pregnancy would be critical for both maternal and fetal well-being. In our post hoc analysis using multivariable log-binomial regression of maternal 25(OH)D status during pregnancy, McDonnell et al. [44] reported that in women who attained a maternal 25(OH)D concentration ≥ 40 ng/mL (100 nmol/L) compared to those who remained with a concentration ≤ 20 ng/mL (50 nmol/L), adjusted for covariates, their risk of preterm birth was reduced by 59%. Based on cellular, animal studies and genetic analyses, it appears that early vitamin D status may have greater bearing on pregnancy outcomes than later status [45, 46], but dissecting the factors that influence these early processes has been a challenge [47, 48]. At the very least, a woman who is considering becoming pregnant or who becomes pregnant should be vitamin D sufficient as defined by the ki-

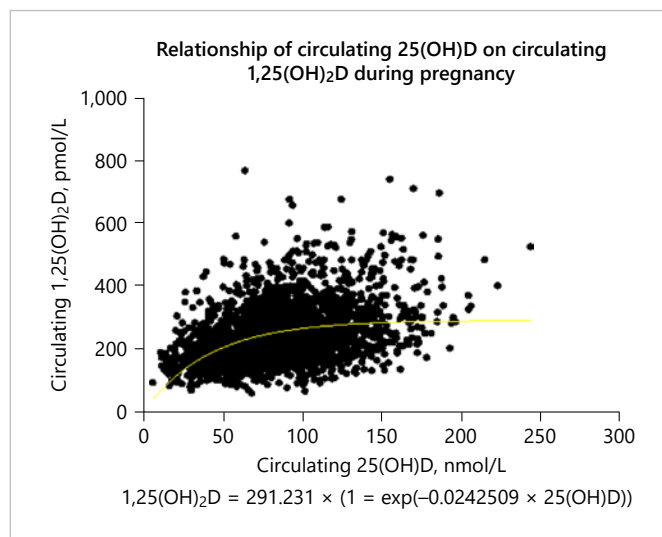


Fig. 2. The relationship of circulating 25(OH)D to control the production of 1,25(OH)₂D during pregnancy. All data points for all subjects in all groups were included in this analysis. (Hollis et al. [34], 2011, with permission.)

netics curve of Figure 2, attaining at least a circulating 25(OH)D concentration of 40 ng/mL (100 nmol/L) as early as possible during pregnancy.

From Birth into Infancy – Achieving Vitamin D Sufficiency during Lactation in Both Mother and Infant

In numerous articles published during the last 3 decades, it is stated that breast milk has a relatively low vitamin D concentration and, as a result, all babies who are breastfed should receive a vitamin D supplement of 400 IU/day to prevent vitamin D deficiency that can lead to osteopenia and rickets in the exclusively breastfed infant [49–51]. This recommendation is based on the observations since the 1930s and beyond that infants and children who received one teaspoon of cod liver oil (which contains about 400 IU/teaspoon) had minimal risk of developing rickets. It does not address how we evolved as a species with such low concentrations of vitamin D. Most young infants today in technologically dependent societies are not exposed to direct sunlight until well after 6 months, and so their ability to use ultraviolet light to synthesize vitamin D endogenously is thwarted. If we look at groups throughout the world who live in sun-rich environments, we see a pattern that differs from those who live at higher latitudes – maternal vitamin D status is better if the mother is exposed to the sun [52], and, therefore, her milk anti-rachitic activity – the total

amount of vitamin D moieties in human milk – is better. It is critical to understand that human milk is deficient in vitamin D only when the mother herself is deficient [53]. We know that during pregnancy, maternal vitamin D status is closely linked with fetal and neonatal vitamin D status. That connection and relationship continues during lactation.

That if a mother had an improved vitamin D status, then her milk anti-rachitic activity would be improved and that of her recipient infant, obviating the need for infant vitamin D supplementation

