Scandinavian Journal of Clinical & Laboratory Investigation, 2012; 72(Suppl 243): 154–162

ORIGINAL ARTICLE

Key questions in Vitamin D research

PATSY M. BRANNON

Cornell University, Ithaca, NY, USA

Abstract

Despite interest and expanding research on non-bone health outcomes, the evidence remains inconclusive concerning the causal role of vitamin D in the non-bone health outcomes. To improve our understanding of its role, research needs to address five key areas related to vitamin D: 1) its physiology and molecular pathways. 2) its relationship to health outcomes. 3) its exposure-response relationships, 4) its interactions with genotype and other nutrients and 5) its adverse effects. Its metabolism needs to be elucidated including extra-renal activation and catabolism, distribution and mobilization from body pools, kinetics of this distribution, and their regulation during pregnancy and lactation. Rigorous, well-designed randomized clinical trials need to evaluate the causal role of vitamin D in a diverse array of non-bone health and chronic disease outcomes across the life cycle and reproductive states. Critically needed is the determination of the exposure-response, inflection and threshold of serum 25(OH)D concentrations relative to functional and health outcomes. The dose-response relationships of standardized measures of serum 25(OH)D need to be understood in response to low and high doses of total vitamin D with careful consideration of confounding factors including catabolic rates. How do relevant genetic polymorphisms, dietary calcium and phosphate and potentially dietary cholesterol interact with vitamin D exposure on its bioavailability, transport, distribution in body pools, metabolism and action as well as on bone and non-bone health outcomes? The nature and mechanisms of U-shaped risk relationships with adverse health outcomes at higher exposure to vitamin D needs elucidated across the life cycle and reproductive stages.

Key Words: *1,25-dihydroxyvitamin D, 25-hydroxyvitamin D, vitamin D intake*

Introduction

The active metabolite of vitamin D, $1,25(OH)_2D$ produced in the kidney by 1α-hydroxylase (CYP27B1), regulates calcium-phosphorous homeostasis and bone health through its classic hormonal actions in concert with its nuclear transcriptional factor, vitamin D receptor (VDR), on target genes containing the VDR response element. Discovery of VDR expression in many tissues not involved in these classical actions as well as vitamin D's regulation of a wide array of cellular processes including proliferation, immune response, and inflammation expanded the investigation of vitamin D's role in human health beyond its classical endocrine function in bone health. Even though interest is keen and research on non-bone health outcomes has exploded, the evidence remains inconclusive concerning the causal role of vitamin D in the prevention and etiology of cardiovascular disease, cancer, infectious disease, autoimmune disorders, diabetes, cognitive disorders, falls, adverse maternal and fetal pregnancy outcomes, etc. despite the plausible biological roles of vitamin D relevant to these non-bone health outcomes [1].

In addition, widespread extra-renal expression of 1α-hydroxylase and *in vitro* evidence suggest the intracrine, autocrine and paracrine action of locally-produced $1,25(OH)_2D$. Extra-renal production appears primarily responsive to the availability of its precursor metabolite, 25-hydroxyvitamin D produced in the liver from vitamin D. Thus, the circulating level of 25-hydroxyvitamin D clearly established as the best biomarker of total exposure to vitamin D from endogenous and dietary sources is increasingly regarded by some as a biomarker of effect. However, evidence validating circulating concentration of 25-hydroxyvitamin D as a biomarker of effect remains absent. Even definitive thresholds for rickets or osteomalacia below which all individuals exhibit these frank deficiencies cannot be determined [1,2].

This paper draws on two interdisciplinary panels [1,3] and two recent critical review [4,5] that identified:

Corresponding author: P. M. Brannon, 225 Savage Hall, Cornell University, Ithaca, NY 14853, USA. E-mail: pmb22@cornell.edu

ISSN 0036-5513 print/ISSN 1502-7686 online © 2012 Informa Healthcare
DOI: 10.3109/00365513.2012.682895

1) the gaps in our knowledge of vitamin D and human health across the life cycle and reproductive stages and
2) key research questions that need to be answered to advance our understanding of the role of vitamin D in human health.

The Institute of Medicine Committee on the Dietary Reference Intakes for Calcium and Vitamin D [1] described research needs for vitamin D, human health outcomes, intake response, dietary requirement, and intake assessment based on their consideration of the evidence through August, 2010 including two Agency on Healthcare Research and Quality (AHRQ) evidence reviews on vitamin D [2] and vitamin D and calcium and health [6] as well as their assessment of the strengths and gaps in the evidence. The NIH Roundtable on Vitamin D and Human Health [3] discussed the research needs focused on five questions addressed by the AHRQ Evidence Based Review on the Effectiveness and Safety of Vitamin D in Bone Health [2] and the NIH Conference on Vitamin D and health in the 21st Century: An Update [7]. We describe research needed to understand the role of vitamin D in pregnancy and lactation [4]. Cashman and Kiely [5] identify research needs relative to public health. Below, the key research questions for Vitamin D are discussed in five areas:

1) physiology and molecular pathways of vitamin D,
2) health outcomes related to vitamin D,
3) exposure-response relationships,
4) interactions with genotype and other nutrients and
5) adverse effects.

