Minireview: Vitamin D: Is There a Role in Extraskeletal Health?

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In recent years, vitamin D has received increased attention due to the resurgence of vitamin D deficiency and rickets in developed countries together with the identification of extraskeletal vitamin D receptor-mediated actions, suggesting unexpected benefits of vitamin D in health and diseases. Although there is increased awareness of the importance of vitamin D, the role of vitamin D in extraskeletal health has been a matter of debate. In this review, we will summarize what is known and indicate the questions that remain and need to be addressed. (Endocrinology 152: 2930–2936, 2011)

The importance of vitamin D for curing rickets has been known for over 80 yr. In recent years, there has been renewed interest in vitamin D because of its many other suggested roles in the skin, in the immune system, and in cancer prevention and treatment. This review will first briefly summarize the mechanisms by which vitamin D maintains calcium homeostasis followed by a consideration of the role of vitamin D in extraskeletal health.

Vitamin D and the Maintenance of Calcium Homeostasis

Vitamin D is important for the development and maintenance of bone as well as for the maintenance of normal calcium and phosphorus homeostasis (1–3). The causal link between vitamin D deficiency during bone development and rickets and in adults between vitamin D deficiency and secondary hyperparathyroidism that can result in accelerated bone loss and increased risk of fracture is well documented (4, 5). Vitamin D is synthesized in the skin from 7-dehydrocholesterol by UV irradiation. The synthesis of vitamin D in the skin, which is the most important source of vitamin D, depends of the intensity of UV irradiation, which varies with season and latitude (6).

Although vitamin D can also be taken in the diet, few foods (which include fortified dairy products and fish oils) contain appreciable amounts of vitamin D. The hormonally active form of vitamin D, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), is produced by two sequential hydroxylations [25 hydroxylation in the liver, which results in the formation of 25-hydroxyvitamin D₃ (25(OH)D₃), the major circulating form of vitamin D, and by 25-hydroxyvitamin D₃ 1α hydroxylation in the proximal renal tubule] (1–3, 7). 25-Hydroxyvitamin D₃ 24 hydroxylase [CYP 24 or 24(OH)ase] limits the amount of 1,25(OH)₂D₃ by accelerating the catabolism of 1,25(OH)₂D₃ in target tissues to 1,24,25(OH)₃D₃, ultimately resulting in the formation of calcitriol, and also by producing 24,25(OH)₂D₃, thus decreasing the 25(OH)D₃ substrate available for 1α hydroxylation. Elevated parathyroid hormone (PTH) resulting from hypocalcemia induces 1,25(OH)₂D₃ synthesis in the kidney. 1,25(OH)₂D₃ in turn acts at the parathyroid gland to suppress PTH production. 1α(OH)ase is negatively regulated by 1,25(OH)₂D₃. 24(OH)ase is reciprocally regulated [stimulated by 1,25(OH)₂D₃ and inhibited by PTH and low calcium] (7).

The actions of 1,25(OH)₂D₃, similar to other steroid hormones, are mediated by a nuclear receptor [vitamin D receptor (VDR)]. 1,25(OH)₂D₃ occupied VDR het-

Abbreviations: DMBA, 7,12 Dimethylbenz(a)anthracene; EAE, experimental autoimmune encephalomyelitis; EII, inflammatory bowel disease; IFN, interferon; KO, knockout; MS, multiple sclerosis; NMU, N-methyl-N-nitrosourea; NOD, nonobese diabetic; 24(OH)ase, 24 hydroxylase; 25(OH)D₃, 25-hydroxyvitamin D₃; 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; PTH, parathyroid hormone; VDR, vitamin D receptor.
Erodimerizes with the retinoid X receptor and together with coregulatory proteins interacts with vitamin D response elements predominantly, but not exclusively, in the promoter region of target genes and modulates their transcription (1, 2, 8, 9). The phenotype of VDR knockout (KO) mice includes rickets, osteomalacia, and secondary hyperparathyroidism and thus represents a mouse model of vitamin D-dependent rickets type II (10–13). When VDR KO mice are fed a diet high in calcium, phosphorus, and lactose, serum calcium and PTH are normalized and osteomalacia and rickets are prevented (14). These findings in the VDR KO mice suggest that a major defect from the loss of VDR activity is a defect in intestinal calcium and phosphate absorption, which is the primary cause of decreased bone mineralization. Thus, the principal function of vitamin in mineral homeostasis is to increase calcium and phosphorus absorption from the intestine. If normal serum calcium is unable to be maintained by intestinal absorption, increased PTH induces the synthesis of 1,25(OH)₂D₃, and together PTH and 1,25(OH)₂D₃ mobilize bone calcium and increase calcium reabsorption from the renal distal tubule (15, 16). Thus, it is clear that, through these mechanisms, vitamin D is vital for mineral homeostasis. Considering these findings and what has been observed in the VDR KO mice, are there extraskeletal biological systems where 1,25(OH)₂D₃ and VDR generate significant biological responses that can affect health and disease?

