Calcif Tissue Int (2013) 92:128–139
DOI 10.1007/s00223-012-9607-4

ORIGINAL RESEARCH

Vitamin D and Pregnancy: Skeletal Effects, Nonskeletal Effects, and Birth Outcomes

Bruce W. Hollis · Carol L. Wagner

Received: 1 February 2012 / Accepted: 10 April 2012 / Published online: 24 May 2012

Abstract The function and requirement of vitamin D during pregnancy for both mother and fetus have remained a mystery. This fact was highlighted by *The Cochrane Review* in 2000, which reported a lack of randomized controlled trials (RCTs) with respect to vitamin D requirements during pregnancy. Unfortunately, during the past decade only a single RCT has been performed with respect to vitamin D requirements during pregnancy. In this review we will discuss vitamin D metabolism during pregnancy as well as the consequences of vitamin D deficiency on skeletal, nonskeletal, and birth outcomes using birth observational data and data from our recent RCT. New RCT data strongly support previous observational studies in that improving nutritional vitamin D status will improve birth outcomes. The new RCT data indicate that 4,000 IU/day vitamin D_3 during pregnancy will "normalize" vitamin D metabolism and improve birth outcomes including primary cesarean section and comorbidities of pregnancy with no risk of side effects.

Keywords Vitamin D · Bone · Pregnancy · Birth outcome · Infant · 25-Hydroxyvitamin D

The current dietary recommendation for vitamin D during pregnancy remains archaic for a simple reason: fear of vitamin D toxicity [1]. To understand this statement, it is important to look at the history surrounding vitamin D during pregnancy. In 1947, Dr. E. Obermer [2] presented evidence that pregnant women required several thousand international units (IUs) of vitamin D daily during pregnancy. This recommendation had barely "seen the light of day" before vitamin D was erroneously associated with causing supravalvular aortic stenosis syndrome during pregnancy [3–6]. Thus, vitamin D was viewed as teratogenic to the developing fetus during pregnancy, and in response, the medical profession adhered to the largely insignificant 200 IU/day dosing recommendation for adults put forth by the Forbes committee in 1963 [7]. Sadly, at present, a similarly low recommendation largely remains in force as highlighted in the recent Institute of Medicine (IOM) document [8], although a more recent recommendation by the Endocrine Society, recommends higher dosing that takes into account the emerging data surrounding vitamin D's effect on nonskeletal functions [9]. It should be noted that the IOM report is a guide for food manufacturers, while the Endocrine Society report is a guide for the clinical care of patients.

Why are the recommendations between the IOM report and the Endocrine Society report so divergent? The answer is simple: the IOM report refused to make any recommendation not based on a randomized controlled trial (RCT), while the Endocrine Society used a vast combination of all available data including observational trials [9]. In this review, we investigate all avenues of data to derive conclusions.

What Constitutes a "Normal" Level of Circulating 25-Hydroxyvitamin D during Pregnancy?

If we go way back to human origins in Africa, vitamin D would never be considered a nutrient. During almost all of

B. W. H. is an academic consultant to Diasorin. The authors have stated that they have no other conflict of interest.

B. W. Hollis (✉) · C. L. Wagner
Department of Pediatrics, Darby Children's Research Institute, Medical University of South Carolina, 173 Ashley Ave., Room 313, Charleston, SC 29425, USA
e-mail: hollisb@musc.edu

human evolution, vitamin D was derived from solar exposure of the skin. Our human ancestors migrated out of Africa approximately 50,000 years ago to more northern latitudes, and thus, the intensity and duration of solar exposure decreased. A critical point of solar vitamin D generation occurred about 10,000 years ago in Europe when a gene controlling skin pigmentation mutated. This mutation decreased human skin pigmentation and allowed more efficient use of the limited sun exposure available at these northern latitudes [10]. If we progress ahead to the present, we are faced with clothing and other civilized customs that restrict solar exposure of the skin. Thus, now vitamin D truly becomes a vitamin because almost all of us now must obtain it from our diet. There is only one minor problem: vitamin D really does not exist in our food supply to any significant degree. As a result, we now must rely on supplementation to achieve our needs, and herein lies the problem.

Until the 1990s, the criterion for appropriate vitamin D nutrition was simply the absence of overt rickets or osteomalacia [7]. To deal with this problem, the IOM in 1997 recommended that infants and adults receive 400 and 200 IU/day vitamin D, respectively [11]. That dose eliminated rickets in children, but the effect of the adult dose remains a mystery as we do know that a 200 IU/day dose will not increase circulating 25-hydroxyvitamin D (25[OH]D) in adults [12]. Remember, in 1963 we had no idea that vitamin D underwent further metabolism in the human body. As it was discovered that vitamin D was metabolized to more active compounds in the late 1960s, important information began to emerge [13, 14].

In 1971, Haddad and Chyu [15] discovered that 25(OH)D circulated in "normal" adults at about 68 nmol. "Normal" for this study was described as anyone not demonstrating an overt affliction. This study also measured 25(OH)D levels in lifeguards, whose levels were shown to be approximately 175 nmol [15]. So, why not define the lifeguards as "normal" for circulating 25(OH)D and try to achieve those circulating levels of 25(OH)D in other humans? The answer is quite simple: 8 years earlier, the Blumberg report recommended only 200 IU/day vitamin D [7]; since 200 IU/day will put humans nowhere near 175 nmol circulating 25(OH)D, nature must be wrong. In 1971, we did not know how much vitamin D_3 was produced by solar exposure; however, that data were forthcoming [16]. As it turns out, unencumbered natural sun exposure to humans can generate thousands of IUs of vitamin D_3 in a short period of time [17]. Of course, these data did not agree with the Blumberg report of 1963, so their relevance was largely ignored.

The misinformation about vitamin D dosing in humans was just beginning. As mentioned earlier, in 1997, the IOM released a report that reaffirmed the vitamin D requirement for adults as 200 IU/day [11]. Worse, that committee also stated that humans can consume only 2,000 IU/day vitamin D for toxicity reasons [11]. Forget the fact that nature allows us to naturally produce 10,000–20,000 IU/day vitamin D_3 from modest solar exposure [17]. Again, nature must be in error. Thus, not only did medical societies accept that low circulating levels of 25(OH)D were "normal" but the premise was that we now "know" that even modest levels of dietary vitamin D, by natural synthesis standards, can harm you. So what is the truth?

To establish a more encompassing answer about "normal" human levels of circulating 25(OH)D or true vitamin D requirements, let us forget for a moment about the original 1963 Blumberg report [7] with respect to adults and the 1997 IOM toxicity report [11]. Let us return to the levels of circulating 25(OH)D in humans living in their original environment—tribal Africa. We have known for decades that sun-exposed humans in North America can attain high (250 nmol) circulating 25(OH)D [15]. We have also known for decades that nonhuman primates living in the wild possess circulating 25(OH)D levels up to 1,250 nmol/L [18]. Yet, this last point has been dismissed because they are not human. How about tribal, nomadic Africans? A new publication and data provide us clear answers to this question [19] (M. Luxwolda, personal communication).

Luxwolda et al. [19] clearly demonstrated that native tribal Africans achieve an average circulating 25(OH)D level of 115 nmol. But how about during pregnancy? Surely, nature would not allow such levels to occur since the recent IOM report states that such levels would be dangerous to the mother and fetus [8]. It appears that nature did not adhere to the IOM report because the recent study data demonstrates that pregnant women from different native tribes in Africa achieve an average circulating 25(OH)D level of 150 nmol/L throughout pregnancy. How do these natural levels compare with levels of 25(OH)D attained in oral supplementation studies in northern latitudes? In our recent RCT, pregnant women receiving 4,000 IU/day vitamin D_3 attained an average circulating 25(OH)D level of 111 nmol [1], well below the 150 nmol/L in a natural environment (M. Luxwolda, personal communication). This means that in a natural state, in which humans have evolved for more than 1 million years, nature has supplied many times the daily vitamin D recommended by Blumberg et al. [7] or the IOM [8, 11]. Our conclusion is that during pregnancy women should have a circulating 25(OH)D level in excess of 100 nmol/L; however, one achieves it, be it solar exposure and/or diet.

