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# Vitamin D and pregnancy: An old problem revisited

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Vitamin D has historically been considered to play a role solely in bone and calcium metabolism. Human disease associations and basic physiological studies suggest that vitamin D deficiency is plausibly implicated in adverse health outcomes including mortality, malignancy, cardiovascular disease, immune functioning and glucose metabolism. There is considerable evidence that low maternal levels of 25 hydroxyvitamin D are associated with adverse outcomes for both mother and fetus in pregnancy as well as the neonate and child. Vitamin D deficiency during pregnancy has been linked with a number of maternal problems including infertility, preeclampsia, gestational diabetes and an increased rate of caesarean section. Likewise, for the child, there is an association with small size, impaired growth and skeletal problems in infancy, neonatal hypocalcaemia and seizures, and an increased risk of HIV transmission. Other childhood disease associations include type 1 diabetes and effects on immune tolerance. The optimal concentration of 25 hydroxyvitamin D is unknown and compounded by difficulties in defining the normal range. Whilst there is suggestive physiological evidence to support a causal role for many of the associations, whether vitamin D deficiency is a marker of poor health or the underlying aetiological problem is unclear. Randomised controlled trials of vitamin D supplementation with an appropriate assessment of a variety of health outcomes are required.



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## Introduction

Vitamin D deficiency, as expressed by its clinical presentation of rickets has an important historical association with obstetric practice. First described clinically in the mid-17th century, rickets produces soft bones in children and can result in significant, lifelong deformity of weight bearing bones including the pelvis. These pelvic deformities do not prevent conception but do prevent vaginal delivery of a live infant. In Glasgow, in the late 19th century, 1 in 30 deliveries involved a rachitic pelvis and destruction of the fetus by craniotomy. Murdock Cameron, the Regius professor of Midwifery at Glasgow University, introduced successful caesarean section into British obstetric practice in women with a deformed pelvis due to rickets caused by childhood vitamin D deficiency. He reported 14 successful cases (all women with rachitic pelvises) in the BMJ in 1890.

The history of the identification of vitamin D in the early 20th century is fascinating (see http://vitamind.ucr.edu/history.html for a brief summary and references). The prevention of vitamin D deficiency was a major public health issue. One of the authors of this article received regular cod-liver oil as a child to prevent vitamin D deficiency. This public health message has been lost. The recognition that ultraviolet light produces skin cancer, and the public health campaigns directed against excessive exposure to ultraviolet light has resulted in the reappearance of vitamin D deficiency. Rickets is increasing in frequency[1] and if low levels of vitamin D are causally associated with other adverse health outcomes, then once again vitamin D deficiency (with a new definition) will become a major public health issue.

One example of the growing number of reports of the association of low vitamin D levels with various medical problems is the re-emergence of the association between vitamin D deficiency and caesarean section. It now appears that low vitamin D concentrations early in pregnancy may identify women at risk of subsequent caesarean section. In a study of 253 women, 28% of women with a low vitamin D (<37.5 nmol/L) had a caesarean section, compared with only 14% of women with a vitamin D 37.5 nmol/L or greater ($P = 0.012$).[2]

Vitamin D has also been suggested to have a number of other effects during pregnancy and on the infant. The purpose of this article is to review the current knowledge of vitamin D and its potential interactions with pregnancy.

## Vitamin D: physiology

Vitamin D is not a vitamin in sun-exposed people. Rather, 'vitamin D' is a highly regulated steroid hormone system with the potential to regulate up to 3% of the human genome. There are two forms – vitamin $D_2$ (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin $D_2$ is a 28-carbon molecule derived from the plant sterol ergosterol and only occurs in humans when it is ingested as part of the diet. Vitamin $D_3$ is a 27-carbon derivative of cholesterol. In humans, this can be ingested, or formed in the skin from the action of ultraviolet light. Unless otherwise specified, in this article when we use the term vitamin D we mean vitamin $D_3$.

