VITAMIN D AND HUMAN PREGNANCY

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INTRODUCTION

At the end of 2007, Time magazine listed the “benefits of vitamin D” as one of its top 10 medical breakthroughs for that year. Since then there has been a remarkable upsurge of interest in vitamin D, with new research advances seemingly published on a weekly basis. In particular, there has been increasing awareness of the variability of vitamin D status in populations across the globe and, significantly, a growing debate about the need for revised parameters for vitamin D supplementation. Although sub-optimal vitamin D is likely to be a widespread problem for 21st century societies, it is also clear that some groups are at much greater risk of low vitamin D status. Prominent amongst these are pregnant women and the aim of the following review article will be to discuss this problem in further detail with specific emphasis on its potential physiological and clinical impact.

Two key factors have underpinned the recent expansion of interest in vitamin D. The first stems from new insights into the parameters for defining ‘normal’ vitamin D status. Until recently adequate vitamin D status in a patient was defined by the presence or absence of the bone disease rickets (osteomalacia in adults). Based on this guideline, serum levels of the main circulating form of vitamin D (25-hydroxyvitamin D, 25OHD) less than 8 ng/ml (20 nM) were considered to represent vitamin D-deficiency, and concentrations above this were considered to be ‘normal’. However, more recent observations have shown that circulating levels of parathyroid hormone (PTH), and intestinal calcium uptake continue to show correlation with serum 25OHD at concentrations as high as 30 ng/ml (75 nM). Thus, it was concluded that optimal vitamin D status was actually represented by a much higher serum level of 25OHD than previously thought, namely greater than 30 ng/ml. This has prompted the introduction of a new term – vitamin D ‘insufficiency’ – defined by serum levels of 25OHD that are sub-optimal (<75 nM) but not necessarily rachitic (<20 nM).

As a result of the new parameters for vitamin D status, it has become evident that sub-optimal vitamin D status is much more common than previously thought. Although this is likely to be a clinical problem for populations across the globe, it is also clear that some groups will be at greater risk of low serum 25OHD than
others. This includes individuals with darker skin pigmentation for whom epidermal synthesis of vitamin D requires much more exposure to ultra-violet (UV) light than those with lighter skin pigmentation. Likewise, because of the curvature of the Earth, the efficacy of UV-induced cutaneous synthesis of vitamin D will be impaired at more northerly or southerly latitudes. This provides an explanation for the low levels of serum 25OHD that have been reported in countries such as the UK, particularly for those individuals who also have darker skin pigmentation. In addition to geographical and pigmentation considerations, it has been recognized that certain population subgroups are also at greater risk of vitamin D-insufficiency, including elderly subjects and pregnant women. The current review will focus specifically on the latter by discussing the potential impact of vitamin D on maternal and fetal physiology during pregnancy.

The second research advance to shape our current perspective on vitamin D concerns the potential physiological impact of vitamin D-insufficiency. Given the classical actions of vitamin D on calcium homeostasis and bone metabolism, it is likely that sub-optimal vitamin D status will exert some effects on the skeleton, although these may not be identical to the rachitic bone disease observed with frank vitamin D-deficiency. However, recent studies have focused on the so-called ‘non-classical’ effects of vitamin D. These include anticancer and cardiovascular actions but prominent reports have also explored the link between vitamin D and the immune system. It seems likely that vitamin D will induce pluripotent effects during pregnancy that extend far beyond its established actions of calcium homeostasis and bone metabolism.

RENA L AND EXTRA-RENAL VITAMIN D METABOLISM

As outlined in the preceding section and in Figure 1, classical vitamin D endocrinology involves interaction between the parathyroid glands, the kidneys and bone. Under conditions of low extracellular calcium, calcium-sensing receptors on parathyroid cells signal to increase PTH secretion. The resulting rise in circulating levels of PTH serves to stimulate PTH receptors on the surface of proximal tubule epithelial cells and subsequent cAMP signaling then upregulates transcription of the renal enzyme vitamin D-1α-hydroxylase (CYP27B1). Located in the inner mitochondrial membrane, CYP27B1 catalyzes conversion of the major circulating form of vitamin D, 25OHD, to active 1,25-dihydroxyvitamin D (1,25(OH)2D). This process is tightly regulated and involves the facilitated uptake of 25OHD by proximal tubule cells expressing the protein megalin, which acts as a cell surface receptor for the serum vitamin D binding protein (DBP). The latter is an abundant multifunctional protein structurally related to albumin and α-fetoprotein. Almost all vitamin D metabolites circulate bound to either DBP [high affinity for vitamin D ligands] or serum albumin [high abundance but low affinity for vitamin D ligands]. In proximal tubule cells a complex of 25OHD-DBP-megalin is internalized through endocytosis, thereby providing substrate for the renal CYP27B1. The 1,25(OH)2D synthesized in the kidney from 25OHD does
Vitamin D and Human Pregnancy 69

