Reshaping the way we view vitamin D signalling and the role of vitamin D in health

James C. Fleet*, Jie Hong and Zhentao Zhang

Department of Foods and Nutrition and The Interdepartmental Nutrition Program, Purdue University, West Lafayette, IN 47907-2059, USA

Although the biological requirement for vitamin D can be met by epidermal exposure to UV light, there are a number of conditions where this production does not occur or is not sufficient to meet biological needs. When this happens, vitamin D must be consumed and is a nutrient. However, two distinct observations have caused researchers to rethink certain dogma in vitamin D biology. First, it appears that in addition to the hormonally active form of 1,25 dihydroxyvitamin D (1,25(OH)_2D), circulating levels of 25 hydroxyvitamin D have a critical importance for optimal human health. This and other data suggest that extra-renal production of 1,25(OH)_2D contributes to Ca homeostasis and cancer prevention. Second, in addition to its role in the transcriptional activation of genes through the vitamin D receptor there is now compelling evidence that 1,25(OH)_2D has a second molecular mode of action; the rapid activation of second-messenger and kinase pathways. The purpose of this second mode of action is only now being explored. The present review will discuss how these two areas are reshaping our understanding of vitamin D metabolism and action.

Vitamin D: Calcium homeostasis: Cancer prevention: Cell signalling pathways

Abbreviations: nVDR, nuclear vitamin D receptor; 25(OH)D, 25 hydroxyvitamin D; 1,25(OH)_2D, 1,25 dihydroxyvitamin D; PTH, parathyroid hormone; VDR, vitamin D receptor.

* Corresponding author: Dr James C. Fleet, fax +1 765 494 0906, email fleetj@cfs.purdue.edu
they are homebound), in individuals who use strong sunblocks in the summer and who avoid the sun, and individuals who live in the upper or lower parts of the northern or southern hemispheres during the winter. For these individuals, special efforts must be made to ensure vitamin D adequacy. This includes dietary supplementation.

### High 25 hydroxyvitamin D influences both calcium homeostasis and cancer risk

While the preceding discussion highlights the nutritional importance of vitamin D, it implies that the biological importance of vitamin D depends upon an individuals ability to produce 1,25(OH)₂D in the kidney and suggests that adequate health is dependent upon high circulating levels of that metabolite. This is intentional, as most of the science that 1,25(OH)₂D is unstable and that it is hard to measure the two parameters. Another possibility is that high levels of 25(OH)D may bind directly to the VDR leading to activation of gene transcription. Brumbaugh & Haussler (1973) have shown that even though 25(OH)D has a low affinity for the VDR (500–1000-fold lower affinity than 1,25(OH)₂D), high levels of 25(OH)D can displace the receptor. Based upon this hypothesis, 1,25(OH)₂D, whose serum levels are 1000 times greater than serum 1,25(OH)₂D levels, may be a direct, physiologically that it is the serum level of 25 hydroxyvitamin D (25(OH)D), the precursor of 1,25(OH)₂D, that most closely correlates with several favourable outcomes for bone health. For example, although animal studies clearly demonstrate that 1,25(OH)₂D is an important regulator of intestinal Ca absorption (Bronner et al. 1986; Song et al. 2003b), several studies have found that the efficiency of Ca absorption was positively correlated to serum 25(OH)D when levels vary within the normal range (50·1 to 86·5 nmol/l) (Barger-Lux et al. 1995; Heaney et al. 1997, 2003; Devine et al. 2002). Similarly, even though 1,25(OH)₂D is known to suppress parathyroid hormone (PTH) gene transcription (Demay et al. 1992), Thomas et al. (1998) and Chapuy et al. (1997) have shown that it is serum 25(OH)D levels that are associated with lower serum PTH levels (peak suppression at 80 nmol/ml). This protection extends to protection from fractures; Trivedi et al. (2003) showed that the supplementation of elderly men and women with 2500 μg (100 000 IU) vitamin D₃ every 4 months for 5 years significantly increased serum 25(OH)D and reduced the age-associated relative risk of fracture.