Pre-vitamin D is produced in the skin from the action of UV light on 7-dehydrocholesterol. The pre-vitamin D is converted non-enzymatically to vitamin D, which leaves the skin, enters the circulation and is bound to both vitamin D binding protein and albumin. Vitamin D binds to the former tightly, the latter more loosely. In the liver, vitamin D (or ingested $D_2$) is 25-hydroxylated to form 25 hydroxyvitamin D [25 (OH)D]. This reaction is not rate limiting. The half-life of 25 (OH)D is variously reported as 4–8 weeks in the circulation and its concentration is used to assess vitamin D status. The SI units for 25 (OH)D are nanomoles per Litre (1 nmol/L = 0.4006 ng/ml). It is the precursor of the active metabolite 1,25-dihydroxyvitamin D [1,25-$(OH)_2$D]. Circulating 1,25-$(OH)_2$D is almost exclusively released from the kidney where the enzyme 1α-hydroxylase (CYP27B1), a cytochrome P450 enzyme in the proximal tubule, catalyses the hydroxylation. This reaction is tightly controlled by a number of factors that regulate calcium homeostasis namely, 1,25-$(OH)_2$D itself, parathyroid hormone (PTH), serum calcium and phosphate. The classical actions of 1,25-$(OH)_2$D to increase calcium absorption from the gut and inhibit parathyroid hormone secretion from the parathyroid glands are a vital part of the maintenance of a normal serum calcium concentration. It is now clear that 1,25-$(OH)_2$D is also produced locally in a variety of tissues and may act locally.[3]

Vitamin D acts via its vitamin D receptor (VDR), a classical steroid receptor of the nuclear retinoid X receptor (RXR) family. This receptor has been found in 37 different human tissues including those

involved in the regulation of glucose metabolism, skeletal muscle, skin, the cardiovascular system, and components of the immune system.[3] This provides the biological plausibility behind the non-classical actions of vitamin D. Furthermore, it is been suggested that local production of 1,25-$(OH)_2$D from 25 OHD could explain the different concentration requirements for 25 OHD for different endpoints. The kidney is said to produce sufficient 1,25-$(OH)_2$D from 25 OHD to maintain calcium absorption from the gut at concentration as low as 20 nmol/L.[4] It is suggested that higher concentrations of 25 OHD are required for sufficient local production of 1,25-$(OH)_2$D in non-renal tissue. This locally produced 1,25-$(OH)_2$D could modulate effects locally in a paracrine or autocrine manner.

As noted earlier, like the other steroid hormones, vitamin D has a binding protein. There are differences in the binding of 1,25-$(OH)_2D_2$ and 1,25-$(OH)_2D_3$ to vitamin D binding protein in blood and this is thought to account for their different half-lives and could account for apparent differences in potency. 1,25-$(OH)_2D_2$ and 1,25-$(OH)_2D_3$ are similar in their binding to the vitamin D receptor and as such are thought to have comparable biological action.

### *Actions of vitamin D: classical*

The "classic" functions of vitamin D are to increase the intestinal absorption of calcium and phosphorous, to reduce the secretion of parathyroid hormone from the parathyroid glands; and complex effects on bone mineralisation through the alteration of osteoblast function and stimulation of osteoclast activity. This increases bone reabsorption to maintain serum calcium concentration when serum calcium is low but the action is more complex when calcium is abundant. Low concentrations of vitamin D, specifically 1,25-$(OH)_2$D, lead to parathyroid hormone secretion. This stimulates renal 1α-hydroxylase activity increasing the conversion of 25 OHD to 1,25-$(OH)_2$D the combined actions of which restore serum calcium to normal.

### *Actions of vitamin D: non-classical*

A wide variety of tissues express both VDR and the 1α-hydroxylase enzyme. Vitamin D effects have been suggested or shown in skeletal muscle, skin, the cardiovascular system, the modulation of insulin resistance and the immune system. Vitamin D may also have effects in the placenta and decidua.

#### *Associations with mortality*

Low 25 OHD is associated with excess mortality and vitamin D supplementation may reduce all cause mortality (OR 0.93, 95% CI 0.87–0.99) as shown in a recent meta-analysis of 9 randomised clinical trials of vitamin D supplementation in more than 50,000 subjects.[5] The causes of the excess mortality include diabetes, cardiovascular disease, and cancer.[6] Cohort and observational studies also demonstrated a negative association between 25 OHD concentration and all cause mortality.[7–10] There are large observational studies showing a correlation between low vitamin D and increased rates of cardiovascular disease in addition to the increased mortality discussed above.[11–14] There has been no trial of vitamin D supplementation with direct outcome measures such as CVD event rate or CVD mortality.

