Metabolism Clinical and Experimental 98 (2019) 112–120

Contents lists available at ScienceDirect

Metabolism Clinical and Experimental

journal homepage: www.metabolismjournal.com

Review

New developments in our understanding of vitamin D metabolism, action and treatment

Sylvia Christakos [a,*], Shanshan Li [a], Jessica De La Cruz [a], Daniel D. Bikle [b]

[a] Department of Microbiology, Biochemistry and Molecular Genetics, Rutgers, the State University of New Jersey, New Jersey Medical School, Newark, NJ, USA
[b] Departments of Medicine and Dermatology, University of California and VA Medical Center, San Francisco, CA, USA

ARTICLE INFO

Article history:
Received 11 March 2019
Accepted 14 June 2019

Keywords:
Vitamin D
Vitamin D metabolism
Calcium homeostasis
Fibroblast growth factor 23
Parathyroid hormone
Intestine
Kidney
Bone
Randomized controlled clinical trials

ABSTRACT

Although vitamin D has been reported to have pleiotropic effects including effects on the immune system and on cancer progression, the principal action of vitamin D is the maintenance of calcium and phosphate homeostasis. The importance of vitamin D in this process is emphasized by the consequences of vitamin D deficiency which includes rickets in children and osteomalacia in adults. Vitamin D deficiency has also been reported to increase the risk of falls and osteoporotic fractures. Although vitamin D fortification of foods (including dairy products) has contributed to a marked decrease in rickets in the Western world, vitamin D deficiency in children and adults is still prevalent world-wide. This review summarizes new developments in our understanding of vitamin D endocrine system and addresses clinical syndromes related to abnormalities in vitamin D metabolism and action. In addition, the current understanding of the evaluation of vitamin D deficiency and sufficiency and recommendations for achieving vitamin D sufficiency are discussed.

Contents

Abbreviations: $1,25(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 25(OH)D, 25-hydroxyvitamin D_3; AI, adequate intake; CDK, chronic kidney disease; CYP24A1, 25-hydroxyvitamin D_3 24 hydroxylase; CYP27B1, 25-hydroxyvitamin D_3 1 α hydroxylase; DBP, vitamin D binding protein; EAR, estimated average requirement; EFSA, European Food Safety Authority; FGF23, fibroblast growth factor 23; HVDRR, hereditary vitamin D resistant rickets; IOM, Institute of Medicine; PTH, parathyroid hormone; RANKL, receptor activator of nuclear kB ligand; RCT, randomized controlled clinical trial; RDA, required daily requirement; RI, recommended intake; RNA, reference nutrient intake; SACN, Scientific Advisory Committee on Nutrition; TRPV5, transient receptor potential cation channel, subfamily V, member 5; TRPV6, transient receptor potential cation channel, subfamily V, member 6; VDR, vitamin D receptor.

* Corresponding author at: Dept. of Microbiology, Biochemistry and Molecular Genetics, Rutgers, the State University of New Jersey, New Jersey Medical School, 185 South Orange Ave., Newark, NJ 07103, USA.
E-mail address: christak@njms.rutgers.edu (S. Christakos).

https://doi.org/10.1016/j.metabol.2019.06.010
0026-0495/

1. Introduction

Nearly a century ago McCollum et al. [1] identified vitamin D as the factor that cured rickets. In recent years there has been renewed interest in vitamin D due not only to an increased awareness of possible benefits of vitamin D on overall health but also due to the resurgence of vitamin D deficiency in children and adults world-wide. In this article we focus on recent advances in our understanding of the vitamin D endocrine system and clinical syndromes related to dysregulation of vitamin D metabolism and action. The determination of vitamin D deficiency and sufficiency and recommendations for achieving vitamin D sufficiency are also addressed in this review.