The importance of a high 25(OH)D status also appears to be important for a non-traditional action of vitamin D as well; cancer chemoprevention. Schwartz & Hulka (1990) hypothesised that vitamin D deficiency is the underlying factor for increased prostate cancer risk due to advancing age, Black race, and northern latitudes; factors associated with decreased synthesis of vitamin D in the skin (Holick, 1997). This group later confirmed that low prostate cancer rates were associated with high UV radiation exposure in the USA (Hanchette & Schwartz, 1992). Several groups have attempted to establish a direct relationship between high vitamin D status and prostate cancer risk. For example, Ahonen et al. (2000) found a 70 % increased prostate cancer risk in men with 25(OH)D levels below the median, especially in younger men (<52 years) who entered the study with low serum 25(OH)D (adjusted odds ratio 3·5).

### How does a high serum 25 hydroxyvitamin D level offer protection?

The observations regarding 25(OH)D-mediated effects on bone and cancer endpoints might be explained in a number of ways. First, they might be artefacts of the simple fact that 1,25(OH)₂D is unstable and that it is hard to measure long-term exposure to this metabolite. The half-life of 25(OH)D in the serum is about 2 weeks, whereas the half-life of 1,25(OH)₂D is less than 6 h (Haddad & Rojanasathit, 1976; Mason et al. 1980). As a result, a peak in serum 1,25(OH)₂D could initiate a change in intestinal Ca absorption but that peak could be gone by the time intestinal Ca absorption is elevated (i.e. reducing the correlation between the two parameters). Another possibility is that high levels of 25(OH)D may bind directly to the VDR leading to activation of gene transcription. Brumbaugh & Haussler (1973) have shown that even though 25(OH)D has a low affinity for the VDR (500–1000-fold lower affinity than 1,25(OH)₂D), high levels of 25(OH)D can displace 1,25(OH)₂D from the receptor. Based upon this hypothesis, 25(OH)D, whose serum levels are 1000 times greater than serum 1,25(OH)₂D levels, may be a direct, physiologically
relevant activator of gene transcription through activation of the VDR. While this hypothesis fails to take into account the impact of the serum vitamin D-binding protein (i.e. 25(OH)D has >600-fold higher affinity and thus may be less likely to be transferred to tissues), this effect certainly accounts for the ability of vitamin D and 25(OH)D to reverse the phenotype of genetic pseudovitamin D-deficient rickets caused by mutations in the 1α-hydroxylase gene (Glorieux & St-Arnaud, 1997). Still, massive amounts of vitamin D (>20 000 IU or 500 µg/d) and 25(OH)D (>100 µg/d) are needed for this effect. This makes it improbable that the relatively moderate increases in 25(OH)D that suppress PTH or provide protection against cancer are working through this pathway (i.e. 2–3-fold changes).

**Extra-renal production of 1,25 dihydroxyvitamin D is critical for optimal health**

A final hypothesis to explain the beneficial effects of high serum 25(OH)D levels is that there may be local production of 1,25(OH)2D in a wide variety of tissues, i.e. 1,25(OH)2D is working as an autocrine or a paracrine regulator rather than an endocrine regulator. Although textbooks teach that the production of 1,25(OH)2D is exclusively renal, there has been evidence to support extra-renal production for some time. Dusso et al. (1988) and Jongen et al. (1984) both found that supplementing anephric subjects with 25(OH)D increased serum 1,25(OH)2D levels from very low levels to low normal ranges. Consistent with these observations, Zehnder et al. (2001) found extra-renal expression of the 1α-hydroxylase protein by immunohistochemistry in skin, lymph nodes, pancreatic islets, brain, placenta, and in colonic epithelial cells.