### Extraskelatal Effects of Vitamin D

The possibility of extraskeletal effects of 1,25(OH)₂D₃ was noted with the discovery in 1979 through the 1980s of the presence of VDR in tissues and cells that were not involved in maintaining calcium homeostasis, including pancreas, skin, placenta, brain, and activated T cells (1, 17–19). VDR is not found in every cell or tissue (for example, VDR has been reported to be undetectable in striated and smooth muscle) (20, 21), supporting a role for VDR at specific extraskeletal sites. VDRs were also noted in a number of cancer cells, including breast, prostate, and colon cancer cells (22–25). When these cancer cells were incubated with 1,25(OH)₂D₃, their cellular proliferation was inhibited (24, 25). Leukemia cells were also found to express VDR, and when incubated with 1,25(OH)₂D₃, they differentiated to normal macrophages (26). In addition, in 1979, evidence of extrarenal 1α(OH)ase was first found in placenta followed by the identification of 1α hydroxylation in macrophages (27, 28). The question that remained was the biological significance of the presence of VDR and 1α(OH)ase in different tissues.

### Extrarenal 1α(OH)ase

#### Monocytes/macrophages

It had been thought that the hypercalcemia and hypercalciuria in patients with sarcoidosis was due to enhanced responsiveness of the intestine to vitamin D. When elevated serum 1,25(OH)₂D₃ occurred in a hypercalcemic anephric patient with sarcoidosis, this established an extrarenal site for the production of 1,25(OH)₂D₃ (29). Subsequently, it was demonstrated that macrophages from patients with sarcoidosis are the source of 1,25(OH)₂D₃ and that monocyte/macrophage 1α(OH)ase is regulated differently than renal 1α(OH)ase [it is not suppressed by elevated 1,25(OH)₂D₃ or calcium] (28, 30). These findings indicated the in vivo significance of 1α(OH)ase in macrophages and the mechanisms responsible for the hypercalcemia of granulomatous disorders.

#### Placenta

In placenta, 1α(OH)ase is expressed in both fetal trophoblasts and maternal decidual cells beginning early in gestation (31). Maternal killer cells from the decidua show decreased synthesis of cytokines, such as TNF and IL-6 in response to 1,25(OH)₂D₃, suggesting that 1,25(OH)₂D₃ may act as an autocrine/paracrine regulator of immunity at the fetal-maternal interface (32). Induction of the mRNA for cathelicidin, an antimicrobial peptide, by 1,25(OH)₂D₃ in decidual cells has also been reported (33). It has been suggested that the immunosuppressive effects of 1,25(OH)₂D₃ allow for proper trophoblast invasion without a maternal immune response and thus for successful implantation (32). Although the placenta was one of the first documented sources of extrarenal 1α(OH)ase activity and 1,25(OH)₂D₃ has been shown to modulate decidual immune cell function, the role of placental 1α(OH)ase is speculative at this time. It is of interest, however, that in 1α(OH)ase KO mice, in addition to rickets, reproductive and immune defects have been noted (34), supporting the suggested role for 1,25(OH)₂D₃ as an autocrine/paracrine regulator of immunity during pregnancy. Although it has been reported that 1,25(OH)₂D₃ can be synthesized at sites other than kidney, macrophages, and placenta, the role of 1α(OH)ase under normal conditions at other extrarenal sites has been a matter of debate.