2. Vitamin D metabolism

2.1. The vitamin D metabolic pathway production and regulation of 1,25(OH)$_2$D$_3$

Vitamin D is produced in the skin from its substrate 7-dehydrocholesterol [2]. This is a non-enzymatic process involving UV light and heat and is the most important source of vitamin D since few foods, which include fatty fish and fortified dairy products, contain vitamin D. The next step is the conversion of vitamin D to 25-hydroxyvitamin D$_3$ (25(OH)D$_3$) in the liver. Recent reports indicate that there are a number of enzymes with 25-hydroxylase activity [3]. However, evidence indicates that CYP2R1 appears to be the dominant 25-hydroxylase in humans [4–6]. 25(OH)D$_3$ is then metabolized in the kidney, as well as in a number of extra-renal sites, to its most biologically active form 1,25-dihydroxyvitamin D$_3$ (1,25(OH)$_2$D$_3$) by the CYP27B1 (25(OH)D$_3$ 1-α hydroxylase) [7–10]. Controlling the levels of 1,25(OH)$_2$D$_3$ and 25(OH)D$_3$ is the enzyme CYP24A1 (25(OH)D$_3$ 24-hydroxylase). CYP24A1 is induced by 1,25(OH)$_2$D$_3$ and is widely distributed, being expressed in most cells. Thus 1,25(OH)$_2$D$_3$ regulates its own metabolism protecting against hypercalcemia [7–10] (Fig. 1). Mutations in each of these enzymes can cause human disease [11]. The production of 1,25(OH)$_2$D$_3$ in the kidney is under stringent control (Fig. 1). Parathyroid hormone (elevated in response to hypocalcemia) stimulates CYP27B1 resulting in enhanced 1,25(OH)$_2$D$_3$ production. 1,25(OH)$_2$D$_3$ acts in turn to suppress PTH production in the parathyroid glands by increasing serum calcium and by upregulating the expression of the calcium sensing receptor, thus sensitizing the parathyroid gland to calcium inhibition [12]. Direct inhibition of PTH expression by 1,25(OH)$_2$D$_3$/VDR has also been reported [13]. 1,25(OH)$_2$D$_3$ also suppresses its own production by directly inhibiting CYP27B1 at least in the kidney [7–10]. Fibroblast growth factor 23 (FGF23), produced primarily from osteoblasts and osteocytes, promotes phosphate excretion and together with its cofactor α-klotho regulates vitamin D metabolism by inhibiting renal 1,25(OH)$_2$D$_3$ production and increasing expression of CYP24A1 [14,15] (Fig. 1). 1,25(OH)$_2$D$_3$ in turn stimulates FGF23 expression. In extrarenal tissues where CYP27B1 is also expressed, regulation differs from that in the kidney. In particular, sites such as epithelial cells and immune cells, regulation is primarily by cytokines and not by PTH or FGF23 [16]. This accounts for the hypercalcemia and inappropriately elevated 1,25(OH)$_2$D$_3$ levels seen in a variety of granulomatous diseases and epithelial cancers.

With age there is a decline in the ability of the kidney to hydroxylate 25(OH)D$_3$ to 1,25(OH)$_2$D$_3$ [17]. An increase in renal CYP24A1 expression as well as an increase in the clearance of 1,25(OH)$_2$D$_3$ with aging