Figure 1  Vitamin D metabolism: classical endocrinology versus placental function. Schematic representation showing key pathways associated with the metabolism and action of vitamin D in the setting of normal renal endocrinology and in the placenta during pregnancy. The vitamin D-activating enzyme 1α-hydroxylase (CYP27B1) is expressed in the kidney proximal tubules, and in decidua and trophoblast. Renal CYP27B1 is induced by parathyroid hormone (PTH), and converts 25-hydroxyvitamin D (25OHD) to 1,25-dihydroxyvitamin D (1,25(OH)₂D). The latter is released into the general circulation but also induces renal 24-hydroxylase (CYP24A1) activity, leading to feedback synthesis of the less active metabolites, 1,24,25-trihydroxyvitamin D (1,24,25(OH)₃D) and 24,25-dihydroxyvitamin D (24,25(OH)₂D). Raised serum 1,25(OH)₂D acts to: enhance phosphate and calcium uptake in the intestine; modulate bone-forming osteoblasts (OB) and bone-resorbing osteoclasts (OC); suppress synthesis of PTH by the parathyroid glands. 1,25(OH)₂D also stimulates expression of fibroblast growth factor 23 (FGF23) which suppresses renal CYP27B1 activity. In the placenta, 25OHD is converted to 1,25(OH)₂D in both maternal and fetal tissues. Lack of CYP24A1 activity in the placenta (particularly in the fetal trophoblast) leads to enhanced local concentrations of 1,25(OH)₂D, and possible spill-over into maternal circulation.

Several things. Within the kidney, 1,25(OH)₂D can induce expression of the vitamin D feedback control enzyme vitamin D-24-hydroxylase (CYP24A1) which converts 1,25(OH)₂D or 25OHD to the less active metabolites 1,24,25-trihydroxyvitamin D (1,24,25(OH)₃D) and 24,25(OH)₂D respectively. Renal CYP27B1 activity also enhances circulating levels of 1,25(OH)₂D which can then act on peripheral tissues such as the intestine and skeleton. These endocrine actions of 1,25(OH)₂D are dependent on expression of the intracellular vitamin D receptor (VDR) in target cells, with the liganded VDR then acting as a transcription factor when bound to vitamin D.
D response element DNA motifs within gene promoters\textsuperscript{21}. VDR-mediated responses also provide another level of feedback control for the vitamin D system, with serum 1,25(OH)\textsubscript{2}D acting to negatively regulate production of PTH\textsuperscript{21}. Moreover, in another feedback control mechanism, 1,25(OH)\textsubscript{2}D stimulates expression of fibroblast growth factor 23 (FGF23) in bone, with the resulting rise in serum FGF23 acting as a potent suppressor of renal CYP27B1 expression\textsuperscript{22} [see Figure 1].

Whilst the kidney clearly acts as the main endocrine source of 1,25(OH)\textsubscript{2}D, studies over the last twenty five years have shown that many other tissues express the CYP27B1 enzyme. Awareness of extra-renal synthesis of 1,25(OH)\textsubscript{2}D initially stemmed from studies of patients with granulomatous diseases such as sarcoidosis, where macrophages were shown to act as an extra-renal source of CYP27B1\textsuperscript{23}. In this instance the localized production of 1,25(OH)\textsubscript{2}D in peripheral tissues affected by the disease was sufficient to spill-over into the general circulation and, in some instances, promote dysregulation of calcium homeostasis\textsuperscript{24}. However, expression of CYP27B1 has also been reported for other extra-renal tissues in the absence of any disease\textsuperscript{25}. Historically, the placenta was one of the first of these extra-renal tissues shown to be capable of synthesizing 1,25(OH)\textsubscript{2}D, with CYP27B1 activity being detectable in both maternal decidua and fetal trophoblast\textsuperscript{26,27}. Since then we have characterized the spatio-temporal organization of placental CYP27B1 and VDR across gestation, confirming that the enzyme and receptor are localized to both the maternal decidua and fetal trophoblast.\textsuperscript{28,29} The physical proximity of these proteins is consistent with a localized function of vitamin D in the placenta, with 1,25(OH)\textsubscript{2}D synthesized by decidual or trophoblastic cells acting in an autocrine or paracrine fashion\textsuperscript{29}. Such a mechanism would be similar to that previously described for the expression of CYP27B1 and VDR within cells from the immune system\textsuperscript{15}.