#### *Associations with cancer*

Vitamin D deficiency has been associated with increased risk for some cancers including bladder, breast, colon, endometrial, oesophageal, gallbladder, gastric, ovarian, pancreatic, rectal, and both Hodgkin's and non-Hodgkin's lymphoma.

Supplementation of vitamin D has been trialled in several types of malignancy with some encouraging results in RCTs of both primary[15,16] and secondary[17,18] prevention of malignancy. Others have shown no benefit.[19,20] Various VDR polymorphisms show an association with cancer risk as well as prognosis in some cancers.[21]

#### *Associations with diabetes*

A number of case-control and observational studies have suggested vitamin D deficiency is associated with an increased risk of type 2 diabetes mellitus[22] and gestational diabetes, confounded by the association of vitamin D deficiency with ethnic groups who have a high prevalence of both type 2 and

gestational diabetes.[23] There are two broad mechanisms by which vitamin D may have a role in glucose tolerance – through regulation of insulin release and/or insulin sensitivity. The evidence for the role of vitamin D in both of these processes is variable and has been reviewed recently by Alvarez and Ashraf.[24] The pancreatic β-cells have both VDR and 1α-hydroxylase, providing possible pathways for vitamin D, including 1,25-$(OH)_2$D produced locally. Polymorphism in the VDR has been linked to types 1 and 2 diabetes as well as to fasting glucose in young men with low levels of physical activity. The association with type 1 diabetes has been debated.[25] Variants in DBP are associated with differences in glucose tolerance and fasting insulin.[26]

An association between vitamin D deficiency and impaired insulin sensitivity or insulin release in response to glucose has been demonstrated in several studies. In three studies 25 OHD concentration was correlated with insulin sensitivity.[27–29] However, in a study of 39 obese non-diabetic subjects, while there was a significant univariant correlation ($r = 0.43$; $P < 0.01$) between 25 OHD and insulin sensitivity, in multivariate analysis this was lost. BMI was the most powerful predictor of 25 OHD concentration ($r = -0.52$; $P < 0.01$).[30] It may be that the well documented association between obesity and low vitamin D is the primary relationship, and that insulin sensitivity is related primarily to the obesity rather than the vitamin D level.

Therapy with vitamin D has not reliably shown efficacy in preventing or controlling diabetes. An RCT of vitamin D supplementation in 100 centrally obese, non-diabetic men, using three doses of 120,000 IU fortnightly, showed an improvement in post prandial insulin sensitivity but no change in other measures of insulin sensitivity or β cell function.[31] A trial of 25 OHD replacement using 4000 IU/day or placebo for six months in a group of insulin resistant South Asian women living in New Zealand found that there was a significant improvement in insulin sensitivity ($P = 0.003$) and insulin resistance ($P = 0.02$), with a decrease in fasting insulin ($P = 0.02$) in women given vitamin D with the greatest improvement in insulin resistance seen when 25 OHD was >80 nmol/L.[32] In *post hoc* analysis of a bone study in which subjects were randomised to either vitamin D and calcium or placebo for 3 years, those with IFG at baseline who were randomised to vitamin D and calcium had a lower rise in fasting plasma glucose compared with the placebo group.[22]

In a trial of supplementation with 40,000 IU per week of vitamin D there was no improvement in diabetic subjects with normal baseline 25 OHD levels.[33] A post hoc analysis of the RECORD trial (an RCT in older people receiving 25 OHD and/or calcium supplementation for secondary prevention of osteoporotic fractures) found no difference in the development of diabetes or escalation of hypoglycaemic therapy.[34] An RCT of 100,000 units by intramuscular injection of vitamin $D_2$ in subjects with type 2 diabetes showed improvement in flow mediated endothelial function when measured 8 weeks after the dose but no change in glycaemic control or insulin sensitivity.[35] Another trial of 100,000 IU vitamin D given as 2 doses 2 weeks apart in healthy adults demonstrated no change in insulin sensitivity or glucose tolerance.[36]

Pittas, in a meta-analysis in 2007 examined the evidence for an association between vitamin D and diabetes, as well as treatment with vitamin D.[22] He concluded "The available evidence is limited…".[22] We agree with Bouillon that "more data are needed to confirm the independent effect of vitamin D on glucose and insulin metabolism.".[37] Whether the associations discussed above have a cause-and-effect relationship is not clear at the present time.