Finally, a comment on the upper safe intake limit (UL) for vitamin D during pregnancy, or anyone for that matter, deserves some attention. The practice of perpetuating a scientific myth has no better example than the toxicity

"myth" of vitamin D. This process for vitamin D has been eloquently documented by Vieth [12]; however, one particular point deserves to be highlighted for the harm it has caused. In 1997, the IOM committee on vitamin D and calcium decided to set a UL for vitamin D [11]. The intent was noble, but the result was a disaster because it was based on a single obscure study that today is regarded as invalid [20]. This resulted in setting the UL for vitamin D at 2,000 IU/day, which crippled clinical vitamin D trials for more than a decade. Why? It is because institutional review boards (IRBs) would not approve vitamin D studies that exceeded that intake. For example, to conduct our vitamin D pregnancy and lactation trials in 2003 [1], we had to write a full investigational drug application to obtain an investigational new drug (IND) number from the U.S. Food and Drug Administration (FDA). One would think that back in 1997 the IOM committee would have asked the following question: if humans can endogenously produce 10,000–20,000 IU vitamin D_3/day from natural sun exposure, how can an oral dose of 2,000 IU/day be of any harm? This same question should also have occurred to the 2010 IOM committee when they set the UL at 4,000 IU/day [8]. There is hope, however, because the 2011 Endocrine Society recommendation apparently did take all this into account when they assigned a 10,000 IU/day UL [9].

RCT of Vitamin D Supplementation during Pregnancy

Table 1 lists all of the RCTs with respect to vitamin D supplementation during pregnancy. There are some stark realities here. First, all but one was performed more than 25 years ago, primarily using vitamin D_2 as a supplement. Second, except for the Hollis et al. [1] study, all the trials used supplemental levels of vitamin D that had limited impact on rising circulating 25(OH)D levels. Finally, all the studies except that of Hollis et al. [1] were small and collected limited data; thus, conclusions are very difficult to extract.

A brief history of the older studies is described here. Initial vitamin D supplementation studies during pregnancy were carried out in the early 1980s. Brooke et al. [21], who studied British mothers of Asian descent, found a greater incidence of small-for-gestational-age (SGA) infants born to mothers who received placebo than for mothers who received 1,000 IU (25 μg) vitamin D_2/day during the final trimester of pregnancy. Neonates in the placebo group also had a greater fontanelle area than did the supplemented group. It must be noted that the placebo group in this study showed profound hypovitaminosis D. Follow-up studies by Brooke et al. [22] were conducted in Asian mothers who again were provided with either placebo or 1,000 IU

Table 1 Summary of RCT vitamin D supplementation studies during pregnancy

Reference	Number of subjects	Vitamin D dose (IU/day)	Therapy duration (months)	Initial 25(OH)D (nmol/L)	End-point 25(OH)D (nmol/L)
Brooke et al. [21][a]	67 Control	0	3	16.3	–
	59 Supplemented	1,000 D_2	3	20.0	168.0
Cockburn et al. [23]	82 Control	0	4	32.5	–
	82 Supplemented	400 D_2	4	39.0	42.8
Brooke et al. [22]	67 Control	0	3	–	–
	59 Supplemented	1,000 D_2	3	–	–
Maxwell et al. [24]	67 Control	0	3	–	–
	59 Supplemented	1,000 D_2	3	20.0	–
Marya et al. [96]	75 Control	0	3	–	–
	25 Supplemented	1,200 D_2	3	–	–
Delvin et al. [97]	15 Control	0	3	17.5 (cord)	–
	15 Supplemented	1,000 D_3	3	–	45.0 (cord)
Mallet et al. [28]	27 Control	0	3	9.5	–
	21 Supplemented	1,000 D_2	3	–	25.3
Hollis et al. [1][b]	111 Supplemented	400 D_3	6	61.5	79.0
	122 Supplemented	2,000 D_3	6	58.3	98.3
	117 Supplemented	4,000 D_3	6	58.3	111.0

[a] It is very likely that the wrong dose of supplementation was given or the assay for 25(OH)D was invalid. The response observed is one that would be expected after supplementation with 10,000 IU/day vitamin D_3 for 3 months [22]

[b] It is important to note that the earlier studies, with the exception of Hollis et al. [1], were conducted with the control group receiving 0 IU vitamin D/day. Since the standard of care for the past three decades in the United States is to give pregnant women 400 IU vitamin D/day included in the prenatal vitamin, it would be unethical to conduct a vitamin D supplementation trial involving pregnant women in the United States today with 0 IU vitamin D/day. Therefore, 400 IU vitamin D/day is the control group

vitamin D_2/day during the last trimester of pregnancy. The follow-up data provided evidence that, during the first year of life, the infants of the maternal placebo group gained less weight and had a lower rate of linear growth than did the infants of the maternal supplemented group.

Cockburn et al. [23] undertook a large vitamin D supplementation study of >1,000 pregnant subjects in the United Kingdom who were supplemented with 400 IU (10 μg) vitamin D_2/day or received a placebo from week 12 of gestation onward. At this level of supplementation, serum concentrations of 25(OH)D in the supplemented group were only slightly higher than those in the placebo group. A defect in dental enamel formation was observed in a higher proportion of the children at 3 years of age in the maternal placebo group. Maxwell et al. [24] conducted a double-blind trial of vitamin D (1,000 IU/day) during the last trimester of pregnancy in Asian women living in London. They found that the supplemented mothers had greater weight gain and, at term, had significantly higher plasma concentrations of retinol-binding protein and thyroid-binding prealbumin, which indicated better protein-calorie nutrition. Almost twice as many infants of the unsupplemented group weighed <2,500 g at birth (the definition of low birth weight) and had significantly lower retinol-binding protein concentrations than did infants of the supplemented mothers.

Supplementation with 1,000 IU (25 μg) vitamin D/day during the last trimester of pregnancy has produced mixed results. The initial study by Brooke et al. [22] described a dramatic increase, from 125 to 150 nmol/L in circulating 25(OH)D, in both mothers and neonates at term. However, these results are highly suspect in light of later and current work and are consistent with a dose response obtained after consumption of 10,000 IU (250 μg) vitamin D/day for 3 months [25]. There also is the possibility that the 25(OH)D assay method used in this study was flawed, as was common during this early period of investigation [26, 27]. Consistent with more recent data, Mallet et al. [28] reported that vitamin D supplementation (1,000 IU/day or 25 μg/day) during the last trimester of pregnancy resulted in an increase in circulating 25(OH)D concentrations of only 12.5–15 nmol/L in maternal and cord serum.

As concluded by *The Cochrane Review* in 2000 [29], these early studies provided an insufficient basis to make any recommendations about vitamin D supplementation during pregnancy. As a result, in 2004, our laboratory initiated a National Institute of Child Health and Human Development–sponsored 6 year randomized, double-blind, placebo-controlled trial of vitamin D supplementation during pregnancy to assess safety and pregnancy outcomes with an approved investigational drug application from the FDA (66,346). The logical question is why it took 25 years before this trial was performed, and the answer is twofold. First, it was expensive, costing about $6 million to perform. Second, until we wrote and received the IND from the FDA, the IRB at our university (representative of other IRBs) would not allow the study to commence, fearing we would do harm by administering the amount of vitamin D (4,000 IU/day) we were proposing. Once the IND was in place, the study could be conducted. The results of our completed study have been published [1] and will be discussed in detail below.