The importance of placental CYP27B1 as a feature of the vitamin D system during pregnancy has been underlined by studies of the CYP27B1 knockout mouse. In these animals the CYP27B1 gene is replaced with a beta-galactosidase reporter construct linked to the endogenous gene promoter for CYP27B1, making it possible to visually demonstrate CYP27B1 transcription in mouse tissues by simply staining for beta-galactosidase activity\textsuperscript{30}. Studies carried out in this way confirmed expression of CYP27B1 in the kidney as would be expected from classical vitamin D endocrinology. However, only one other tissue showed significant levels of CYP27B1 transcription, and this was the placenta\textsuperscript{30}. The capacity for highly efficient synthesis of 1,25(OH)\textsubscript{2}D in the placenta has been underlined by studies of the feedback control enzyme CYP24A1 which acts to catabolize vitamin D metabolites to less potent forms\textsuperscript{31}. Expression studies have shown decreased levels of mRNA for CYP24A1 during early stages of gestation\textsuperscript{29}. An explanation was provided by more recent DNA analyses indicating that the CYP24A1 gene is highly methylated in the placenta, resulting in the silencing of CYP24A1 expression\textsuperscript{32}. Further studies revealed that this effect is very selective, and suggests that the placenta is one of the few tissues in which feedback regulation of 1,25(OH)\textsubscript{2}D response is effectively switched off\textsuperscript{32}. The net effect of suppressing CYP24A activity in such a way would be to enhance placental
concentrations of 1,25(OH)2D, but it is also possible that the rise in placental 1,25(OH)2D levels will be significant enough to spill-over into the fetal or maternal circulation. Thus, opposing patterns of transcription for placental CYP27B1 and CYP24A1 may provide a potent system for optimizing vitamin D activity during pregnancy (see Figure 1).

VITAMIN D AND THE REGULATION OF MINERAL HOMEOSTASIS DURING PREGNANCY

Adults depend heavily on circulating concentrations of vitamin D metabolites for metabolism of calcium and bone. Without the active form of vitamin D, 1,25(OH)2D, the body cannot absorb calcium and phosphorus adequately, resulting in hypocalcemia and secondary hyperparathyroidism associated with skeletal calcium absorption. However, calcium demands in pregnancy are high, to aid in both fetal and neonatal development and growth. As a consequence, many maternal adaptations are observed in pregnancy that supports the need for this extra calcium. In contrast to the situation in adult, the regulation of mineral homeostasis during pregnancy appears to be less dependent on vitamin D33.

Calcium is actively transported across the placenta34,35 and human fetuses typically accrete 21g-30g of calcium by term, and 80% of this is accumulated in the third trimester, indicating a daily transfer requirement of approximately 200mg of calcium33,36. To attain the required amount of calcium and regulate the fetal calcium level, the fetus makes use of the placenta, kidneys, bone and intestine. The fetal-placental unit is seen to function relatively independently of the mother, and the fetal blood calcium level is maintained at a higher level than in the maternal circulation37–39. Evidence from rat and mouse models suggests that the fetus sets a blood calcium level rather than establishing a particular gradient; this level is independent of the maternal calcium level40. The site of the active transport is likely to be at the fetus-facing basement membrane of the syncytiotrophoblast cells in the human and at the trophoblast cells and the basal surface of the endoderm of the intraplacental yolk sac in rodents41,42.

Doubling of intestinal calcium absorption starts in the first trimester of early human pregnancy43–45, and remains increased in all trimesters46,47. This was initially thought to be mediated by 1,25(OH)2D, as it is known that serum levels of 1,25(OH)2D increase early in pregnancy and peak at twice the non-pregnant range33,43,48–53. However, free concentrations of 1,25(OH)2D appear to increase only during the third trimester, and the peak in intestinal calcium absorption occurs well before this54,55. The increase in maternal 1,25(OH)2D is primarily mediated by increased renal activity of the enzyme CYP27B1, but expression and activity of CYP27B1 has also been reported in extra-renal tissues, notably in maternal decidua, placenta and also in the fetal kidneys26,27,56–61. Reports differ as to how much these sources contribute to the circulating levels of 1,25(OH)2D, but it is generally thought to be minimal54.

Animal studies have shown that 1,25(OH)2D clearance during pregnancy remains consistent with pre-pregnancy levels, indicating that the raised maternal levels are
due to increased production of the hormone rather than decreased excretion. This increase of 1,25(OH)₂D is independent of a rise in maternal PTH which remains in the low-normal range in the 1st trimester. Studies have shown that CYP27B1 activity can be regulated by other sources including parathyroid hormone-related protein [PTHrP], oestradiol, prolactin [PRL] and placental lactogen, all of which are increased in pregnancy and which may help explain the rise in 1,25(OH)₂D independent of PTH responses. Interestingly, 1,25(OH)₂D does not readily cross the placenta. Fetal levels are generally lower than maternal levels and are associated with low concentrations of fetal PTH and high phosphorus. Other studies have shown that 1,25(OH)₂D does not readily cross the placenta in rats, suggesting that circulating levels of 1,25(OH)₂D in the fetus are derived largely from fetal sources.