It was hypothesized by Hollis and Wagner [54] more than 2 decades ago that if a mother had an improved vitamin D status, then her milk anti-rachitic activity would be improved and that of her recipient infant, obviating the need for infant vitamin D supplementation. Maternal vitamin D supplementation would effectively treat both the mother and her breastfeeding infant. This was studied in 2 pilot studies by our group [54, 55] and then in a larger trial sponsored by the National Institutes of Health [56] that has since been repeated by Dawodu et al. [57] in another region of the world – the Middle East – where there is profound vitamin D deficiency. In the various trials, mothers at 1 month postpartum were randomized to receive 1 of 3 treatments: 400, 2,400, or 6,400 IU vitamin D/day. Infants of mothers in the 400-IU group received the standard of care of 400 IU/day, while infants of mothers in the 2,400- and 6,400-IU group received 0 IU/day (placebo). Maternal supplementation with 2,400 IU vitamin D/day with infants on placebo resulted in higher rates of infant insufficiency and that arm of the study was stopped early-on in the study. Mothers in the 6,400-IU group had improved vitamin D status, milk anti-rachitic activity, and their infants had circulating 25(OH)D concentrations that were comparable to infants receiving 400 IU/day direct supplementation [55, 56]. There were no safety issues noted in these studies except with the 2,400 IU arm and the higher rates of infant deficiency, but no issues with toxicity from vitamin D. Similar results were reported by Dawodu et al. [57].

Oberhelman et al. [58] studied 40 exclusively breastfeeding mothers and infants who were randomized to receive either daily maternal vitamin D supplementation of 5,000 IU/day versus a single large bolus of 150,000 IU once as a higher bolus vitamin D, with the primary outcome at 28 days being maternal and infant vitamin D status. The daily versus single bolus were comparable at 28 days; however, the mother and infant pair who received the single bolus had a large increase in their circulating 25(OH)D that rapidly declined but was still improved compared to baseline.

A systematic review and meta-analysis by O’Callaghan et al. [59], reviewing relevant studies on the topic of alternatives to daily infant vitamin D supplementation through September 2018, identified 28 relevant papers of which 5 were randomized clinical trials that met inclusion criteria for the analysis. The meta-analysis suggests that the results are promising, with the need for larger studies in diverse groups of women necessary to be carried out before policy changes can be made [59].

While application of alternatives to infant supplementation are being discussed, a major issue complicating recommendations is that compliance by parents to give their breastfeeding infants daily vitamin D drops is low in many regions of the world [60, 61]. In the USA, reports of compliance with the recommendation of infant vitamin D supplementation range from 9 to 20%, leaving most breastfeeding infants in the USA deficient, dependent on their mothers who are often themselves deficient [62–64]. These are less than satisfying statistics. At the end of the day, where maternal compliance with taking a vitamin D supplement is much greater than that of parental adherence with infant supplementation, maternal vitamin D supplementation alone remains as a viable alternative to infant vitamin D supplementation that safely and effectively treats both the mother and her breastfeeding infant.

