10.5005/jp-journals-10009-1408

ORIGINAL ARTICLE

Maternal Vitamin D Deficiency and Fetal Growth

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ABSTRACT

There is increasing incidence of vitamin D deficiency in pregnancy in developed countries. Dark skinned women who have migrated and live in higher latitudes are at greatest risk. Vitamin D supplementation is now recommended in several countries, yet its impact on fetal growth remains unclear. Observational studies suggest a possible correlation between maternal serum vitamin D and birth weight. However, differences in birth weight can be confounded by soft-tissue growth and placental function. The effect on vitamin D on bone mineral indices using dual energy X-ray absorptiometry is difficult to ascertain in the neonatal period and therefore remains unclear. Prenatal ultrasound is a safe and practical modality for assessing skeletal growth, yet very few studies have investigated fetal growth in the context of vitamin D status: one study has demonstrated no correlation with femur length, whereas two studies have shown positive correlations with femur length and femur volume respectively. The effect of vitamin D supplementation on birth weight has been investigated in seven interventional studies. However, there is considerable methodological heterogeneity and high risk of bias among some of them. Meta-analysis of well conducted randomized controlled trials (RCTs) has not demonstrated a significant effect on birth weight. The effect of vitamin D supplementation on ultrasound markers of fetal growth has not been reported to date. In summary, there is weak evidence that maternal vitamin D status may have a positive association with measures of fetal skeletal growth. However, if such an association exists, it is not clear whether it is causal or spurious. Randomized controlled trials of vitamin D supplementation are needed, where fetal ultrasound and neonatal bone mineral indices will be reported as primary outcome measures.

Keywords: 3D ultrasound, Biometry, Femur, Vitamin D.

How to cite this article: Ioannou C. Maternal Vitamin D Deficiency and Fetal Growth. Donald School J Ultrasound Obstet Gynecol 2015;9(3):223-229.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

There has been an increased incidence of clinical and subclinical vitamin D deficiency in developed countries in recent years.[1] Observational[2] and interventional studies[3] have suggested that such deficiency in pregnancy may lead to an increased risk of obstetric complications, for instance, pre-eclampsia, gestational diabetes and primary cesarean section. However, its impact on the developing fetus is much less clear. It is possible that lack of maternal vitamin D may contribute to suboptimal skeletal growth during intrauterine life, which in turn may predispose to a raised lifetime risk of osteoporosis, as a result of fetal programming.[4]

The objectives of this review are (1) to summarize the epidemiology and physiological effects of vitamin D during pregnancy and (2) to investigate the effect of maternal deficiency and supplementation on the developing fetus.

MAIN RESULTS

Physiology of Vitamin D

Vitamin D3 (or cholecalciferol) is synthesized by human skin cells, through ultraviolet B (UVB)-induced photo conversion of 7-dehydrocholesterol.[5] It can also be obtained through a diet rich in animal and fish fat. Vitamin D2 (or ergocalciferol) is a similar compound of plant origin, which is at least three times less biologically active compared with D3, when given in supplement form.[6]

Whether endogenously synthesized or acquired through diet, vitamin D is then transformed into 25 (OH) D by the addition of a hydroxyl group at the 25′ position.[7] Although 25 (OH) D is not biologically active, it represents the body's main store of vitamin D and it is also the main circulating form which is usually bound to a carrier, the vitamin D binding protein (DBP). 25-hyxroxyvitamin D can easily be measured in human serum, using widely available biologic assay kits and it is the molecule which is commonly referred to as 'vitamin D' in everyday clinical practice.

