Update on vitamin D: More than just a nutrient

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Abstract

An increasing number of pregnancy complications have been ascribed or at least associated with vitamin D deficiency. Vitamin D has undergone a metamorphosis from nutrient to hormone and more recently has been recognised to have broader biological relevance. This includes potential immunogenic, oncogenic and metabolic actions. This review outlines the roles of vitamin D, the problems in accurate measurement and determining normal ranges and how this is important in an understanding of vitamin D in normal and abnormal pregnancy.

Keywords

Pregnancy, reference values, vitamin D

Introduction

When Edward Mellanby discovered vitamin D 90 years ago,¹ he would not have foreseen the broader implications this molecule would have in human biology and its potential role as a modulator of disease beyond that he initially assigned to its role in bone cell biology; deficiency of which he had proven caused rickets. At that time the concept that a dietary factor was an essential vital nutrient, deficiency of which could cause disease, was a novel concept arriving as it did after a 'Pasteurian era' where the bulk of human disease studied at the time had been attributed to microbial causes. In the years immediately preceding that discovery and for several decades thereafter, numerous vitamins were characterised and studied for their role in human biology and disease states. Thus, vitamin D remained classified as a nutrient for the first few decades after its discovery. Indeed the fact that one teaspoon of cod liver oil containing approximately 5 µg of vitamin D (200 IU) was known to be sufficient to prevent childhood rickets meant that for most of the 20th century, the Recommended Dietary Intake (RDI) for vitamin D was somewhere in the range of 200–400 IU. In reality, RDI was in some ways a misnomer as it was really a minimum, not maximum, recommendation. Only in recent years has the RDI been changed and increased two to fourfold over that earlier limit and even that recommendation is still the subject of considerable controversy in adults and children, let alone pregnant women. It is instructive however to read the reports from the UK in the 1950s that described the epidemic of the previously rare 'idiopathic infantile hypercalcaemia' attributed to the widespread fortification of milk and cereal with vitamin D such that infants and newborns were receiving 4000 IU per day.² The lay concept that 'vitamins are good for you' and that toxicity is rare if not impossible is misplaced and yet prone to exploitation.

Actions of vitamin D

Unlike other molecules, vitamin D underwent a metamorphosis from 'nutrient' to 'hormone' with the advent of dialysis and the realisation that those patients who no longer died from chronic kidney disease developed osteodystrophy. That condition was characterised by a number of different pathologies but encompassed a degree of hypo-mineralisation and hypocalcaemia reminiscent of that seen in vitamin D-deficient individuals. The discovery that vitamin D was metabolised to the active hormonal form of 1,25-dihydroxy vitamin D3 [1,25(OH)2D3]^{3,4} by a tightly regulated negative feedback loop in the kidney firmly placed the further study of this hormone and its role in normal human growth and development under the umbrella of endocrinologists and nephrologists for the next two or three decades.

The realisation following advances in molecular biology that the receptor for 1,25(OH)2D3 (VDR) was widely expressed in tissues other than that considered to be part of the classic kidney/bone/gut/parathyroid axis forced a further change in the way clinicians and scientists thought about vitamin D and its role in human biology and disease.⁵ Further evidence that there is also ubiquitous expression of the 24-hydroxylase⁶ that is responsible for the catabolic destruction of 1,25(OH)2D3 also supported hypotheses that this molecule has a broader biological relevance. Subsequent proof that tissues such as macrophages, monocytes, activated lymphocytes and antigen presenting cells possess an intrinsic ability to synthesise their own active form of the molecule through local tissue production and expression of the 1-hydroxylase⁷ pointed towards a potential immunogenic role while a host of tissues such as breast, colon, prostate and pancreas also implicate an oncogenic or metabolic role.⁸ In vitro studies support a broad array of genomic and non-genomic mechanisms by which 1,25(OH)2D3 can influence cytodifferentiation and immunomodulation in an autocrine and paracrine fashion.^{9–11} These studies are supported by epidemiological associations with infectious, malignant and autoimmune disease^{12,13} to provide a sufficient body of evidence warranting further investigation and large-scale interventional studies.