Recently, several papers have provided data that support a role for the local production of 1,25(OH)2D in prostate cancer prevention. Hsu et al. (2001) examined eighteen cell lines of normal prostatic epithelial cells, eight cell lines of cells from patients with benign prostatic hypertrophy cells, fifteen adenocarcinoma cell lines, and four established prostate cancer cell lines (LNCaP, PC-3, DU145 and MDA-PCa 2b). They found that normal prostate epithelial cells displayed a high level of 1α-hydroxylase activity (2.20 ± 0.61 pmol/mg protein per h). This activity was impaired in benign prostatic hypertrophy cells (1.48 ± 0.18 pmol/mg protein per h), primary cultures of prostate cancer cells (0.32 ± 0.15 pmol/mg protein per h) and prostate cancer cell lines (0.17 ± 0.16 pmol/mg protein per h). They subsequently showed that the percentage of growth inhibition in response to treatment of cells with 25(OH)D was dependent upon the 1α-hydroxylase activity. As a result, while the prostate cancer cell line LNCaP was responsive to 1,25(OH)2D treatment, their lack of 1α-hydroxylase activity caused them to be unresponsive to 25(OH)D treatment. Whitlatch et al. (2002) later demonstrated that the anti-proliferative effects of 25(OH)D could be restored in LNCaP cells transfected with a transgene conferring 1α-hydroxylase activity.

These data provide strong support for the role of autocrine or paracrine regulation in prostate cancer prevention. This model has been generalised, integrated with the more traditional renal mode of 1,25(OH)2D production, and is presented in Fig. 2. Still, it must be noted that there has not yet been a report of 1α-hydroxylase activity in the vitamin D-responsive, Ca-transporting cells of the small intestine. Similarly, it has not been demonstrated that the loss of 1α-hydroxylase activity in the prostate leads to increased prostate cancer or that the loss in the intestine leads to reduced efficiency of intestinal Ca absorption. With the development of global and conditional 1α-hydroxylase knockout mice (St Arnaud et al. 2003), the ability to test these questions directly (as well as the quantitative assessment of 25(OH)D as a direct activator of the VDR) is within reach.

**The two modes of 1,25 dihydroxyvitamin D action**

The bulk of the research on 1,25(OH)2D action has focused on the activation or repression of gene transcription that is mediated through the VDR. This and other data comprise a significant body of evidence supporting a critical role for the VDR in the Ca-regulating actions of 1,25(OH)2D (see Fig. 3 for a current model of the mechanism of vitamin D-mediated gene activation). For example, mutations in the VDR cause genetic rickets (vitamin D-resistant rickets or type II rickets) and alopecia in affected individuals. Also, serum PTH levels are dramatically elevated and intestinal Ca absorption is lower in nuclear VDR (nVDR) knockout mice compared with wild-type littermates (van Cromphau...

![Fig. 2. Model for local production and autocrine action of 1,25 dihydroxyvitamin D₃ (1,25(OH)₂D₃). Traditionally, consumption of a low-Ca diet leads to increased renal production of 1,25(OH)₂D₃ and endocrine signalling leading to activation of the vitamin D receptor (VDR)-mediated gene transcription in vitamin D target tissues (for additional details, see Fig. 1). Alternately, the presence of extra-renal 1α-hydroxylase activity in several non-traditional vitamin D target tissues suggests that autocrine signalling may occur within cells due to elevated serum 25(OH)D (25(OH)₂D₃) levels that result from feeding a diet with high dietary vitamin D content or after high exposure to UV light in the proper wavelength. Although not shown, locally produced 1,25(OH)₂D₃ could also be released and act locally as a paracrine signal. PTH, parathyroid hormone.](https://www.cambridge.org/core/services/asset/55c53a5e43d14f6f8c77336e0f45f9c0/image)
et al. 2001; Song et al. 2003a). The VDR is also critical for the growth-inhibitory properties of 1,25(OH)\textsubscript{2}D; prostate cancer cells with little or no VDR are not growth inhibited in response to 1,25(OH)\textsubscript{2}D treatment (Miller et al. 1995; Hedlund et al. 1996a,b; Zhuang et al. 1997).

While research on VDR-mediated gene transcription continues to be fruitful, there is now compelling evidence for the existence of rapid activation of signal-transduction pathways by 1,25(OH)\textsubscript{2}D within various cell types (Nemere & Farach-Carson, 1998; Sitrin et al. 1999). Fig. 4 summarises the kinase and second-messenger pathways that have been shown to be activated through rapid 1,25(OH)\textsubscript{2}D-mediated signalling. This form of signalling has been more closely associated with the mechanism of action of peptide hormones and growth factors yet is being recognised as important for the action of other steroid hormones as well (Harvey et al. 2002). Nonetheless, the discovery of a second mechanism for vitamin D action raises several interesting questions: i.e. ‘why do we need two distinct vitamin D signalling pathways?’, ‘are there independent biological processes regulated by each pathway?’ and ‘how does vitamin D signal the cell to rapidly initiate signal-transduction pathways?’