The biologically active compound 1,25 $(OH)_2$ D is generated by the addition of a second hydroxyl group at the 1′ position, under the action of a renal hydroxylase.[8] The production of 1,25 $(OH)_2$ D is tightly coupled with its inactivation into 24,25 $(OH)_2$ D by a different 24′ hydroxylase, forming a regulatory feedback loop. As a fat soluble vitamin, 1,25 $(OH)_2$ D can enter the cell membrane through passive diffusion and then binds onto an intracellular vitamin D receptor (VDR).[9] This vitamin—receptor complex acts as a gene transcription factor[10] which promotes gene expression in several target

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tissues. The main ensuing effects include the increased provision of mineral, via intestinal absorption of calcium and phosphorus[11] and calcium retention at the kidneys;[11] a direct effect on the growth plate of long bones which promotes maturation and calcification;[12] activation of osteoblasts—which increase the cellular bone component—and of osteoclasts which are necessary for bone remodelling;[11] and finally non-skeletal effects, such as modulation of immune and inflammatory responses.[9] Those physiological actions stimulate the growth of long bones, through endochondral ossification at the level of the growth plate.

Defining an 'optimal' level of serum vitamin D has been the subject of considerable debate. In the literature deficiency and insufficiency have variously been defined using arbitrary cut-offs of <12.5 nmol/l[13], <15 nmol/l[14], <25 nmol/l[15], <27.5 nmol/l[16], <40 nmol/l[17] and <50 nmol/l[18]. There is little doubt that rickets can manifest when serum levels fall below 25 nmol/l; a syndrome characterized by growth failure and the typical appearance is costochondral beading, swelling of epiphyses and bowing of weight bearing bones, mainly of the legs. Rickets is now uncommon in the developed world with 2.9 new cases per 1,00,000 children per year in Canada.[19] Most authorities agree that 25 nmol/l is the threshold of such clinical deficiency. However, it is also well documented that an individual can maintain levels of 100 nmol/l or over, by means of adequate sunlight exposure alone.[20] Levels between 25 and 100 nmol/l therefore represent a 'gray area', where the threshold level of subclinical deficiency or 'insufficiency' is difficult to establish. There are now increasing rates of such insufficiency, which is not severe enough to manifest as rickets, but may still result in suboptimal skeletal mineralization during childhood and puberty.[11,21]

An alternative strategy for defining insufficiency is indirectly through the metabolic effects of vitamin D supplementation. Individuals with low levels of vitamin D can have reduced serum calcium and raised parathyroid hormone (PTH) levels, known as secondary hyperparathyroidism. The therapeutic effect of vitamin D administration in those individuals can be assessed by measuring the reduction of PTH following supplementation; a study has shown that there is no added benefit from supplementation when the baseline vitamin D exceeds 50 nmol/l.[22] Levels between 25 and 50 nmol/l are therefore often regarded as insufficiency. To add further confusion to this debate, recent studies have suggested that the target range of serum vitamin D for optimal health outcomes outside pregnancy should be 75 to 100 nmol/l.[23,24]

There is huge geographical, cultural and interpersonal variation in natural sunlight exposure, dietary habits and clothing practices. As a result of this variation, the relative importance of dietary acquisition over endogenous vitamin D production is different for different populations. Reduced endogenous production can be caused by decreasing UVB exposure, as in the case of higher latitude, living indoors and clothing habits that limit skin exposure. Dietary acquisition of vitamin D then becomes an increasing necessity in order to maintain optimal bone health. Yet the dietary sources of vitamin D are fairly limited: oily fish, fortified margarines and some breakfast cereals, as well as smaller amounts in red meat and egg yolk.[25] Populations at risk include dark skinned individuals[26] who migrate and live at higher latitude;[27] strict vegetarians;[28] those who systematically avoid direct sunlight exposure;[26] non-supplemented, exclusively breastfed infants;[29] and pregnant women,[11] especially those from any of the above risk groups.

