Vitamin D deficiency and impaired placental function: potential regulation by glucocorticoids?

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Abstract

Maternal vitamin D deficiency has been implicated in a range of pregnancy complications including preeclampsia, preterm birth and intrauterine growth restriction. Some of these adverse outcomes arise from alterations in placental function. Indeed, vitamin D appears critical for implantation, inflammation, immune function and angiogenesis in the placenta. Despite these associations, absence of the placental vitamin D receptor in mice provokes little effect. Thus, interactions between maternal and fetal compartments are likely crucial for instigating adverse placental changes. Indeed, maternal vitamin D deficiency elicits changes in glucocorticoid-related parameters in pregnancy, which increase placental and fetal glucocorticoid exposure. As in utero glucocorticoid excess has a well-established role in eliciting placental dysfunction and fetal growth restriction, this review proposes that glucocorticoids are an important consideration when understanding the impact of vitamin D deficiency on placental function and fetal development.


Introduction

As the sole conduit between maternal and fetal systems, the placenta mediates fetal exposure to nutritional, hormonal and other physiological cues throughout gestation. In addition to facilitating the direct exchange of oxygen and nutrients between mother and fetus, the placenta functions in a specialized endocrine capacity to produce hormones essential for maternal physiological adaptations to pregnancy (Evain-Brion & Malassine 2003). As such, a healthy placenta is vital for normal fetal growth and development, whereas impaired placental function disrupts fetal growth outcomes and may also result in long-term health complications in offspring via developmental programming mechanisms (Burton et al. 2016, Sferruzzi-Perri & Camm 2016).

Maternal vitamin D deficiency has become a significant problem in modern day obstetrics; the rates of vitamin D deficiency have increased in recent decades (Looker et al. 2008), and it is estimated that between 18% and 84% of pregnant women worldwide are vitamin D deficient (Dawodu & Wagner 2007). Although vitamin D has traditionally been associated with bone health through its regulation of calcium and phosphate absorption, it also plays central roles in cellular proliferation and differentiation (Samuel & Sitrin 2008), vascular function (Tare et al. 2011, Ni et al. 2014) and immune regulation (Prietl et al. 2013). As a consequence of these pleiotropic functions, vitamin D metabolism is important for a range of key developmental events, including decidualization (Halhali et al. 1991), modulation of maternal immune function (Tamblyn et al. 2015) and fetal bone formation (Young et al. 2012). Accordingly, maternal vitamin D deficiency has been linked to pregnancy complications such as preeclampsia (Bodnar et al. 2007, Achkar et al. 2015), bacterial vaginosis (Bodnar et al. 2009), preterm birth (Qin et al. 2016) and gestational diabetes mellitus (Burris & Camargo 2014). Moreover, gestational vitamin D deficiency is associated with intrauterine growth restriction (IUGR) in infants (Gernand et al. 2014, Chen et al. 2015a) and adverse postnatal health outcomes in offspring, including increased rates of asthma (Zosky et al. 2014), hypertension (Tare et al. 2011) and impaired neurodevelopment (Whitehouse et al. 2012, Eyles et al. 2013, Hawes et al. 2015, Pet & Brouwer-Brolsma 2016).

There are several forms of vitamin D, but vitamin D₃ is the naturally occurring form in animals. To elicit biological effects, vitamin D, which is obtained through the diet or synthesized in the skin via UVB exposure must be converted to its active form, 1α,25-dihydroxyvitamin D (1,25(OH)₂D). To achieve this, circulating vitamin D is first converted to 25-hydroxyvitamin D (25(OH)D) by hepatic 25-hydroxylase, which is then hydroxylated to 1,25(OH)₂D via the enzymatic activity of 1α-hydroxylase, encoded by the CYP27B1
Although the latter conversion typically occurs in the kidney, the placenta also exhibits abundant expression and activity of 1α-hydroxylase (Zehnder et al. 2002), which likely indicates a functional importance for local placental vitamin D metabolism during gestation (Fig. 1). Circulating 1,25(OH)2D elicits genomic effects by binding to the vitamin D receptor (VDR), which dimerizes with the retinoid X receptor (RXR) and induces subsequent gene transcription through vitamin D response element (VDRE) binding to target gene promoter regions (Haussler et al. 2013). However, as with many steroid hormones, vitamin D can also exert rapid non-genomic effects, likely via VDR located within the plasma membrane (Norman et al. 2002, Menegaz et al. 2011). VDR is also abundantly expressed in placental tissue, thus allowing for localized placental 1,25(OH)2D binding and subsequent gene transcription (Shahbazi et al. 2011). Interestingly, the precursor 25-(OH)D readily crosses the placenta; however, 1,25(OH)2D is unable to be transported across the placenta, and thus is found in relatively low levels in fetal circulation (Kovacs 2008).