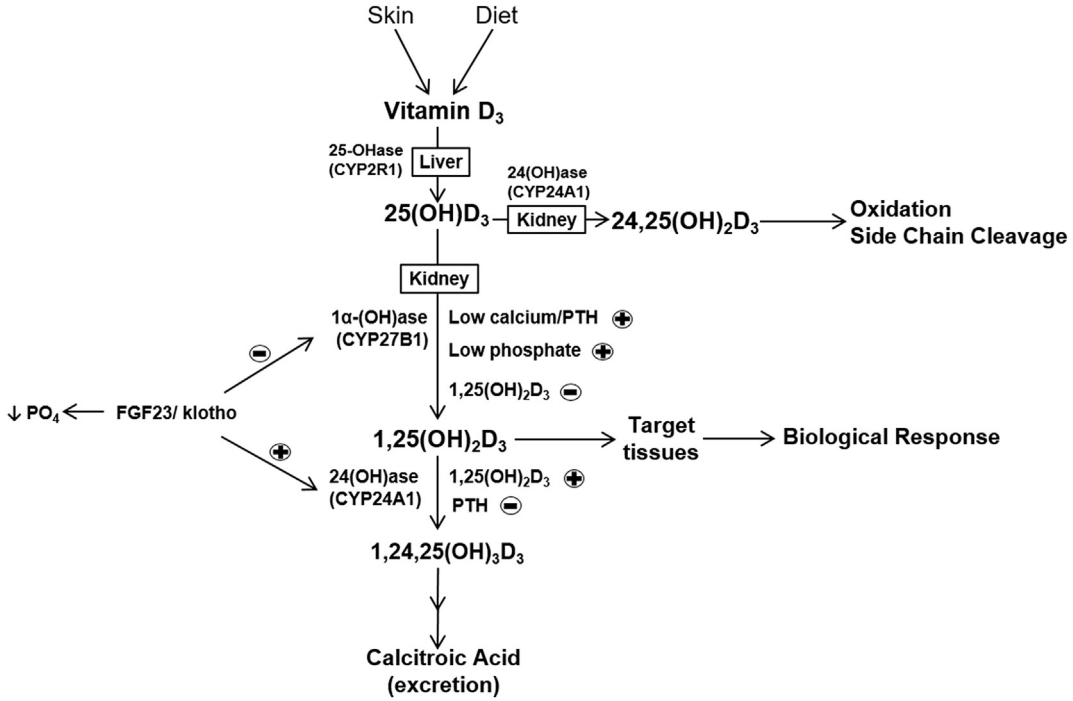

Fig. 1. Vitamin D metabolic pathway. The production of 1,25(OH)$_2$D$_3$ in the kidney is under stringent control by PTH, 1,25(OH)$_2$D$_3$, FGF23/klotho and low dietary calcium and phosphate.

have also been reported [17–20]. Thus the combined effect of a decline in the ability of the kidney to synthesize $1,25(OH)_2D_3$ and an increase in the catabolism of $1,25(OH)_2D_3$ by CYP24A1 may contribute to age related bone loss.

2.2. Mutations in the vitamin D hydroxylases and human disease

Evidence that CYP2R1 is the key vitamin D 25-hdyroxylase is indicated from studies showing that patients with functional mutations in CYP2R1 develop vitamin D- dependent rickets with high PTH and alkaline phosphatase levels and very low 25(OH)D levels [5–7,21]. This form of rickets is currently called vitamin D-dependent rickets, type 1B (VDDR1B). These patients respond well to physiologic doses of calcifediol ($25(OH)D_3$) [21]. Mutations in the renal 1α-hydroxylase (CYP27B1), diagnosed generally in the first year of life, result in skeletal defects of classic rickets, muscle weakness, and growth retardation in spite of normal intake of vitamin D [7]. These findings emphasize the importance of the CYP27B1 enzyme. Patients with this disease were first diagnosed by Fraser et al. [22]. Laboratory assessment includes low $1,25(OH)_2D_3$ levels, hypocalcemia, hypophosphatemia, high PTH but normal levels of 25(OH)D. Physiological levels of calcitriol ($1,25(OH)_2D_3$) are curative. This disease can be distinguished from vitamin D deficiency since vitamin D deficiency presents with low 25(OH)D levels and often normal $1,25(OH)_2D_3$ levels. The crucial role of CYP24A1 in the catabolism of $1,25(OH)_2D_3$ in humans was noted in recent studies of children with inactivating mutations in CYP24A1 [23]. These children present with hypercalcemia, hypercalciuria, decreased PTH, low $24,25(OH)_2D_3$ and normal to high $1,25(OH)_2D_3$ levels. It was suggested that this mutation was the probable cause of at least some cases of idiopathic infantile hypercalcemia, which can manifest after vitamin D supplementation. Stopping vitamin D supplementation, implementing a low calcium diet and limiting sunlight exposure have been suggested as therapy. It should be noted that adults with this syndrome have also been described [24,25]. Patients with a history of hypercalcemia and kidney stones may warrant screening for CYP24A1 mutations. Very recently a gain of function mutation in CYP3A4, a P450 enzyme involved in drug metabolism located in liver and intestine, has been described leading to rickets with decreased serum calcium and phosphate and elevated PTH and alkaline phosphatase. CYP3A4 was found to inactivate $25(OH)D_3$ and $1,25(OH)_2D_3$ [26]. This is a distinct form of vitamin D dependent rickets since it does not involve a defect in synthesis of vitamin D metabolites but rather is due to accelerated inactivation of vitamin D metabolites. The authors called this vitamin D dependent rickets type 3 and suggest that accelerated vitamin D inactivation by genetic or induced CYP3A4 activity may be a risk factor for vitamin D deficiency.