The fetal kidneys and placenta express CYP27B1 and can thus metabolize 25-OHD to active 1,25(OH)₂D. Fetal nephrectomy has been shown to lower fetal 1,25(OH)₂D levels in sheep and rats, suggesting a significant contribution from the fetal kidneys to circulating levels of active vitamin D. Fetal blood levels of 1,25(OH)₂D are typically lower than maternal levels in humans, but umbilical artery levels of 1,25(OH)₂D are slightly higher than umbilical venous levels, endorsing the contribution of the fetal kidneys. Precursor 25OHD readily crosses the haemochorial placentas of rats, so that fetal blood levels of 1,25(OH)₂D are typically lower than maternal levels in humans, but umbilical artery levels of 1,25(OH)₂D are slightly higher than umbilical venous levels, endorsing the contribution of the fetal kidneys.

Evidence from several animal models indicates that 1,25(OH)₂D is not necessary for normal fetal calcium homeostasis and bone mineralization. In pregnant rats, sheep and pigs that were hypocalcemic due to severe vitamin D deficiency, the fetuses maintained completely normal blood calcium and phosphate levels and had fully mineralized skeletons at term. The Hannover pig model showed that the fetuses of homozygous 1,25(OH)₂D-deficient sows also maintained completely normal blood calcium and phosphate levels and fully mineralized their skeletons. Also, fetal mice that lack the gene encoding the VDR are born with normal skeletons. Human studies have also shown that at term the cord blood calcium and skeletal mineralization is completely normal in fetuses of vitamin D-deficient mothers. The role of 1,25(OH)₂D in fetal-placental calcium transport is also unclear. Receptors for 1,25(OH)₂D [VDR] are present in the placentas of humans, rats, and sheep and might therefore have a role in placental calcium physiology. In placental perfusion models in rats, guinea pigs and sheep, pharmacological doses of 1,25(OH)₂D or 1α,25(OH)₂D increased the fetal blood calcium, transport of calcium across the placenta, and the mineral content of fetuses. Prior nephrectomy in fetal sheep reduced calcium transfer in the placental perfusion model which could only be partly restored by administration of 1,25(OH)₂D.

Although the fetal parathyroid glands are capable of synthesizing PTH early in gestation, intact PTH does not cross the placenta of primates, sheep, and rodents and probably does not cross the human placenta. Fetal parathyroidectomy in sheep and fetal decapitation in rats caused hypocalcemia, indicating that the fetal parathyroid glands contribute to calcium homeostasis, by secreting PTH and PTHrP. PTHrP is believed to be a prohormone which is processed into several
different circulating fragments or hormones and activates the common PTH/PTHrP receptor. Amino-terminal forms of PTHrP (PTHrP 1–34, 1–86 or 1–141) resemble PTH in their actions on kidney and bone and a mid-molecular form of PTHrP stimulates placental calcium transport in the fetus. The carboxy-terminal portion of PTHrP is able to inhibit osteoclastic bone reabsorption in some in-vitro assays, and rats in vivo. This fragment of PTHrP is therefore thought to have a role in protecting the maternal skeleton during pregnancy. PTHrP levels are significantly higher in human cord blood than in maternal circulation at term. PTH cannot cross the placenta and it has been suggested that PTHrP cannot either, though circulating levels are found in the fetus, as it is produced at many sites throughout the developing embryo and fetus, including the fetal parathyroid glands, skeletal growth plate, trophoblast cells of the placenta, amnion, chorion, umbilical cord. All of these sites may contribute to the circulating levels of PTHrP in the fetus and may therefore be relevant to fetal calcium and bone metabolism. Higher venous umbilical PTHrP levels have been identified in comparison to arterial umbilical levels in pigs, indicating that the placenta may be an important source of PTHrP in the fetus. PTHrP thus appears to regulate the fetal blood calcium as well as fetal-placental calcium transport.

The rise in intestinal absorption of calcium occurs by mid-pregnancy in rats, before the onset of rapid skeletal mineralization in the fetus. This early increase in intestinal calcium absorption may allow the pregnant mother to accrete calcium before the peak fetal demand late in pregnancy. Inadequate accumulation of calcium early in pregnancy may lead to a net loss of maternal skeletal calcium in later pregnancy. Maternal vitamin D deficiency has been found to cause maternal skeletal demineralization by the end of pregnancy. The markers of bone formation and reabsorption in humans indicate that bone turnover is probably low in the first half of pregnancy, but maybe increased in the third trimester. The third trimester increase in bone turnover corresponds to the time of the peak rate of calcium transfer to the fetus and may result from mobilization of skeletal calcium stores to help supply the fetus.