Physiology and molecular pathways of vitamin D

Despite the considerable advancement of our understanding of the metabolism of vitamin D (Figure 1), the distribution of vitamin D and 25(OH)D in body pools including their storage and mobilization is 'notably lacking' [1]. We need to elucidate the body pools for the parent and major circulating metabolite [1,2]. Does adipose tissue merely sequester vitamin D and its metabolites or does it function as a storage pool from which vitamin D and its metabolites can be mobilized [1,2]? If the latter, what factors influence its storage and mobilization? How does this relate to adiposity of an individual and the level of the existing stores? What is the relationship with exposure either from dietary or endogenous sources [1,3]? Understanding this relationship for dietary versus endogenous vitamin D is important because the initial transport to the liver and interaction with peripheral tissues differ between these (Figure 1). Endogenously produced vitamin D enters the blood, binds to the vitamin D binding protein (DBP) and is transported to the liver whereas dietary vitamin D is absorbed, packaged into chylomicrons, released from the lymph into the peripheral tissue where the chylomicrons are metabolized by lipoprotein lipase and then transported in the remnant particles to the liver. Does this difference in initial transport alter the distribution of dietary versus endogenously produced vitamin D among body pools, particularly the storage pools in adipose tissue? Do storage concentrations affect the response of circulating 25(OH)D concentrations to intake of vitamin D? What is also unclear, but needs to be assessed is whether storage pools relate to health outcomes – either beneficially or adversely.

We also need to understand the flux and kinetics among these pools for 25(OH)D and the factors that affect these dynamics. 25(OH)D can be taken up by tissues through one of two mechanisms (Figure 1). Free 25(OH)D can diffuse across cell membranes, which appears to be nearly a ubiquitous mechanism. The amount of DBP and its effect on free versus bound 25(OH)D could inversely influence the available free 25(OH)D for uptake as demonstrated recently in cultured human monocytes [8]. In the kidney, mammary epithelial cell, hepatic stellate cells, osteoblasts and malignant B-lympohcytes, the coreceptors megalin and cubulin can bind and internalize DBP with its bound 25(OH)D [9–13]. Interestingly, the uptake of DBP is low in normal quiescent B-lymphocytes unless activated with mitogen [10] and variable among breast cancer cell lines [11]. Megalin expression also varies among specific tissues and during development [9]. Thus, this endocytotic mechanism may be limited to specific tissues in specific physiologic, pathophysiologic or developmental states. In contrast to the inverse effect of DBP concentrations on diffusion, the amount of DBP could directly influence the uptake 25(OH)D via the endocytotic mechanism. Exemplifying the complexity these relationships may pose is the recent nested case-control report that circulating DBP concentrations inversely relate to pancreatic cancer risk whereas 25(OH)D concentrations positively associate with pancreatic risk [14]. However, DBP and 25(OH)D concentrations interact such that the higher combined concentrations of DBP and 25(OH)D associate with reduced risk whereas high 25(OH)D concentrations and low DBP concentrations associate with increased risk. We need to understand better the impact of development and physiologic or pathophysiologic states of specific tissues on the expression of megalin/cubulin and the relative uptake by each pathway both in terms of the effects on the dynamics of the distribution of 25(OH)D and on health outcomes.

The nature and significance of extra-renal production of $1,25(OH)_2D$ needs to be determined and its impact non-bone health outcomes needs to be elucidated [1]. Critical to our understanding is the relevance of vitamin D nutriture and serum