While the recent observations on rapid 1,25(OH)\textsubscript{2}D signalling are interesting, the central importance of VDR-mediated signalling appears to leave little room for an independent effect of these pathways. To date, the area that best demonstrates the importance of the rapid activation of kinase pathways by 1,25(OH)\textsubscript{2}D is the modulation of growth-zone chondrocyte biology (Boyan et al. 1994, 1999, 2003). Treatment of growth-zone chondrocytes with 1,25(OH)\textsubscript{2}D activates phospholipase A\textsubscript{2} and causes an increase in membrane fluidity, activates phospholipase C leading to the activation of protein kinase C and release of intracellular Ca, and indirectly activates protein kinase A through production of prostaglandin E\textsubscript{2}. These changes are important for the activation of growth factors (i.e. latent transcriptional growth factor-H9252 in matrix), proteoglycan degradation, and matrix mineralisation. The fact that these effects occur in the nucleus-free matrix vesicles released from chondrocytes, as well as in intact chondrocytes, clearly demonstrates that the events are independent of transcriptional activation. The final aspect of this system that bears noting is that chondrocytes have the ability to produce 1,25(OH)\textsubscript{2}D from 25(OH)D (Schwartz et al. 1992). This is consistent with the idea of an autocrine or paracrine signalling pathway that was developed in the first part of the present review.

**Do rapid and nuclear 1,25 dihydroxyvitamin D signalling pathways interact?**

It is not essential that rapid vitamin D signalling be independent of the VDR or VDR-mediated gene activation. In fact, there are two lines of evidence that suggest the rapid activation of kinases by 1,25(OH)\textsubscript{2}D and the function of the VDR are related. A model for these interactions is presented in Fig. 5.

**Modulation of vitamin D receptor function by kinase activation.** Several studies support the hypothesis that signal-transduction pathways are important regulators of nVDR-mediated gene expression. For example, the
suppression of protein kinase C activity with staurosporine or H7 inhibited 1,25(OH)2D-regulated 25-hydroxyvitamin D 24-hydroxylase (CYP24) gene expression in proliferating, small-intestine crypt-like, rat IEC-6 cells (Koyama et al. 1994) and the activation of protein kinase C with phospholipase C; src, receptor tyrosine kinase; MARRS, membrane-associated rapid response steroid-binding protein; PKC, protein kinase C; PLC, phospholipase C; src, receptor tyrosine kinase; nVDR, nuclear VDR.

The mechanism for this interaction between 1,25(OH)2D-induced kinase pathways and VDR-mediated gene transcription is unclear but is probably due to the modulation of protein–protein interactions that are responsible for the recruitment of co-activators necessary for the disruption of higher-order chromatin structure and transcriptional activation (see Fig. 3). For example, Paredes et al. (2002) showed that access to vitamin D response elements in the osteocalcin gene promoter is limited when the DNA is present in a chromosomal context. As a result, protein–protein interactions mediated by the nVDR are critical for chromosomal unwinding. That is, before transcriptional activation, the nVDR–retinoid X receptor dimer recruits cAMP-response element-binding protein–binding protein and steroid receptor co-activator-1 to make a complex with histone acetyl transferase activity that acetylates histones H3 and H4 and relieves the constraints imposed by chromatin structure (Chen et al. 1999; Freedman, 1999). Both cAMP-response element-binding protein–binding protein and steroid receptor co-activator-1 are targets of mitogen-activated protein kinase-mediated phosphorylation and this may lead to increased histone acetyl transferase activity of the complex (Rowan et al. 2000; Lee et al. 2003). In addition, after chromosomal unwinding, the nVDR–retinoid X receptor dimer recruits the mediator D complex (also known as DRIP) and utilises it to recruit and activate the basal transcription unit containing RNA polymerase II (Rachez et al. 1999; Chiba et al. 2000). Barletta et al. (2002) showed that the association between the nVDR and a member of the mediator complex, DRIP205, was enhanced following the treatment of cultured bone and kidney cells with the phosphatase inhibitor okadaic acid. This interaction enhanced 1,25(OH)2D-mediated reporter gene