**ALTERNATIVE ACTIONS FOR VITAMIN D DURING PREGNANCY**

It was assumed for many years that the rise in maternal serum 1,25(OH)₂D that occurs at the end of the first trimester of pregnancy is linked to maternal calcium homeostasis. However, studies of CYP27B1-deficient animals and an anephric pregnant woman suggest that the maternal renal vitamin D system is still likely to be the major contributor to the gestational rise in serum 1,25(OH)₂D. Instead, the presence of VDR in the placenta has highlighted potential autocrine or paracrine functions for vitamin D at the fetal-maternal interface. One possible explanation is that 1,25(OH)₂D acts as a locally synthesized regulator of placental calcium transport, but an immunmodulatory function has also been proposed. The latter is supported by other facets of placental vitamin D function. For example,
within maternal decidua, expression of CYP27B1 is not restricted to decidualized stromal cells but is also detectable in decidual macrophages, endorsing a possible immunomodulatory function for localized synthesis of 1,25(OH)\textsubscript{2}D\textsuperscript{28}. Indeed, the heterogeneous cells that make up the placenta suggest that there may be many other immunological targets for vitamin D within this tissue. Maternal and fetal cells are able to mediate innate\textsuperscript{111–113} and adaptive\textsuperscript{114,115} immunity, and thus it is possible that vitamin D will exert effects on a variety of responses during pregnancy, including implantation, as well as responses to infection and inflammation. Indeed, expression of VDR and CYP27B1 has been reported in many tissues that can be broadly termed ‘barrier sites’\textsuperscript{116,117}, indicating that localized responses to vitamin D may be a key feature of these tissues.

Despite its long-standing association with rickets and osteoporosis, vitamin D has increasingly become recognized as a pluripotent regulator of biological functions beyond its classical effects on bone/calcium homeostasis. In particular, 1,25(OH)\textsubscript{2}D has been shown to be a key modulator of immune responses\textsuperscript{118,121}. Both the receptor for 1,25(OH)\textsubscript{2}D, VDR,\textsuperscript{119,122,123} and CYP27B1,\textsuperscript{117,118,124} are expressed by cells from the immune system. The presence of CYP27B1 in macrophages\textsuperscript{125} and dendritic cells\textsuperscript{126} indicates that local (autocrine or paracrine) synthesis of 1,25(OH)\textsubscript{2}D is a pivotal feature of vitamin D action within the immune system. Until recently, studies have focused on the ability of 1,25(OH)\textsubscript{2}D to modulate adaptive immunity: promotion of more benign, humoral, type 2 helper T-cell (Th2) responses at the expense of potentially detrimental cellular Th1 responses\textsuperscript{127,128}. Vitamin D may also enhance tolerogenic immunity through the generation of immunosuppressive regulatory T-cells (Treg)\textsuperscript{129}. At the same time vitamin D may also promote more effective T-cell-mediated adaptive immunity through the regulation of tissue-specific T-cell homing\textsuperscript{130}.

Perhaps the most prominent facet of immunity to be linked with vitamin D stems from the ability of vitamin D to promote innate immune responses, specifically antimicrobial activity\textsuperscript{118,131}. Pathogens such as Mycobacterium tuberculosis (\textit{M. tb}) stimulate immune responses via cell surface pathogen-recognition receptors such as the toll-like receptor (TLR) system. In 2006, studies by Robert Modlin and his group at UCLA showed that expression of VDR and CYP27B1 by monocytes is potently induced following activation of TLR2/1 by \textit{M. tb}\textsuperscript{131}. The resulting rise in localized production of 1,25(OH)\textsubscript{2}D is then able to trigger endogenous interaction with the monocyte VDR, with the liganded receptor complex acting to regulate transcription of a wide variety of target genes. Prominent amongst these is the antimicrobial protein, cathelicidin, which acts by killing bacteria such as \textit{M. tb}\textsuperscript{132}, whilst providing feedback regulation of innate immunity via attenuation of TLR expression\textsuperscript{133}. Since then, other antibacterial proteins such as β-defensin 2 have also been shown to be stimulated by 1,25(OH)\textsubscript{2}D\textsuperscript{134}. Furthermore, vitamin D has also been shown to act as a promoter of autophagy, a cytosolic process that is essential for the killing of pathogens within autophagosomes\textsuperscript{135}.

Crucially, the localized tissue specific synthesis of 1,25(OH)\textsubscript{2}D that drives all of the antibacterial effects outlined above is dependent on the availability of substrate for CYP27B1, namely 25OHD. In view of the fact that 25OHD is the major circulating
form of vitamin D, it became clear that innate antimicrobial effects of vitamin D may be highly influenced by patient vitamin D status. Unlike the PTH/FGF23-responsive CYP27B1 classically observed in the kidney, extra-renal sources of the enzyme do not appear to be subject to classical feedback control, so that the synthesis of 1,25(OH)₂D by cells such as monocytes is more likely to reflect the availability of precursor 25OHD. This was confirmed by studies using monocytes cultured in medium supplemented with serum from vitamin D-sufficient Caucasian donors which showed much higher levels of M. tb-induced cathelicidin expression relative to monocytes cultured with serum from vitamin D-insufficient African-Americans. Similar observations have also been made using serum from vitamin D-insufficient subjects supplemented with vitamin D to restore optimal vitamin D status. Importantly, the effect of vitamin D supplementation is dependent on elevated circulating levels of 25OHD, with no change in serum levels of 1,25(OH)₂D being observed. Although these studies have shown that vitamin D status is a key determinant of the magnitude of innate immune responses to infection, it is likely that other factors will also influence this activity. Notably, recent studies have shown that the bioavailability of 25OHD for local conversion to 1,25(OH)₂D is influenced by the serum DBP, which is known to show distinct genotypic variations.