Roles of vitamin D

1. Nutrient-bone health
2. Hormone-1,25-dihydroxy vitamin D3 activity and negative feedback regulation
3. Cytokine-cytodifferentiation, immunomodulation

Vitamin D and reproduction

In pregnancy, the hypotheses regarding the importance of vitamin D are no less compelling. The evolutionary observation that skin pigmentation decreases with increasing latitudinal distance from the equator provides circumstantial evidence for the theory that exposure to sunlight has the capacity to exert a selective pressure to human populations. Vitamin D is not the only molecule synthesised in response to

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Figure 1. 1,25(OH)₂D₃ levels and calcium metabolism in pregnancy.

ultraviolet light exposure and we must always keep in mind the possibility that vitamin D is a marker for an as yet to be characterised effect arising from ultraviolet light exposure that is independent of vitamin D. Nonetheless for the reasons outlined above, vitamin D is perhaps the most compelling candidate that could contribute to this effect. Apart from well-characterised seasonal differences in vitamin D levels, modern migration patterns prove that pigmented individuals who now live at higher latitudes or those who through habit or custom shield themselves from ultraviolet light exposure have significantly lower vitamin D levels.¹⁴ In the era of the 'classic' bone-calcium paradigm, the potential for vitamin D to exert a selective pressure on a population's ability to reproduce was reasoned to be due to the inability of the rachitic skeleton to support childbearing and/or breastfeeding. Presumably this hypothetical effect would become more dramatic with increasing parity and would have to invoke two simultaneous deficiencies; specifically, a deficiency of both vitamin D and dietary calcium.

Calcium absorption from the gut does so via two distinct processes; one a passive, saturable, para-cellular diffusion driven by electrochemical gradients across the lumen and the second an active trans-cellular vitamin D-dependent process which is age and diet dependent. Vitamin D primarily exerts its effect at the gut to enhance gut calcium absorption in the face of dietary calcium restriction where active calcium transport is necessary to meet daily requirements.¹⁵ In the face of adequate dietary calcium, the requirement for active gut transport through a vitamin D-mediated process is less dominant.¹⁶ As humans of different race and colour cherished and acquired skills in animal husbandry, milk became a staple food source in many cultures. However, as will be outlined by other reviewers associated with this publication, other plausible mechanisms could be proposed by which vitamin D deficiency could predispose to population- or pregnancy-specific disorders that would threaten the survival of the mother and/or her child (Figure 1).¹⁷

Normal range of vitamin D in pregnancy

Instrumental in facilitating the exponential increase in vitamin D testing of pregnant women has been two changes in the way we define and measure vitamin D deficiency in human populations. First, there were changes to the way that 'normal' vitamin D levels in populations were defined.¹⁸ Second, technical changes to the way that vitamin D levels were measured were compatible with automation of vitamin D assays and wider dissemination of 'abnormal' results. Once again a discussion of the validity with which normal ranges can be defined by threshold levels in human populations is tangential to this article but it is nonetheless valid to note that there is considerable debate and controversy as to where

the inflection point for 'normality' occurs and for which endpoint. Given the known association between vitamin D, calcium and parathyroid hormone levels, the basis for most thresholds have been the level of vitamin D at which there is an inflection point for suppression of parathyroid hormone. This level varies with age, gender and populations in different geographic areas (Figure 2).¹⁹

Proponents of higher thresholds for normal vitamin D argue that this dependence upon a classic vitamin D endocrine system ignores the emerging data supporting a role for the local tissue production of 1,25(OH)₂D₃ that is substrate dependent but cannot yet provide population-based data that defines that threshold of sufficiency. Irrespective of any controversy about the definition of vitamin D sufficiency in the normal population, a recommendation to advocate screening for vitamin D deficiency and its replacement in pregnancy based on extrapolation from non-pregnant populations will fail to take into account the unique set of adaptations that occur in pregnant women. It is already known that vitamin D metabolism is markedly altered in pregnancy. There is an increase in 1,25(OH)₂D₃ production through stimulation of renal and placental 1-hydroxylase activity, relative suppression of parathyroid hormone levels and stimulation of PTHrP at a tissue level.²⁰ At the same time, there is also an increase in urinary calcium excretion that mirrors the increase in gut calcium absorption. There is a change in weight and body composition which in non-pregnant populations influences 25OHD levels; these effects in pregnancy have not been extensively studied. Similarly, there are pregnancy-related increases to the circulating levels of the protein, Gc protein, charged with the responsibility of binding vitamin D and carrying it throughout the body.²¹ Gc protein circulates at levels that provide an excess of binding sites for circulating 25OHD and 1,25(OH)₂D₃ but its role in the tissue delivery of vitamin D is still being clarified. The fact that genotypic differences in Gc protein have been associated with different binding affinity that could influence the kinetics and availability of vitamin D at a tissue level implies that a great deal about vitamin D metabolism in pregnancy remains to be clarified before we can conclude anything about its association with disease in pregnancy.²²

Irrespective of whether there is any advance in our understanding of the role of vitamin D in human disease and biology beyond that of the classic 'bone-calcium' axis, it would be of paramount importance to first obtain a consensus about any data that seeks to define 'normal' vitamin D levels in pregnancy.

