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Vitamin D Activities for Health Outcomes

Howard A. Morris, Ph.D.

School of Pharmacy and Medical Sciences, University of South Australia, Chemical Pathology Directorate and Hanson Institute, SA Pathology, Adelaide, Australia

Reports describing significant health risks due to inadequate vitamin D status continue to generate considerable interest amongst the medical and lay communities alike. Recent research on the various molecular activities of the vitamin D system, including the nuclear vitamin D receptor and other receptors for 1,25-dihydroxyvitamin D and vitamin D metabolism, provides evidence that the vitamin D system carries out biological activities across a wide range of tissues similar to other nuclear receptor hormones. This knowledge provides physiological plausibility of the various health benefits claimed to be provided by vitamin D and supports the proposals for conducting clinical trials. The vitamin D system plays critical roles in the maintenance of plasma calcium and phosphate and bone mineral homeostasis. Recent evidence confirms that plasma calcium homeostasis is the critical factor modulating vitamin D activity. Vitamin D activities in the skeleton include stimulation or inhibition of bone resorption and inhibition or stimulation of bone formation. The three major bone cell types, which are osteoblasts, osteocytes and osteoclasts, can all respond to vitamin D via the classical nuclear vitamin D receptor and metabolize 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D to activate the vitamin D receptor and modulate gene expression. Dietary calcium intake interacts with vitamin D metabolism at both the renal and bone tissue levels to direct either a catabolic action on the bone through the endocrine system when calcium intake is inadequate or an anabolic action through a bone autocrine or paracrine system when calcium intake is sufficient.

Key Words: Vitamin D, Metabolic bone diseases, Osteomalacia, Osteoporosis, Bone fractures, Calcium, Dietary, 25-hydroxyvitamin D

INTRODUCTION

Reports describing significant health risks due to an inadequate vitamin D status continue to generate considerable interest in the medical field as well as in other lay communities. The number of scientific publications on vitamin D indexed by the PubMed database has been increasing by 15-20% every year since 2009. Many of them describe the associations between a low vitamin D status and increased risk of various diseases. The highest level of evidence (systematic review of the results from randomized controlled trials [1]) indicates that an adequate vitamin D status protects against rickets in children (or osteomalacia in adults) [2], osteoporotic fractures [3], falls [4], and premature mortality [5].

The efficacy of adequate vitamin D status in reducing the risk of the latter three conditions has been particularly demonstrated in weak elderly individuals. A recent meta-analysis demonstrated that even in people younger than 65 yr, vitamin D status remained inversely related to mortality, although the impact was not as high as that in older subjects [5]. Lower level evidence, including randomized controlled trials, comparative studies with or without concurrent controls, and case series, suggest that a low vitamin D status is associated with an increased risk of cancer, cardiovascular disease, respiratory infections, autoimmune diseases, and health service utilization and costs [6].

There continues to be considerable skepticism and controversy regarding such wide-ranging effects from what some clini-

cians consider a simple nutrient. Such skepticism particularly arises from efficacy claims made on the basis of weak evidence [7]. However, as the complex cell biology of vitamin D, including its metabolism and molecular modes of action, continues to be elucidated, the plausibility of such wide-ranging actions is increased [8]. Vitamin D activity requires at least two elements, metabolism to synthesize the biologically active metabolite 1,25-dihydroxyvitamin D (1,25D) [9] and a receptor protein of which at least two have been well described. These include the classical nuclear receptor [10] and a receptor strongly associated with membranes [11]. It is quite possible that other receptors may be discovered in the future along with other physiologically relevant ligands for these receptors. Our current understanding of vitamin D metabolism formed in the skin following exposure to ultraviolet-B (UVB) light, sequential hydroxylation at the carbon 25- and the 1- positions of vitamin D to form 1,25D is well described [12]. The critical serum 25-hydroxyvitamin D levels for prevention of rickets in children and osteomalacia is 20 nmol/L, which is lower than that required for reducing the risk of osteoporotic fractures that are reduced at levels greater than 60 nmol/L in combination with an adequate dietary calcium intake [13].