Vitamin D Insufficiency during Pregnancy

The dual role of vitamin D during pregnancy is to maintain maternal skeletal health while at the same time facilitating the mobilization of mineral in order to support the developing fetus. Maternal serum levels of 25 (OH) D generally remain stable during gestation in the absence of supplementation.[1,30] In contrast, the concentration of the biologically active form 1,25 $(OH)_2$ D in the mother doubles between the first and third trimesters.[30] Increased availability of the active form drives increased maternal mineral absorption, which in turn supports the fetal calcium accrual, from approximately 50 mg/day at around 20 weeks, to an average of 250 mg/day during the third trimester.[31]

It is difficult to estimate the net effect of these changes on the maternal skeleton. Whilst a study of pre- and post-pregnancy bone mineral density measured by dual emission X-ray absorptiometry (DEXA) did not show any significant difference,[30] a study of maternal bone density using quantitative calcaneal ultrasound (QUS) demonstrated a slight but significant bone mass reduction during the third trimester.[32]

Observational studies of serum vitamin D concentrations during gestation are indicative of high prevalence of subclinical vitamin D insufficiency in developed countries. Dark skinned women living at higher latitudes are at greater risk: 83% of pregnant Pakistani women living in Oslo have a vitamin D less than 30 nmol/l;[27] similarly 80% of veiled or dark-skinned pregnant women at their first antenatal appointment in Melbourne have concentrations less than 22.5 nmol/l.[26] In the context of an interventional study of vitamin D supplementation in South Wales, 50% of a cohort of non-caucasian pregnant women had levels <20 nmol/l prior to supplementation.[33] These reports are

heavily selected as they contain samples of women at the highest risk of antenatal vitamin D deficiency.

Unselected population studies in the US[1], UK[34] and Australia[35] demonstrate an overall prevalence of vitamin D deficiency in pregnancy (<25 nmol/l) between 7 and 18%; vitamin D insufficiency and deficiency combined (<50 nmol/l) range from 33 to 49%. Amongst non-supplemented pregnant women in the US, the rate of insufficiency reaches 66%, compared with 49% in matched, non-pregnant controls.[1] This demonstrates that pregnancy itself puts an added burden on the low vitamin D stores of a significant proportion of women in childbearing age.

It is evident that pregnant women may benefit from vitamin D supplementation, with the objective of optimizing their own skeletal health during pregnancy. Data from interventional[36-38] as well as observational studies[1] confirm that vitamin D supplementation during pregnancy increases maternal serum and neonatal cord vitamin D concentrations. Adequate supplementation for deficient women is likely to necessitate at least 25 µg (1000 IU) daily and should start ideally in early, rather than late, gestation.[33] The Canadian Pediatric Society now recommends routine supplementation for pregnant and lactating women with 50 µg (2000 IU) of vitamin D daily.[39] In 2008 the UK National Institute for Clinical Excellence endorsed supplementation of high-risk pregnant women with 10 µg (400 IU) daily;[25] this recommendation initially applied to women of South Asian, African, Caribbean or Middle Eastern family origin; those who have limited exposure to sunlight (housebound or covered when outdoors); those whose diet is low in oily fish, eggs, meat, vitamin D-fortified margarine or breakfast cereal; and those with a prepregnancy BMI of above 30.[25] However, in 2012 the Chief Medical Officers in all four UK countries recommended that all pregnant women should receive 10 µg (400 IU) daily.