Although recent studies of vitamin D-induced antibacterial activity have focused primarily on monocytes and macrophages, it is important to recognize that a wide range of cell types express TLRs and have the necessary machinery to respond to infection. These include granulocytic cells such as neutrophils which provide a rapid response to infection and inflammation, and which also show 1,25(OH)₂D-induced antibacterial activity. Other studies have demonstrated the induction of CYP27B1 in keratinocytes as part of a mechanism to support 1,25(OH)₂D-induced epidermal antimicrobial responses following wounding of the skin. In a similar fashion, CYP27B1 expression has been reported in the gastrointestinal (GI) tract, and this may play a role in mediating immune responses and protection against inflammation within the GI tract. With these observations in mind, several previous reviews have postulated that CYP27B1 may fulfill a similar barrier function during pregnancy. The remainder of this section will review the evidence to support this proposal.

Vitamin D and implantation

Potential beneficial effects of vitamin D on implantation in animals were first postulated more than half a century ago, although later studies in which hypervitaminosis D was induced reported potential detrimental effects on fetal development. Other studies took a more mechanistic approach, showing that administration of active 1,25(OH)₂D promoted endometrial decidualization in rats. Likewise, differential gene analysis indicated that calbindin-D9K, a vitamin D target gene, was also important to the process of implantation, although later studies have questioned the importance of calbindin-D9K as an essential feature of implantation.
in primates\textsuperscript{147}. As a result of these and other observations, the first putative model for vitamin D effects on implantation was proposed in which cytokines produced at the fetal-maternal interface were proposed to act as stimulators of CYP27B1 expression\textsuperscript{142}. The resulting synthesis of 1,25(OH)\textsubscript{2}D thus produced a soluble factor capable of promoting effects important to implantation. In particular, this review indicated that responses to vitamin D during implantation may be diverse and they highlighted novel effects of 1,25(OH)\textsubscript{2}D on the HOXA10 gene, known to be involved in embryonic development\textsuperscript{148}. Moreover, it was suggested that a pivotal effect of vitamin D during the early stages of fetal development may stem from the potent anti-inflammatory effects of 1,25(OH)\textsubscript{2}D. With the latter in mind, it was proposed that 1,25(OH)\textsubscript{2}D could provide a potential treatment for recurrent spontaneous abortion\textsuperscript{149}. The most recent comment on the link between vitamin D and implantation has come from studies showing that women with higher vitamin D status were more likely to become pregnant following in vitro fertilisation-embryo transfer\textsuperscript{150}.

**Vitamin D and infection and inflammation during pregnancy**

In common with other barrier sites the placenta and reproductive tissues express a wide range of antibacterial\textsuperscript{151–153} and antiviral factors\textsuperscript{154}. It therefore seems likely that the high levels of local 1,25(OH)\textsubscript{2}D production within the placenta will impact on these antimicrobials. Studies in-vitro using human placenta tissue have shown that 25OHD and 1,25(OH)\textsubscript{2}D induce expression of cathelicidin in both maternal decidua\textsuperscript{155} and fetal trophoblast\textsuperscript{156}. In the latter case, vitamin D-induced cathelicidin expression promoted intracellular killing of *Escherichia coli* (*E. coli*), underlining the potential importance of vitamin D as a key mediator of placental response to infection\textsuperscript{156}. It is interesting to note studies showing that maternal vitamin D-insufficiency is associated with increased rates of bacterial vaginosis in the first trimester of pregnancy. In a similar vein, other recent studies have described an association between vitamin D status in pregnant women and risk of maternal to child transmission (MCTC) of human immunodeficiency virus (HIV)\textsuperscript{158}. In this study the authors propose that the lower levels of MCTC in vitamin D-sufficient women may be due to improved innate immune response to infection. Given the fundamental role of the placenta in vertical transmission of HIV from mother to the fetus\textsuperscript{159}, it is possible to speculate that vitamin D-induced innate immune responses within the placenta may play a role in combating MCTC of viral infections.