In reference to more recent publications, one of the most puzzling developments was the omission of our RCT pregnancy study from the 2012 Cochrane review of vitamin D supplementation for women during pregnancy [30]. One of us (C. L. W.) was invited to present our published RCT results to the World Health Organization in the fall of 2011. She was informed that the study would not be considered in the upcoming Cochrane review because we did not have a "control" group, meaning a group of pregnant women receiving 0 IU vitamin D during pregnancy. Such a group would be unethical in many regions of the world today because it violates the "standard of care" of giving 400 IU vitamin D/day as part of the prenatal vitamin. Zero IU dosing of vitamin D to those women therefore would never be approved by any knowledgeable IRB in those regions of the world, including the United States, Canada, and most of Europe. For this reason, the applicability of the latest Cochrane review to a major sector of the world is limited.

Vitamin D Metabolism during Pregnancy

Vitamin D metabolism during pregnancy is vastly different from any other time in human physiology, and this point has gone largely unappreciated. With respect to the conversion of vitamin D to 25(OH)D, this metabolic conversion appears to be similar in pregnant and nonpregnant states and follows first- and zero-order enzyme kinetics (Fig. 1) [1, 31]. The similarities, however, end there. It has been known for decades that during pregnancy 1,25-dihydroxyvitamin D ($1,25[OH]_2D$) levels become extremely elevated [32–34]. This increase in circulating $1,25(OH)_2D$ levels has in particular been attributed to an increase in the serum vitamin D-binding protein (DBP) that would regulate the amount of "free" $1,25(OH)_2D$ available in the circulation [33]. While this rise in DBP during pregnancy has been shown to be 46–103 %, depending on the assay employed [35], it cannot account for the nearly three- to-fourfold increase in circulating $1,25(OH)_2D$ in our recent study [1]. Bikle et al. [34] clearly demonstrated that free $1,25(OH)_2D$ levels are increased during pregnancy despite the significant increase in DBP levels, and our recent data agree with this premise [1]. In fact, the new data from our

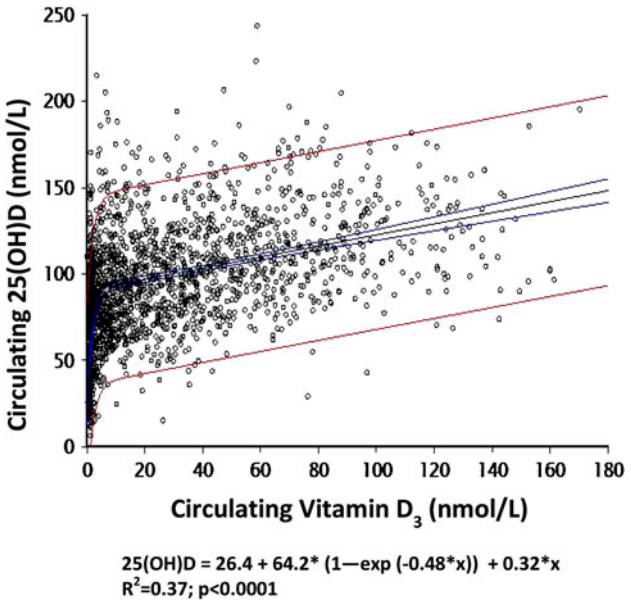

Fig. 1 The relationship between circulating vitamin D to control the production of 25(OH)D during pregnancy [1]

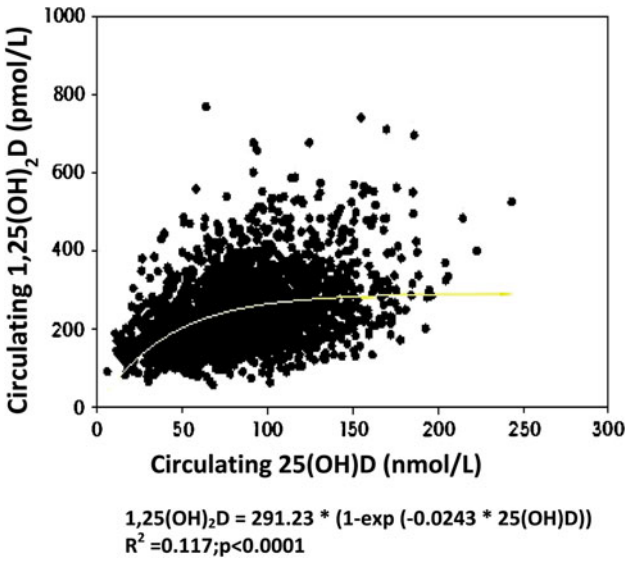

Fig. 2 Relationship of circulating 25(OH)D to circulating $1,25(OH)_2D$ during pregnancy [1]

study demonstrate that a circulating 25(OH)D level of approximately 40 ng/mL (100 nmol/L) is required to optimize production of $1,25(OH)_2D$ during human pregnancy through renal and/or placental production of the hormone (Fig. 2) [1]. Again, this relationship exhibits first- and zero-order enzyme kinetics. It is also of great interest that production of circulating $1,25(OH)_2D$ in the fetus is linked directly to circulating 25(OH)D [36].

Vitamin D metabolism is greatly altered during pregnancy, and pregnancy itself is the primary driver for these extraordinary circulating $1,25(OH)_2D_3$ levels. From our data, it is evident that production of $1,25(OH)_2D_3$ is really not under the control of the classic regulators of calcium, phosphorus, and PTH. The dramatic rise in maternal circulating $1,25(OH)_2D_3$ following conception is remarkable for many reasons: by 12 weeks of gestation, maternal circulating $1,25(OH)_2D_3$ levels are already triple those of a nonpregnant female [1]. From that point in gestation, $1,25(OH)_2D_3$ levels rise much higher and are driven by substrate—25(OH)D—availability (Fig. 2). This substrate dependence of $1,25(OH)_2D_3$ production is never observed in normal human physiology driven by classic calcium homeostasis.

Another remarkable factor in pregnant women is how they can attain supraphysiologic levels of $1,25(OH)_2D_3$, sometimes exceeding 700 pmol/L in our study, and yet never exhibit hypercalciuria or hypercalcemia [1]. These supraphysiological circulating levels of $1,25(OH)_2D_3$ during pregnancy are possibly of placental origin or from the renal 1-α-hydroxylase that would have to be uncoupled from feedback control and for reasons other than maintaining calcium homeostasis. The second scenario is most likely because women with nonfunctional renal 1-α-hydroxylase and normal placental function fail to increase circulating $1,25(OH)_2D_3$ during pregnancy [37]. The increased levels of $1,25(OH)_2D_3$ may be due to methylation of the catabolic *CHP24A1* placental gene [38]. Calcitonin may be a contributor to this process in that it rises during pregnancy [39], is known to stimulate the renal 1-α-hydroxylase gene independently of calcium levels [40, 41], and protects by opposing hypercalcemia [42]. Another possible stimulator of 1-α-hydroxylase during pregnancy is prolactin [43]. If prolactin were a major contributor, however, the effect should continue into lactation, which we do not see, and would be accompanied by elevated circulating $1,25(OH)_2D_3$ levels, which also are not seen [44]. Clearly, vitamin D metabolism during pregnancy is unique in human physiology; but what is its purpose?

What Constitutes Vitamin D Deficiency during Pregnancy?

What circulating level of 25(OH)D [9] does a pregnant woman require to be considered replete? The 2010 IOM report states that if one exhibits a circulating 25(OH)D level of 50 nmol/L, then that individual should be considered replete [8]. That document also stated that individuals consuming an adequate diet would receive enough vitamin D from that diet without consideration of race, latitude, or season and would not require a dietary supplement. Yet, there is a plethora of data from numerous studies throughout the world in the last decade that

suggests otherwise [9]. Fortunately, new clinical guidelines have recently been released by the Endocrine Society that provide serious guidance to the vitamin D deficiency problem [9].