ENDOCRINE AND AUTOCRINE/PARACRINE METABOLISM OF VITAMIN D

The renal activity of the enzyme 25-hydroxyvitamin D-1α hydroxylase (CYP27B1) is responsible for synthesizing plasma 1,25D, and in healthy, non-pregnant subjects, renal synthesis appears to be the sole source of plasma 1,25D [14]. The endocrine activities of plasma 1,25D are strongly linked with the regulation of plasma calcium and phosphate homeostasis as well as imparting protection against rickets, a metabolic bone disease, in children and osteomalacia in adults [13]. Renal synthesis of plasma 1,25D is tightly regulated largely through regulation of the renal expression of the gene coding for 25-hydroxyvitamin D-1α hydroxylase (CYP27B1), in which the parathyroid hormone, FGF 23, and plasma calcium through the calcium-sensing receptor play key roles [12].

1,25D also exerts autocrine or paracrine activities, because it is synthesized in a wide range of tissues through the expression of CYP27B1 [15]. There is no evidence that synthesis of 1,25D in these non-renal tissues significantly contributes to plasma 1,25D levels [13]. The autocrine/paracrine activities of 1,25D and their physiological significance have been well described in the skin where it is responsible for the regulation of the proliferation and maturation of keratinocytes, including formation of the per-

meability barrier in the skin, as well as in innate immunity, hair follicle cycling, and suppression of tumor formation [16]. Each of these activities requires a nuclear vitamin D receptor (VDR).

Autocrine/paracrine activities have been well described within both rodent and human bone cells demonstrating that local synthesis of 1,25D from 25-hydroxyvitamin D inhibits osteoblast proliferation and stimulates osteoblast maturation and mineral deposition *in vitro* [17]. The effects of 1,25D on osteoblast-like cells are dependent on their maturation stage, for example, stimulation of osteoblast expression of the receptor activator of nuclear factor- kappaB ligand (RANKL) by 1,25D only occurs in immature osteoblasts [18]. There is also *in vivo* evidence from a rodent model supporting the concept that bone cell 1,25D synthesis regulates bone mineral homeostasis by down-regulating mRNA levels of RANKL in whole bone and increases the time required for osteoblast bone formation [19]. Considerable clinical data suggest that maintaining serum 25-hydroxyvitamin D levels in the elderly in combination with adequate dietary calcium intake reduces the risk of fractures [3], thereby supporting the view that this is applicable to humans.

It is likely that such autocrine/paracrine activities of 1,25D are exerted in other tissues, thereby regulating specific tissue physiology with data available for the colon [20] and breast [21] tissues. The one mechanism common in these various tissues is the inhibition of cell proliferation and enhancement of cell maturation. Clearly, such activities could be associated with the risk of cancer.

MOLECULAR ACTIONS OF VITAMIN D AND VITAMIN D RECEPTOR

1. Regulation of gene transcription

Vitamin D exerts one of its biological activities through binding of the 1,25D metabolite to the classical nuclear VDR, which acts as a nuclear transcription factor similar to other steroid hormones. The VDR was found to be expressed in 31 out of 39 tissues harvested from young mice, although only 7 of these tissues demonstrated high levels of VDR mRNA [22]. A hierarchical clustering of nuclear receptor tissue expression indicates that VDR is most closely related to nuclear receptors regulating bile acids and xenobiotic metabolism. VDR acts to stimulate gene transcription after binding to 1,25D by forming a heterodimer with the retinoid-X receptor (RXR) protein, which binds to a VDR-specific gene sequence [23]. Vitamin D responsive genes are defined by the genetic coding of a specific control element known as the vitamin D response element (VDRE) in the regulatory re-

gion of the genome, which is often but not always situated close to the transcriptional start site of the gene. Binding of the 1,25D-VDR-RXR complex to the VDRE initiates recruitment and assembly of a very large complex of coactivator proteins. This complex remodels the locally condensed chromatin by the actions of enzymes, which either add or remove acetyl or methyl groups from histones. The complex recruits the RNA polymerase II enzyme to the transcriptional start site, which initiates mRNA synthesis of the vitamin D-responsive gene.