A recent study shows that placental VDR expression is downregulated in pregnancies complicated by fetal growth restriction (Nguyen et al. 2015b) and is associated with abnormal trophoblast expression of cell-cycle regulatory genes in vitro (Nguyen et al. 2015a). This indicates that placental vitamin D metabolism regulates fetal growth and development; however, relatively little is known of the mechanisms behind such regulatory processes, and whether they may be compromised during vitamin D-deficient pregnancies. Interestingly, recent evidence shows that placental-specific ablation of Vdr expression does not have overt effects on placental phenotype or fetal growth outcomes (Wilson et al. 2015). This suggests that vitamin D homeostasis during pregnancy likely involves communication between maternal, placental and fetal compartments. Indeed, recent research has shown an interaction between vitamin D pathways and glucocorticoids ('stress' hormones) in the context of the placenta, brain, lung and other tissues (Obradovic et al. 2006, Hidalgo et al. 2011, Chambers et al. 2015, Tesic et al. 2015, Kassi et al. 2016). The current review aims to discuss the potential mechanisms by which placental function may be implicated in the adverse outcomes associated with vitamin D-deficient pregnancies. The key focus of this review is to propose

![Vitamin D metabolic pathways during pregnancy across maternal, fetal and placental compartments.](image-url)

**Figure 1** Vitamin D metabolic pathways during pregnancy across maternal, fetal and placental compartments. Vitamin D is absorbed into maternal circulation via UVB-catalyzed synthesis in the skin or dietary intake (1) and is then converted to 25(OH)D via hepatic 25-hydroxylase (2). This is subsequently hydroxylated to the active form of the metabolite in various tissues, including the kidney and placenta (3). The precursor 25(OH)1D can also directly cross the placenta (6), where it is hydroxylated to the active metabolite in the fetal kidney (4). The active 1,25(OH)1D then elicits genomic effects through binding to the vitamin D receptor (VDR) in target tissues, including the placenta (5). This induces the transcription of target genes via vitamin D response element (VDRE), thus enabling the regulation of a range of important processes during gestation (6).
that the involvement of factors extrinsic to vitamin D pathways, particularly the action of glucocorticoids, is crucial for eliciting placental effects.

Vitamin D deficiency and placental development and function

Vitamin D metabolic pathways in the placenta may facilitate the transport of calcium and phosphate across the placenta, enabling fetal skeletal development. Changes in fetal femoral development with maternal vitamin D insufficiency can be observed as early as 19-week gestation using 3D ultrasound (Mahon et al. 2010) and such changes in bone structure may occur well into early childhood (Javaid et al. 2006). Although vitamin D may have a role in fetal skeletal development, other dominant pregnancy hormones such as prolactin, estradiol and lactogen are also key to this process (Kirby et al. 2013). There is evidence that the increased maternal intestinal calcium absorption that occurs in pregnancy and calcium supply to the fetus is regulated by mechanisms independent of the typical vitamin D pathway. Thus, maternal vitamin D deficiency is associated with reduced fetal cord blood calcium and bone mineralization in some (Cockburn et al. 1980, Maghbooli et al. 2007), but not all (Javaid et al. 2006) studies. Indeed, a systematic review of vitamin D supplementation in pregnancy found limited evidence for an association between maternal vitamin D status and offspring birthweight, bone mass or serum calcium concentrations (Harvey et al. 2014). Furthermore, mice with genetic removal of Vdr (which therefore eliminates 1,25(OH)2D3 signaling) do not exhibit differences in placental calcium transfer or fetal skeletal mineralization (Kovacs et al. 2005). This raises the possibility that the key function of vitamin D in pregnancy is beyond calcium/bone effects. Indeed, maternal vitamin D deficiency is implicated in pregnancy complications associated with placental dysfunction such as preeclampsia, preterm birth and IUGR. This, in conjunction with the presence of a local vitamin D metabolic pathway, implies that vitamin D has a critical role in placental development and function. So what are the underlying biological mechanisms that contribute to the effects of vitamin D on placental development and function? The key mechanisms include effects on implantation, inflammation, immune function and angiogenesis.