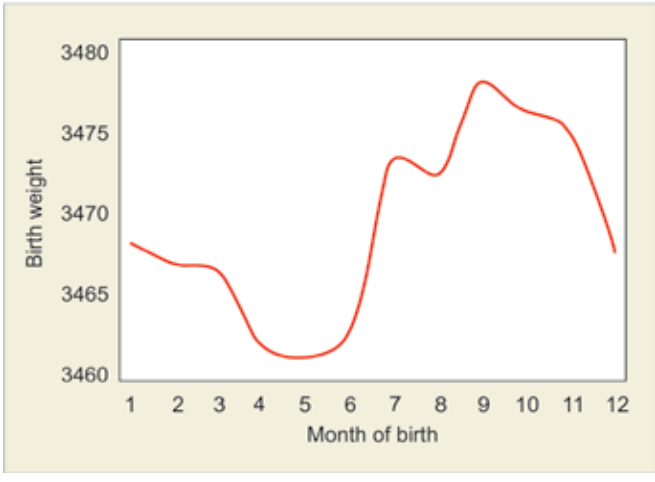
The Impact of Vitamin D on Fetal Growth

Although vitamin D insufficiency is common during pregnancy, there are limited data about its effect on the fetus. Potential markers of fetal skeletal growth include neonatal indices, such as birth weight and neonatal bone mineral content (BMC) or bone mineral density (BMD); and prenatal ultrasonographic markers, such as femur length (FL) or more recently the femur volume (FV).

Birth weight has often been used as a surrogate marker of neonatal skeletal growth, given that there is a strong correlation between birth weight and BMC.[40] There is a well-established seasonal variation of birth weight which could be explained by seasonal changes in sunlight exposure and maternal vitamin D levels. Studies from the northern and southern hemispheres, have demonstrated that population birth weights vary throughout the year, with a seasonal periodicity of approximately 30 gm. For instance, data from the Queensland perinatal register[41] indicate that babies born in the winter months (June to October) are heavier than those born during the summer (January to May); similarly, babies born in Northern Ireland during the winter months (January to April) are heavier than those born during the summer (June to September) (Graph 1).[42] It can be inferred that sunlight exposure in early pregnancy—rather than at the time of birth—replenishes the maternal vitamin D stores and subsequently stimulates fetal skeletal growth during the remainder of the pregnancy. A survey of 971 pregnant women in Australia[35] demonstrated such a seasonal periodicity of their serum vitamin D levels; higher mean concentrations were noted in the sunny months of January to May. Babies born to vitamin D deficient mothers also had lower birth weight, with an adjusted mean difference of 151 gm.

The gold standard measure of neonatal skeletal growth is BMC and BMD, which can be assessed postnatally using dual energy X-ray absorptiometry (DEXA).[43] However, this radiological technique involves a small amount of radiation exposure and has not been widely used in the literature to date. A cohort of 198 mothers and their children were followed-up postnatally at the age of 9, where a DEXA scan of the child was performed; it was demonstrated that maternal vitamin D insufficiency during pregnancy was associated with reduced BMC in childhood.[34] It is not clear whether this difference could be attributed to altered growth as a result of intrauterine lack of vitamin D; or whether it was the consequence of postnatal nutritional deficiencies.

Femur length (FL) is the most widely used ultrasonographic marker of fetal bone growth, but no correlation between FL and maternal vitamin D level was found on a cohort of 424 women from Southampton, UK.[44] However



Graph 1: Yearly variation of birth weights according to the month of birth in Queensland, Australia; a peak is noted during the winter months June to December

a positive correlation was demonstrated more recently in 171 pregnant adolescents from the US[45] where higher maternal vitamin D levels were associated with longer fetal femurs.

Femur volume (FV) measurement can be performed using multiplanar tracing on 3D ultrasound.[46] However, this technique has been shown to have poor interobserver agreement.[47] A simple alternative method has been proposed and validated,[48] which consists of three linear measures: the FL obtained from the standard 2D femoral plane (a plane of the 3D volume), the proximal metaphysis diameter (PMD) and mid-shaft diameter (MSD) obtained from the reconstructed C plane and a volume equation. Using this method, a positive correlation was demonstrated between maternal vitamin D and FV; this correlation was mediated primarily through a significant positive effect on the PMD, but not the FL. This finding suggests that a possible fetal anabolic effect of vitamin D may be more evident in long bone girth that length.

Interventional Studies: The Fetal Effect of Vitamin D Supplementation

Animal experimental data support the hypothesis that vitamin D supplementation may improve fetal skeletal growth: administration of vitamin D to female pregnant rats was shown to increase the dry tibial weight, tibial ash weight and the whole body weight of the 28-day old offspring.[49]

However, the evidence in humans is less clear. There is no evidence at present to suggest that vitamin D supplementation has any measurable impact of fetal ultrasound indices.