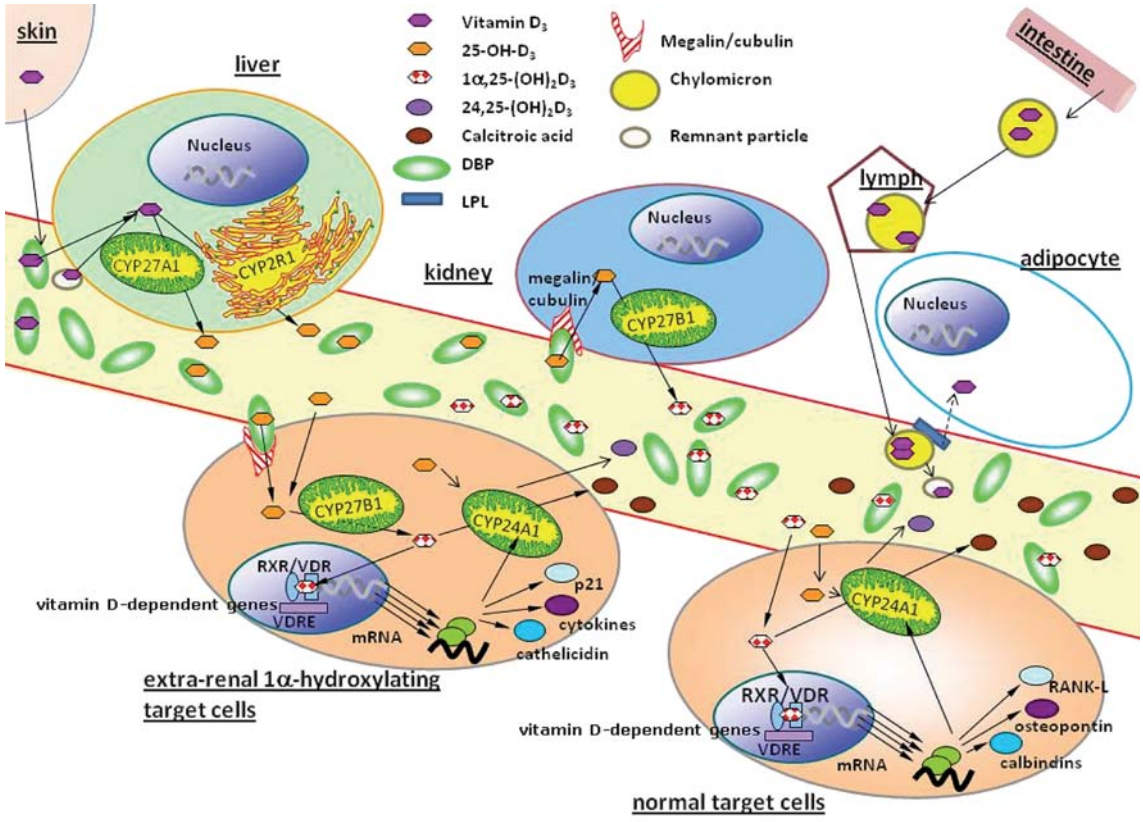

Figure 1. Vitamin D metabolism, transport and body pools. Endogenously produced vitamin D diffuses into the blood, binds to the vitamin D binding protein (DBP) and transports to the liver. In contrast, dietary vitamin D is first packaged into chylomicrons after intestinal absorption, transported to the lymph into the peripheral blood where the chylomicrons are metabolized by lipoprotein lipase (LPL) and then transported to the liver in the rsulting remnant particles. Regardless of source, vitamin D is hydroxylated in the liver to 25-hydroxyvitamin D [25(OH)D], which is transported in the blood bound to DBP. In the kidney and possibly a wide-variety of extra-renal tissues, 25(OH)D is again hydroxylated to the active 1,25 dihydroxyvitamin D [1,25$(OH)_2$D]. Both 1,25$(OH)_2$D and 25(OH)D can be catabolized to the inactive calcitroic acid or 24,25$(OH)_2$D, respectively, by the 24-hydroxylase (CYP 24A1) that is also widely expressed. Circulating 25(OH)D can be taken up by one of two mechanisms, the ubiquitous diffusion of free 25(OH)D or the DBP-receptor mediated endocytosis by megalin/cubulin, which is selectively expressed in a tissue, developmental and pathophysiologic specific manner (adapted from Jones [44]).

25(OH)D to this production and local action *in vivo*. Identifying and validating biomarkers of effect in relevant models for use in human studies is also essential to demonstrate conclusively that this extra-renal production and local action seen *in vitro* occurs *in vivo*. Studies using tissue specific knockout models for VDR, 1α-hydroxylase and the catabolic 24-hydroxylase would be helpful to understand and characterize role of extra-renal production and local action and its relationship to health outcomes [3].

Controversy exists concerning the bioavailability and bioequivalence of vitamin D3 and D2. The physiological responses, metabolism, distribution in body pools, comparative kinetics of metabolism, including catabolism by 24-hydroxylase, and safety risks need to be determined [1].

During pregnancy and lactation, vitamin D metabolism also is not well understood. The mechanisms whereby well-documented physiologic changes in maternal vitamin D metabolism during pregnancy are unknown [4]. Specifically, researchers need to determine the mechanisms through which maternal DBP and serum 1,25$(OH)_2$D concentrations increase. The relative contributions of the kidney and placenta to increased 1,25$(OH)_2$D need to be understood. Changes in the distribution of vitamin D and its metabolites during pregnancy are also unknown. In lactation, the mechanism of transfer of vitamin D, its metabolites and DBP across mammary epithelium also needs to be determined. Such understanding might improve our approaches to enhancing the low content of vitamin D and its metabolites in human milk without exposing the lactating woman to dietary intakes in excess of the Tolerable Upper Intake Level (UL) of 100 μg/d [1].

In summary, key research needs exist for understanding the metabolism including extra-renal activation and catabolism, distribution and mobilization from body pools, kinetics, and regulation of the dynamics including changes during pregnancy and lactation and impact of dietary intake and sources of vitamin D.

Health outcomes related to vitamin D

The IOM Committee [1], the NIH Roundtable [3] and we [4] all emphasized the pressing need to determine the causal role of vitamin D in non-bone health outcomes, including but not limited to falls, infectious disease, autoimmune disorders, chronic diseases (total and site-specific cancers, cardiovascular disease, diabetes, cognitive disorders, etc.), its developmental programming in utero and the early postnatal period, and maternal and fetal adverse outcomes (preeclampsia, gestational diabetes, obstructed labor/Caesarian section, small for gestational age, gestational duration, etc.) [1,3,4]. Intriguing evidence links low serum 25(OH)D concentrations with increased risk of these adverse health outcomes; but as evaluated by the IOM Committee [1] and the AHRQ evidence review on vitamin D and health outcomes [6], the evidence is conflicting, primarily observational in nature and lacks causality. Thus, the role of vitamin D in non-bone health outcomes is presently inconclusive. The IOM Committee [1], the NIH Roundtable [3] and we [4] point to the need for rigorous and well-designed randomized clinical trials to evaluate the causal role of vitamin D in non-bone health outcomes across the life cycle and reproductive states. High quality studies with strong causal designs are needed that adequately consider key confounders including but not limited to baseline vitamin D status and intakes, interaction with dietary calcium and phosphate, baseline muscle quality and function, physical activity, adiposity and body composition, skin pigmentation, etc. A number of such trials are underway, and their results are eagerly awaited. We highlighted, in particular, the need to examine the role of vitamin D in maternal infectious disease, the only pregnancy outcome for which consistent observational associations exist [4]. The IOM Committee [1] also highlighted the need to determine appropriateness of 25(OH)D as a biomarker of effect, which is lacking presently as discussed above.

Despite the considerable and causal evidence for the role of vitamin D in bone health, gaps in our knowledge still exist for younger ages, during menopause and in dark-skinned individuals. The IOM committee [1] identified the need to clarify threshold effects of vitamin D on skeletal health outcomes by life stage and for different racial/ethnic groups. The NIH Roundtable [3] noted the need for research on subclinical rickets and osteomalacia in terms of their prevalence, improved surveillance and biomarkers indicative of early pathology in US population, in particular. More broadly, Cashman and Kiely [5] identified the greatest immediate need to be the definition of the threshold serum 25(OH)D value for vitamin D sufficiency by 'an accountable, transparent and systematic evidence-based consensus development process, engaging all the relevant stakeholders'.