Conflict of Interest Statement

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References

- 1 Racicot K, Kwon JY, Aldo P, Silasi M, Mor G. Understanding the complexity of the immune system during pregnancy. *Am J Reprod Immunol*. 2014 Aug;72(2):107–16.
- 2 Mor G, Cardenas I. The immune system in pregnancy: a unique complexity. *Am J Reprod Immunol*. 2010 Jun;63(6):425–33.
- 3 Mor G, Cardenas I, Abrahams V, Guller S. Inflammation and pregnancy: the role of the immune system at the implantation site. *Ann N Y Acad Sci*. 2011 Mar;1221(1):80–7.
- 4 Bikle DD. Vitamin D: an ancient hormone. *Exp Dermatol*. 2011 Jan;20(1):7–13.
- 5 Wagner CL, Eidelman AI. The impact of vitamin D on the maternal and infant epigenome: the role of pregnancy and breastfeeding. *Breastfeed Med*. 2018 Jun;13(5):305–6.
- 6 Bikle DD. Vitamin D regulation of immune function. *Vitam Horm*. 2011;86:1–21.
- 7 Bikle DD. Vitamin D metabolism and function in the skin. *Mol Cell Endocrinol*. 2011 Dec;347(1-2):80–9.
- 8 Hollis BW. Comparison of equilibrium and disequilibrium assay conditions for ergocalciferol, cholecalciferol and their major metabolites. *J Steroid Biochem*. 1984 Jul;21(1):81–6.
- 9 Bhattacharyya MH, DeLuca HF. Subcellular location of rat liver calciferol-25-hydroxylase. *Arch Biochem Biophys*. 1974 Jan;160(1):58–62.
- 10 Hollis BW, Pittard WB 3rd. Evaluation of the total fetomaternal vitamin D relationships at term: evidence for racial differences. *J Clin Endocrinol Metab*. 1984 Oct;59(4):652–7.
- 11 Hollis BW, Wagner CL. Clinical review: The role of the parent compound vitamin D with respect to metabolism and function: Why clinical dose intervals can affect clinical outcomes. *J Clin Endocrinol Metab*. 2013 Dec;98(12):4619–28.
- 12 Hollis BW, Pittard WB 3rd, Reinhardt TA. Relationships among vitamin D, 25-hydroxyvitamin D, and vitamin D-binding protein concentrations in the plasma and milk of human subjects. *J Clin Endocrinol Metab*. 1986 Jan;62(1):41–4.
- 13 Bikle DD, Gee E, Halloran B, Kowalski MA, Ryzen E, Haddad JG. Assessment of the free fraction of 25-hydroxyvitamin D in serum and its regulation by albumin and the vitamin D-binding protein. *J Clin Endocrinol Metab*. 1986 Oct;63(4):954–9.
- 14 Bikle DD, Halloran BP, Gee E, Ryzen E, Haddad JG. Free 25-hydroxyvitamin D levels are normal in subjects with liver disease and reduced total 25-hydroxyvitamin D levels. *J Clin Invest*. 1986 Sep;78(3):748–52.
- 15 Kumar R, Thompson JR. The regulation of parathyroid hormone secretion and synthesis. *J Am Soc Nephrol*. 2011 Feb;22(2):216–24.
- 16 Bouillon R, Bikle D. Vitamin D Metabolism Revised: fall of Dogmas. *J Bone Miner Res*. 2019 Nov;34(11):1985–92.
- 17 Bouillon R, Marcocci C, Carmeliet G, Bikle D, White JH, Dawson-Hughes B, et al. Skeletal and Extraskeletal Actions of Vitamin D: Current Evidence and Outstanding Questions. *Endocr Rev*. 2019 Aug;40(4):1109–51.
- 18 Hollis BW. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr*. 2005 Feb;135(2):317–22.
- 19 Nykjaer A, Dragun D, Walther D, Vorum H, Jacobsen C, Herz J, et al. An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3. *Cell*. 1999 Feb;96(4):507–15.
- 20 Marzolo MP, Farfán P. New insights into the roles of megalin/LRP2 and the regulation of its functional expression. *Biol Res*. 2011;44(1):89–105.
- 21 Holick MF. Vitamin D deficiency. *N Engl J Med*. 2007 Jul;357(3):266–81.
- 22 Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. *Chem Biol*. 2014 Mar;21(3):319–29.
- 23 Holick MF. Vitamin D: evolutionary, physiological and health perspectives. *Curr Drug Targets*. 2011 Jan;12(1):4–18.
- 24 Gallagher JC, Bikle DD. Vitamin D: Mechanisms of Action and Clinical Applications. *Endocrinol Metab Clin North Am*. 2017 Dec;46(4):xvii–xviii.
- 25 Hollis BW, Wagner CL. New insights into the vitamin D requirements during pregnancy. *Bone Res*. 2017 Aug;5(1):17030.
- 26 Bikle DD, Schwartz J. Vitamin D Binding Protein, Total and Free Vitamin D Levels in Different Physiological and Pathophysiological Conditions. *Front Endocrinol (Lausanne)*. 2019 May;10:317.
- 27 Hacker-Thompson A, Schloetter M, Sellmeyer DE. Validation of a dietary vitamin D questionnaire using multiple diet records and the block 98 health habits and history questionnaire in healthy postmenopausal women in northern California. *J Acad Nutr Diet*. 2012 Mar;112(3):419–23.
- 28 Kumar R, Cohen WR, Silva P, Epstein FH. Elevated 1,25-dihydroxyvitamin D plasma levels in normal human pregnancy and lactation. *J Clin Invest*. 1979 Feb;63(2):342–4.
- 29 Carneiro RM, Prebehalla L, Tedesco MB, Sereika SM, Hugo M, Hollis BW, et al. Lactation and bone turnover: a conundrum of marked bone loss in the setting of coupled bone turnover. *J Clin Endocrinol Metab*. 2010 Apr;95(4):1767–76.
- 30 Ritchie LD, Fung EB, Halloran BP, Turnlund JR, Van Loan MD, Cann CE, et al. A longitudinal study of calcium homeostasis during human pregnancy and lactation and after resumption of menses. *Am J Clin Nutr*. 1998 Apr;67(4):693–701.
- 31 Hollis BW, Wagner CL. Assessment of dietary vitamin D requirements during pregnancy and lactation. *Am J Clin Nutr*. 2004 May;79(5):717–26.
- 32 Olsen SF, Secher NJ. A possible preventive effect of low-dose fish oil on early delivery and pre-eclampsia: indications from a 50-year-old controlled trial. *Br J Nutr*. 1990 Nov;64(3):599–609.
- 33 Bikle DD, Gee E, Halloran B, Haddad JG. Free 1,25-dihydroxyvitamin D levels in serum from normal subjects, pregnant subjects, and subjects with liver disease. *J Clin Invest*. 1984 Dec;74(6):1966–71.
- 34 Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. *J Bone Miner Res*. 2011 Oct;26(10):2341–57.