### *Other non-classical effects of vitamin D*

#### *Skeletal muscle and skin*

Vitamin D has been suggested or shown to have actions in skeletal muscle, skin, and the immune system. Severe vitamin D deficiency is associated with myopathy and muscle pain which is corrected with vitamin D therapy. Muscle biopsies from vitamin D deficient humans show type II muscle fibre atrophy as well as increased interfibrillar spaces and infiltration of fat[38] which is improved with therapy. The VDR is found in skeletal muscle.[39] Vitamin D supplementation in vitamin D deficient humans results in an increase in type II muscle fibre diameter and in the proportion of type II fibres.[39] A meta-analysis concluded that that supplementation with vitamin D reduces falls in nursing home patients.[40] However, a systematic review examining the effect of vitamin D supplementation on balance and muscle strength, as well as falls, in community dwelling women found that six of the eight

studies show no benefit in balance or gait in this group. Five of eight observational studies found a positive association between low vitamin D and falls.[41] Another meta-analysis focussing on falls in those older than 65 years found that a dose of 700–1000 IU per day of vitamin D reduced the risk of falls by 19%[42] These reviews show several points – it is more difficult to demonstrate an effect of vitamin D in healthier populations; intervention studies are less often positive than observational studies; and the dose used in many trials may be insufficient to produce the desired clinical effect.

Vitamin D analogues are used as a topical treatment for psoriasis. This is the first non-classical effect leading to a definitive therapy.

## Physiology of vitamin d during pregnancy and lactation

During pregnancy and lactation, maternal physiology is altered which facilitates the transfer of calcium to the fetus and neonate. The fetus requires about 30 g of calcium during development, the majority in the third trimester. Parathyroid hormone-related protein (PTHrP) increases during pregnancy. This is expressed in the fetal parathyroid glands, myometrium, and in the placenta and other fetal membranes and increases 1α-hydroxylase activity in the kidney and placenta, resulting in an increase in the production of 1,25-$(OH_2)$D. The rise in 1,25-$(OH_2)$D causes maternal PTH levels to fall.[43] Overall, parathyroid hormone bioactivity is increased but serum calcium remains normal as calcium is transferred to the fetus and lost in the urine.

In normal pregnancy, maternal 1,25-$(OH_2)$D increases progressively from the first trimester, to a peak of twice the non-pregnancy level in the third trimester. Pregnancy does not alter the clearance of 1,25-$(OH_2)$D. The increase in maternal serum level is due to increased production as well as to an increase in vitamin D binding protein.[44] The increased production is primarily due to elevated 1α-hydroxylase activity in the maternal kidney, with some input from the fetal kidney, placenta and decidua.

Fetal calcium levels are higher than maternal throughout gestation. There is active transport of calcium across the placenta. Fetal vitamin D concentrations are up to 20% lower than maternal as measured in cord blood.[45] Thus vitamin D deficiency is vertically transmitted. Vitamin D is not essential for fetal skeletal mineralisation although subtle skeletal differences have been demonstrated. Low vitamin D predisposes to hypocalcaemia in the immediate postpartum period, and then rickets over the next few months. Breast milk vitamin D correlates well with maternal vitamin D concentrations so breast fed infants are at risk of persistent vitamin D deficiency if the mother is deficient. This has obvious clinical implications. The US Center for Disease Control recommends that all lactating women have 400 IU of vitamin D per day, but this dose is likely to be inadequate in those with an existing deficiency particularly if sun exposure is limited.[46]

### *Lactation*

Lactating women secrete approximately 280–400 mg/day of calcium in breast milk. PTHrP and possibly the calcium sensing receptor in the mammary gland control milk calcium content which is independent of maternal vitamin D.[47,48] Supplementation during lactation has been trialled both in the mother and the infant, with either route proving effective at increasing the infant vitamin D level.[46]

## Impact of vitamin D deficiency on maternal and infant health

Vitamin D deficiency during pregnancy has been linked with a number of fetal, neonatal and maternal health problems: for the child; small size, neonatal hypocalcaemia and seizures, impaired growth, skeletal problems including rickets and low BMD. Other possible disease associations include type 1 diabetes, asthma, atopy, an increased risk of HIV transmission, as well as schizophrenia. For the mother, infertility, a lower success rate of IVF, preeclampsia, gestational diabetes and an increased rate of caesarean section have been suggested. Some of these associations are stronger than others.