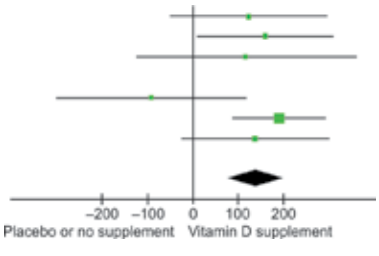
A single, non-randomized, cohort study assessed the neonatal BMC between supplemented and non-supplemented pregnant women; an unvalidated method of radioisotope absorptiometry of the baby's forearm was used, but this did not demonstrate any difference between the study groups.[50]

The only existing evidence regarding the fetal effect of supplementation in pregnancy is using birth weight as outcome measure: we have identified 7 interventional studies[3, 36-38, 50-52] and one Cochrane systematic review.[53] The latter has concluded that there is no significant effect of vitamin D supplementation on birth weight. Considerable methodological heterogeneity, conflicting results and a high risk of bias among some of the primary studies are making their interpretation difficult.

One of the primary studies reported no significant difference in birth weight but did not provide the birth weight data.[37] A meta-analysis of birth weight data of all remaining six studies would indicate a significant increase of birth weight in the group of vitamin D supplemented women, with a mean difference of 138 gm (Table 1). However, only three of the included studies are well-described randomized controlled trials (RCTs)[3,36,38] whereas the other three are cohort or interventional studies[50-52] with high risk of bias. Separate analysis of those three RCTs (Table 2) does not show any significant difference in birth weights between babies of supplemented mothers and those receiving placebo: one study found a non-significant trend for reduced birth weight in the supplementation group[38] and two studies showed a trend for increased birth weight.[3,36] In one study there was a significant increase of infant weight at 1 year of age in children from supplemented pregnancies at their postnatal follow-up.[54] There was also a significant reduction of the anterior fontanelle surface area measured clinically at birth,[36] suggesting that babies after supplementation had better ossified fontanelles. This is an interesting finding since there is

Table 1: Meta-analysis of trials of vitamin D supplementation; all studies included

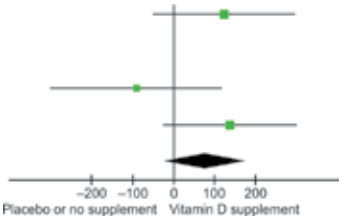
Study or subgroup	Vitamin D supplement Mean	SD	Total	Placebo or no supplement Mean	SD	Total	Weight	Mean difference IV, fixed, 95% CI	Years	Mean difference IV, Fixed, 95% CI
Brooke (1000 IU)	3,157	468	59	3,034	523	67	13.2%	123.00 (–50.04, 296.04]	1980	
Marya (1200 IU)	2,890	320	25	2,730	360	75	17.6%	160.00 [10.43, 309.57]	1981	
Congdon (1000 IU)	3,173	471	19	3,056	396	45	6.8%	117.00 [–124.33, 358.33]	1983	
Delvin (1000 IU)	0	0	0	0	0	0		Not estimable	1986	
Mallet (1000 IU)	3,370	367	21	3,460	377	29	9.1%	–90.00 [–298.48, 118.48]	1986	
Marya (600,000 IU)	2,990	360	100	2,800	370	100	38.5%	190.00 [88.82, 291.18]	1988	
Hollis (2000 IU)	3,360	585	122	3,222	675	111	14.9%	138.00 [–24.92, 300.92]	2011	
Total (95% CI)			346			427	100.0%	137.82 [75.03, 200.62]		



Heterogeneity: $Chi^2 = 5.75$, df = 5 (p = 0.33); $I^2 = 13\%$; Test for overall effect: Z = 4.30 (p < 0.0001)

