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SKELETAL REGULATIONS (D GADDY, SECTION EDITOR)

# Vitamin D and Bone

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**Abstract** All cells comprising the skeleton—chondrocytes, osteoblasts, and osteoclasts—contain both the vitamin D receptor and the enzyme CYP27B1 required for producing the active metabolite of vitamin D, 1,25 dihydroxyvitamin D. Direct effects of 25 hydroxyvitamin D and 1,25 dihydroxyvitamin D on these bone cells have been demonstrated. However, the major skeletal manifestations of vitamin D deficiency or mutations in the vitamin D receptor and CYP27B1, namely rickets and osteomalacia, can be corrected by increasing the intestinal absorption of calcium and phosphate, indicating the importance of indirect effects. On the other hand, these dietary manipulations do not reverse defects in osteoblast or osteoclast function that lead to osteopenic bone. This review discusses the relative importance of the direct versus indirect actions of vitamin D on bone, and provides guidelines for the clinical use of vitamin D to prevent/treat bone loss and fractures.

**Keywords** Vitamin D · Vitamin D receptor · CYP27B1 · 25hydroxyvitamin · 1,25 dihydroxyvitamin D · 24,25 dihydroxyvitamin D · Bone · Chondrocytes · Osteoblasts · Osteoclasts

## Introduction

Rickets became a public health problem with the movement of the population from the farms to the cities during the Industrial Revolution. Various foods such as cod liver oil and irradiation of other foods including plants were found to prevent or cure this disease, leading eventually to the discovery of the active principle—vitamin D. Given that rickets in children and osteomalacia in adults are the most striking manifestations of vitamin D deficiency, and that the major phenotype in humans and mice lacking a functional vitamin D receptor (VDR) or the enzyme CYP27B1 (25OHD 1$\alpha$-hydroxylase) producing the active metabolite of vitamin D $1,25(OH)_2D$, vitamin D signaling is clearly established as critical for bone health. But are its effects direct or indirect? VDRs are found in all cell types of the skeleton: chondrocytes, osteoblasts, osteocytes, and osteoclasts. Likewise, CYP27B1 is also expressed in these cells. Moreover, hundreds of publications have shown direct effects of $1,25(OH)_2D$ and 25OHD on various bone cells making the case for a direct effect. On the other hand, vitamin D deficiency as well as animals (and humans) lacking a functional VDR or CYP27B1, can be successfully treated by increasing the calcium and phosphate content of the diet. Following a brief introduction to the vitamin D endocrine system, I review the data examining the issue of whether the effects of vitamin D on bone are direct or indirect, then conclude with a discussion of current guidelines for the use of vitamin D, not only to prevent rickets and osteomalacia, but the more subtle problems of osteoporosis and fractures.

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## Overview of Vitamin D Production, Metabolism, and Function

### Vitamin D Production, Metabolism to Active Forms, and Serum Transport

Vitamin $D_3$ is produced in the skin from 7-dehydrocholesterol by ultraviolet (UV) irradiation, which breaks the B ring to form pre-$D_3$. Pre-$D_3$ isomerizes to $D_3$ or with continued UV

irradiation to tachysterol and lumisterol [1]. $D_3$ is preferentially removed from the skin, bound to vitamin D binding protein (DBP). Vitamin D is also found in small quantities in the diet, but is a common supplement. This can be in the form of $D_2$ or $D_3$, which differ in their side chains impacting both their affinity for DBP and subsequent metabolism, but which for the sake of this discussion will be treated as equivalent. The liver and other tissues metabolize vitamin D, whether from the skin or oral ingestion, to 25OHD, the principal circulating form of vitamin D, by several enzymes of which CYP27A1 (mitochondrial) and CYP2R1 (microsomal) are the best studied. 25OHD is then further metabolized to $1,25(OH)_2D$ principally in the renal proximal tubule by the enzyme CYP27B1, although other cells such as epidermal keratinocytes, parathyroid gland, intestinal epithelium, macrophages, and various bone cells and chondrocytes contain this enzyme [2•].