Let us compare the two report recommendations for vitamin D with respect to pregnancy. As mentioned earlier, the IOM recommended a circulating level of 25(OH)D of 50 nmol/L, whereas the Endocrine Society recommends a level of more than 75 nmol/L [8, 9]. To achieve the 50 nmol/L, the IOM recommends 400–600 IU/day, which it states can be obtained through dietary means without supplementation [8]. In contrast, the Endocrine Society recommends an intake of 1,500–2,000 IU/day to achieve a circulating 25(OH)D level of more than 75 nmol/L [9]. How could these recommendations be so divergent? The IOM chose to use only RCT data limited to skeletal integrity, whereas the Endocrine Society chose to use a combination of peer-reviewed basic and clinical scientific publications [8, 9]. The reader will have to decide which report is best to adopt and use for patient guidance.

To examine the actual vitamin D deficiency rates during pregnancy, two recent publications provide some important insight. Even when applying the recent IOM normative 25(OH)D range of 50 nmol/L to the data [8], Hamilton et al. [45] and Johnson et al. [46] both provide shocking deficiency rates during pregnancy in a sunny climate. This work confirms an earlier study by Lee et al. [47] that documented high deficiency rates in mothers consuming prenatal vitamins. This deficiency problem is especially severe in minority populations. The IOM report claims that individuals obtain enough vitamin D from their diet to achieve the circulating 25(OH)D level minimum of 50 nmol/L. This statement is in direct conflict with two recent supplementation studies during pregnancy that failed to achieve this minimum circulating requirement for 25(OH)D [47, 48].

Finally, we have proposed to use mathematical models based on clinical data to dictate what would constitute vitamin D deficiency in the pregnant subject [1]. This is actually a simple task if one inspects Figs. 1, 2 and chooses the inflection point between first- and zero-order enzyme kinetics. These models give the level of at least 100 nmol/L circulating 25(OH)D to support maximal $1,25(OH)_2D$ production during pregnancy by overcoming "substrate limitation."

Consequences of Vitamin D Deficiency during Pregnancy

Calcium Homeostasis and Skeletal Integrity

At the top of any list concerning vitamin D deficiency, calcium homeostasis and skeletal integrity remain a top priority. What is really surprising is the relative dearth of information that exists between this topic and its effect on the pregnant woman and her fetus. There are no RCT data to guide us here, and thus, we must rely on observational data. A superb observational study by Yorifuji et al. [49] provides data to suggest that craniotabes in the newborn infant is the earliest sign of subclinical vitamin D deficiency during pregnancy. At 1 month of age, infants with craniotabes had significantly higher serum alkaline phosphatase, intact PTH, and lower circulating 25(OH)D levels than infants not exhibiting craniotabes. Several other recent observational studies have linked poor nutritional vitamin D status to abnormalities in both maternal and fetal skeletal markers and skeletal integrity itself [50–53]. It is also worth mentioning an animal model study that suggests pregnancy itself upregulates intestinal calcium absorption and skeletal mineralization independently of vitamin D, suggesting that vitamin D is not required for the skeletal adaptations during pregnancy [54]. However, this study was performed in mice, and its relevance to human physiology remains to be established. One additional observation is that in utero vitamin D levels may impart a positive impact on skeletal integrity later in life [55].

Data from our recent RCT tend to support the relative lack of effect of vitamin D on the calcium and skeletal homeostatic systems as implied in the animal model [1, 54]. As discussed earlier in this review, circulating $1,25(OH)_2D$ levels in the pregnant human elevate to supraphysiologic levels without any classic stimulus such as low serum calcium or increased PTH [1]. Further, in spite of these huge circulating $1,25(OH)_2D$ levels, serum and urinary calcium levels are normal [1]. The only observed changes in classic calcium homeostatic parameters we observed in our patients were due to normalizing circulating 25(OH)D levels, and they were (1) lowering PTH levels in vitamin D deficient African Americans and (2) a normalization of urinary calcium excretion [1].

Alterations in Immune Function

The control of immune function, both adaptive and innate by nutritional vitamin D status, is a very active area of investigation with regard to pregnancy. Liu et al. [56], using the mouse as an experimental animal for assessing vitamin D's role in the regulation of placental inflammation, determined that maternal and fetal vitamin D levels play a pivotal role in controlling placental inflammation. Does vitamin D play a similar role in human pregnancy? Current data suggest that it does. Walker et al. [36] have shown that cord blood vitamin D status in human participants controls the innate immune response. This study demonstrated that cord blood vitamin D deficiency, by its effect on toll-like receptor–induced antimicrobial production, altered in vitro monocyte responses [36]. The result of

this defect would be decreased barrier protection against invading pathogens. Actual observational data suggest that this is, in fact, the case.

Belderbos et al. [57] demonstrated that nutritional vitamin D deficiency in otherwise healthy neonates is associated with subsequent increased risk of respiratory syncytial viral bronchiolitis. Further, maternal vitamin D deficiency is associated with bacterial vaginosis, and this deficiency may contribute to the strong racial disparity in the prevalence of bacterial vaginosis [58, 59]. Similar mechanisms, with respect to vitamin D deficiency and innate immune function, likely contribute to periodontal disease during pregnancy [60]. Low circulating 25(OH)D levels also have been linked to the risk of respiratory infection, wheezing, and asthma [61–63] and have an apparent impact on the markers of severity of childhood asthma [64], possibly by altering T-regulatory cells [65]. Our recent RCT, using the intent-to-treat model, failed to demonstrate a relationship with vitamin D supplementation and infection [1] (Table 2). Finally, one of the most important aspects of vitamin D's proposed interactions with the adaptive immune system involves its potential to alter multiple sclerosis susceptibility of the infant later in life by improving nutritional vitamin D status during pregnancy [66–70].

Complications and Outcomes of Pregnancy

Complications of pregnancy include preeclampsia, gestational diabetes, and hypertension. Although these complications of pregnancy are well known and contribute to morbidity and mortality during pregnancy, their association with nutritional vitamin D status is a new area of investigation. Preeclampsia is a multisystem disorder that complicates 3–8 % of pregnancies in Western countries and constitutes a major source of morbidity and mortality worldwide [68, 69]. Overall, 10–15 % of maternal deaths are directly associated with preeclampsia and eclampsia.

Some epidemiological findings support the hypothesis of a genetic and immunological etiology. The risk of preeclampsia is two- to fivefold higher in pregnant women with a maternal history of this disorder. Depending on ethnicity, the incidence of preeclampsia ranges 3–7 % in healthy nulliparas and 1–3 % in multiparas. Other risk factors have been identified, including a medical history of chronic hypertension, kidney disease, diabetes, obesity, birthplace in Africa, age $\geq$35 years, pregnancy characteristics such as twin or molar pregnancy, previous preeclampsia, and fetal congenital abnormality [71, 72].

Preeclampsia may be life-threatening for both mother and child, increasing both fetal and maternal morbidity and mortality and often leading to preterm delivery of the fetus due to worsening preeclampsia [69]. In the mother, preeclampsia may cause premature cardiovascular disease, such as chronic hypertension, ischemic heart disease, and stroke, later in life [73]. Children born after preeclamptic pregnancies and who are relatively small at birth have an increased risk of stroke, coronary heart disease, and metabolic syndrome in adult life [74–76].