- 35 Hollis BW, Wagner CL. Vitamin D and pregnancy: skeletal effects, nonskeletal effects, and birth outcomes. *Calcif Tissue Int*. 2013 Feb;92(2):128–39.
- 36 Karras SN, Wagner CL, Angeloudi E, Kotsa K. Maternal vitamin D status during pregnancy in Europe: the two sides of the story. *Eur J Nutr*. 2017 Sep;56(6):2207–8.
- 37 Karras SN, Wagner CL, Castracane VD. Understanding vitamin D metabolism in pregnancy: from physiology to pathophysiology and clinical outcomes. *Metabolism*. 2018 Sep;86:112–23.
- 38 Al-Garawi A, Carey VJ, Chhabra D, Mirzakhani H, Morrow J, Lasky-Su J, et al. The Role of Vitamin D in the Transcriptional Program of Human Pregnancy. *PLoS One*. 2016 Oct;11(10):e0163832.
- 39 Litonjua AA, Carey VJ, Laranjo N, Harshfield BJ, McElrath TF, O'Connor GT, et al. Effect of Prenatal Supplementation with Vitamin D on Asthma or Recurrent Wheezing in Offspring by Age 3 Years: The VDAART Randomized Clinical Trial. *JAMA*. 2016 Jan;315(4):362–70.
- 40 Baca KM, Govil M, Zmuda JM, Simhan HN, Marazita ML, Bodnar LM. Vitamin D metabolic loci and vitamin D status in Black and White pregnant women. *Eur J Obstet Gynecol Reprod Biol*. 2018 Jan;220:61–8.
- 41 Barchitta M, Maugeri A, La Rosa MC, Magnano San Lio R, Favara G, Panella M, et al. Single Nucleotide Polymorphisms in Vitamin D Receptor Gene Affect Birth Weight and the Risk of Preterm Birth: Results From the "Mamma & Bambino" Cohort and A Meta-Analysis. *Nutrients*. 2018 Aug;10(9):10.
- 42 Newton DA, Baatz JE, Kindy MS, Gattoni-Celli S, Shary JR, Hollis BW, et al. Vitamin D binding protein polymorphisms significantly impact vitamin D status in children. *Pediatr Res*. 2019 Nov;86(5):662–9.
- 43 Heaney RP. Guidelines for optimizing design and analysis of clinical studies of nutrient effects. *Nutr Rev*. 2014 Jan;72(1):48–54.
- 44 McDonnell SL, Baggerly KA, Baggerly CA, Aliano JL, French CB, Baggerly LL, et al. Maternal 25(OH)D concentrations ≥ 40 ng/mL associated with 60% lower preterm birth risk among general obstetrical patients at an urban medical center. *PLoS One*. 2017 Jul;12(7):e0180483.
- 45 Mirzakhani H, Litonjua AA, McElrath TF, O'Connor G, Lee-Parritz A, Iverson R, et al. Early pregnancy vitamin D status and risk of preeclampsia. *J Clin Invest*. 2016 Dec;126(12):4702–15.
- 46 Weiss ST, Litonjua AA. Vitamin D in Host Defense: Implications for Future Research. *Am J Respir Cell Mol Biol*. 2017 Jun;56(6):692–3.
- 47 Hewison M. The earlier the better: preconception vitamin D and protection against pregnancy loss. *Lancet Diabetes Endocrinol*. 2018 Sep;6(9):680–1.
- 48 Hewison M, Wagner CL, Hollis BW. Vitamin D Supplementation in Pregnancy and Lactation and Infant Growth. *N Engl J Med*. 2018 Nov;379(19):1880–1.
- 49 Hollis BW, Roos BA, Lambert PW. Vitamin D compounds in human and bovine milk. *Adv Nutr Res*. 1982;4:59–75.
- 50 Wagner CL, Greer FR; American Academy of Pediatrics Section on Breastfeeding; American Academy of Pediatrics Committee on Nutrition. Prevention of rickets and vitamin D deficiency in infants, children, and adolescents. *Pediatrics*. 2008 Nov;122(5):1142–52.