In summary, evaluating the causal role of vitamin D in non-bone health outcomes would not only advance our understanding of its role, but would also clarify its potential to improve public health through the reduction of chronic disease. In addition, we need consensus and evidence-based definition of the threshold 25(OH)D concentration indicative of sufficiency.

Exposure-response relationships

Critically needed in the judgment of both the IOM Committee [1] and the NIH Roundtable [2] is the determination of the inflection and threshold of serum 25(OH)D relative to functional and health outcomes for which vitamin D plays a causal role at several life and reproductive states in order to understand vitamin D status and requirements. In particular, such research needs to include dark-skinned individuals, infants, adolescents, and reproductive-aged, pregnant and lactating women [3]. We need to identify specific health outcomes in relation to graded and fully measured intakes of vitamin D including baseline intakes [1].

Indeed, the nature of the dose-response relationship between intake and achieved serum 25(OH)D concentrations needs to be clarified. Assuming minimal sun exposure, the IOM Committee found a curvilinear relationship between total intake and achieved 25(OH)D concentrations in a simulated dose-response curve [1]. The committee also found a curvilinear response to total intake at lower latitudes ($>40\,^{\circ}$N $<49.5\,^{\circ}$N) during the winter, where sun exposure is widely regarded as insufficient for providing vitamin D synthesis in the winter. However, the achieved 25(OH)D concentration was approximately 25 % higher at these lower northern latitudes than at the more northern latitudes, raising questions about whether sun exposure is as minimal between $40\,^{\circ}$N and $49.5\,^{\circ}$N during the winter as assumed.

Cashman and colleagues [15] report from a systematic review and meta-regression a linear relationship with total intakes <35 μg/d but a curvilinear relationship when intakes >35 μg/d are included. Both the IOM [1] and Cashman and colleagues [15] find no effect of age on the dose-response relationship. Cashman and colleagues [15] also report higher achieved 25(OH)D level by 8 %, at the lower latitudes in the winter. The difference in the curvilinear relationship including higher intakes and the linear relationship restriced to intakes ≤ 35 μg/day exemplifies our need to understand the effect of low and high intake on the metabolism and distribution of vitamin D as discussed above. A recent study in postmenopausal women reports a curvilinear (quadratic) relationship of achieved 25(OHD) concentrations with dietary intakes from 10 to 120 μg/d [16]. Neither the

IOM nor the meta- analysis included pregnant or lactating women, but a recent study [17] reports a curvilinear relationship of circulating vitamin D with 25(OH)D concentrations in pregnant women supplemented with 10 to 100 μg/d. We need to understand the metabolism and dose-response relationships of exposure to achieved 25(OH)D concentrations at low and high vitamin D intakes described above. Exemplifying the need to understand the metabolism is the recent finding that the initial $24,25(OH)_2D$ to 25(OH)D concentrations predict the response of 25(OH)D to supplemental vitamin D intake [18], suggesting that catabolism is an important factor in the response to vitamin D. How habitual vitamin D exposure and usual 25(OH)D concentrations affect catabolism needs to be understood in order to model and interpret the dietary exposure-response relationships.

We need to clarify the influence of age, body weight and body composition on the variability of the response of 25(OH)D concentrations to intake/exposure [1,3]. In addition, the impact of body stores on the response of 25(OH)D concentrations to intake exposure also needs to be clarified. The NIH Roundtable noted the need to assess whether different fortificant delivery systems and food production methods affect bioavailability, bioequivalence and safety of vitamin D [3]. Both the IOM Committee and NIH Roundtable described the need to understand whether there is a level of sun exposure that poses minimal UVB risk for cancer and enables sufficient vitamin D production [1,3].

Standardization of 25(OH)D measurement remains a critical need [1,3]. Use of the Standard Reference Material from the National Institute of Standards and Technology would ensure comparability of 25(OH)D measures across studies and to facilitate synthesis and meta-analysis of results across studies. Recent studies continue to document the variable performance among different assay methodologies [19–21]. Although immunoassay methods correlate with LC-MS methods, systematic negative and positive biases are reported [19–21]. Further, immunoassays are affected by DBP concentration [21]. The lack of standardization of the measurement of 25(OH)D concentrations reduces the internal validity of studies of vitamin D and, thus, the strength of the evidence for vitamin D's role in human health.

In summary, the dose-response relationships of standardized measures of serum 25(OH)D needs to be understood in response to low and high doses of total vitamin D considering confounding factors including catabolic rates.

Interactions with genotype and other nutrients

Emerging evidence highlights the potential interaction of relevant genetic polymorphisms in key genes with vitamin D exposure to impact bioavailability, transport, distribution in body pools, metabolism and action of vitamin D [1,3]. How these interactions impact vitamin D's role in human health is critical to understand. The IOM Committee [1] noted the need to elucidate the effect of genetic variation among racial/ethnic groups. Evidence supports the impact of polymorphisms in DBP/GC, VDR, 25-hydroxylase, 24-hydroxylase and 7-dehydrocholesterol reductase (or near it) on variability in serum 25(OH)D concentrations [22–26]. As an example, the 25-hydroxylase (CYP 2R1) SNP RS12794714-AA allele associates with a 15.7 % lower serum 25(OH)D concentration than the GG allele [24]. Emerging evidence also associates these relevant genetic polymorphisms with health outcomes [27–32].