While the sole curative treatment of preeclampsia is delivery, management of the preeclamptic woman must continuously balance the risk to benefit ratio of induced preterm delivery and maternal–fetal complications. Further, no drug intervention is known to prevent preeclampsia. There are intriguing possibilities that vitamin D is integral in maintaining normal placental integrity and function [77]. Bodnar et al. [78] first described the relationship between poor vitamin D status and risk of preeclampsia. Additional observational studies have strengthened this observation in the past year. Baker et al. [79], using a nested case–control study, found that maternal mid-gestation vitamin D deficiency was associated with increased risk of severe preeclampsia. Robinson et al. [80], utilizing a case–control investigation with gestation-matched contemporaneous control participants, determined that circulating 25(OH)D levels were significantly decreased in early-onset severe preeclamptic individuals. This group further demonstrated that 25(OH)D levels are lower among SGA patients in early-onset severe preeclampsia than those infants without growth retardation [81]. It was concluded from this study that vitamin D status may impact fetal growth through placental mechanisms. Data from our RCT do not suggest a positive or negative effect between vitamin D and birth weight (Table 2) [1]. Finally, Wei et al. [82] recently published a longitudinal study that clearly demonstrated an inverse relationship between circulating 25(OH)D during pregnancy and preeclampsia.

In contrast to prior studies, Bodnar et al. [83] published an observational study linking both low and high nutritional vitamin D status to an increased risk of SGA births in only white women. Data from our RCT do not support this premise (Table 3). In fact, data from our study do not demonstrate any risk of SGA associated with vitamin D supplementation in our Caucasian population at the higher levels of vitamin D (Table 3). It is still possible that lower levels of vitamin D may contribute to SGA births, but this cannot be ascertained from our data. As for African Americans and Hispanics, our data are not clear and offer no trends. What is clear is that an RCT would be necessary for this relationship to be assessed, the cost and time of which would be enormous. Along the spectrum of hypertensive disorders of pregnancy, additional evidence of vitamin D's role during pregnancy comes from an observational study by Ringrose et al. [84], who found a high prevalence of vitamin D deficiency in pregnant women in

Table 2 Pregnancy characteristics and outcomes by vitamin D supplementation group controlling for race

Characteristic	400 IU ($n = 111$)	2,000 IU ($n = 122$)	4,000 IU ($n = 117$)	p	p, controlling for race
Maternal age at delivery (years) (mean ± SD)	27.4 ± 5.7	28.0 ± 5.7	27.1 ± 5.5	0.49	0.2
Baseline 25(OH)D (nmol/L) (mean ± SD)	61.2 ± 27.1	57.6 ± 22.4	59.8 ± 25.4	0.53	0.8
Gestational age (weeks) at delivery (mean ± SD)	38.6 ± 2.2	38.8 ± 1.8	39.1 ± 1.8	0.17	0.1
Birth weight (g) at delivery (mean ± SD)	3,222 ± 675	3,360 ± 585	3,285 ± 598	0.23	0.2
Mode of delivery[a], *n* (%)					
Uncomplicated vaginal	69 (62.2 %)	81 (66.4 %)	81 (69.8 %)		
Assisted vaginal	2 (1.8 %)	4 (3.3 %)	9 (7.8 %)		
C/S after labor	23 (20.7 %)	19 (15.6 %)	19 (16.4 %)		
C/S without labor	17 (15.3 %)	18 (14.8 %)	7 (6.0 %)		
Vaginal	71 (74.7 %)	85 (79.4) %	90 (85.7 %)		
Primary C/S	24 (25.3 %)	22 (20.6) %	15 (14.3 %)	0.15	0.046
Previous PTB, *n* (%)	20 (18.0 %)	32 (26.2 %)	23 (19.7 %)	0.27	0.9
PTB < 37 weeks, *n* (%)	9 (8.1 %)	5 (4.1 %)	7 (6.0 %)	0.44	0.5
PTL < 37 weeks in this pregnancy, *n* (%)	16 (14.4 %)	22 (18.0 %)	14 (12.0 %)	0.41	0.4
PTL/PTB < 37 weeks, *n* (%)	23 (20.7 %)	24 (19.7 %)	20 (17.1 %)	0.77	0.4
Gestational diabetes, *n* (%)	8 (7.2 %)	5 (4.1 %)	3 (2.6 %)	0.25	0.1
Preeclampsia/eclampsia/gest. hypertension	9 (8.1 %)	6 (4.9 %)	3 (2.6 %)	0.16	0.05
Infection, *n* (%)	47 (42.3 %)	60 (49.2 %)	44 (37.6 %)	0.19	0.4
Any					
Bacterial	36 (32.4 %)	44 (36.1 %)	32 (27.4 %)	0.35	0.3
Viral	8 (7.2 %	6 (4.9 %)	6 (5.1 %)	0.71	0.4
Fungal	13 (11.7 %)	22 (19.0 %)	13 (11.1 %)	0.23	0.8
Comorbidity (PTB), *n* (%) (infection, PTB, gestational diabetes, preeclampsia/hypertension/HELLP)	63 (56.8 %)	67 (54.9 %)	53 (45.3 %)	0.17	0.06
Comorbidity (PTL/PTB), *n* (%) (infection, PTL/PTB < 37 weeks, gestational diabetes, preeclampsia/hypertension/HELLP)	70 (63.1 %)	72 (59.0 %)	59 (50.4 %)	0.14	0.03
Pill count pills taken: pills issued (median)	0.47	0.49	0.50	0.70	0.9

[a] Mode of delivery was categorized a priori as either vaginal (defined as spontaneous or assisted vaginal delivery, which included use of forceps or vacuum extraction) or cesarean section (C/S; further subdivided as cesarean following labor, cesarean without labor, and repeat elective cesarean). Primary cesarean section included women who had undergone a cesarean section with or without labor for either a maternal or a fetal indication and did not include women who underwent a repeat, elective cesarean section

PTB preterm birth, *PTL* preterm labor

From Hollis et al. [1]

Canada and that this deficiency was independently linked to hypertension in these women that may be regulated by flow-mediated dilation [85].

A recent observational study by Lau et al. [86] has provided us with a link between low vitamin D levels and gestational diabetes mellitus. Lau et al. [86] found that circulating 25(OH)D levels were inversely associated with fasting, 2 h blood glucose levels during glucose tolerance testing and glycated hemoglobin levels (HbA_{1c}). Multivariate analysis identified 25(OH)D and glucose levels as independent predictors of HbA_{1c}. Thus, low 25(OH)D levels were associated with poor glycemic control during pregnancy. A recent study by Parlea et al. [87] suggests that vitamin D may influence glucose tolerance during pregnancy and supports studies of vitamin D as a potential intervention to prevent gestational diabetes. Also, recent observational studies have linked vitamin D deficiency to an increase in primary cesarean section [88] and premature delivery [89].

The risk of adverse events during pregnancy was not increased in the higher-dose vitamin D supplementation groups in our recent clinical trial [1]. We evaluated women

Table 3 Number and percentage of small-for-gestational-age (SGA) infants by vitamin D treatment group

Racial group	Number of subjects	400 IU/day	2,000 IU/day	4,000 IU/day
Caucasian	111	1(0.9 %)	0	0
African American	97	6 (6.2 %)	4 (4.1 %)	5 (5.2 %)
Hispanic	137	6 (4.4 %)	1 (0.7 %)	4(2.9 %)

Data derived from Hollis et al. [1]

monthly throughout their pregnancies starting at 12 weeks' gestation. To be eligible for the study, women had to be in good general health at the time of enrollment without a history of hypertension or diabetes. When reviewing combined or cumulative comorbidities of pregnancy, the data from our RCT strongly suggest that vitamin D supplementation during pregnancy can significantly decrease complications of pregnancy including primary cesarean section ($p = 0.046$), hypertensive disorders of pregnancy ($p = 0.05$), and comorbidities of pregnancy ($p = 0.03$) [1] (Table 2).