### The Role of VDR in Tissues Not Involved in Maintaining Calcium Homeostasis

#### VDR in cancer cells: Is there a role for vitamin D in cancer prevention and treatment?

In addition to the presence of VDR in cancer cells and the inhibition of proliferation by 1,25(OH)₂D₃, compel-
ling evidence for a role for vitamin D and 1,25(OH)₂D₃ in cancer prevention and treatment is from animal studies. It has been demonstrated that rats fed diets low in vitamin D and calcium develop significantly more mammary tumors when treated with 7,12 dimethylbenz(a)anthracene (DMBA) than rats fed control diets with adequate vitamin D and calcium (35). In other in vivo studies using N-methyl-N-nitrosourea (NMU), an inhibition of the progression of mammary tumor growth is observed in rats treated with 1,25(OH)₂D₃ or analogs of 1,25(OH)₂D₃ after NMU treatment (36). When rats are treated with 1,25(OH)₂D₃ or analogs of 1,25(OH)₂D₃ before treatment with NMU, tumor incidence is reduced or prevented (37). In addition, the incidence of preneoplastic mammary lesions and the development of estrogen receptor negative tumors in response to DMBA is higher in VDR KO mice compared with wild-type mice (38). In response to DMBA, VDR KO mice also display increased sensitivity to development of a variety of skin tumors and tumors in the lymph nodes (38, 39). The studies in the VDR KO mice provide direct evidence in vivo that VDR ablation can enhance sensitivity to tumor-ogenesis. 1,25(OH)₂D₃ has also been shown to delay the development of prostate interepithelial neoplasms in the Nkx3.1;Pten mutant mouse (a putative model for prostate carcinogenesis) (40) and to have tumor inhibitory activity in models of colorectal adenoma (41). 1,25(OH)₂D₃ and 1,25(OH)₂D₃ analogs have been reported to potentiate the antitumor actions of traditional anticancer agents (42–44). A number of cellular mechanisms have been proposed for the anticancer activity of 1,25(OH)₂D₃ (42, 44). These preclinical studies provide evidence supporting tumor inhibitory activity of 1,25(OH)₂D₃. At the least, understanding the mechanisms involved can potentially lead to the identification of new targets for anticancer treatment. However, the role of vitamin D, 1,25(OH)₂D₃, or 1,25(OH)₂D₃ analogs to treat cancer patients is uncertain at this time. The number of completed clinical trials is limited. Most of the clinical trials have been conducted in prostate cancer patients (fewer studies have been conducted in patients with other cancers) and in patients with advanced cancer that have not responded to traditional anticancer therapy (42, 44). A limitation of previous clinical trials is that it may not be possible to observe significant vitamin D anticancer effects in patients with very advanced disease who have failed other therapies. In the future, well designed, large scale clinical trials are needed to assess whether or not dietary vitamin D, 1,25(OH)₂D₃, or 1,25(OH)₂D₃ analogs, perhaps in combination with traditional chemotherapy agents and early in disease, have a role as anticancer agents. The evidence in the laboratory indicates that 1,25(OH)₂D₃ generates biological responses that result in the inhibition of the disease process of cancer. To demonstrate the suggested benefit of vitamin D, new, large scale clinical trials are needed.

**VDR in keratinocytes**

Studies in keratinocytes indicated that 1,25(OH)₂D₃ causes a marked decrease in proliferation and an increase in differentiation (45). This led to the concept that 1,25(OH)₂D₃ and/or its less calcemic analogs could be used to treat psoriasis. Topically applied, 1,25(OH)₂D₃ and 1,25(OH)₂D₃ analogs have been developed as a therapy for psoriasis (46). Thus, at least for psoriasis, 1,25(OH)₂D₃ and its analogs do have therapeutically relevant effects on the skin.

**Vitamin D and the cardiovascular system**

Studies in VDR KO mice and 1α(OH)ase KO mice have shown that these mice develop hypertension and cardiac hypertrophy, which is associated with an increase in renin (47, 48). Data obtained in the 1α(OH)ase KO mice indicate that the protective role of 1,25(OH)₂D₃ against cardiovascular abnormalities involves repression of renin biosynthesis by a calcium and phosphorus-independent mechanism (48). Thus, these findings in mice indicate that vitamin D does have a beneficial effect on the cardiovascular system. Clinical and epidemiological studies are also suggestive of an effect of vitamin D on cardiac function. In most of these studies, associations are reported (for example, between low serum 25(OH)D and hypertension) (49, 50), and large scale intervention studies have not been done. Thus, conclusive clinical evidence of a role of vitamin D in cardiovascular health is not yet available.