$1,25(OH)_2D$ is the principal hormonal form of vitamin D, responsible for most of its biologic actions. The production of $1,25(OH)_2D$ in the kidney is tightly controlled, being stimulated by parathyroid hormone (PTH), and inhibited by calcium, phosphate, and fibroblast growth factor 23 (FGF23). Extrarenal production of $1,25(OH)_2D$ as in keratinocytes, macrophages, and osteoblasts is under different control, being stimulated primarily by cytokines such as tumor necrosis factor-α, interferon-γ, and interleukin (IL)-1β [2•, 3]. $1,25(OH)_2D$ reduces $1,25(OH)_2D$ levels in cells by decreasing production or by stimulating its catabolism through the induction of CYP24A1, the 24-hydroxylase [4]. 25OHD and $1,25(OH)_2D$ are hydroxylated in the 24 position by this enzyme to form $24,25(OH)_2D$ and $1,24,25(OH)_3D$, respectively. This 24-hydroxylation is generally the first step in the catabolism of these active metabolites, although $24,25(OH)_2D$ and $1,24,25(OH)_3D$ have their own biologic activities, and in particular $24,25(OH)_2D$ may be the preferred metabolite regulating resting zone chondrocyte function [5]. CYP24A1 is induced by $1,25(OH)_2D$, which serves as an important feedback mechanism to avoid vitamin D toxicity. The vitamin D metabolites are transported in blood bound to DBP and albumin. Very little circulates as the free form [6, 7]. The liver produces DBP and albumin, and these proteins may be lost in protein losing enteropathies or the nephrotic syndrome. Thus individuals with liver, intestinal, or renal diseases that result in low levels of these transport proteins may have low total levels of the vitamin D metabolites without being vitamin D deficient as their free concentrations may be normal [7].

### Vitamin D Mechanism of Action

VDR is a transcription factor regulating the expression of genes that mediate its biologic activity [8]. VDR is a member of a rather large family of nuclear hormone receptors that includes the receptors for glucocorticoids, mineralocorticoids, sex hormones, thyroid hormone, and vitamin A metabolites or retinoids. VDR is widely distributed, and is not restricted to those tissues considered the classic target tissues of vitamin D. VDR upon binding to $1,25(OH)_2D$ heterodimerizes with other nuclear hormone receptors, in particular the family of retinoid X receptors. This complex then binds to special DNA sequences called vitamin D response elements (VDREs) in the promoters (and other regions) of genes that it regulates. A variety of additional proteins called coactivators bind to the activated VDR/retinoid X receptor (RXR) heterodimers to form a bridge from the VDR/RXR complex to the proteins responsible for transcription such as RNA polymerase II or to help unravel the chromatin at the site of the gene via recruitment of histone acetyl transferases (HATs), allowing transcription to proceed [9]. The vitamin D receptor interacting protein complex (DRIP or Mediator) is an example of the first type of coactivator complex; the steroid receptor coactivator family (SRC 1–3) are examples of the latter type of coactivator. Different genes vary in their regulation by these coactivators. In addition to coactivators there are a number of corepressors. Corepressors typically work by recruiting histone deacetylases to the gene, which reverse the actions of HAT, leading to a reduction in access to the gene by the transcription machinery. Like the coactivators, the corepressors can be specific for different genes, and in either case different cells differentially express these coregulators, providing some specificity for the actions of $1,25(OH)_2D$ and VDR.

In addition to regulating gene expression, $1,25(OH)_2D$ has a number of nongenomic actions including the ability to acutely stimulate various signaling pathways and calcium transport across the plasma membrane. The mechanisms mediating these nongenomic actions and their physiologic significance remain unclear, although both a membrane form of the VDR [10] as well as a distinct membrane protein originally called MARRS (membrane-associated rapid response steroid binding protein) now referred to as PD1A3 (protein disulfide isomerase associated protein 3) [11•, 12] have been implicated. Similarly, it is not clear that all actions of the VDR require the ligand $1,25(OH)_2D$. The best example of this is the hair loss in animals and patients with certain VDR mutations [13, 14] but not in animals and patients with mutations in CYP27B1 [15, 16]. As mentioned, the VDR is widely distributed, and the actions of $1,25(OH)_2D$ are quite varied. This review focuses on bone.

## Vitamin D Effects on Bone

### Overview of Bone Formation

Bone develops intramembranously (eg, skull) or from cartilage (endochondral bone formation; eg, long bones with growth plates). Intramembranous bone formation occurs