*Effect of vitamin D on the fetus and neonate*

*Infant size*

Low maternal 25 OHD has been correlated with low birthweight, birth length and growth to one year in various studies.[49] In data from the Amsterdam ABCD cohort including 3730 women with a live singleton pregnancy, women with vitamin D deficiency (25 OHD <29.9 nmol/L) had infants with lower birth weights (−114.4 g, 95% CI −151.2, −77.6) and a higher risk of SGA (OR 2.4, 95% CI 1.9, 3.2) compared with women with a 25 OHD >50 nmol/L.[50]

A recent nested case-control study of nulliparous pregnant women with a singleton pregnancy who delivered a small for gestational age (SGS) infant demonstrated that in white women there was a U shaped relation between maternal 25 OHD and the risk of an small infant. This relationship was not seen in black women although maternal 25 OHD <37.5 nmol/L was more common. In the white women, the lowest risk of an SGA infant was at 25 OHD concentrations of 60–80 nmol/L.[51] Several single nucleotide polymorphisms in the vitamin D receptor are linked to SGA risk.[51]

*Infant bone*

Infant bone mass/bone mineral density is influenced by maternal vitamin D status and effects can be seen *in utero*. Maternal vitamin D deficiency is associated with subtle fetal bone abnormalities. A study of 424 women examined fetal femur length, distal metaphyseal cross-sectional area and the ratio of femoral metaphyseal cross-sectional area to femur length, in relation to maternal 25 OHD concentration. Low maternal vitamin D status was associated with greater femoral metaphyseal cross-sectional area and a higher femoral splaying index at 19 and 34 weeks gestation. In fetuses from mothers with a 25 OHD <25 nmol/L, the mean femoral splaying index increased from 0.074 to 0.084.[52] A study of newborn infants examined knee-heel length and found that maternal vitamin D <28 nmol/L was associated with a shorter knee-heel length (4.3 mm smaller (95% CI −7.3, −1.3)).[45]

A recent longitudinal cross-sectional study of 125 women–infant pairs with a median maternal vitamin D of 42.6 nmol/L used to define two groups, showed that those infants born to women with a higher vitamin D had greater bone mineral content and cross-sectional tibial area.[53] A different measure of bone mineral density – infant tibial speed of sound – has also been shown to correlate with maternal and infant serum vitamin D.[54] The influence of maternal pregnancy 25 OHD on fetal bone may persist over the longer term. Reduced maternal vitamin D in late pregnancy has been associated with reduced whole body and lumbar spine bone mineral content in children at 9 years.[55]

*Neonatal morbidity*

Neonates with low vitamin D may experience hypocalcaemic seizures, particularly if they have any other insult that predisposes to seizures and/or hypocalcaemia.[1]

An English study of infants with severe cardiomyopathy related to hypocalcaemia, found a mean infant vitamin D level of 18.5 nmol/L. All these infants had been breast fed.[56] Similarly, a ten year record review at the Children's National Medical Center in Washington DC, found four infants (all exclusively breast fed) presenting with dilated cardiomyopathy and hypocalcaemic rickets, with resolution of the cardiomyopathy with replacement of vitamin D and calcium.[57] Another examined ECGs and echo-cardiograms in 27 infants with rickets. In those infants most severely affected, an abnormality in left ventricular posterior wall thickness normalised after treatment.[58]

Maternal vitamin D status has also been linked to measures of immune tolerance[59] during childhood. Maternal vitamin D supplementation during pregnancy is associated with an increase in gene expression of immunoglobulin-like transcripts (ILT 3 and 4) in the cord blood, which are linked to immune tolerance.[60] Maternal vitamin D deficiency and low vitamin D intake in pregnancy have been associated with childhood wheeze, eczema and asthma. A study of 2000 healthy women–infant pairs showed maternal vitamin D intake in the highest quartile was associated with lower risk of wheeze in five year old children.[61]

In 763 Japanese mother–child pairs, children whose mothers had consumed 4.3 ug/day of vitamin D or more in their diet had a significantly reduced risk of wheeze and eczema (adjusted ORs [95% CIs] 0.64 [0.43–0.97] and 0.63 [0.41–0.98], respectively).[62] In an observational study of 1194 mother–child pairs in the north-east United States with a mean total maternal vitamin D intake of 548 (SD167) IU/day, those mothers in the highest quartile of daily intake showed a lower risk of having a child with

recurrent wheeze, OR 0.39 [0.25–0.62], when compared with those in the lowest quartile. In addition, every 100 unit increase in maternal vitamin D intake was associated with a lower childhood risk of recurrent wheeze, OR 0.81 [0.74–0.89].[63]