Implantation, inflammation and immune function

Vitamin D has potent effects on immune responses (Liu et al. 2006) and influences both the innate and adaptive arms of the immune system (Hewison 2011). Immune adaptations are vital for successful pregnancy outcome and vitamin D likely acts to promote implantation due to its role in inflammatory pathways and immune function. Thus, administration of 1,25(OH)2D3 in rats promotes decidualization (Halhali et al. 1991), and vitamin D likely has a key role to play in the immune function of the decidua (for an extensive review see Tamblyn et al. 2015), given its important immunomodulatory effects on trophoblasts. Indeed, vitamin D metabolites enhance extravillous trophoblast invasion in vitro (Chan et al. 2015) and act in a paracrine manner to suppress cytokine secretion in uterine natural killer cells (Evans et al. 2006) and villous trophoblast (Diaz et al. 2009, Noyola-Martinez et al. 2013).

In light of this, it seems that the anti-inflammatory actions of vitamin D likely play a critical role in the prevention of placental pathologies. Primary human trophoblast cell cultures treated with antiphospholipid antibodies (as a model of antiphospholipid syndrome, an autoimmune disorder associated with pregnancy loss and preeclampsia) demonstrate a dampened inflammation response in the presence of vitamin D (Gysler et al. 2015), whereas vitamin D treatment inhibits trophoblast expression and secretion of IL-10 in placental tissue obtained from both healthy and pre-eclamptic pregnancies (Barrera et al. 2012). The actions of vitamin D extend into enhancing antibacterial responses to Escherichia coli in trophoblast culture (Liu et al. 2009). Genetic ablation of Cyp27b1 stimulates placental inflammation when challenged with LPS; yet, in wild-type placenta, the presence of vitamin D metabolites suppresses the inflammatory response to LPS (Liu et al. 2011). Placental LPS-induced inflammation can be inhibited by pre-administration of vitamin D, preventing IUGR, which appears at least partly due to increasing interactions of the VDR and the nuclear factor kappa B p65 subunit (Chen et al. 2015b). Therefore, it appears that vitamin D has generally positive effects in regulating placental immune function and providing protective effects against inflammation and bacterial infection.

Placental angiogenesis and nutrient transport

There is some evidence for maternal vitamin D deficiency altering placental vascular development. Thus, dietary vitamin D-restricted mice exhibited decreased vascular diameter (Liu et al. 2013) and decreased fetal capillary length and volume (Tesic et al. 2015) in the labyrinth zone, which is the highly vascular zone of the rodent placenta responsible for nutrient and waste exchange. Mechanistic evidence for vitamin D effects on angiogenesis have been provided by studies in endothelial colony-forming cells. Thus, vitamin D promotes the formation of capillary-like structures in conjunction with an upregulation of angiogenic factor vascular endothelial growth factor (Vegf) (Grundmann et al. 2012). These observed changes in Vegf are supported by the observation that maternal
vitamin D deficiency is associated with a reduction of Vegf in the highly vascular labyrinth zone of mouse placenta (Tesic et al. 2015).

In addition to altered angiogenic pathways, there is also an association between vitamin D status and placental nutrient transport. Indeed, Cleal and coworkers (Cleal et al. 2015) found that human placental gene expression of amino acid transporters correlated with maternal vitamin status as well as vitamin D-binding protein. This implies that while placental vitamin D is important for supplying amino acids to the fetus, delivery of vitamin D to the placenta is also crucial for this process (Cleal et al. 2015). Supporting associations have also been found in mechanistic studies using primary human trophoblast cell culture, whereby 1,25-dihydroxy vitamin D₃ was shown to regulate system A amino acid transporter activity via increased expression of the SNAT2 amino acid transporter (Chen et al. 2017).