**Vitamin D and the immune system**

A role for vitamin D in the immune system was suggested by early studies indicating the presence of VDR in activated T cells (19). 1,25(OH)₂D₃ inhibits lymphocyte proliferation and activation and IL-2 and interferon (IFN)γ are decreased after activated T cells are exposed to 1,25(OH)₂D₃ (51). 1,25(OH)₂D₃ has also been shown to inhibit the differentiation and survival of dendritic cells, resulting in impaired alloreactive T cell activation (51). The inhibition of maturation and differentiation of dendritic cells results in a decrease in IL-12 and an increase in IL-10 secretion (51). IL-17, which is involved in the pathogenesis of autoimmune inflammation and has been implicated in numerous autoimmune diseases, is inhibited by 1,25(OH)₂D₃ (52). These in vitro studies provide evidence that 1,25(OH)₂D₃ is a modulator of the immune system. With regard to in vivo physiological significance, animal studies have shown that 1,25(OH)₂D₃ can protect against a number of experimental autoimmune diseases, including experimental autoimmune encephalomyelitis (EAE) (the murine model of mul-
Multiple sclerosis (MS), systemic lupus erythematosus, inflammatory bowel disease (IBD), and autoimmune thyroiditis (53, 54). At least in EAE, systemic lupus erythematosus, and IBD, dietary calcium is required for the 1,25(OH)₂D₃ suppressive effects (53, 55). 1,25(OH)₂D₃ inhibits induction of EAE when given before immunization (56, 57). In addition, when EAE mice with paralysis are treated with 1,25(OH)₂D₃, treatment results in a reversal of paralysis, and this improvement is observed throughout the course of treatment (Fig. 1) (57, 58). Inhibition of EAE by 1,25(OH)₂D₃ has been reported to be dependent on IL-10 and to be associated with an inhibition of IL-12 and IL-17 (58–60). Studies in VDR KO mice have indicated that VDR is necessary for 1,25(OH)₂D₃ to suppress EAE (61). Hypercalcemia itself, induced by PTH, blocks EAE in female but not male mice (62). Further, UV irradiation suppresses EAE independent of vitamin D (63). Although vitamin D may play a protective role, the reduction of MS at high sunlight regions may not be via production of vitamin D (63, 64). VDR KO mice also develop more severe IBD (65). IBD is due, at least in part, to an immune-mediated attack that results in IL-17 and IFNγ overproduction. The enhanced severity of IBD in the VDR KO mice has been associated with increased numbers of IL-17 and IFNγ secreting T cells and a reduction in regulatory T cells in the KO mice (66). 1,25(OH)₂D₃ and 1,25(OH)₂D₃ analogs also prevent autoimmune diabetes in nonobese diabetic (NOD) mice and inhibit the progression of autoimmune diabetes when administered after disease onset (Fig. 2) (53, 67, 68). Decreased numbers of effector T cells and induction of regulatory T cells have been associated with the protective effect of 1,25(OH)₂D₃ in the NOD mouse model (68). These in vivo studies in mouse models are convincing and suggest that vitamin D has a role in protection against these diseases. Whether vitamin D, 1,25(OH)₂D₃, or vitamin D analogs are effective in humans with autoimmune diseases in reducing symptoms perhaps together with traditional therapies or if vitamin D supplementation is protective if given early in life is not known at this time. Recent clinical studies are suggestive of a protective role of vitamin D. For example, in a study by Munger et al. (69), there was an inverse relationship between 25(OH)D₃ levels and MS, and this relationship was particularly strong when 25(OH)D levels were measured before the age of 20, suggesting that vitamin D supplements in adolescents and young adults may be important for those with a family history of MS.

**Conclusion**

Recent studies have suggested that vitamin D can also modulate innate immunity. 1,25(OH)₂D₃ has been shown to induce the antimicrobial peptide cathelicidin with subsequent killing of bacteria, including mycobacterium tuberculosis (70). Whether there is a beneficial effect of vitamin D in tuberculosis patients or a subset of tuberculosis patients remains to be determined.

**Innate immunity**

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**Conclusion**

The recent Institute of Medicine recommendation related to calcium and vitamin D supported their key role in skel-
et al. health but concluded that “it is not yet compelling that either nutrient confers benefits for extraskeletal health” (71). As indicated in this review, the evidence in the laboratory, including the use of animal models, indicates that 1,25(OH)₂D₃ generates a number of extraskeletal biological responses. These responses include inhibition of cancer progression, effects on the cardiovascular system and the skin, and inhibition of certain autoimmune diseases. Although there are many major differences between animal models and human disease, it is likely that many genes function similarly in humans and animals. In addition, findings related to 1,25(OH)₂D₃ effects in animal models may suggest mechanisms involving similar pathways in humans that could lead to the identification of new therapies. Although large-scale clinical trials are needed and, unlike vitamin D deficiency and rickets, a causal link between vitamin D deficiency and specific diseases, including cancer and autoimmune diseases, has not yet been proven, convincing evidence in the laboratory of beneficial effects of 1,25(OH)₂D₃ beyond bone cannot be dismissed.

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