Such transcriptional complexes have been identified for all steroid hormone nuclear receptors investigated, and this transcriptional complex defines the specificity and sensitivity of many of their biological responses. It appears that vitamin D also regulates a multiplicity of biological responses through a wide range of tissues in this manner. Currently, we have understood the contribution of at least four elements of the transcriptional complex [24]. In the case of vitamin D, the nuclear receptor ligand, 1,25D, identifies the physiological specificity of the response. The VDRE identifies the genetic specificity of the response. The various co-activators and other proteins complexing to the liganded VDR-RXR heterodimer bound to the VDRE identify the cell or tissue specificity of the response. Finally, the transcription and translation of the gene and the specific gene product activity identifies the physiological response.

2. Rapid actions of vitamin D (non-genomic activities)

Over time, the ability of 1,25D to exert biological effects over periods of time considerably shorter than those required to detect products of gene transcription have been recognized. These activities take minutes and have been termed as "non-genomic actions," although very often gene transcription levels are enhanced by such activities. They take place in the cytoplasm of the cell rather than in the nucleus and often involve modulation of intracellular calcium levels as well as activation of intracellular signals through phosphate kinases and phosphatases, the pathways vary among different cell types [25, 26].

One study has demonstrated that 1,25D acts through a distinct membrane-associated, rapid response steroid binding receptor (MARRS) [11] to initiate such rapid activities. This protein belongs to a superfamily of multifunctional glucose-regulated and redox-sensitive proteins that have been previously implicated in binding thyroid hormones and estrogens in glycoprotein biosynthesis and in immune responses [27]. The classical nuclear VDR also elicits rapid responses, which are considered to require the association of the VDR with plasma cell membrane constituents [25, 28].

3. Actions of the vitamin D receptor through intracellular protein binding

Recent data suggests that a further mode of action of the vitamin D system is by direct binding of the classical nuclear VDR to intracellular proteins. Many of these VDR-binding proteins are transcriptional co-activators or co-repressors involved in the transcriptional complex required for genomic actions or act as transcription factors themselves. One such protein is β-catenin, and interaction with VDR modulates expression of β-catenin-responsive genes in some cells and vitamin D-responsive genes in others [29]. Some of these complexes require 1,25D binding to the VDR, while others do not require 1,25D. In skeletal cell physiology the wingless integration (Wnt) signaling pathway including β-catenin regulates bone formation as indicated by the inhibition of bone formation by the actions of the Wnt signaling pathway antagonist sclerostin. Inhibitors of sclerostin are currently under investigation for use in the treatment for postmenopausal osteoporosis [30]. Preliminary *in vitro* experimentation with a human osteoblast-like osteosarcoma cell line indicates that VDR stimulates β-catenin activity in this cell line apparently independent of 1,25D [10].

THE ROLE OF VITAMIN D RECEPTOR IN CALCIUM AND BONE MINERAL HOMEOSTASIS

Each of the molecular mechanisms of the vitamin D system described above contributes to maintaining plasma calcium and bone mineral homeostasis. VDR expression in intestinal, renal, and bone tissues is essential for maintaining plasma calcium and phosphate homeostasis. VDR is expressed by all three major bone cell types: osteoblasts, osteoclasts, and osteocytes [12]. Ablation of the VDR gene produces hereditary vitamin-D-resistant rickets (HVDRR), a rare autosomal recessive condition characterized by significantly elevated levels of 1,25D, alopecia, hypocalcaemia, hypophosphatemia, and rickets, which is a bone tissue mineralization abnormality in children [31-33]. Global VDR-ablated mouse models (global-VDR [-/-]) demonstrate rachitic bone changes analogous to HVDRR when fed a standard diet; however, feeding these animals high calcium, phosphorus, and lactose diets until 10 weeks of age corrects plasma calcium and phosphate levels preventing the development of rachitic bone changes, which achieved normal bone volume and strength [34, 35]. Specific ablation of VDR in the intestine with concomitantly reduced calcium absorption, increased serum 1,25D, and parathyroid hormone (PTH) levels associated with trabecular and cortical bone loss and cortical porosity sufficiently severe to initi-