Lack of in utero vitamin D has also been associated with abnormal brain development in experimental animals [90]. In humans, vitamin D during pregnancy has been associated with risk of schizophrenia at both low and higher levels of circulating 25(OH)D [91]. In this study, the highest quintile of circulating 25(OH)D was 51 nmol/L, and to suggest that these levels would result in increased schizophrenia rates would be puzzling indeed since levels in native Africans are at least three times this level and have been for hundreds of thousands of years [19]. In the latest evidence, with respect to offspring and neurocognitive development, Whitehouse et al. [92] demonstrated that maternal vitamin D insufficiency during pregnancy is significantly associated with offspring language impairment.

Summary

Meaningful research with regard to vitamin D supplementation in pregnant women has been hampered for decades by misconceived dietary recommendations and fear of toxicity, which have been refuted [93]. Current vitamin D intake recommendations during pregnancy range from 400–600 IU/day from the IOM report [8] to 1,500–2,000 IU/day from the Endocrine Society report [9]. The American College of Obstetricians and Gynecologists (ACOG) has chosen to follow the IOM report [94]: the ACOG document states that, "Vitamin D screening and supplementation during pregnancy is not required 'unless' women live in cold climates, reside in northern latitudes, wear sunscreen and protective clothing, are ethnic minorities, or are vegetarian." This "unless" group basically defines the entire North American and European populations. Many recent observational studies now exist that present strong evidence of the positive effects that vitamin D can provide by improving birth outcomes [78–88]. When we conceived our vitamin D supplementation pregnancy RCT in 2001, we simply asked the question, how much vitamin D would be required to increase circulating 25(OH)D levels to achieve levels that mimic those obtained due to significant solar exposure and provide levels that have been inherent in humans for most of our evolution [19, 95]? We anticipated that amount to be several thousand IUs per day. After convincing the FDA that our proposed study was designed to establish efficacy and effectiveness while minimizing risk, we received investigation drug approval, and the study was undertaken and completed. However, one of the most puzzling developments was the omission of our RCT pregnancy study from the most recent Cochrane review on vitamin D supplementation for women during pregnancy [30]. Without inclusion of our RCT, which included a control group appropriate for a large section of the world today, the world literature and the recommendations for vitamin D supplementation continue to remain at odds.

How did our assumption about natural historical human levels and supplementation turn out? The results actually are quite remarkable and provide strong evidence of the positive effects of vitamin D on birth outcomes without any hint of adverse effects. The daily intake of vitamin D to accomplish these results was 4,000 IU. Our 4,000 IU/day group achieved 111.0 nmol/L circulating 25(OH)D (Table 1), while values in traditionally living populations in East Africa are known to be 115 nmol/L [19] but significantly less than is observed in native African women who are pregnant (M. Luxwolda, personal communication). We believe that this is the circulating level of 25(OH)D we should aspire to attain, not a level based on geometric means from populations that live in sun-restricted environments, covered with clothing, and told to avoid the sun at all costs to the point of a skin mutation that maximizes limited solar irradiation [10, 16]. We believe that one should assimilate solid historical data with data generated from modern techniques [1, 19] and not be wedded to ideas that came from intuition that has simply carried forth for decades [7]. Our rigorous and well-designed RCT accessing vitamin D supplementation during pregnancy, overseen by both the National Institutes of Health and the FDA, provides clear evidence of vitamin D's role in nonskeletal health outcomes in pregnancy. Basically, it is our perspective with regard to vitamin D and pregnancy that our genome has developed to >60 ng 25(OH)D/mL (150 nmol/L) since the beginning of humankind, but we are so arrogant as to believe that levels in sun-starved humans are

"normal." Worse, we think these levels in which our genome evolved are actually harmful in some way, so we have endless data safety and monitoring boards to evaluate 25(OH)D levels that are totally natural but now in some way doing us harm. The next step in this saga will be to evaluate the evidence based on historical precedence and accumulating data that will en masse supersede previously held dogma.

References

1. Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL (2011) Vitamin D supplementation during pregnancy: double-blind, randomized clinical trial of safety and effectiveness. J Bone Miner Res 26(10):2341–2357
2. Obermer E (1947) Vitamin-D requirements in pregnancy. Br Med J 2(4535):927
3. Black J, Bonham-Carter J (1963) Association between aortic stenosis and facies of severe infantile hypercalcemia. Lancet 2:745–749
4. Friedman WF, Mills L (1969) The relationship between vitamin D and the craniofacial and dental anomalies of the supravalvular aortic stenosis syndrome. Pediatrics 43:12–18
5. Taussig HB (1966) Possible injury to the cardiovascular system from vitamin D. Ann Intern Med 65:1195–1200
6. Friedman WF (1967) Vitamin D as a cause of the supravalvular aortic stenosis syndrome. Am Heart J 73:718–720
7. Blumberg R, Forbes G, Fraser D (1963) The prophylactic requirement and the toxicity of vitamin D. Pediatrics 31:512–525
8. Food and Nutrition Board, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (2010) Dietary reference intakes for vitamin D and calcium. National Academies Press, Washington, DC
9. Holick MF, Binkley NC, Bischoff-Ferrari HA et al (2011) Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 96(7):1911–1930
10. Lamason RL, Mohideen MA, Mest JR et al (2005) SLC24A5, a putative cation exchanger, affects pigmentation in zebrafish and humans. Science 310(5755):1782–1786
11. Food and Nutrition Board, Standing Committee on the Scientific Evaluation of Dietary Reference Intakes (1997) Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. National Academies Press, Washington, DC
12. Vieth R (1999) Vitamin D supplementation, 25-hydroxy-vitamin D concentrations, and safety. Am J Clin Nutr 69:842–856
13. Blunt JW, DeLuca HF, Schnoes HK (1968) 25-Hydroxycholecalciferol: a biologically active metabolite of vitamin D_3. Biochemistry 7(10):3317–3322
14. Lawson DE, Fraser DR, Kodicek E, Morris HR, Williams DH (1971) Identification of 1,25-dihydroxycholecalciferol, a new kidney hormone controlling calcium metabolism. Nature 230(5291):228–230
15. Haddad JG, Chyu K (1971) Competitive protein-binding radioassay for 25-hydroxycholecalciferol. J Clin Endocrinal Metab 33:992–995
16. Hollis B (2005) Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. J Nutr 135:317–322
17. Clemens TL, Adams JS, Henderson SL, Holick MF (1982) Increased skin pigment reduces the capacity of skin to synthesise vitamin D_3. Lancet 1(8263):74–76
18. Vieth R (2003) Effects of vitamin D on bone and natural selection of skin color: how much vitamin D nutrition are we talking about? In: Agarwal SC, Stout SD (eds) Bone loss and osteoporosis: an anthropological perspective. Kluwer Academic/Plenum, New York, pp 139–154
19. Luxwolda MF, Kuipers RS, Kema IP, Kema IP, Janneke Dijck-Brouwer DA, Muskiet FA (2012) Traditionally living populations in East Africa have a mean serum 25-hydroxyvitamin D concentration of 115 nmol/l. Br J Nutr 23:1–5
20. Narang N, Gupta R, Jain M, Aaronson K (1984) Role of vitamin D in pulmonary tuberculosis. J Assoc Physicians India 32:185–186
21. Brooke OG, Brown IRF, Bone CDM et al (1980) Vitamin D supplements in pregnant Asian women: effects on calcium status and fetal growth. Br Med J 1:751–754
22. Brooke OG, Butters F, Wood C (1981) Intrauterine vitamin D nutrition and postnatal growth in Asian infants. Brit Med J 283:1024
23. Cockburn F, Belton N, Purvis R et al (1980) Maternal vitamin D intake and mineral metabolism in mothers and their newborn infants. Br Med J 231:1–10
24. Maxwell J, Ang L, Brooke O, Brown I (1981) Vitamin D supplements enhance weight gain and nutritional status in pregnant Asians. Br J Obstet Gynaecol 88:987–991
25. Heaney R, Davies K, Chen T, Holick M, Barger-Lux M (2003) Human serum 25-hydroxycholecalciferol response to extended oral dosing with cholecalciferol. Am J Clin Nutr 77:204–210
26. Hollis BW (1984) Comparison of equilibrium and disequilibrium assay conditions for ergocalciferol, cholecalciferol and their major metabolites. J Steroid Biochem 21:81–86
27. Hollis BW (2005) Detection of vitamin D and its major metabolites. In: Feldman D, Glorieux F, Pike J (eds) Vitamin D. Academic Press, New York, pp 932–950
28. Mallet E, Gugi B, Brunelle P, Henocq A, Basuyau J, Lemeur H (1986) Vitamin D supplementation in pregnancy: a controlled trial of two methods. Obstet Gynecol 68:300–304
29. Mahomed K, Gulmezoglu AM (2000) Vitamin D supplementation in pregnancy. Cochrane Database Syst Rev 2:CD000230
30. De-Regil LM, Palacios C, Ansary A, Kulier R, Pena-Rosas JP (2012) Vitamin D supplementation for women during pregnancy. Cochrane Database Syst Rev 2:CD008873
31. Heaney RP, Armas LA, Shary JR, Bell NH, Binkley N, Hollis BW (2008) 25-Hydroxylation of vitamin D_3: relation to circulating vitamin D_3 under various input conditions. Am J Clin Nutr 87(6):1738–1742
32. Kumar R, Cohen WR, Silva P, Epstein FH (1979) Elevated 1,25-dihydroxyvitamin D plasma levels in normal human pregnancy and lactation. J Clin Invest 63(2):342–344
33. Bouillon R, Van Assche FA, Van Baelen H, Heyns W, DeMoor P (1981) Influence of the vitamin D–binding protein on serum concentrations of $1,25(OH)_2D$. J Clin Invest 67:589–596
34. Bikle DD, Gee E, Halloran B, Haddad JG (1984) Free 1,25-dihydroxyvitamin D levels in serum from normal subjects, pregnant subjects, and subjects with liver disease. J Clin Invest 74(6):1966–1971
35. Haughton M, Mason R (1992) Immunonephelometric assay of vitamin D–binding protein. Clin Chem 38:1796
36. Walker VP, Zhang X, Rastegar I et al (2011) Cord blood vitamin D status impacts innate immune responses. J Clin Endocrinol Metab 96(6):1835–1843
37. Greer FR, Hollis BW, Napoli JL (1984) High concentrations of vitamin D_2 in human milk associated with pharmacologic doses of vitamin D_2. J Pediatr 105:61–64