There have been several studies reporting a possible relationship between vitamin D supplementation during pregnancy or infancy and the development of diabetes-related autoantibodies and/or type 1 diabetes.[64,65] A meta-analysis including five observational studies of vitamin D supplementation documented a reduced risk of developing type 1 diabetes in infants who were supplemented with vitamin D (OR 0.71 [0.60–0.84]).[66] The All Babies in Southeast Sweden (ABSS) study examined the development of islet autoantibodies and found no relation between supplementation with vitamin D and islet antibodies, but a reduced rate of GAD or IA-2 antibodies.[65]

One study of 1669 children with the haplotype HLA-DQB, which confers susceptibility to type 1 diabetes, showed that maternal intake of vitamin D from food (but not supplements) was negatively associated with risk of asthma – HR 0.8 [0.64–0.99]. The women were ingesting 204 (SD 104) IU vitamin D from food and 56 (SD 104) IU from supplements/day.[67]

The prevalence of vitamin D deficiency in the non-pregnant HIV positive population is variously quoted as between 30 and 50% and may influence disease progression and survival in the non-pregnant population.[68] There is one Tanzanian study of vitamin D in pregnant women with HIV, which found that a maternal vitamin D <80 nmol/L (<32 ng/mL) was associated with a 50% higher risk (CI 2–120%) of maternal to child transmission of HIV at 6 weeks. Children born to women with a low vitamin D level had a 61% higher risk of dying during follow-up (95% CI, 25–107%).[69]

### *Associations with maternal problems in pregnancy*

#### *Gestational diabetes*

There is an association between low maternal vitamin D and gestational diabetes as might be expected from the association with insulin resistance and type 2 diabetes. In a cross-sectional study of 741 pregnant women, the prevalence of severe vitamin D deficiency (<12.5 nmol/L) was found to be higher in those with GDM (44% vs 23.5%) and there was a correlation between insulin resistance and vitamin D level ($r = -0.02$; $P = 0.002$).[70] An Australian study of 307 pregnant women found maternal 25 OHD concentrations inversely related to maternal fasting glucose.[23] The problem with these and many other studies is that it is not clear what role confounding factors such as ethnicity and obesity play in this relationship. Multivariate analysis with its assumption of a linear relationship between the confounders may not be enough to answer this question.

The one trial of supplementation with intravenous and oral 1,25-$(OH_2)$D in 12 women with GDM showed no change in glucose but decreased insulin levels following supplementation, suggesting an increase in insulin sensitivity.[71] Not all studies find an association between 25 OHD and GDM.

#### *Preeclampsia*

Preeclampsia occurs more frequently in those women who are vitamin D deficient or become so during the course of pregnancy. In a nested case-control study of women followed from less than 16 weeks gestation to delivery, a 50 nmol/L decline in 25 OHD over the course of the pregnancy increased the risk of preeclampsia by an adjusted odds ratio of 2.4 (95% CI, 1.1–5.4).[72] Low VEGF and increased pro-inflammatory cytokines have been associated with preeclampsia. Vitamin D has been shown to influence their expression which could underlie the association.[73–75]

One population study examined estimated vitamin D intake and preeclampsia risk and found a reduced odds ratio (0.76, 95% CI 0.60–0.95) for the development of preeclampsia in women with intake 600–800 IU/day compared with less than 200 IU/day. The reduction from supplement intake alone was 27% (OR 0.73, 95% CI 0.58–0.92) for women taking 400–600 IU/day compared with no supplements. There was no association between vitamin D from dietary intake alone and preeclampsia.[76] In a randomised trial of 400 women, 200 were given calcium 375 mg/day and vitamin D 1200 IU/day from 20 to 24 weeks pregnancy onwards (there was no placebo control). Blood pressure was significantly lower at 32 and 36 weeks in the supplemented group but the incidence of toxaemia (defined as BP > 140 mmHg systolic and/or 90 mmHg diastolic with urinary protein >300 mg/24 h) was unchanged.[77]