ate spontaneous bone fractures [36]. When an intestinal-specific transgene for VDR was expressed in the global-VDR (-/-) mouse, thereby inducing VDR expression only in the intestine of this mouse model, calcium absorption was restored accompanied by restoration of plasma calcium homeostasis preventing the rachitic phenotype of VDR knockout mice [37]. Such data clearly show that any defect in bone mineralization is largely dependent on the availability of plasma calcium and phosphate via VDR-mediated intestinal absorption.

Regulation of plasma calcium homeostasis is also maintained by 1,25D-mediated renal reabsorption of calcium, which requires expression of VDR in the kidney. Global-VDR (-/-) mice show increased renal excretion of calcium whether on a normal diet with hypocalcaemia and marked increase in the PTH levels or on high calcium and phosphate diets with normal plasma calcium and PTH levels [38]. Clinical evidence confirms that serum 1,25D regulates renal tubular reabsorption of calcium in humans so that in case of mild renal failure, 1,25D acts to maintain plasma calcium homeostasis [39].

VDR-mediated activities within bone cells are also critical in regulating plasma calcium and bone mineral homeostasis. Studies conducted with *in vitro* and *in vivo* models have shown that vitamin D activity can either promote or inhibit bone formation and stimulate or inhibit bone mineral catabolism. These actions on the bone mineral appear most likely to support the maintenance of plasma calcium homeostasis under varying physiological circumstances. However, definitive proof of such a relationship between vitamin D actions on bone cells and plasma calcium homeostasis is yet to be demonstrated. Evidence is available to support the plausibility of the concept summarized here.

Global-VDR (-/-) mice fed a standard calcium diet develop hypocalcemia and hypophosphatemia with markedly raised PTH levels. However, there is a failure to increase osteoclastic activity, which is mostly likely due to inadequate levels of RANKL, a VDR-mediated factor produced by osteoblasts and osteocytes required for osteoclastogenesis [40]. Even when global-VDR (-/-) mice were fed the high calcium and phosphate diet, animals at 16 weeks of age demonstrated marked osteopenia despite normalization of serum calcium and phosphate levels. The reduced trabecular bone volume was a result of impaired mineral apposition in these global-VDR (-/-) mice and not increased bone resorption. According to the authors, the importance of stimulation of bone formation may increase with aging. Recent data with an osteoblast-specific VDR knockout mouse model demonstrated impaired RANKL expression and activity, thereby confirming the essential role of VDR in osteoblasts for the regulation of osteo-

clastogenesis [41]. There were no apparent changes in the bone formation parameters observed in these 16-week-old osteoblast-specific VDR knockout mice, suggesting that the VDR activity in osteoblasts mainly stimulates bone resorption.

The role of VDR has also been examined in osteocytes *in vivo* with an osteocyte-specific VDR deletion mouse model [42]. Under normal dietary conditions, the absence of VDR in osteocytes did not appear to be essential for osteocyte function or to bone mineral status. With pharmacological levels of plasma 1,25D, osteocyte expression of genes involved in inhibition of mineralization was upregulated in Wild Type mice only, and the localized regions of under-mineralized bones around the osteocyte lacunae were detected. Data generated from this model indicate that high 1,25D plasma levels can act on osteocytes to inhibit bone mineral deposition in addition to stimulating bone resorption by way of increasing RANKL expression by osteoblasts and possibly osteocytes. Such actions would maintain or even increase calcium within the plasma compartment through the action of plasma 1,25D and osteocyte VDR.