38. Novakovic B, Sibson M, Ng HK et al (2009) Placenta-specific methylation of the vitamin D 24-hydroxylase gene: implications for feedback autoregulation of active vitamin D levels at the fetomaternal interface. J Biol Chem 284(22):14838–14848
39. Stevenson JC, Hillyard CJ, MacIntyre I, Cooper H, Whitehead MI (1979) A physiological role for calcitonin: protection of the maternal skeleton. Lancet 2(8146):769–770
40. Shinki T, Ueno Y, DeLuca HF, Suda T (1999) Calcitonin is a major regulator for the expression of renal 25-hydroxyvitamin D_3-1alpha-hydroxylase gene in normocalcemic rats. Proc Natl Acad Sci USA 96(14):8253–8258
41. Zhong Y, Armbrecht HJ, Christakos S (2009) Calcitonin, a regulator of the 25-hydroxyvitamin D_3 1alpha-hydroxylase gene. J Biol Chem 284(17):11059–11069
42. Zaidi M, Moonga BS, Abe E (2002) Calcitonin and bone formation: a knockout full of surprises. J Clin Invest 110(12): 1769–1771
43. Ajibade DV, Dhawan P, Fechner AJ, Meyer MB, Pike JW, Christakos S (2010) Evidence for a role of prolactin in calcium homeostasis: regulation of intestinal transient receptor potential vanilloid type 6, intestinal calcium absorption, and the 25-hydroxyvitamin D_3 1 alpha hydroxylase gene by prolactin. Endocrinology 151(7):2974–2984
44. Carneiro RM, Prebehalla L, Tedesco MB et al (2010) Lactation and bone turnover: a conundrum of marked bone loss in the setting of coupled bone turnover. J Clin Endocrinol Metab 95(4):1767–1776
45. Hamilton SA, McNeil R, Hollis BW et al (2010) Profound vitamin D deficiency in a diverse group of women during pregnancy living in a sun-rich environment at latitude 32 degrees N. Int J Endocrinol 2010:917428
46. Johnson DD, Wagner CL, Hulsey TC, McNeil RB, Ebeling M, Hollis BW (2011) Vitamin D deficiency and insufficiency is common during pregnancy. Am J Perinatol 28(1):7–12
47. Lee JM, Smith JR, Philipp BL, Chen TC, Mathieu J, Holick MF (2007) Vitamin D deficiency in a healthy group of mothers and newborn infants. Clin Pediatr 46(1):42–44
48. Sahu M, Das V, Aggarwal A, Rawat V, Saxena P, Bhatia V (2009) Vitamin D replacement in pregnant women in rural north India: a pilot study. Eur J Clin Nutr 63(9):1157–1159
49. Yorifuji J, Yorifuji T, Tachibana K et al (2008) Craniotabes in normal newborns: the earliest sign of subclinical vitamin D deficiency. J Clin Endocrinol Metab 93(5):1784–1788
50. Haliloglu B, Ilter E, Aksungar FB et al (2011) Bone turnover and maternal 25(OH) vitamin D_3 levels during pregnancy and the postpartum period: should routine vitamin D supplementation be increased in pregnant women? Eur J Obstet Gynecol Reprod Biol 158(1):24–27
51. Mahon P, Harvey N, Crozier S et al (2010) Low maternal vitamin D status and fetal bone development: cohort study. J Bone Miner Res 25(1):14–19
52. Viljakainen HT, Saarnio E, Hytinantti T et al (2010) Maternal vitamin D status determines bone variables in the newborn. J Clin Endocrinol Metab 95(4):1749–1757
53. Viljakainen HT, Korhonen T, Hytinantti T et al (2011) Maternal vitamin D status affects bone growth in early childhood—a prospective cohort study. Osteoporos Int 22(3):883–891
54. Fudge NJ, Kovacs CS (2010) Pregnancy up-regulates intestinal calcium absorption and skeletal mineralization independently of the vitamin D receptor. Endocrinology 151(3):886–895
55. Javaid M, Crozier S, Harvey N et al (2006) Maternal vitamin D status during pregnancy and childhood bone mass at 9 years: a longitudinal study. Lancet 367:36–43
56. Liu NQ, Kaplan AT, Lagishetty V et al (2011) Vitamin D and the regulation of placental inflammation. J Immunol 186(10): 5968–5974
57. Belderbos ME, Houben ML, Wilbrink B et al (2011) Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. Pediatrics 127(6):e1513–e1520
58. Bodnar LM, Krohn MA, Simhan HN (2009) Maternal vitamin D deficiency is associated with bacterial vaginosis in the first trimester of pregnancy. J Nutr 139(6):1157–1161
59. Hensel KJ, Randis TM, Gelber SE, Ratner AJ (2011) Pregnancy-specific association of vitamin D deficiency and bacterial vaginosis. Am J Obstet Gynecol 204(1):e41–e49
60. Boggess KA, Espinola JA, Moss K, Beck J, Offenbacher S, Camargo CA (2010) Vitamin D status and periodontal disease among pregnant women. J Periodontol 82(2):195–200
61. Camargo CA Jr, Ingham T, Wickens K et al (2011) Cord-blood 25-hydroxyvitamin D levels and risk of respiratory infection, wheezing, and asthma. Pediatrics 127(1):e180–e187
62. Erkkola M, Kaila M, Nwaru BI et al (2009) Maternal vitamin D intake during pregnancy is inversely associated with asthma and allergic rhinitis in 5-year-old children. Clin Exp Allergy 39(6): 875–882
63. Carroll KN, Gebretsadik T, Larkin EK et al (2011) Relationship of maternal vitamin D level with maternal and infant respiratory disease. Am J Obstet Gynecol 205(3):e211–e217
64. Brehm JM, Celedon JC, Soto-Quiros ME et al (2009) Serum vitamin D levels and markers of severity of childhood asthma in Costa Rica. Am J Respir Crit Care Med 179(9):765–771
65. Chi A, Wildfire J, McLoughlin R et al (2011) Umbilical cord plasma 25-hydroxyvitamin D concentration and immune function at birth: the Urban Environment and Childhood Asthma study. Clin Exp Allergy 41(6):842–850
66. Ramagopalan SV, Link J, Byrnes JK et al (2009) HLA-DRB1 and month of birth in multiple sclerosis. Neurology 73(24):2107–2111
67. Willer CJ, Dyment DA, Sadovnick AD, Rothwell PM, Murray TJ, Ebers GC (2005) Timing of birth and risk of multiple sclerosis: population based study. BMJ 330(7483):120
68. Salzer J, Svenningsson A, Sundstrom P (2010) Season of birth and multiple sclerosis in Sweden. Acta Neurol Scand 122(1): 70–73
69. Duley L (2009) The global impact of pre-eclampsia and eclampsia. Semin Perinatol 33(3):130–137
70. Carty DM, Delles C, Dominiczak AF (2010) Preeclampsia and future maternal health. J Hypertens 28(7):1349–1355
71. Barton JR, Sibai BM (2008) Prediction and prevention of recurrent preeclampsia. Obstet Gynecol 112(2 Pt 1):359–372
72. Rijhsinghani A, Yankowitz J, Strauss RA, Kuller JA, Patil S, Williamson RA (1997) Risk of preeclampsia in second-trimester triploid pregnancies. Obstet Gynecol 90(6):884–888
73. Meads CA, Cnossen JS, Meher S, et al. (2008) Methods of prediction and prevention of pre-eclampsia: systematic reviews of accuracy and effectiveness literature with economic modelling. Health Technol Assess 12(6):iii–iv, 1–270
74. Osmond C, Kajantie E, Forsen TJ, Eriksson JG, Barker DJ (2007) Infant growth and stroke in adult life: the Helsinki Birth Cohort Study. Stroke 38(2):264–270
75. Forsen T, Eriksson JG, Tuomilehto J, Osmond C, Barker DJ (1999) Growth in utero and during childhood among women who develop coronary heart disease: longitudinal study. BMJ 319(7222):1403–1407
76. Barker DJ, Martyn CN, Osmond C, Hales CN, Fall CH (1993) Growth in utero and serum cholesterol concentrations in adult life. BMJ 307(6918):1524–1527
77. Liu NQ, Hewison M (2011). Vitamin D, the placenta and pregnancy. Arch Biochem Biophys. doi:10.1016/j.abb.2011.11.018
78. Bodnar LM, Catov JM, Simhan HN, Holick MF, Powers RW, Roberts JM (2007) Maternal vitamin D deficiency increases the risk of preeclampsia. J Clin Endocrinol Metab 92(9):3517–3522