- 51 Dawodu A, Tsang RC. Maternal vitamin D status: effect on milk vitamin D content and vitamin D status of breastfeeding infants. *Adv Nutr*. 2012 May;3(3):353–61.
- 52 Luxwolda MF, Kuipers RS, Kema IP, van der Veer E, Dijck-Brouwer DA, Muskiet FA. Vitamin D status indicators in indigenous populations in East Africa. *Eur J Nutr*. 2013 Apr;52(3):1115–25.
- 53 Wagner C. Current concepts in vitamin D requirements for mother and her breastfeeding infant. In: Lawrence R, editor. International Society for Research in Human Milk and Lactation. Breastfeeding Medicine. Trieste, Italy; 2013. pp. 561–562.
- 54 Hollis BW, Wagner CL. Vitamin D requirements during lactation: high-dose maternal supplementation as therapy to prevent hypovitaminosis D for both the mother and the nursing infant. *Am J Clin Nutr*. 2004 Dec;80(6 Suppl):1752S–8S.
- 55 Wagner CL, Hulsey TC, Fanning D, Ebeling M, Hollis BW. High-dose vitamin D3 supplementation in a cohort of breastfeeding mothers and their infants: a 6-month follow-up pilot study. *Breastfeed Med*. 2006;1(2):59–70.
- 56 Hollis BW, Wagner CL, Howard CR, Ebeling M, Shary JR, Smith PG, et al. Maternal Versus Infant Vitamin D Supplementation During Lactation: A Randomized Controlled Trial. *Pediatrics*. 2015 Oct;136(4):625–34.
- 57 Dawodu A, Salameh KM, Al-Janahi NS, Bener A, Elkum N. The Effect of High-Dose Postpartum Maternal Vitamin D Supplementation Alone Compared with Maternal Plus Infant Vitamin D Supplementation in Breastfeeding Infants in a High-Risk Population. A Randomized Controlled Trial. *Nutrients*. 2019 Jul;11(7):11.
- 58 Oberhelman SS, Meekins ME, Fischer PR, Lee BR, Singh RJ, Cha SS, et al. Maternal vitamin D supplementation to improve the vitamin D status of breast-fed infants: a randomized controlled trial. *Mayo Clin Proc*. 2013 Dec;88(12):1378–87.
- 59 O'Callaghan KM, Taghivand M, Zuchniak A, Onoyovwi A, Korsiak J, Leung M, et al. Vitamin D in Breastfed Infants: Systematic Review of Alternatives to Daily Supplementation. *Adv Nutr*. 2020 Jan;11(1):144–59.
- 60 Oberhelman S, Cozine E, Umaretiya P, Maxson J, Thacher T. Vitamin D and the breastfeeding infant: family medicine clinicians' knowledge, attitudes, and practices. *J Hum Lact*. 2018 May;34(2):331–6.
- 61 Wagner CL, Hollis BW. Commentary on "Vitamin D and the Breastfeeding Infant: Family Medicine Clinicians' Knowledge, Attitudes, and Practices" by Oberhelman et al. *J Hum Lact*. 2018 May;34(2):337–9.
- 62 Perrine CG, Sharma AJ, Jefferds ME, Serdula MK, Scanlon KS. Adherence to vitamin D recommendations among US infants. *Pediatrics*. 2010 Apr;125(4):627–32.
- 63 Weernink MG, van Wijk RM, Groothuis-Oudshoorn CG, Lanting CI, Grant CC, van Vlimmeren LA, et al. Insufficient vitamin D supplement use during pregnancy and early childhood: a risk factor for positional skull deformation. *Matern Child Nutr*. 2016 Jan;12(1):177–88.
- 64 Ahrens K, Rossen L, Simon A. Adherence to vitamin D recommendations among US infants aged 0 to 11 Months, NHANES, 2009 to 2012. *Clin Pediatr (Phila)*. 2016 Jun;55(6):555–6.