In another trial of supplementation with either calcium or calcium with vitamin D, where the provision of supplementation was stratified according to the risk of preeclampsia, those receiving vitamin D and calcium together were in the highest preeclampsia risk group. Of 666 women managed conventionally, 113 developed preeclampsia (16.9%). In the treatment group, overall, 210 women were managed with calcium and/or calcium and vitamin D supplementation and 10.9% developed preeclampsia. Those ($n = 7$) receiving calcium with vitamin D had a preeclampsia rate of 42.9%, but this result is difficult to interpret because the women receiving the combined treatment were at a higher baseline risk and the numbers are small. The rate for the two medium risk groups receiving calcium alone and the low risk group who received no treatment were 20%, 18%, 8.8% respectively.[78]

Gestational diabetes and preeclampsia are associated, and treatment of gestational diabetes directed at reducing hyperglycaemia significantly reduced the risk of preeclampsia in two large trials.[79,80] Vitamin D, but not calcium alone may reduce the risk of preeclampsia. These complex interactions make interpretation of the role and possible mechanisms of vitamin D in this setting very difficult.

*Infertility treatment*

Vitamin D levels in the serum and follicular fluid of women undergoing IVF treatment have been shown to be strongly associated with success of the treatment – each nanomole increase in follicular fluid vitamin D increased the likelihood for achieving clinical pregnancy by 2.4%.[81] Additionally, in a pilot study of 60 women with PCOS, those treated with calcium and vitamin D supplementation in addition to metformin had a higher rate of ovarian follicle formation over three months in comparison to metformin alone.[82]

## Measurement of vitamin D

Serum 25 OHD concentration is the accepted biochemical indicator of vitamin D status. It is not an easy analyte to measure.[83] The assays currently available include competitive protein-binding assays, radio-immuno assays (RAI), and direct detection measures including gas chromatography/mass spectrometry (GC/MS), high-performance liquid chromatography (HPLC), and liquid chromatography-tandem mass spectroscopy (LC–MS/MS).[83,84] Whilst at present, there is no single accepted "gold standard" measurement the most likely to become so are gas or liquid chromatography with mass spectrometry.[84]

The primary problem for the clinician is that the laboratory measurement of vitamin D is not standardised, and there is a large inter and intra, laboratory and assay, variability. The Vitamin D External Quality Assessment Scheme confirms this. There is currently a standard reference material under development in an attempt to bring a degree of cohesion.[83–85] The clinician should ensure that the laboratory that they use participates in an appropriately validated quality control scheme and that the assay has been standardised against a reference method. Hopefully in the future appropriate standards will improve the current situation.

## What is the normal range for vitamin D?

Historically, vitamin D deficiency was defined on clinical grounds – the presence of rickets in children or osteomalacia in adults. The US Centre for Disease Control still uses this definition (http://www.Cdc.Gov/cfs/cfsglossary.Htm#v). However, the majority of clinicians now use the concentration of 25 OHD to define the vitamin D status of a subject. The risk of rickets or osteomalacia probably begins at about 20 nmol/L 25 OHD. Bone health is impaired at 25 OHD level above that which gives rise to rickets/osteomalacia. The initial definition of mild vitamin D deficiency known as vitamin D insufficiency, was based on levels of 25 OHD which led to poor quality bone and an increased risk of minimal trauma fracture.[86]

A position statement from the Australian and New Zealand Bone and Mineral Society, the Endocrine Society of Australia, and Osteoporosis Australia[87] gives a balanced opinion on this controversial issue. The range is based on the association between bone health and various concentrations of 25 OHD. Values of 25 OHD less than 50 nmol/L are said to be low. Abnormal levels of vitamin D not sufficient to cause rickets are called vitamin D insufficiency (25–49 nmol/L). More recently, others[88] have redefined vitamin D

insufficiency as vitamin D deficiency based on the 25 OHD levels below which parathyroid hormone is inversely associated with 25 OHD concentration, and fractional calcium absorption may increase with vitamin D supplementation. This latter claim has been challenged.[4] Fracture risk is associated with low vitamin D levels[89] but the benefit of vitamin D in preventing fracture has been difficult to demonstrate, and the beneficial effect of vitamin D and calcium in combination is rather modest.

Low levels of vitamin D have also been associated with a variety of adverse health outcomes. The idea of low vitamin D as a disease entity in itself is beginning to take hold and a vitamin D deficiency syndrome has been described (http://www.Vitamindcouncil.Org/vdds.Shtml). However, these associations have not been established as causal in large RCT's. The lower limit of vitamin D thought to indicate normality ranges from 50 to 80 nmol/L and is based on associations with surrogate biochemical or clinical endpoints.