**Fig. 4.** Rapid activation of signal-transduction pathways by 1,25 dihydroxyvitamin D3 (1,25(OH)2D3). Recent evidence indicates that in addition to the classical mode of action utilising the vitamin D receptor (see Fig. 3), 1,25(OH)2D3 can rapidly activate various kinase and second-messenger pathways: protein kinase A (PKA), NF-κB, mitogen-activated protein kinases (MAPK), and protein kinase C (PKC). Please note that not all of the pathways listed have been described in all cell types. SRC, receptor tyrosine kinase; P13K, phosphatidylinositol 3 kinase; PIP2, phosphatidylinositol-4,5 bisphosphate; PIP3, phosphatidylinositol-3,4,5 trisphosphate; AKT, protein kinase B; IK2B, inhibitor of NFκB; Raf1, mitogen activated kinase kinase kinase; AP, activated protein; PLC, phospholipase C; DAG, diacylglycerol; IP3, inositol trisphosphate.

**Fig. 5.** A model of cross-talk between 1,25 dihydroxyvitamin D3 (1,25(OH)2D3) signalling pathways. The model here depicts simplified versions of the classical vitamin D receptor (VDR)-mediated transcriptional activation pathway and the rapid activation of kinases by 1,25(OH)2D3. The larger, grey arrows indicate potential points of cross-talk between the two pathways that are supported by the literature. This cross-talk includes a role of kinase pathways in modulating the transcriptional activity of the VDR as well as a non-transcriptional role for the VDR in transducing the signal to initiate kinase activity. PKC, protein kinase C; PLC, phospholipase C; src, receptor tyrosine kinase; MARRS, membrane-associated rapid response steroid-binding protein; MAPK, mitogen-activated protein kinase; RXR, retinoid X receptor; nVDR, nuclear VDR.
activity. These and other data suggest that 1,25(OH)₂D-mediated activation of various kinase pathways could lead to the phosphorylation of critical co-activators and this in turn could increase the transcriptional activation of vitamin D target genes by increasing VDR co-activator interactions. However, while this suggests that cross-talk between the two vitamin D signal pathways exists, more work is needed to solidify the connections between the rapid actions of 1,25(OH)₂D and the classical activation of the VDR by 1,25(OH)₂D.

Involvement of vitamin D receptor in the rapid activation of signal-transduction pathways. There is considerable controversy regarding the mechanism used by 1,25(OH)₂D to transduce a signal across the membrane of cells and activate kinase pathways. One line of evidence suggests that there may be a unique membrane receptor for 1,25(OH)₂D. This protein has been termed the membrane-associated rapid response steroid-binding protein (Farach-Carson & Nemere, 2003). This protein binds 1,25(OH)₂D with high affinity, is distinct from the classical VDR, and is found on the membrane of a number of cells that respond to 1,25(OH)₂D treatment by activating kinase pathways and stimulating Ca fluxes. However, in addition to this protein, several recent papers have indicated that the classical VDR may be involved in at least some of the rapid 1,25(OH)₂D signalling pathways.

Buitrago et al. (2000, 2001b) have demonstrated that in myocytes 1,25(OH)₂D treatment activates the membrane-associated tyrosine kinase, src kinase, and this coincides with an increased association of VDR with src kinase as well as tyrosine phosphorylation of the VDR, i.e. the VDR is a target of src kinase activation. Bettou et al. (2003) found that in Caco-2 cells VDR is part of a ternary complex with the protein phosphatase PP1c and the p70 S6 kinase. This indicates that rapid signalling requiring the VDR to inactivate the VDR in a non-traditional role can lead to the growth arrest of proliferating Caco-2 cells in part through the inactivation of p70 S6 kinase. Finally, Erben et al. (2002) found that the 1,25(OH)₂D-mediated activation of Ca fluxes is blocked in osteoblasts from mice expressing a nVDR that lacks the DNA-binding domain. Although this observation conflicts with data generated by Wali et al. (2003) in VDR-null mice, coupled with the other data from cell systems, it suggests that the VDR may participate in at least some rapid signalling pathways.