- 65 Amraei M, Mohamadpour S, Sayehmiri K, Mousavi SF, Shirzadpour E, Moayeri A. Effects of Vitamin D Deficiency on Incidence Risk of Gestational Diabetes Mellitus: A Systematic Review and Meta-analysis. *Front Endocrinol (Lausanne)*. 2018 Feb;9:7.
- 66 Bi WG, Nuyt AM, Weiler H, Leduc L, Santamaria C, Wei SQ. Association Between Vitamin D Supplementation During Pregnancy and Offspring Growth, Morbidity, and Mortality: A Systematic Review and Meta-analysis. *JAMA Pediatr*. 2018 Jul;172(7):635–45.
- 67 Fang K, He Y, Mu M, Liu K. Maternal vitamin D deficiency during pregnancy and low birth weight: a systematic review and meta-analysis. *J Matern Fetal Neonatal Med*. 2019 Jul 8;1–7.
- 68 Fogacci S, Fogacci F, Banach M, Michos ED, Hernandez AV, Lip GYH, Blaha MJ, Toth PP, Borghi C, Cicero AFG; Lipid and Blood Pressure Meta-analysis Collaboration (LBPMC) Group. Vitamin D supplementation and incident preeclampsia: A systematic review and meta-analysis of randomized clinical trials. *Clin Nutr*. 2020 Jun;39(6):1742–52.
- 69 Gallo S, McDermaid JM, Al-Nimr RI, Hakeem R, Moreschi JM, Parkeener M, et al. Supplementation during Pregnancy: An Evidence Analysis Center Systematic Review and Meta-Analysis. *J Acad Nutr Diet*. 2020 May;120(5):898–924.e4.
- 70 Li W, Qin Z, Gao J, Jiang Z, Chai Y, Guan L, et al. Vitamin D supplementation during pregnancy and the risk of wheezing in offspring: a systematic review and dose-response meta-analysis. *J Asthma*. 2019 Dec;56(12):1266–73.
- 71 Maugeri A, Barchitta M, Blanco I, Agodi A. Effects of Vitamin D Supplementation During Pregnancy on Birth Size: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients*. 2019 Feb;11(2):E442.
- 72 Ojo O, Weldon SM, Thompson T, Vargo EJ. The Effect of Vitamin D Supplementation on Glycaemic Control in Women with Gestational Diabetes Mellitus: A Systematic Review and Meta-Analysis of Randomised Controlled Trials. *Int J Environ Res Public Health*. 2019 May;16(10):E1716.
- 73 Pacheco-González RM, García-Marcos L, Morales E. Prenatal vitamin D status and respiratory and allergic outcomes in childhood: A meta-analysis of observational studies. *Pediatr Allergy Immunol*. 2018 May;29(3):243–53.
- 74 Santamaria C, Bi WG, Leduc L, Tabatabaei N, Jantchou P, Luo ZC, et al. Prenatal vitamin D status and offspring's growth, adiposity and metabolic health: a systematic review and meta-analysis. *Br J Nutr*. 2018 Feb;119(3):310–9.
- 75 Shen SY, Xiao WQ, Lu JH, Yuan MY, He JR, Xia HM, et al. Early life vitamin D status and asthma and wheeze: a systematic review and meta-analysis. *BMC Pulm Med*. 2018 Jul;18(1):120.
- 76 Shi D, Wang D, Meng Y, Chen J, Mu G, Chen W. Maternal vitamin D intake during pregnancy and risk of asthma and wheeze in children: a systematic review and meta-analysis of observational studies. *J Matern Fetal Neonatal Med*. 2019 May 7:1–7.
- 77 Tous M, Villalobos M, Iglesias L, Fernández-Barrés S, Arija V. Vitamin D status during pregnancy and offspring outcomes: a systematic review and meta-analysis of observational studies. *Eur J Clin Nutr*. 2020 Jan;74(1):36–53.
- 78 Yuan Y, Tai W, Xu P, Fu Z, Wang X, Long W, Guo X, Ji C, Zhang L, Zhang Y, Wen J. Association of maternal serum 25-hydroxyvitamin D concentrations with risk of preeclampsia: a nested case-control study and meta-analysis. *J Matern Fetal Neonatal Med*. 2019 Jul 17:1–10.
- 79 Zhang Y, Gong Y, Xue H, Xiong J, Cheng G. Vitamin D and gestational diabetes mellitus: a systematic review based on data free of Hawthorne effect. *BJOG*. 2018 Jun;125(7):784–93.