when osteoprogenitor cells proliferate and produce osteoid, a type I collagen-rich matrix. The osteoprogenitor cells differentiate into osteoblasts, which then deposit calcium phosphate crystals into the matrix to produce woven bone. This bone is remodeled into mature lamellar bone. Endochondral bone formation is initiated by the differentiation of mesenchymal stem cells into chondroblasts that produce the proteoglycan-rich type II collagen matrix. These cells continue to differentiate into hypertrophic chondrocytes that shift from making type II collagen to producing type X collagen. These cells also initiate the degradation and calcification of the matrix by secreting matrix vesicles filled with degradative enzymes such as metalloproteinases and phospholipases, alkaline phosphatase (thought to be critical for the mineralization process by hydrolyzing the mineralization inhibitor pyrophosphate), and calcium phosphate crystals. Vascular invasion and osteoclastic resorption are stimulated by the production of vascular endothelial growth factor (VEGF) and other chemotactic factors from the degraded matrix. The hypertrophic chondrocytes also begin to produce markers of osteoblasts such as osteocalcin, osteopontin, and type I collagen resulting in the initial deposition of osteoid. Terminal differentiation of the hypertrophic chondrocytes and the subsequent calcification of the matrix are markedly impaired in vitamin D deficiency leading to the flaring of the ends of the long bones and the rachitic rosary along the costochondral junctions of the ribs, classic features of rickets. Although supply of adequate amounts of calcium and phosphate may correct most of these defects in terminal differentiation and calcification, the vitamin D metabolites, $1,25(OH)_2D$ and $24,25(OH)_2D$, have been shown to exert distinct roles in the process of endochondral bone formation.

The VDR makes its first appearance in the fetal rat at day 13 of gestation in the condensing mesenchyme of the vertebral column [17], then subsequently in osteoblasts and the proliferating and hypertrophic chondrocytes by day 17 [17]. However, fetal development is quite normal in vitamin D–deficient rats [18] and VDR knockout mice [13], suggesting that vitamin D and the VDR are not critical for skeletal formation. Rickets develops postnatally, becoming most manifest after weaning.

## Vitamin D-Regulated Bone Formation

As noted in the introduction nutritional vitamin D deficiency, altered vitamin D responsiveness such as VDR mutations (hereditary vitamin D-resistant rickets), and deficient production of $1,25(OH)_2D$ consequent to CYP27B1 mutations (pseudo-vitamin D deficiency) all have rickets as their main phenotype. This indicates that vitamin D, and in particular $1,25(OH)_2D$, is of critical importance to bone. Furthermore, both VDR and CYP27B1 are found in bone cells [19, 20], as is CYP27B1 [3, 21–24, 25•, 26•]. However, the rickets resulting from vitamin D deficiency or VDR mutations (or knockouts) can be corrected by supplying adequate amounts of calcium and phosphate by infusions or orally [27–33], or in the case of the VDR knockout by expressing the VDR solely in the intestine [34•]. This would suggest that vitamin D metabolites are unimportant for bone, or that substantial redundancy has been built into the system. I prefer the latter explanation given that numerous examples exist demonstrating the close interaction between calcium and vitamin D with respect to their compensatory/synergistic actions, and bone is no exception [35•].

A further complicating factor in determining the role of vitamin D metabolites in bone is the multitude of effects these metabolites have on systemic calcium homeostatic mechanisms, which themselves impact on bone. The lack of vitamin D results in hypocalcemia and hypophosphatemia, which as implied above is sufficient to cause rickets. Moreover, part of the skeletal phenotype in vitamin D deficiency is also due to the hyperparathyroidism that develops in the vitamin D-deficient state as PTH has its own actions on bone and cartilage. Furthermore, vitamin D metabolites can alter the responsiveness of bone to growth hormone [36], and the expression and/or secretion of a large number of skeletally derived factors including insulin-like growth factor-1 [37], its receptor [38], and binding proteins [39, 40], transforming growth factor-β [41], VEGF [42], IL-6 [43], IL-4 [44], and endothelin receptors [45], all of which can exert effects on bone of their own as well as modulate the actions of the vitamin D metabolites on bone.

Understanding the impact of vitamin D metabolites on bone is additionally complicated by species differences, differences in responsiveness of bone and cartilage cells according to their states of differentiation, and differences in responsiveness in terms of the vitamin D metabolite being examined. For example, $1,25(OH)_2D$ added in the early stages of osteoblast cultures inhibited the expression of collagen 1 and alkaline phosphatase but stimulated their expression when added to more differentiated cultures [46]. Similarly, it has been shown that osteoblasts obtained from VDR knockout mice show increased expression of alkaline phosphatase, bone sialoprotein, and osteocalcin, and mineralize more readily than osteoblasts from wild-type mice in vitro [47] and in vivo [48]. However, other studies have shown an increase in osteoblast differentiation markers with $1,25(OH)_2D$ or 25OHD [3, 21, 49], and overexpressing the VDR in mature osteoblasts also leads to increased bone [50, 51]. Osteocalcin and osteopontin in human and rat cells have well-described VDREs in their promoters [52–54], but the mouse does not [55]. Moreover, alkaline phosphatase and the *COL1A1* and *COL1A2* genes producing type I collagen do not have clearly defined VDREs, so it remains unclear how these genes are regulated

by 1,25$(OH)_2$D. These maturation-dependent effects of 1,25 $(OH)_2$D on bone cell function may explain the surprising ability of excess 1,25$(OH)_2$D to block mineralization leading to hyperosteoidosis [56, 57], as such doses may prevent the normal maturation of osteoblasts.