Table 2: Meta-analysis of trials of vitamin D supplementation; only studies at low risk of bias included

Study or subgroup	Vitamin D supplement Mean	SD	Total	Placebo or no supplement Mean	SD	Total	Weight	Mean difference IV, fixed, 95% CI	Years	Mean difference IV, fixed, 95% CI
Brooke (1000 IU)	3,157	468	59	3,034	523	67	35.5%	123.00 (–50.04, 296.04]	1980	
Marya (1200 IU)	2,890	320	25	2,730	360	75	0.0%	160.00 [10.43, 309.57]	1981	
Congdon (1000 IU)	3,173	471	19	3,056	396	45	0.0%	117.00 [–124.33, 358.33]	1983	
Delvin (1000 IU)	0	0	0	0	0	0		Not estimable	1986	
Mallet (1000 IU)	3,370	367	21	3,460	377	29	24.5%	–90.00 [–298.48, 118.48]	1986	
Marya (600,000 IU)	2,990	360	100	2,800	370	100	0.0%	190.00 [88.82, 291.18]	1988	
Hollis (2000 IU)	3,360	585	122	3,222	675	111	40.0%	138.00 [–24.92, 300.92]	2011	
Total (95% CI)			202			207	100.0%	76.92 [–26.18, 180.02]		



Heterogeneity: $Chi^2 = 3.27$, df = 2 (p = 0.19); $I^2 = 39\%$; Test for overall effect: Z = 1.46 (p = 0.14)

no direct physiological evidence to suggest that vitamin D plays any role in endomembranous bone homeostasis; however it is also known that aggressive treatment with vitamin D in X-linked hypophosphatemic rickets can cause premature suture obliteration.[55] It is possible that vitamin D may exert an effect on fontanelle size indirectly as a result of increased mineral provision.

DISCUSSION

This review has demonstrated that, according to observational studies,[35,45,48] a positive correlation may exist between maternal vitamin D and fetal skeletal growth. Nevertheless, interventional studies of vitamin D supplementation in pregnancy[3,36-38,50-52] have not yet conclusively demonstrated any such effect on fetal indices.

One of the difficulties is how the effects of maternal vitamin D status on fetal development may be measured in order to be identified. A postnatal outcome measure, such as birth weight is easy to obtain, but it is a crude measure and can be confounded by the effect of soft-tissue growth and placental function. Conversely, specific skeletal markers, such as BMC or BMD should represent the gold standard method of assessing bone size and mineralization in the neonate. However, DEXA scans in the neonatal period are technically challenging; the earliest these could be performed is several days or weeks after the birth and therefore their results can be confounded by postnatal weight gain and nutrition; excessive movement of the newborn may also introduce considerable measurement error.

Ultrasound measures represent a suitable alternative, free of radiation exposure and acceptable to pregnant women. A correlation between vitamin D and FL[45] or FV[48] has been demonstrated in observational studies; however it is always difficult to establish the causal pathway in such observational associations. Furthermore when attributing increased bone length or bone volume to the action of maternal vitamin D, an underlying assumption is made that such an increase is beneficial. However it is possible that bigger size does not always correspond to improved mineralization. In other words, one may speculate that if skeletal size increases in the absence of sufficient mineralization, then bone 'quality' may be inferior. Finally, it should be highlighted that the effect of vitamin D on FL or FV—whether real or spurious—is likely to be clinically small. Nevertheless, ultrasound measures, such as FL and FV are simple, non-invasive biometric indices; they do not carry radiation risk; and they could be used as outcome measures in interventional trials of vitamin D supplementation aimed at optimizing skeletal health in pregnancy. The paucity of ultrasound data in existing vitamin D trials is the unfortunate reflection of the fact that almost all of these were published in the early 1980s, before FL became an established tool for everyday fetal biometry. As the argument strengthens for vitamin D supplementation, more randomized trials are needed in order to answer the question regarding its effect on the developing fetus.

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