79. Baker AM, Haeri S, Camargo CA Jr, Espinola JA, Stuebe AM (2010) A nested case–control study of midgestation vitamin D deficiency and risk of severe preeclampsia. J Clin Endocrinol Metab 95(11):5105–5109
80. Robinson CJ, Alanis MC, Wagner CL, Hollis BW, Johnson DD (2010) Plasma 25-hydroxyvitamin D levels in early-onset severe preeclampsia. Am J Obstet Gynecol 203(4):e361–e366
81. Robinson CJ, Wagner CL, Hollis BW, Baatz JE, Johnson DD (2011) Maternal vitamin D and fetal growth in early-onset severe preeclampsia. Am J Obstet Gynecol 204(6):556.e1–556.e4
82. Wei SQ, Audibert F, Hidiroglou N et al (2012) Longitudinal vitamin D status in pregnancy and the risk of pre-eclampsia. BJOG 119:832–839. doi:10.1111/j.1471-0528.2012.03307.x
83. Bodnar LM, Catov JM, Zmuda JM et al (2010) Maternal serum 25-hydroxyvitamin D concentrations are associated with small-for-gestational age births in white women. J Nutr 140(5): 999–1006
84. Ringrose JS, PausJenssen AM, Wilson M, Blanco L, Ward H, Wilson TW (2011) Vitamin D and hypertension in pregnancy. Clin Invest Med 34(3):E147–E154
85. Harris RA, Pedersen-White J, Guo DH et al (2011) Vitamin D_3 supplementation for 16 weeks improves flow-mediated dilation in overweight African-American adults. Am J Hypertens 24(5):557–562
86. Lau SL, Gunton JE, Athayde NP, Byth K, Cheung NW (2011) Serum 25-hydroxyvitamin D and glycated haemoglobin levels in women with gestational diabetes mellitus. Med J Aust 194(7): 334–337
87. Parlea L, Bromberg IL, Feig DS, Vieth R, Merman E, Lipscombe LL (2011) Association between serum 25-hydroxyvitamin D in early pregnancy and risk of gestational diabetes mellitus. Diabet Med. doi:10.1111/j.1464-5491.2011.03550.x
88. Merewood A, Mehta SD, Chen TC, Bauchner H, Holick MF (2009) Association between vitamin D deficiency and primary cesarean section. J Clin Endocrinol Metab 94(3):940–945
89. Shibata M, Suzuki A, Sekiya T et al (2011) High prevalence of hypovitaminosis D in pregnant Japanese women with threatened premature delivery. J Bone Miner Metab 29(5):615–620
90. Kesby JP, Eyles DW, Burne TH, McGrath JJ (2011) The effects of vitamin D on brain development and adult brain function. Mol Cell Endocrinol 347(1–2):121–127
91. McGrath JJ, Eyles DW, Pedersen CB et al (2010) Neonatal vitamin D status and risk of schizophrenia: a population-based case–control study. Arch Gen Psychiatry 67(9):889–894
92. Whitehouse AJ, Holt BJ, Serralha M, Holt PG, Kusel MM, Hart PH (2012) Maternal serum vitamin D levels during pregnancy and offspring neurocognitive development. Pediatrics 129(3): 485–493
93. Hollis B, Wagner C (2004) Assessment of dietary vitamin D requirements during pregnancy and lactation. Am J Clin Nutr 79:717–726
94. ACOG Committee on Obstetric Practice (2011) ACOG Committee opinion no. 495: Vitamin D: screening and supplementation during pregnancy. Obstet Gynecol 118(1):197–198
95. Halliday TM, Peterson NJ, Thomas JJ, Kleppinger K, Hollis BW, Larson-Meyer DE (2011) Vitamin D status relative to diet, lifestyle, injury, and illness in college athletes. Med Sci Sports Exerc 43(2):335–343
96. Marya R, Rathee S, Lata V, Mudgil S (1981) Effects of vitamin D supplementation in pregnancy. Gynecol Obstet Invest 12:155–161
97. Delvin EE, Salle BL, Glorieux FH, Adeleine P, David LS (1986) Vitamin D supplementation during pregnancy: effect on neonatal calcium homeostasis. J Pediatr 109(2):328–334