Relative to pregnancy, the IOM Committee [1] highlights our need to understand the interaction of these genetic polymorphisms and epigenetic regulation of vitamin D on developmental outcomes. We [4] noted the need to understand genetic variants in the vitamin D metabolic pathway, particularly in *Vdr*, 1α-hydroxylase and 24-hydroxylase relative to their impact on risk to the maternal and fetal outcomes and interaction with maternal vitamin D status. Such interactions are reported for VDR polymorphisms for birth weight and size [30–31], 1α-hydroxylase and 24-hydroxylase and the risk of gestational diabetes [32]. Further, the possibility for genotypic interaction in the paracrine action of vitamin D exists because of the juxtaposition of the fetal genotype in the placental trophoblasts with the maternal genotype in the decidual tissue [33] with the same exposure to serum 25(OH)D in maternal blood that bathes both tissues.

The NIH Roundtable [3] described our need to understand the influence of calcium and phosphate on regulation of vitamin D activation and catabolism through parathyroid hormone and fibroblast growth factor 23. Specifically, research needs to identify the pathways that regulate vitamin D activation and catabolism in order to understand these interactions on its metabolism.

Emerging evidence also suggests other possible nutrient interactions with vitamin D. First, retinoic acid upregulates megalin, cubulin and DBP endocytosis in a mammary cancer cell line [34], second, recent evidence [35] documents the absorption of vitamin D by the two intestinal receptors involved in cholesterol absorption—scavenger receptor B1 and Nieman Pick C1 Like 1 receptor—and its reduction by both cholesterol and the drug, ezetimibe. Thus, vitamin A status could affect vitamin D distribution and tissue uptake in specific tissues whereas dietary cholesterol and the widely-used drug ezetimibe could affect dietary vitamin D's bioavailability.

In summary, the interaction of relevant genetic polymorphisms in key genes, dietary calcium and phosphate and potentially cholesterol and the drug,ezetimibe, with vitamin D exposure needs to be determined on the bioavailability, transport, distribution in body pools, metabolism and action of vitamin D as well as on bone and non-bone health outcomes.

Adverse effects

Frank toxicity with vitamin D occurs with very high intakes >250 μg/d [1], but emerging evidence of U-shaped risk curves exists for higher serum 25(OH)D concentrations within what is widely considered 'physiologic' and a variety of health outcomes including all-cause mortality, selected cancers, CVD, falls and fractures at high exposures [1], Based on this emerging evidence, the IOM DRI Committee found that serum 25(OH)D concentrations >125–150 nmol/L associate with increased adverse risk of these outcomes [1]. Since 2010 additional evidence of U-shaped risk has been reported for SGA [36], fetal femur length [37], developmental programming of schizophrenia [38] and eczema [39], biomarkers of inflammation such as CRP [40]. and frailty in older women [41]. Adequate consideration is needed for potential confounders such as recent weight loss, supplement-taking individuals with chronic illness etc. We need to assess the relationship of higher exposure to vitamin D with adverse health outcomes. Typical analyses may not detect U-shaped pattern with standard parametric multivariate modeling using linear or logistic analyses. Researchers need to consider alternative analyses such as non-parametric or spline-analyses to evaluate the relationship of vitamin D exposure/25(OH)D concentrations with health outcomes. Given the ethical concerns, innovative methods are needed to identify and assess these adverse outcomes [1], especially for non-bone health outcomes [3].

The data are limited on the safety of long-term high serum 25(OH)D concentrations achieved through supplementation, so we need to understand the long-term effects of high dose intakes of vitamin D with health outcomes [1,3]. Particularly challenging will be assessing the long-term adverse outcomes of developmental programming of high maternal 25(OH)D concentrations during pregnancy because confounding factors during pregnancy may persist postnatally. Rigorous studies in relevant animal models could inform our understanding of the long term impact of high level exposure to vitamin D across the life cycle. Also important to understand is the interrelationship between dietary calcium and vitamin D toxicity [3].

We also need to understand the biological basis for U-shaped risk relationships. The NIH Roundtable also noted our need to understand the metabolic fate and dynamics of high doses of vitamin D and whether these dynamics are problematic in the long-term [2]. Three mechanisms have been proposed. Tuohimaa and colleagues propose excessive catabolism of tissue-generated 1,25(OH)2D by 24-hydroxylase induced by high 25(OH)D concentrations [42]. Vieth [43] proposes that cyclic seasonal slow declines in 25(OH)D create an imbalance in the local production and degradation of $1,25(OH)_2D$ resulting in sub-optimal $1,25(OH)_2D$ tissue concentrations and adverse outcomes. Alternatively, McGrath and colleagues [22] propose that optimal serum 25(OH)D concentrations to reduce chronic disease vary by genotypes positing that both low-dose and high-dose respondents in the same population sample can lead to the observed U-shaped risk responses. As noted above, plausible evidence does suggest that SNP's in DBP (GC),VDR, 1α-hydroxylase (CYP 27B1), 24 hydroxylase (CYP 2R) and near 7 dehydrocholesterol reductase associate with serum 25(OH)D concentrations. Such genotype-nutrient interactions could not just affect the requirement for vitamin D as noted previously but could also affect the susceptibility to adverse outcomes at higher exposures.

In summary, we need to understand the biological basis for the emerging U-shaped risk curves and evaluate their public health significance. We also need to assess the long-term effects of high dietary exposure through supplementation on adverse health outcomes.