The specific role of placental vitamin D receptor

So how many of these observed effects of vitamin D on placental development and function are mediated via binding of the vitamin D receptor in the placenta? Initial studies in Vdr-knockout (Vdr⁻/⁻) dams demonstrated decreased fertility and a reduction in fetal weight in comparison to heterozygous (Vdr⁺/-) dams (Kovacs et al. 2005); however, the role of the placenta in this study was not assessed. Elegant investigations of placentas from Vdr⁻/⁻ matings have proven to be more definitive in aiding our understanding of the significance of placental Vdr, as this eliminates maternal pathophysiology and generates each of the three possible placental–fetal genotypes within the one pregnancy (Vdr⁺⁺, Vdr⁺⁻ and Vdr⁻⁻). Thus, in response to an LPS challenge to simulate maternal infection, inflammatory markers were upregulated in the Vdr⁻⁻ placentas in comparison to wild type (Vdr⁺⁺) (Liu et al. 2011), further implicating a role for vitamin D as a regulator of placental inflammation. Microarray and morphology comparisons of heterozygous-bred Vdr⁺⁺ and Vdr⁻⁻ placentas by Wilson and coworkers (Wilson et al. 2015) have revealed that although there were some differences in gene expression, this did not manifest in altered placental morphology or placental function, as fetal and placental weights were unaltered. Of those genes that did change, Cyp24a1, which encodes the enzyme responsible for the catabolism of 1,25(OH)₂D₃, was robustly downregulated in Vdr⁻⁻ placentas, whereas other altered genes are implicated in pathways such as autophagy, cell signaling and cytoskeletal modifications and mammalian target of rapamycin (mTOR) signaling. It should be noted that, in the studies mentioned (Liu et al. 2011, Wilson et al. 2015), whole placental gene expression was assessed and therefore, whether there are differential gene expression responses between the junctional and labyrinth zone of Vdr⁻⁻ placentas is yet to be established. On the whole, however, the lack of a robust placental phenotype in heterozygous-bred Vdr⁻⁻ placentae highlights that maternal vitamin D status and the associated physiological response is critical for causing the placental phenotype, as opposed to direct actions of vitamin D on the placental vitamin D receptor.

The potential physiological responses to maternal vitamin D deficiency are myriad. Our group has recently begun to focus on the potential interactions between vitamin D and glucocorticoids during pregnancy; however, there are many other key candidates of interest. Given the multifaceted role of vitamin D in immune pathways, there remains much work to be done elucidating how maternal vitamin D status influences appropriate immune and inflammation response in pregnancy. Furthermore, the observation that mTOR expression is altered in Vdr⁻⁻ placentas is of particular interest as placental mTOR signaling plays a pivotal role as a maternal nutrient sensor and thus is critically implicated in fetal growth and development (for a review see Dimasuay et al. 2016). Herein, we discuss how vitamin D deficiency alters glucocorticoid-related pathways in the maternal, fetal and placental compartments and how this may elicit the observed changes in placental development and function.

Glucocorticoids and pregnancy

Glucocorticoids regulate many physiological pathways including cellular differentiation, immunoregulation, inflammation and metabolism. During pregnancy, activity of the hypothalamic–pituitary–adrenal (HPA) axis markedly increases and accordingly, free glucocorticoids (cortisol and corticosterone in humans and rodents respectively) rise. This upregulation of glucocorticoids enables the mother to meet the metabolic demands of pregnancy (Seckl & Holmes 2007, Wharfe et al. 2016) and plays an essential role in the maturation of fetal tissues in preparation for birth (Cole et al. 1995a,b, Diaz et al. 1998, Surbek et al. 2012). The issue arises, however, when fetal glucocorticoid exposure is excessive as this results in placental dysfunction, fetal growth restriction and altered organ development (Benediktsson et al. 1993, Lindsay et al. 1996a, Nyirenda et al. 1998, Ain et al. 2005, Hewitt et al. 2006, Holmes et al. 2006, Wyrwoll et al. 2009, Cuffe et al. 2011, Vaughan et al. 2012). Furthermore, the effects of early-life glucocorticoid exposure on development resonate throughout the lifespan with adverse effects on adult health outcomes including cardiometabolic and neuropsychiatric disorders (Benediktsson et al. 1993, Lindsay et al. 1996a,b).