3. Vitamin D: molecular mechanism of action

The actions of $1,25(OH)_2D_3$ are mediated by the vitamin D receptor, a ligand dependent nuclear receptor. The modular structure of VDR is comprised of an A/B domain, a conserved zinc finger DNA-binding domain (DBD), a flexible hinge region and a multifunctional ligand binding domain (LBD). VDR heterodimerizes with the retinoid X receptor (RXR). VDR/RXR interacts with specific DNA sequences, leading to activation or repression of transcription. For transcriptional activation, in general the vitamin D response element (VDRE) in target genes is composed of two direct repeats of the hexonucleotide sequence AGGTCA separated by 3 base pairs (bp) although variations on this hexonucleotide sequence are numerous [9,10,27]. $1,25(OH)_2D_3$ promotes heterodimerization of VDR with RXR and also recruits coregulatory complexes that participate in the regulation of transcription of VDR target genes., including SRC-2 (GRIP1) and CBP that display histone acetylase activity and Mediator complex, which functions to recruit RNA polymerase II [9,10,27] (Fig. 2). SRCs (SRC- 1, -2 and -3) are primary coactivators that bind to VDR and recruit secondary coactivators such as CBP. Brahma-related gene 1 (BRG-1), an ATPase that is a component of the SWI/SNF chromatin remodeling complex, has also been reported to play a fundamental role in $1,25(OH)_2D_3$ induced transcription [28] (Fig. 2). Thus VDR mediated gene transcription is a multistep process requiring a combination of transcriptional coactivators in a cell type and gene specific manner. At least 49 different mutations in the VDR in over 100 patients have been described, and occur throughout the VDR [29]. These mutations result in early onset of rickets, low calcium and phosphate, high PTH and growth retardation, indicating the essential role of VDR as the mediator of vitamin D action [29]. From recent genome wide studies we have obtained a new perspective on VDR mediated transcription. VDR binding sites are not only located at proximal promoters, as previously reported, but are situated also within introns and at distal intergenic regions many kilobases upstream or downstream of regulated genes [10,30]. In addition, recent evidence indicated that $1,25(OH)_2D_3$ results in an increase in levels of acetylation at H4K5, H3K9 and H3K27 which can define sites of action of $1,25(OH)_2D_3$ [10,31]. Epigenetic histone changes within enhancers of certain VDR target genes can play a crucial role in $1,25(OH)_2D_3$ mediated transcriptional activation. Understanding the multiple factors involved in VDR mediated transcription can lead to the design of drugs that can selectively modulate $1,25(OH)_2D_3$ responses in specific tissues, resulting in new approaches to sustain calcium balance and perhaps enhance suggested anti-inflammatory and anti-tumor activities of $1,25(OH)_2D_3$.