Summary

To improve our understanding of vitamin D's role in human health, research needs to answer questions in five areas: 1) physiology and molecular pathways of vitamin D, 2) health outcomes related to vitamin D, 3) exposure-response relationships, 4) interactions with genotype and other nutrients and 5) adverse effects. The metabolism including extra-renal activation and catabolism, distribution and mobilization from body pools, kinetics, and regulation of the dynamics including changes during pregnancy and lactation and impact of dietary intake and sources of vitamin D needs to be elucidated. Rigorous, well-designed randomized clinical trials need to evaluate the causal role of vitamin D in a diverse array of non-bone health and chronic disease outcomes across the life cycle and reproductive states. Critically needed is the determination of the exposure-response, inflection and threshold of serum 25(OH)D relative to functional and health outcomes. The dose-response relationships of standardized measures of serum 25(OH) D needs to be understood in response to low and high doses of total vitamin D considering confounding factors including catabolic rates. How do relevant genetic polymorphisms in key genes, dietary calcium and phosphate and potentially

cholesterol and the drug, ezetimibe, interact with vitamin D exposure on its bioavailability, transport, distribution in body pools, metabolism and action as well as on bone and non-bone health outcomes? The nature and mechanisms U-shaped risk relationships with adverse health outcomes at higher exposure to vitamin D needs elucidated across the life cycle and reproductive stages.

Questions and answers

H Morris, Australia

One issue, the paper on road accident victims and the relationship between osteoid volume and 25(OH) D has been mentioned several times during the conference and it is unfortunate that the term 'osteomalacia' was used in that paper. There is no evidence of osteomalacia in those patients. An increase in osteoid volume can occur with an increase in bone turnover, so an increased osteoid volume is seen in primary and secondary hyperparathyroidism. Therefore, in this study, the increased osteoid volume indicates not osteomalacia, but increased bone turnover.

P Brannon

I think that is a very important point, which is why I note it as 'as defined in this study' because not everyone would have defined it that way.

I Young, UK

As a clinical chemist, I was particularly interested in the paper from Denis O'Reilly's group, looking at vitamin D following knee arthroplasty. For a lot of other micronutrients, we are used to them being fairly strong negative acute phase reactors and that suggests that for vitamin D there might be a similar effect. It seemed to me that if you were dealing with measuring at vitamin D in an unwell population that was potentially a very major confounder in terms of saying whether or not people were deficient and making recommendations around supplementation or treatment. Many clinicians will not understand that at all and I was wondering if you had any views on how that should be handled. Is the evidence robust enough for us to say it is a real problem and if it is, how should we respond?

P Brannon

You raise a good point and for several years I have been asking people if there is an inflammatory response of DBP, because I think that is what we would want to know and the O'Reilly study does not tell us. The other question I have been asking is whether protein-energy malnutrition influences DBP production by the liver. Both of these are very important questions. In countries like India, where there is sun exposure, the population has extraordinarily low 25(OH)D concentrations and they also have a problem with protein-energy malnutrition. The paper hints that there might be an inflammatory response and if that is the case, most of the biomarkers that are influenced by inflammation and also indicators of nutritional status have been deemed to be of limited use unless coupled to another biomarker which is not affected by inflammation. We don't know the answer.

I Young

We are used to seeing profound falls in micronutrients in a short space of time as part of the acute phase response, so when I saw the data, I was not surprised by the magnitude or rapidity of the change, given what we observe with other similar parameters and it may well be protein-related, I agree.

P Brannon

That is speculation, but I wish they had measured DBP in their patients.

I Young

What concerned me was that if we measure vitamin D in any hospitalised population, most patients will have some kind of acute inflammatory process, would the results be reliable? Can it be interpreted on the cut-offs recommended for a healthy population?

P Brannon

And it is really a question for any consensus development evidence based process of defining clinical guidelines, because we are going to have to distinguish between those patients who are in an acute inflammatory state and those who are not.

R Vieth, Canada

Regarding the surgery paper, John Haddad, who was an eminent DBP researcher, established that the protein has a half-life of about 72 hours and that the binding proteins have multiple functions in the body, one in being actin scavengers, so if there is muscle trauma, actin will increase in the circulation, it will be sequestered by DBP and the half-life of the DBP in the circulation will be shortened. It would be excellent to measure DBP following surgery.

Conflict of interest: The author has no conflict of interest.

References

[1] Institute of Medicine. Dietary reference intakes for calcium and Vitamin D. Ross AC, Taylor CL, Yaktine AL, Del Vale HB (eds). National Academies Press, Washington DC. 2011.
[2] Cranney A, Horsley T, O'Donnell S, Weiler H, Puil L, Ooi D, Atkinson S, Ward L, Moher D, Hanley D, Fang M, Yazdi F, Garritty C, Sampson M, Barrowman N, Tsertsvadze A,

Mamaladze V. Effectiveness and safety of vitamin d in relation ot bone health. Evidence report/technology/assessment no 158. AHRQ publication no -7-EO13. Rockville, MD: Agency for Healthcare Research and Quality, 2007. Internet: http://www.ahrq.gov/downloads/pub/evidence/pdf/vitamind/vitad.pdf (accessed 2/20/2012).

[3] Brannon PM, Yetley EA, Regan BL, Picciano MF. Summary of roundtable discussion on vitamin D research needs. Am J Clin Nutr 2008;88(suppl):587S–592S.

[4] Brannon PM, Picciano MF. Vitamin D in pregnancy and lactation in humans. Annu Rev Nutr 2011; 21;31:89–115.

[5] Cashman KD, Kiely, M. Towards prevention of vitamin D deficiency and beyond: knowledge gaps and research needs in vitamin D nutrition and public health. Br J Nutr 2011;106:1617–1627.

[6] Chung M, Balk EM, Brendel M, Ip S, Lau J, Lee J, Lichtenstein A, Patel K, Raman G, Tatsioni A, Terasawa T, Trikalinos TA. Vitamin D and calcium: a systematic review of health outcomes. Evidence Report No. 183. (Prepared by the Tufts Evidence-based Practice Center under Contract No. HHSA 290–2007–10055-I.) AHRQ Publication No. 09-E015. Rockville, MD: Agency for Healthcare Research and Quality. August, 2009. Internet: http://www.ahrq.gov/downloads/pub/evidence/pdf/vitadcal/vitadcal.pdf (accessed 2/20/2012).