Given the potentially detrimental effects of glucocorticoid excess, it is critical that fetal exposure to glucocorticoids is tightly regulated. Indeed, fetal glucocorticoid levels remain up to 10-fold lower than those of maternal levels during the majority of gestation (Beitins et al. 1973, Edwards et al. 1993). This marked differential arises largely due to the presence of the enzyme 11\(\beta\)-hydroxysteroid dehydrogenase type 2 (Hsd11b2) in placental and fetal tissues. Hsd11b2 catalyzes the conversion of cortisol and corticosterone into their inactive forms, cortisone and 11-dehydrocorticosterone respectively. In humans, Hsd11b2 is highly expressed in the syncytiotrophoblast (Stewart et al. 1995) at the interface between maternal and fetal circulations. Similar patterns of Hsd11b2 expression are seen within the labyrinth zone of the rodent placenta (Brown et al. 1996, Burton et al. 1996). Expression of placental Hsd11b2 decreases at the end of gestation, presumably to facilitate fetal organ maturation (Burton et al. 1996).

Environmental perturbation in pregnancy can increase fetal glucocorticoid exposure by altering placental transfer of glucocorticoids. 10–20% of maternal glucocorticoids reach the fetus unaltered (Benediktsson et al. 1997), and therefore, stressors (i.e., psychological stress, dietary insults, inflammation and hypoxia) that increase maternal glucocorticoid levels can increase fetal glucocorticoid exposure. Furthermore, these stressors generally reduce placental Hsd11b2 expression and/or activity and thus increase fetal glucocorticoid exposure (Langley-Evans et al. 1996, Mairesse et al. 2007, Seckl & Holmes 2007, Vieau et al. 2007). Therefore, given the high incidence of maternal vitamin D deficiency in pregnancy, could this act as a physiological stressor and exert effects on pathways involved in fetal glucocorticoid exposure?

**Vitamin D and glucocorticoid interactions**

The possibility of vitamin D and glucocorticoid pathway interactions are a relatively unexplored consideration; however, it seems likely from other studies that there is scope for substantial cross-talk between these two hormones. Indeed, vitamin D exerts antagonistic effects to the exposure of the synthetic glucocorticoid dexamethasone in the hippocampus. Thus, dexamethasone increases viable cells, decreases apoptotic cells and reduces neurite outgrowth in hippocampal cell culture with each change mitigated by concomitant administration of vitamin D (Obradovic et al. 2006). In addition, vitamin D appears to downregulate glucocorticoid receptor levels (Obradovic et al. 2006). Similar observations have been made in a squamous cell carcinoma model whereby dexamethasone induces de novo transcription of the vitamin D receptor in a glucocorticoid receptor-dependent manner (Hidalgo et al. 2011). Furthermore, vitamin D also appears to have direct effects on the glucocorticoid sensitivity of peripheral blood mononuclear cells, with evidence of direct downregulation of glucocorticoid receptor expression and inhibition of GR translocation to the nucleus (Kassi et al. 2016). Moreover, in patients with steroid-resistant asthma, vitamin D administration beneficially alters the clinical response to glucocorticoids (Chambers et al. 2015).

So what occurs to glucocorticoid-related parameters in models of gestational vitamin D deficiency? There are multiple pathways that influence fetal exposure to glucocorticoids with vitamin D deficiency (Fig. 2). Maternal vitamin D deficiency in a mouse model of pregnancy elevates circulating maternal glucocorticoids as well as adrenal weight, indicating possible chronic

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**Figure 2** Proposed effects of developmental vitamin D deficiency on glucocorticoid metabolism and levels in the mother and fetus.