Summary and conclusions

The purpose of the present review was to demonstrate that certain dogma that has been used to explain the biology of vitamin D action is falling under question. In the first part of the review, we discussed how in some circumstances the circulating level of 25(OH)D is a better marker of vitamin D action than serum 1,25(OH)₂D levels (the exception being severe dietary Ca insufficiency) even though the traditional thinking suggests that vitamin D acts in an endocrine fashion only after being converted to 1,25(OH)₂D in the kidney. Other data indicate that this is possible because extra-renal production of 1,25(OH)₂D is a critical component of the protection mediated by high serum 25(OH)D levels. This puts 1,25(OH)₂D in a new role as an autocrine or paracrine mediator rather than an endocrine hormone. In the second part of the review, we examined the evidence that rapid activation of signal-transduction pathways by 1,25(OH)₂D constitutes a new, and important mode of vitamin D action apart from the traditional activation of gene transcription mediated through the VDR. With the possibility that a paracrine role exists for 1,25(OH)₂D, we can begin to make physiological sense of these rapid actions for 1,25(OH)₂D. It will be interesting to learn whether regional fluxes in 1,25(OH)₂D levels due to local production could be occurring and signalling cells, even though serum levels of the hormone do not change. These data, while complicating our model of vitamin D action, provide new life to the study of the nutritional role of vitamin D and stress the importance of high vitamin D status for maximising the protective effects of vitamin D on Ca homeostasis and for cancer prevention.

References


growth plate chondrocytes via membrane receptor-mediated protein kinase C by a mechanism that involves changes in phospholipid metabolism and the action of arachidonic acid and PGE2. Steroids 64, 129–136.


Brumbaugh PF & Haussler MR (1973) 1Alpha, 25-dihydroxyvita-
mion D3 receptor: competitive binding of vitamin D analogs. Life Sciences 13, 1737–1746.


Buitrago C, Vazquez G, de Boland AR & Boland RL (2000) Activation of Src kinase in skeletal muscle cells by 1, 1,25-
(OH)(2)-vitamin D(3) correlates with tyrosine phosphorylation of the vitamin D receptor (VDR) and VDR-Src interaction. Journal of Cellular Biochemistry 79, 274–281.


Chiba N, Suldan Z, Freedman L & Parvin J (2000) Binding of lig-
anded vitamin D receptor to the vitamin D receptor interacting protein coactivator complex induces interaction with RNA polymerase II holoenzyme. Journal of Biological Chemistry 275, 10719–10722.


turbing phosphorylation pathways by okadaic acid and stau-


Dwivedi PP, Hii CS, Ferrante A, Tan J, Der CJ, Omdahl JL, Morris HA & May BK (2002) Role of MAP kinases in the 1,25-
dihydroxyvitamin D3-induced transactivation of the vitamin D receptor cytochrome P450C24 (CYP24) promoter. Specific functions for ERK1/ERK2 and ERK5. Journal of Biological Chemistry 277, 29643–29653.

mimin D. Molecular Endocrinology 16, 1524–1537.


Heaney RP, Dowell MS, Hale CA & Bendich A (2003) Calcium absorption varies within the reference range for serum 25-

Hedlund TE, Moffatt KA & Miller GI (1996a) Stable expression of the nuclear vitamin D receptor in the human prostatic carci-
coma cell line JCA-1: evidence that the antiproliferative effects of 1 alpha, 25-dihydroxyvitamin D3 are mediated exclusively through the genomic signaling pathway. Endocrinology 137, 1554–1561.

Hedlund TE, Moffatt KA & Miller GI (1996b) Vitamin D receptor expression is required for growth modulation by 1 alpha,25-


Lee KC, Li J, Cole PA, Wong J & Kraus WL (2003) Transcriptional activation by thyroid hormone receptor-beta involves chromatin remodeling, histone acetylation, and syner-