### Vitamin D-Regulated Chondrocyte Differentiation and Function

The impairment of endochondral bone formation observed in vitamin D deficiency is associated with decreased alkaline phosphatase activity of the hypertrophic chondrocytes [58], alterations in the lipid composition of the matrix [59] perhaps secondary to reduced phospholipase activity [60], and altered proteoglycan degradation [61] due to changes in metalloproteinase activity [61]. Both 1,25$(OH)_2$D and 24,25 $(OH)_2$D appear to be required for optimal endochondral bone formation [62]. However, in the CYP24A1 knockout mouse, which fails to produce any 24-hydroxylated metabolites of vitamin D, the skeletal lesion is defective mineralization of intramembranous (not endochondral) bone [63]. Furthermore, the skeletal abnormality appears to be due to high circulating 1,25$(OH)_2$D levels in that crossing this mouse with one lacking the VDR corrects the problem [63]. Whether this reflects species differences between mice and other species (most studies demonstrating the role of 24,25$(OH)_2$D in bone and cartilage have used rats and chicks) remains unknown. Chondrocytes from the resting zone of the growth plate of rats tend to be more responsive to 24,25$(OH)_2$D than 1,25$(OH)_2$D, whereas the reverse is true for chondrocytes from the growth zone with respect to stimulation of alkaline phosphatase activity [64], regulation of phospholipase A2 (stimulation by 1,25$(OH)_2$D, inhibition by 24,25$(OH)_2$D) [65], changes in membrane fluidity (increased by 1,25$(OH)_2$D, decreased by 24,25$(OH)_2$D) [66], and stimulation of protein kinase C activity [67]. These actions of 1,25$(OH)_2$D and 24,25$(OH)_2$D do not require the VDR and are nongenomic in that they take place with isolated matrix vesicles and membrane preparations from these cells [64]. Evidence for a specific receptor for 24,25 $(OH)_2$D in chondrocytes is gaining strength (St. Arnaud, personal communication), and the existence of a functional membrane receptor for 1,25(OH)2D in these cells is supported by the observation that an antibody against PD1A3 (one of the putative membrane receptors for 1,25$(OH)_2$D) blocks these effects of 1,25$(OH)_2$D on these cells [68].

### Vitamin D-Regulated Bone Resorption

In addition to its role in promoting bone formation, 1,25 $(OH)_2$D promotes bone resorption by increasing the number and activity of osteoclasts [69]. These effects may be direct in part, in that the osteoclast contains the VDR and CYP27B1 [24, 25•], and 25OHD promotes their differentiation in the presence of macrophage colony-stimulating factor (m-CSF) and receptor activator of nuclear factor-κB ligand (RANKL) [25•]. However, these same studies showed an inhibition of their resorptive capacity [25•]. Better established is the stimulation of osteoclastogenesis by 1,25$(OH)_2$D via the osteoblast [70]. Osteoblasts produce membrane-associated RANKL that activates RANK on osteoclasts and their hematopoietic precursors. This cell-to-cell contact in combination with m-CSF also produced by osteoblasts stimulates the differentiation of precursors to osteoclasts, and promotes their activity. 1,25$(OH)_2$D regulates this process by inducing RANKL [71], as does PTH, prostaglandin E2, and IL-11, all of which stimulate osteoclastogenesis. 1,25$(OH)_2$D requires the VDR in osteoblasts for this purpose, although the other hormones and cytokines do not. Osteoblasts from VDR knockout mice fail to support 1,25$(OH)_2$D-induced osteoclastogenesis, whereas osteoclast precursors from VDR knockout mice can be induced by 1,25$(OH)_2$D to form osteoclasts in the presence of osteoblasts from wild-type animals [72], indicating that it is the osteoblast that is the key cell responding to 1,25$(OH)_2$D with respect to osteoclast formation.