A strong body of evidence has implicated infection in the pathogenesis of prematurity, particularly early preterm delivery. This includes animal studies where administration of microorganisms or microbial antigens induces preterm delivery\textsuperscript{160}. In addition, there are extensive clinical data showing increased risk of prematurity in pregnant women with systemic infection such as kidney infection with *E. coli*, malaria and typhoid fever as well as intrauterine infection\textsuperscript{161}. More recently, periodontal infection has been postulated to be a risk factor for preterm delivery\textsuperscript{162}. Preterm delivery in the setting of infection is believed to result from the actions of
pro-inflammatory cytokines secreted as part of the maternal and/or fetal host response to microbial invasion\textsuperscript{163–166}. Romero and colleagues have argued that infection-related preterm labor and premature rupture of membranes (PROM) is due to the activation of host-defense systems which disturb the quiescence of pregnancy\textsuperscript{164}. Enhanced expression of pro-inflammatory cytokines such as interleukin (IL)-1, IL-6 and tumor necrosis factor \(\alpha\) (TNF\(\alpha\)) is associated with preterm delivery\textsuperscript{167}, and these factors have been detected in elevated concentrations in the amniotic fluid, serum and gestational tissues of women with preterm delivery\textsuperscript{168}. The pro-inflammatory cytokines can be induced by a number of stimuli, including bacterial endotoxins, and they have been shown to promote spontaneous labor and PROM via their actions on gestational tissues\textsuperscript{169–171}.

Several studies have reported that PROM and early preterm births are more likely to involve intraamniotic infection (chorioamnionitis)\textsuperscript{172–175}. As a result, it has been concluded that the presence of chorioamnionitis significantly elevates the risk of early preterm delivery\textsuperscript{172}, and that chorioamnionitis is inversely related to gestational age at birth\textsuperscript{175}. Maintenance of normal pregnancy requires efficient coordination of anti-microbial and anti-inflammatory responses within the fetal-placental unit\textsuperscript{164,165}. It is therefore possible to speculate that vitamin D plays a significant role in modulating both of these processes. Studies using cultured human decidual cells have shown that treatment with either 25OHD or 1,25(OH)\textsubscript{2}D acts to suppress expression of a wide range of cytokines, underlining the potential for local (autocrine) inhibition of placental inflammation\textsuperscript{155}. Other studies have confirmed the anti-inflammatory actions of 1,25(OH)\textsubscript{2}D on trophoblastic cells whilst also showing that inflammatory cytokines such as TNF\(\alpha\) can compromise VDR-mediated anti-inflammatory action by promoting expression of the catabolic enzyme CYP24A1\textsuperscript{176}.

**Vitamin D-insufficiency and pregnancy**

In view of the new parameters for interpretation of vitamin D status outlined previously, the 13\textsuperscript{th} Workshop on Vitamin D concluded that vitamin D insufficiency was a global epidemic\textsuperscript{177}. Nevertheless, it is also clear that some groups are much more at risk of vitamin D insufficiency than others, and prominent amongst these are pregnant women. In the USA, the 1988–1994 National Health and Nutrition Examination Survey revealed that 42\% of African-American women of child-bearing age had serum 25OHD levels that were lower than 37.5 nM, half the current optimal target level. This compares with only 4\% of white women\textsuperscript{178}. More recent studies of vitamin D insufficiency during pregnancy and lactation have served to underline the magnitude of the problem\textsuperscript{11,178–182}. Data from Bodnar et al\textsuperscript{11} showed that 74–95\% of pregnant US black women and 46–62\% of pregnant white women were vitamin D insufficient. Notably, almost 45\% of the African-American mothers who experienced preterm birth (<32 weeks) had serum 25OHD levels that were less than 37.5 nM.

The seminal observations by Bodnar and colleagues of vitamin D-insufficiency during pregnancy have been endorsed by a wide array of subsequent reports. These include studies of other populations in the USA\textsuperscript{10,183}, Canada\textsuperscript{184,185}, the UK\textsuperscript{186,187},
Table 1 Maternal and fetal health problems that have been associated with vitamin D status or intake.

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<th>Clinical problem</th>
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<th>Fetal/Neonatal</th>
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<td>Preeclampsia</td>
<td>198, 199, 201</td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis</td>
<td>157, 195</td>
<td></td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>190, 214, 216</td>
<td></td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>216, 217</td>
<td></td>
</tr>
<tr>
<td>Fetal skeleton/bone</td>
<td>218</td>
<td></td>
</tr>
<tr>
<td>Neonatal bone mass</td>
<td>219, 220</td>
<td></td>
</tr>
<tr>
<td>Childhood bone mass</td>
<td>197, 221</td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td>211, 212, 222</td>
<td></td>
</tr>
<tr>
<td>Type 1 diabetes</td>
<td>208, 209</td>
<td></td>
</tr>
<tr>
<td>Multiple Sclerosis</td>
<td>210</td>
<td></td>
</tr>
<tr>
<td>Autism</td>
<td>223</td>
<td></td>
</tr>
<tr>
<td>Maternal-fetal HIV transfer</td>
<td>158</td>
<td></td>
</tr>
</tbody>
</table>