(A) In gestational vitamin D deficiency, maternal circulating active glucocorticoids (GCs) and GC release in response to stress is elevated, likely increasing placental GC exposure and transport.

(B) In the placenta, 11\(\beta\)-Hsd2 (a key enzyme that inactivates GCs) is decreased by vitamin D deficiency, which decreases the conversion of active GCs to inactive forms.

(C) In the fetus, the combination of increased maternal GC levels and decreased GC inactivation due to vitamin D deficiency leads to increased fetal GC exposure.

(D) Ultimately, vitamin D-deficient fetuses exhibit a likely increase in GC exposure in the brain, as indicated by increases in the GC-responsive gene Tsc22d3.
HPA activation (Fig. 2A, Tesic et al. 2015). HPA axis hyperactivation and altered feedback is supported by studies in rats showing that vitamin D-deficient pregnant rats have exaggerated serum corticosterone levels in response to restraint stress (Eyles et al. 2006). In addition to elevated maternal glucocorticoids, vitamin D deficiency also modifies placental Hsd11b2 expression. Thus, in mouse pregnancy at E14.5 (approximately 2/3 of mouse gestation), maternal vitamin D deficiency results in a 32% reduction in Hsd11b2 expression in placentas of male, but not female fetuses (Tesic et al. 2015). This reduction in Hsd11b2 presumably increases the placental transfer of glucocorticoids across to the fetus. Although this has not been directly measured, this notion is supported by the observation that the glucocorticoid-sensitive gene Tsc22d3 is increased in fetal brains at E14.5 and E17.5 from vitamin D-deficient dams (Tesic et al. 2015). This implies that some of the well-documented effects of early-life vitamin D deficiency on adverse adult health outcomes may in part, be attributed to fetal glucocorticoid exposure. Indeed by E14.5, glucocorticoid receptors are expressed in many organ systems including the brain, heart, lung, kidney and adrenal glands (Thompson et al. 2004, Rog-Zielinska et al. 2013), and therefore, many aspects of fetal development can be disturbed by excessive glucocorticoid exposure.

Although the direct level of fetal exposure to glucocorticoids remains unclear, the reduced placental Hsd11b2 (Tesic et al. 2015), elevated maternal circulating glucocorticoids (Tesic et al. 2015), increased maternal HPA axis response to stress (Eyles et al. 2006) and upregulated Tsc22d3 in the brains of deficient offspring (Tesic et al. 2015) all point to increased fetal exposure to glucocorticoids in vitamin D-deficient mothers. Reinforcing this notion, are the observations that vitamin D deficiency in mouse pregnancy perturbs placental vascular development (Liu et al. 2013, Tesic et al. 2015) recapitulating (albeit to a lesser extent) that seen in rat and mouse models of glucocorticoid excess (Hewitt et al. 2006, Wyrwoll et al. 2009, Vaughan et al. 2013). Although rodent offspring from mothers vitamin D deficient until parturition show normal glucocorticoid restraint response (Eyles et al. 2006), we have recent evidence to suggest this may not be the case when mothers and offspring are deficient until weaning (unpublished data). This supports a growing body of evidence suggesting that the timing and duration of vitamin D deficiency are important in determining effects on offspring (Burne et al. 2004a,b). Extensive work is required, however, to tease apart the interactions between vitamin D and glucocorticoids in the context of pregnancy. Indeed, it will be of great interest to see whether similar concomitant effects of vitamin D, glucocorticoids and their respective receptors occur in placental cell lines as they do in other cell-based studies.

Conclusions

Although the diverse effects of vitamin D on placental function may explain many complications associated with vitamin D deficiency in pregnancy, these effects are likely reliant on factors beyond immediate vitamin D pathways. There remains considerable work to be done in teasing apart the exact mechanisms, but the effects of vitamin D deficiency on glucocorticoid metabolism may be an important component for eliciting adverse placental effects. In the wider context, it is tempting to propose that vitamin D supplementation may improve multiple aspects of placental function and maternal physiology. However, there are considerable challenges around appropriate human levels of vitamin D (Lucas & Neale 2014), and thus, this remains an important ongoing area of research.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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