[7] Brannon PM, Yetley, EA, Bailey RL, Picciano MF. Overview of the conference "Vitamin D and Health in the 21st Centurey: An Update." Am J Clin Nutr 2008;88(suppl): 483S-90S.

[8] Chun RF, Lauridsen AL, Suon L, Zella La, Pike JW, Modlin RL, Marineau AR, Wilkinson RJ, Adams J, Hewison M. Vitamin D binding protein directs moncyte responses to 25hydroxy- and 1,25-dihydroxyvitamin D. J Clin Endocrinol Metab 2010;95:3368–3376.

[9] Willnow TE, Nykiaer A. Cellular uptake of steroid carrier proteins- mechanisms and implications. Mol Cell Endocrinol 2010;316:93–102.

[10] Esteban C, Genakens M, Ena JM, Mishal Z, Machot A, Torres JM, Uriel J. Receptor-mediated uptake and processing of vitamin D binding protein in human B-lymphoid cells. J Biol Chem 1992;267:10177–83.

[11] Rowling MJ, Kemmis CM, Taffany DA, Welsh J. Megalin-mediated endocytosis of vitamin D binding protein correlates with 25-hydroxycholecalciferol actions in human mammary cells. J Nutr 2006;136:2754–60.

[12] Gressner OA, Lahme B, Gressner AM. Gc-globulin (vitamin D binding protein) is synthesized and secreted by hepatocytes and internalized by hepatic stellate cells through Ca(2+)-dependent interaction with the megalin/gp330 receptor. Clin Chim Acta 2008;390(1–2):28–37.

[13] Atkins GJ, Anderson PH, Findlay DM, Welldon KJ, Vincent C, Zannettino AC, O'Loughlin PD, Morris HA. Metabolism of vitamin D3 in human osteoblasts: evidence for autocrine and paracrine activities of 1 alpha,25-dihydroxyvitamin D3. Bone 2007;40(6):1517–28.

[14] Weinstein SJ, Stolzenberg-Solomog RZ, Kopp W, Rager H, Virtano J, Allbanes D. Impact of circulating vitamin D binding protein levels on the association between 25-hydroxyvitamin D and pancreatic cancer risk: a nested case-control study. Cancer Res 2012;72:1–9.

[15] Cashman KD, Fitzgerald AP, Kiely M, Seamans KM. A systematic review and meta-regression analysis of the vitamin D intake-serum 25-hydroxyvitamin D relationship to inform European recommendations. Br J Nutr 2011; 106(11):1638–48.

[16] Gallagher JC, Sai A, Templin T 2nd, Smith L. Dose response to vitamin d supplementation in postmenopausal women: a randomized trial. Ann Intern Med 2012;156:425–437.

[17] Hollis BW, Johnson D, Hulsey TC, Ebeling M, Wagner CL. Vitamin D supplementation during pregnancy: Double blind, randomized clinical trial of safety and effectiveness. J Bone Miner Res 2011;18(6):371–5.

[18] Wagner D, Hanwell HE, Schnabl K, Yazdanpanah M, Kimball S, Fu L, Sidhom G, Rousseau D, Cole DE, Vieth R. The ratio of serum 24,25-dihydroxyvitamin D(3) to 25-hydroxyvitamin D(3) is predictive of 25-hydroxyvitamin D(3) response to vitamin D(3) supplementation. J Steroid Biochem Mol Biol 2011;126:72–7.

[19] Farrell CJ, Martin S, McWhinney B, Straub I, Williams P, Hermann M. State of the art vitamin D assays: a comparison of automated immunoassays with liquid chromatography tandem mass spectrometry methods. Clin Chem 2012; 58:531–42.

[20] Mann HW, Cho JH, Hur M, Song J, Oh GY, P CM, Yun YM, Kim JQ. Comparison of four current 25-hydroxyvitamin D assays. Clin Biochem 2012;45:326–30.

[21] Heijboer AC, Blankenstein MA, Kema IP, Buijs MM. Accuracy of 6 routine 25-hydroxyvitamin D assays influence: influence of vitamin D binding protein concentration. Clin Chem 2012;58:543–8.

[22] McGrath JJ, Saba S, Burne THH, Eyles DW. A systematic review of the associationbetween common single nucleotide polymorphisms and 25-hydroxyvitamin D concentrations. J Steroid Biochem Mol Biol 2010;471–477.

[23] Hibler EA, Hu C, Jurutka PW, Martinez ME, Jacobs ET. Polymorphic variation in the GC and CASR Genes and associations with vitamin D metabolite concentration and, metachronous colorectal neoplasia. Cancer Epidemiol Biomarkers Prev 2012;21:368–75.

[24] Bu FX, Armas L, Lappe J, Zhou Y, Gao G, Wang HW, Recker R, Zhan LJ. Comprehensive association analysis of nine candidate genes with serum 25-hydroxy vitamin D levels among healthy Caucasian subjects. Hum Genet 2010; 128:549–556.

[25] Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, Kiel DP, Streeten EA, Ohlsson C, Koller DL, Peltonen L, Cooper JD, O'Reilly PF, Houston DK, Glazer NL, Vandenput L, Peacock M, Shi J, Rivadeneira F, McCarthy MI, Anneli P, de Boer IH, Mangino M, Kato B, Smyth DJ, Booth SL, Jacques PF, Burke GL, Goodarzi M, Cheung CL, Wolf M, Rice K, Goltzman D, Hidiroglou N, Ladouceur M, Wareham NJ, Hocking LJ, Hart D, Arden NK, Cooper C, Malik S, Fraser WD, Hartikainen AL, Zhai G, Macdonald HM, Forouhi NG, Loos RJ, Reid DM, Hakim A, Dennison E, Liu Y, Power C, Stevens HE, Jaana L, Vasan RS, Soranzo N, Bojunga J, Psaty BM, Lorentzon M, Foroud T, Harris TB, Hofman A, Jansson JO, Cauley JA, Uitterlinden AG, Gibson Q, Järvelin MR, Karasik D, Siscovick DS, Econs MJ, Kritchevsky SB, Florez JC, Todd JA, Dupuis J, Hyppönen E, Spector TD. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 2010;7;376:180–8.