Ireland\(^{188}\), Europe\(^{189}\), The Middle East and Asia\(^{190–193}\), and Australia\(^{187, 194}\). In several of these studies it was observed that pregnant women with darker skin pigmentation were at even greater risk of low vitamin D status when compared to pregnant women with lighter pigmented skin\(^{11, 187, 189, 195}\). The increasing evidence for global vitamin D-insufficiency during pregnancy raises the question of the likely physiological and clinical consequences of impaired maternal vitamin D status during pregnancy. A summary of the various clinical problems that have been linked to maternal vitamin D-insufficiency during pregnancy is shown in Table 1. These include effects on both maternal and fetal physiology and incorporate classical and non-classical responses to vitamin D. However, the role of vitamin D during pregnancy can also be viewed in relation to its predominant activities:

1. effects on fetal skeletal development;
2. regulation of placental function;
3. contributions to childhood illness and fetal programming.

The classical effects of vitamin D on fetal skeletal development are perhaps best illustrated by Cyrus Cooper and colleagues who reported a study of 424 pregnant women who were stratified according to their vitamin D status\(^{196}\). High-resolution 3D ultrasound analysis showed that sub-optimal vitamin D status was associated with increased fetal femur metaphyseal cross-sectional area and femur splaying index, whereas femur length showed no changes. These data were the first to show in-utero changes in skeletal morphology associated with vitamin D status, but also add to previous studies by the authors showing that children born to mothers with vitamin...
D insufficiency during pregnancy exhibit deficits in bone mineral content at 9 years of age\textsuperscript{197}. Although this association cannot be automatically assumed to be causal, the observations are nevertheless provocative. Notably, the authors demonstrated differences in skeletal development associated with vitamin D status as early as week 19 of gestation, coincident with the rise in maternal levels of active $1,25(OH)_2\text{D}$ that occurs early in gestation. As described above, the precise mechanism by which maternal 25OHD levels exert effects on the fetal skeleton remain unclear, but may include localized autocrine effects on cells within the growth plate.

Epidemiology suggests that there is a strong link between vitamin D-insufficiency and preeclampsia, a prevalent problem associated with pregnancy\textsuperscript{198,199}. Preeclampsia is a collection of symptoms during pregnancy that are linked to maternal hypertension. The precise mechanism by which vitamin D may influence the pathophysiology is at present unclear, although dysregulation of the vitamin D-activating enzyme CYP27B1 has been described in trophoblastic cells from the placentas of preeclampsia mothers\textsuperscript{200}. Significantly, preeclampsia is one of the few disorders to be studied with respect to the possible beneficial effects of vitamin D supplementation. Studies of a cohort of nulliparous pregnant women in Norway showed a 27\% reduction in risk of preeclampsia in women taking vitamin D supplements relative to those who did not take supplements\textsuperscript{201}. However, as yet, there have been no blinded prospective trials to assess the possible protective effects of enhanced maternal vitamin D status with respect to preeclampsia.

A possible role for gestational vitamin D-insufficiency in fetal programming of adult disease has been recognized for many years, most notably in relation to osteoporotic bone disease in later life (reviewed in\textsuperscript{202}). However, more recently this has been expanded to include non-classical actions of vitamin D and childhood illness. These include brain development and adult mental health\textsuperscript{203–207}, autoimmune disease\textsuperscript{208–210}, and asthma\textsuperscript{211–213}. At present the hypothesized role of vitamin D in fetal programming is based entirely on epidemiology. However, in the coming years it is highly likely that the fetal impact of vitamin D will be clarified by prospective studies aimed at supplementing pregnant women with doses of vitamin D that will effectively achieve the new targets for optimal vitamin D status or vitamin D sufficiency. It is also to be hoped that functional studies that explore the cellular and immunological actions of vitamin D at the fetal-maternal interface will shed more light on the mechanisms by which vitamin D metabolism and signaling can exert their effects during pregnancy.

**Conclusions**

There is now increasing evidence that vitamin D plays a pluripotent role during pregnancy. Classical vitamin D endocrinology remains an essential component of maternal calcium homeostasis, but it is becoming clear that effects of vitamin D extend far beyond this. The current review highlights the potential array of maternal and fetal responses that may be influenced by vitamin D and, most importantly, that may be dysregulated by vitamin D insufficiency. An important future development
will be the expansion of clinical trials to assess the clinical benefits of vitamin D supplementation during pregnancy. However, it is also important to recognize that novel mechanisms involving non-classical actions of vitamin D may further expand the role of vitamin D during pregnancy. In recent studies we have shown that vitamin D is a potent stimulator of innate antimicrobial responses to infection in human placental cells, whilst simultaneously inhibiting inflammation\textsuperscript{155,156}. Given the link between infection, inflammation and risk of preterm birth\textsuperscript{164}, it is tempting to speculate that vitamin D may have even broader beneficial effects in the general maintenance of pregnancy. This and other possible effects of vitamin D will form an exciting new set of challenges for researchers in the coming years.

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Vitamin D and Human Pregnancy


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