One very well conducted study of 93 adults with a mean sun exposure of 28 h per week, demonstrated a mean 25 OHD concentration of 78.8 nmol/L (measured by RIA (Diasorin) and reverse phase HPLC and confirmed with liquid chromatography mass spectroscopy). The highest level was 154.7 nmol/L.[90] None had a value below 25 nmol/L, 7% had values below 50 nmol/L. This suggests that with adequate sun exposure vitamin D levels are higher than currently observed in most populations. In our opinion, this study supports the notion that 50 nmol/L is a reasonable lower threshold for the normal range with 150 nmol/L the upper limit of normal. Regardless of the concentration of 25 OHD chosen as the lower limit of normal, the higher that value, the less likely that supplemental vitamin D has been proven to have any beneficial effect.

The normal range in pregnancy and lactation is likewise uncertain. The maternal and infant complications associated with vitamin D deficiency occur more often with a vitamin D level below 50 nmol/L. Vitamin D deficiency is common in women in pregnancy throughout different ethnic and socio-economic groups. The reported prevalence ranges from about 20 to 84%, depending in part on the definition. Those groups with increased skin pigmentation or customs of dress or behaviour that reduce sunlight exposure are at increased risk. Vitamin D concentrations vary a little through pregnancy, increasing in the late second trimester[91] but this change is not as marked as that which occurs between summer and winter.

## How much vitamin D supplement is required to achieve the desired normal range?

This is an important question but difficult to answer. It will vary with the amount of sun exposure and hence season, dietary intake and level of obesity. Several gastroenterological problems including achlorhydria and malabsorption result in low 25 OHD levels even with apparently appropriate sun exposure.[92] The current daily recommended intake is generally in the order of 400 IU/day. This will almost certainly not be sufficient to normalise low 25 OHD levels. Heaney has demonstrated that 5000 IU per day are required to significantly elevate low values.[93]

What level of supplementation is likely to lead to toxicity? Again this is controversial and not well documented. Toxicity is likely to vary with the group being studied and the duration of therapy. Potential toxicity includes hypercalcaemia, hypercalciuria, renal calculi and soft tissue and vascular calcification. Toxicity is likely to worsen with calcium supplementation. Hypercalcaemia, the most direct and worrying problem, is more likely with 25 OHD levels of 200 nmol/L or more unless there is underlying parathyroid autonomy. Vitamin D should be used with caution in hypercalcaemia and chronic renal failure.

## Summary/conclusions

Vitamin D can no longer be regarded as just a bone/calcium hormone. The role of vitamin D in multiple non-classical metabolic processes, while primarily demonstrated by association studies in human populations, has a possible physiological basis. There is considerable evidence that low maternal vitamin D levels are associated with worse outcomes for both mother and fetus in pregnancy, and for the neonate. Whether the association between vitamin D status and a wide range of adverse health outcomes is because 25 hydroxyvitamin D acts as a marker for some other health parameter such as obesity, or there is a direct causal relationship remains to be determined in most instances. The

optimal concentration of 25 hydroxyvitamin D is unclear, or at least controversial. RCTs of vitamin D supplementation with measurement of 25 hydroxyvitamin D to determine the baseline status, the level achieved on supplementation with appropriate documentation of possible confounders and the assessment of a variety of health outcomes are required. In the meantime it seems reasonable to try and achieve a 25 hydroxyvitamin D concentration of greater than 50 nmol/L in most populations, including pregnant women.

## Practice points

- Low vitamin D levels are associated with multiple adverse health outcomes in mother, neonate and child.
- Laboratory measurement of vitamin D can be problematic and attention needs to be given to the assay used.
- The "normal" range of vitamin D is difficult to define but as a minimum level it should probably be >50 nmol/L
- The best method of vitamin D supplementation and the dose required to produce a safe and effective improvement in vitamin D concentration has yet to be defined but will certainly require a larger dose than currently recommended.

## Research agenda

- There is an urgent need for randomised controlled trials of vitamin D supplementation with measurement of 25 hydroxy vitamin D to determine the baseline status, the level achieved on supplementation with appropriate documentation of possible confounders and assessment of a variety of health outcomes.
- An ongoing effort to standardise the vitamin D assay is already in progress.

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