[26] Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, Jacobs EJ, Ascherio A, Helzlsouer K, Jacobs KB, Li Q, Weinstein SJ, Purdue M, Virtamo J, Horst R, Wheeler W, Chanock S, Hunter DJ, Hayes RB, Kraft P, Albanes D. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet 2010;1;19:2739–45.

[27] Kurt O, Yilmaz-Aydogan H, Uyar M, Isbir T, Seyhan MF, Can A. Evaluation of ERα and VDR gene polymorphisms in relation to bone mineral density in Turkish postmenopausal women. Mol Biol Rep 2012;(in press).

[28] Lee YH, Choi SJ, Ji JD, Song GG. Vitamin D receptor ApaI, TaqI, BsmI, and FokI polymorphisms and psoriasis susceptibility: a meta-analysis. Mol Biol Rep 2012; (in press).

[29] Lee YH, Bae SC, Choi SJ, Ji JD, Song GG. Associations between vitamin D receptor polymorphisms and susceptibility to rheumatoid arthritis and systemic lupus erythematosus: a meta-analysis. Mol Biol Rep 2011;38:3643–51.

[30] Morley R, Carlin JB, Pasco JA, Wark JD, Ponsonby AL. Maternal 25-hydroxyvitamin D concentration and offspring birth size: effect modification by infant VDR genotype. Eur J Clin Nutr 63:2009;802–4.
[31] Swamy GK, Garrett ME, Miranda ML, Ashley-Koch AE. Maternal vitamin D receptor genetic variation contributes to infant birthweight among black mothers. Am J Med Genet A 2011;155A:1264–71.
[32] Ramos-Lopez E, Kahles H, Weber S, Kukic A, Penna-Martinez M, Badenhoop K, Louwen F. Gestational diabetes mellitus and vitamin D deficiency: genetic contribution of CYP27B1 and CYP2R1 polymorphisms. Diabetes Obes Metab 2008;10:683–5.
[33] Brannon PM. Vitamin D and adverse pregnancy outcomes: beyond bone health and growth. Proc Nutr Soc 2012; 20:1–8.
[34] Bhlon TM, Taffany DA, Welsh, Rowling, MJ. Retinoids modulate expression of the endocytic partners megalin, cubulin and disabled-2 and uptake of vitamin D binding protein in human mammary cells. J Nutr 2008;138:1323–28.
[35] Reboul E, Goncalves A, Comera C, Bott R, Nowicki M, Landrier JF, Jourdheuil-Rahmani D, Dufour C, Collet X, Borel P. Vitamin D intestinal absorption is not a simple passive diffusion: evidences for involvement of cholesterol transporters. Mol Nutr Food Res 2011;55:691–702.
[36] Bodnar LM, Catov JM, Zmuda JM, Cooper ME, Parrott MS, Roberts JM, Marazita ML, Simhan HN. Maternal serum 25-hydroxyvitamin D concentrations are associated with small-for-gestational age births in white women. J Nutr 2010;140:999–1006.
[37] Mahon P, Harvey N, Crozier S, Inskip H, Robinson S, Arden N, Swaminathan R, Cooper C, Godfrey K; SWS Study Group. Low maternal vitamin D status and fetal bone development: cohort study. J Bone Miner Res 2010;25:14–9.
[38] McGrath JJ, Eyles DW, Pedersen CB, Anderson C, Ko P, Burne TH, Norgaard-Pedersen B, Hougaard DM, Mortensen PB. Neonatal vitamin D status and risk of schizophrenia: a population-based case-control study. Arch Gen Psychiatry 2010;67:889–94.
[39] Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, Godfrey KM, Cooper C; Princess Anne Hospital Study Group. Maternal vitamin D status during pregnancy and child outcomes. Eur J Clin Nutr 2008; 62: 68–77.
[40] Amer M, Qayyum R. elation between serum 25-hydroxyvitamin D and C-reactive protein in asymptomatic adults (from the continuous National Health and Nutrition Examination Survey 2001 to 2006). Am J Cardiol 2012;109:226–30.
[41] Ensrud KE, Ewing SK, Fredman L, Hochberg MC, Cauley JA, Hillier TA, Cummings SR, Yaffe K, Cawthon PM; Study of Osteoporotic Fractures Research Group. Circulating 25-hydroxyvitamin D levels and frailty status in older women. J Clin Endocrinol Metab 2010;95:5266–5273.
[42] Tuohimaa P, Tenkanen L, Ahonen M, Lumme S, Jellum E, Hallmans G, Stattin P, Harvei S, Hakulinen T, Luostarinen T, Dillner J, Lehtinen M, Hakama M. Both high and low levels of blood vitamin D are associated with a higher prostate cancer risk: a longitudinal, nested case-control study in the Nordic countries. Int J Cancer 2004;108:104–8.
[43] Vieth R. Enzyme kinetics hypothesis to explain the U-shaped risk curve for prostate cancer vs. 25-hydroxyvitamin D in nordic countries. Int J. Cancer 2004;111:468.
[44] Jones G. Pharmacokinetics of vitamin D toxicity. Am J Clin Nutr 2008;88(suppl):582S-586S.