In contrast to these catabolic and anti-mineralization activities of plasma 1,25D and VDR in osteoblasts and osteocytes in bone, VDR also mediates anabolic activity within the bone under conditions of adequate dietary calcium intake. Over-expression of VDR, specifically in mature osteoblast lineage cells (OSVDR), demonstrated increased mineral apposition and decreased bone resorption activity resulting in increased cortical and trabecular bone volumes [43]. In addition, the calcium content of the mineralized matrix in both the cortical and trabecular bones was modestly increased in OSVDR mice [44]. Importantly, this increased bone mineral phenotype is lost when OSVDR mice are fed low dietary calcium suggesting that the mechanism by which 1,25D and VDR in mature osteoblasts increases bone volume depends on the adequacy of dietary calcium and possibly on low plasma 1,25D levels.

A similar anabolic action of vitamin D has been demonstrated by dietary manipulation of vitamin D status in rodents, where both adequate serum 25-hydroxyvitamin D combined with adequate dietary calcium intake were required for optimal trabecular and cortical bone mineral volumes [45, 46]. The adequate serum 25-hydroxyvitamin D levels under these conditions reduced bone RANKL expression and bone resorption, while also slightly prolonging the bone formation period of osteoblasts.

CONCLUSION

The 21st century has witnessed an increase in knowledge re-

garding vitamin D metabolism and its modes of action, which provides plausibility to claims of the various organs and disease processes modulated by vitamin D. Expression of VDR and synthesis of 1,25D occurs in many if not most tissues of the body. Two protein receptors for 1,25D have been characterized. Other metabolites of vitamin D such as 24,25-dihydroxyvitamin D have been proposed to exert biological activities possibly through their specific receptor [47], and others have suggested that metabolites of vitamin D other than 1,25D may activate the classical nuclear VDR [16]. The various modes of VDR action described here along with tissue expression indicate that the vitamin D system is part of a hierarchical circuitry that extends beyond individual tissues to form a mega-network governing the physiology at the scale of a whole organism [22]. However, there continues to be considerable difficulty in categorizing these activities within the clinical context for maintaining good health, and recent meta-analyses of clinical trials of vitamin D supplementation suggest that a low vitamin D status is a marker and not a cause of ill health [48].

Current data confirm that vitamin D plays a critical role in maintaining plasma calcium and phosphate homeostasis and bone mineral homeostasis. This knowledge provides a sound basis for improving nutritional strategies for the prevention of osteomalacia (and rickets in children) and osteoporosis and fractures in the elderly. This new knowledge reinforces the concept that the various activities of vitamin D on the bone are dependent on the state of plasma calcium and phosphate homeostasis rather than on the bone mineral status. When plasma calcium levels are low, vitamin D has the capacity to both stimulate the release of calcium and phosphate from bone and inhibit bone mineral deposition. When plasma calcium and phosphate levels are adequate, associated with lower plasma levels of calciotropic hormones in conjunction with an adequate vitamin D status, bone cells, particularly mature osteoblasts and osteocytes, can convert plasma 25-hydroxyvitamin D to 1,25D to enhance calcium and phosphate accrual in bone tissue and improve bone strength.

Further studies are required to determine the various molecular actions of vitamin D on bone cells as well as on the intestine and kidney, which contribute to plasma calcium and phosphate homeostasis. The new data obtained from these studies will enhance the understanding of the interaction of vitamin D status and dietary calcium and phosphate intakes for optimal health, including the optimization of bone health and reduction in the risk of fractures, including skeletal complications of diseases such as seen in chronic kidney disease. Laboratory medicine will continue to make important contributions to the care of these patients through analyses of serum 25-hydroxyvitamin D and other biomarkers.

Authors' Disclosures of Potential Conflicts of Interest

Acknowledgments

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