## Effects on Bone: Direct or Indirect

The degree to which vitamin D directly affects bone versus its indirect actions via 1,25$(OH)_2$D stimulation of intestinal calcium and phosphorus absorption remains a matter of debate, although both are clearly involved. VDR-ablated mice (VDR knockout mice) develop secondary hyperparathyroidism, hypocalcemia, and rickets after weaning [13, 14]; similar changes are seen when CYP27B1 is knocked out [15, 16]. However, as noted previously, when VDR knockout or CYP27B1 knockout mice are fed a rescue diet containing high levels of calcium, phosphorus, and lactose, serum ionized calcium and PTH levels are normalized, and rickets and osteomalacia are prevented [30, 32]. This suggests that a major effect of 1,25$(OH)_2D_3$ is the provision of calcium and phosphate to bone from the intestine, rather than a direct action on bone. Furthermore, transgenic expression of VDR in the intestine of VDR knockout mice results in normalization of serum calcium, bone density, and bone volume [34•]. However, a more extensive analysis of the effect of the rescue diet on the skeleton of VDR knockout, CYP27B1 knockout, and CYP27B1/VDR double knockout mice demonstrated that even when hypocalcemia and secondary hyperparathyroidism are prevented by the rescue diet, not all changes in osteoblast number, mineral apposition rate, and bone volume are rescued [73]. In particular, these studies demonstrated that the width of the growth remained increased in the CYP27B1 knockout and

double knockout. Furthermore, trabecular bone was markedly osteopenic in all knockout models with decreased mineral apposition rates in both cortical and trabecular bone and reductions in alkaline phosphatase expression, all signifying decreased osteoblast number or activity. This conclusion was confirmed by bone marrow stromal cell cultures from these animals demonstrating reductions in colony-forming units and mineralized nodules despite their ingestion of the rescue diet. Osteoclast numbers in the trabeculae were likewise reduced as was expression of RANKL in extracts of the bones. Other studies have selectively deleted VDR [22] or CYP27B1 [26•] in chondrocytes.

These mice would not be expected to have abnormalities in intestinal calcium and phosphate transport or other metabolite abnormalities accompanying the global knockout of these genes. However, while neither mouse model showed a marked alteration in growth plate development, both showed a decrease in vascular invasion at the chondro-osseous junction with decreased osteoclasts and increased poorly mineralized bone in the primary spongiosa. The chondrocyte-specific VDR null mouse had an increase in serum phosphate and $1,25(OH)_2D$ levels, with decreased FGF23 associated with increased expression of CYP27B1 and the sodium phosphate transporter Npt2a in the kidney [22]. A similar decrease in expression of FGF23 in the bone of chondrocyte-specific CYP27B1 knockout was noted (metabolic consequences were not evaluated). These results indicate that vitamin D signaling in the chondrocyte was clearly affecting endochondral bone formation on the one hand and systemic calcium/phosphate homeostasis via changes in FGF23 production on the other.

So to answer the question as to the relative importance of direct versus indirect effects of vitamin D on bone, we can conclude that both are required, and a deficiency in one can be at least partially compensated by the other.

## Clinical Implications

### Nutritional Requirements

Up to this point we have been primarily concerned with the animal and cellular studies. These studies including those of transgenic animal models have taught us much concerning the direct and indirect actions of vitamin D on bone. The animal models in particular have demonstrated that although the more flagrant manifestations of rickets/osteomalacia in states of vitamin D deficiency, pseudo-vitamin D deficiency (CYP27B1 mutations), and hereditary vitamin D-resistant rickets (VDR mutations) can be prevented with high calcium/phosphate diets, the more subtle and long-term deficits leading to osteopenia/osteoporosis may not be. While rickets and osteomalacia remain clinically relevant problems, it is the role of vitamin D in the prevention/treatment of osteopenia/osteoporosis that commands most clinical attention at present in developed countries. Serum 25OHD levels provide the most useful surrogate for assessing vitamin D status, as the conversion of vitamin D to 25OHD is less well controlled (ie, primarily substrate dependent) than the subsequent conversion of 25OHD to $1,25(OH)_2D$. $1,25(OH)_2D$ levels, unlike 25OHD levels, are well maintained until the extremes of vitamin D deficiency because of the secondary hyperparathyroidism, and so do not provide a useful index for assessing vitamin D deficiency at least in the initial stages. Furthermore, as discussed earlier, many tissues including bone express CYP27B1 and may be more dependent on circulating 25OHD than circulating $1,25(OH)_2D$ for their vitamin D requirements [74•].