Measurement of vitamin D in pregnancy

There have also been changes to the way that vitamin D is measured. In addition to the concerns about interference from lipophilic substances in the matrix of human serum, it was previously thought necessary to extract and chromatographically separate vitamin D metabolites before immunoassay because of the abundance of a highly avid vitamin D binding protein. This is still certainly the case with current techniques that use mass spectroscopy (LC-MS/MS) for analytical detection. Problems with sample losses and recovery during extraction and handling and the more cumbersome workflow associated with those steps were bypassed with the advent of automated one-step immunoassays that attempt to measure 25OHD levels in unextracted serum. Once again it is beyond the scope of this article to describe the challenges associated with that technology, but there are sufficient differences between some of the commercially available assays to recommend that clinicians with an interest in measuring vitamin D levels in their patients need to be aware of which assay their service provider uses and the robustness of that test and laboratory to deliver accurate and precise results.²³ Moreover, the ability of these tests to perform as well in pregnant serum where there is an even greater abundance of circulating vitamin D binding protein has not been routinely assessed for all immunoassays. Advances in the speed and efficiency with which serum can be analysed using LC-MS/MS technology have the potential to optimise

Figure 2. Various levels of serum 25OHD (ng/ml) at which serum PTH (pg/ml) plateaus and/or is maximally suppressed. (Reproduced with permission JCEM.)

the analytical precision with which vitamin D levels can be measured in human populations. Notwithstanding some of the technical issues associated with LC-MS/MS, it is probably timely to consider all of the issues that will influence biological variability in pregnant women before a wider rollout of an expensive analytical technique can be advocated.

Vitamin D deficiency and pregnancy

The realisation that vitamin D has a broader immunomodulatory role allows us to revisit the hypothesis concentrating on a number of diseases in pregnancy, either pre-existing or pregnancy specific, that could exert a potent selective effect on the mother and/or her fetus from the moment of conception of the first child. The associations that have been described between vitamin D in pregnancy, its outcomes and disorders affecting the mother and her infant will be presented in a further review in this journal. However, it is appropriate to state that these data should be considered hypothesis generating and this author would encourage the development of well-designed trials that study clinically relevant outcomes to justify the explosion in testing and cost of replacement and monitoring that has occurred in the past decade.²⁴ However, the confidence with which we will be able to scientifically ascertain these associations is critically dependent upon our ability to understand and confidently measure vitamin D's biological and analytical variations.

Conclusion

Thus, many things need to come together simultaneously for the story of vitamin D replacement in pregnancy to advance beyond its current level of evidence. At present, there is unequivocal evidence that giving vitamin D to pregnant women will increase the vitamin D levels in the mothers and their babies.²⁵ Whether that makes a difference to the disease burden in human populations will be harder to establish. That task is made even more difficult by technical and analytical variation that could obscure significant associations. Advocates for vitamin D testing and replacement in all pregnant women may argue the

case that uncertainty as to degree of benefit, if any, justifies replacement on the basis that it is not harmful. Similar arguments may have supported the public health initiative to fortify milk and cereal with vitamin D in the 1950s at a time when a recognised association with vitamin D deficiency, rickets, was more prevalent. More recently, high-dose annual vitamin D therapy which was thought to assist in reducing the burden of osteoporotic fracture in elderly patients was unexpectedly proven to do the exact opposite.^{26,27} With hindsight, these recommendations had to be withdrawn or revised and we should cautiously approach the future with a scientific rigour that seeks to avoid the mistakes of the past. Proper study design of maternal and fetal outcome together with accurate testing of vitamin D in pregnant women is paramount before we will be able to assign normality (and treat) an abnormality in a woman's 25OHD level. At present that is not the case.

Declaration of conflicting interests

The Author declares that there is no conflict of interest.

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