Historically, vitamin D sufficiency was defined as the level of 25OHD sufficient to prevent rickets in children and osteomalacia in adults. Levels of 25OHD below 5 ng/mL (or 12 nM) are associated with a high prevalence of rickets or osteomalacia. However, there is a growing consensus that these lower limits of normal are too low. Recently an expert panel for the Institute of Medicine recommended that a level of 20 ng/mL (50 nM) was sufficient for 97.5 % of the population with respect to prevention of bone disease and fractures, although up to 50 ng/mL (125 nM) was safe [75•]. For individuals between the ages of 1 to 70 years of age, 600 IU of vitamin D was thought to be sufficient to meet these goals, although up to 4,000 IU of vitamin D was considered safe [75•]. These guidelines at least with respect to the lower recommended levels of vitamin D supplementation are unlikely to correct vitamin D deficiency in individuals with obesity, dark complexions, limited capacity for sunlight exposure, or malabsorption. Moreover, the lower end of these recommendations has been considered too low and the upper end too restrictive by a number of vitamin D experts. In a meta-analysis of several randomized clinical trials, Bischoff-Ferrari et al. [76, 77•] concluded that at least 700 to 800 IU of vitamin D per day were required to achieve the 30 ng/mL (75 nM) level of 25OHD that seemed necessary for fracture prevention. Supplemental calcium may enhance the beneficial actions of vitamin D on bone, but calcitriol and its analogues cannot be recommended because of the higher incidence of hypercalcemia/hypercalciuria [78]. Although most studies have focused on postmenopausal females, the recommendations are relevant to males as well [79]. The benefits of vitamin D on fracture prevention are a combination of increased intestinal calcium absorption [80], increased bone mineral density [81], and reduced risks of falls [82].

### Vitamin D Treatment Strategies

Adequate sunlight exposure is the most cost-effective means of obtaining vitamin D. Whole-body exposure to sunlight in

the summertime has been calculated to provide the equivalent of 10,000 IU of vitamin D [83]. A 0.5 minimal erythema dose of sunlight (ie, half the dose required to produce a slight reddening of the skin) or UVB radiation to the arms and legs that can be achieved in 5 to 10 min on a bright summer day in light-skinned individuals has been calculated to be the equivalent of 3,000 IU of vitamin D [84]. However, concerns regarding the association between sunlight and skin cancer and/or solar aging of the skin have limited this approach, perhaps to the extreme, although it remains a viable option for those unable or unwilling to benefit from oral supplementation.

Studies have demonstrated that on average for every 100 IU of vitamin D supplementation administered, the 25OHD levels rise by 0.5 to 1 ng/mL [83, 85]. For obese individuals or those with malabsorption (including after bariatric surgery) much higher doses are likely to be required. Unfortified food contains little vitamin D with the exception of wild salmon and other fish products such as cod liver oil. Milk and other fortified beverages typically contain 100 IU/8-oz serving. Vitamin $D_2$ is more rapidly cleared than vitamin $D_3$, so if vitamin $D_2$ is used, it needs to be given at least weekly. Toxicity due to vitamin D supplementation has not been observed at doses less than 10,000 IU per day in several studies [86].

Although the debate over the optimal level of vitamin D is not likely to subside soon, my own recommendation is to maintain a 25OHD level of 30 ng/mL by a combination of sunshine and supplementation, a level that is universally recognized as safe and effective. The amount of vitamin D supplementation required to achieve this level will vary substantially among individuals based on where they live (latitude), time of year, amount of unprotected sun exposure, skin color, body mass index, what they eat, and absorption efficiency so that one-size-fits-all recommendations are inadequate. As in most aspects of medicine, clinical judgment is required.

## Conclusions

Understanding the relative contributions of direct and indirect actions of vitamin D on bone is complex. The cells in bone and cartilage contributing to skeletal formation and maintenance have both VDR and CYP27B1, and respond directly to both circulating 25OHD and $1,25(OH)_2D$ (circulating and endogenously produced). However, these cells are also responsive to blood levels of calcium and phosphate, elements required for bone formation. Dietary calcium and phosphate can to some extent compensate for deficient vitamin D signaling, and vitamin D can compensate to some extent for deficiencies in calcium and phosphate. But all are involved. Defining the optimal level of vitamin D to maintain bone health remains under debate. But achieving a level of 25OHD around 30 ng/mL is both safe and effective. Additional research will be necessary to determine whether this is the optimal level.

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