4. Vitamin D and bone mineral homeostasis

4.1. Recent advances in our understanding of $1,25(OH)_2D_3$ regulation of intestinal calcium absorption

Vitamin D is a principal factor involved in the control of mineral balance. Vitamin D deficiency during bone development causes growth retardation and rickets and in adults vitamin D deficiency can cause secondary hyperparathyroidism resulting in osteoporosis and/or osteomalacia and increased risk of fracture [32,33]. However, in spite of the importance of vitamin D in mineral homeostasis, understanding vitamin D action and the molecular targets that mediate the impact of vitamin D have remained incomplete and are a subject of continuing investigation. The principal action of vitamin D in maintenance of calcium homeostasis is intestinal calcium absorption [9,34]. This conclusion was made from studies in VDR null mice, an animal model of hereditary vitamin D- resistant rickets (HVDRR; also known as vitamin D dependent rickets type II) [29]. When VDR null mice are fed a diet which includes high calcium, rickets and osteomalacia are prevented as serum calcium and PTH are normalized [35,36]. In addition, intravenous or high dose oral calcium cures rickets and promotes normal mineralization in patients with HVDRR [37]. Transgenic expression of VDR specifically in the intestine of VDR null mice prevents rickets and normalizes serum calcium, further indicating that a major action of $1,25(OH)_2D_3$/VDR in the prevention of skeletal abnormalities is enhancement of intestinal calcium absorption [38]. When the demand for calcium increases under conditions of low dietary calcium intake, during skeletal growth or during pregnancy and lactation, vitamin D mediated intestinal calcium absorption occurs predominantly by an active transcellular process [34,39]. Two of the most pronounced effects of $1,25(OH)_2D_3$ in the intestine are increased synthesis of calbindin, an intracellular calcium binding protein that has been proposed to facilitate calcium movement through the cytoplasm, and increased synthesis of transient receptor potential cation channel, subfamily V, member 6 (TRPV6), an apical epithelial calcium channel that mediates calcium entry into the enterocyte [40]. Evidence for a role of TRPV6 and calbindin in intestinal calcium absorption includes the findings that both proteins are expressed in the duodenum, both are induced at weaning (the time of onset of intestinal calcium transport) and both are induced, in response to $1,25(OH)_2D_3$, prior to the peak of intestinal calcium absorption [40]. However, studies in calbindin-D_{9k} or TRPV6

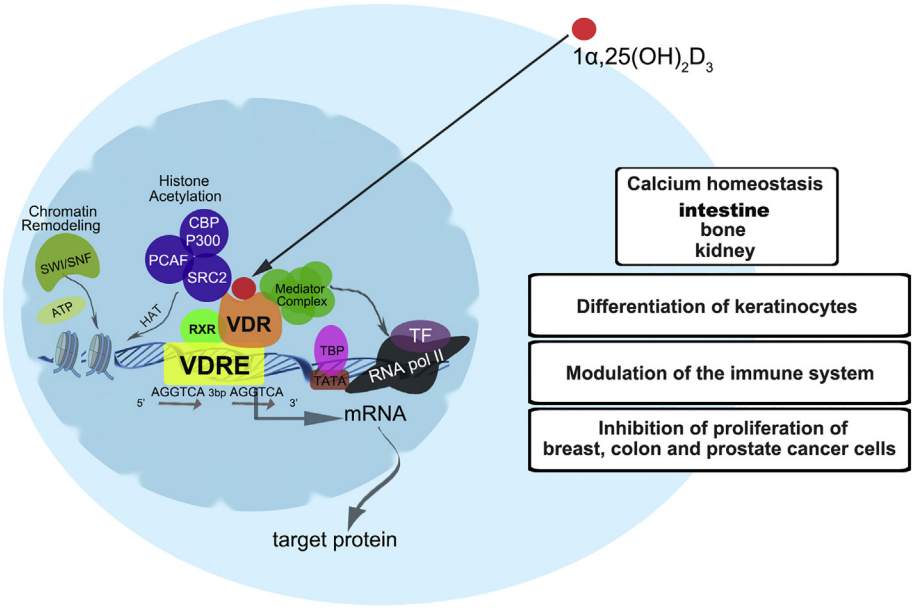

Fig. 2. Mechanism of action of $1,25(OH)_2D_3$. The actions of $1,25(OH)_2D_3$ are mediated by the VDR. $1,25(OH)_2D_3$ binds to VDR which heterodimerizes with RXR. VDR/RXR interacts with vitamin D response elements (shown here as two direct repeats of AGGTCA separated by 3 base pairs; however it should be noted that there are numerous variations of this sequence) in and around target genes. $1,25(OH)_2D_3$-VDR recruits coregulatory complexes including the histone acetyltransferase (HAT) activity containing complex (SRC-2, also known as GRIP1), CBP and PCAF, and mediator complex which facilitates activation of the RNA polymerase II holoenzyme. The SWI/SNF chromatin remodeling complex has also been reported to contribute to $1,25(OH)_2D_3$ induced transcription. The principal action of vitamin D in the maintenance of calcium homeostasis is intestinal calcium absorption. When $1,25(OH)_2D_3$ regulation of intestinal calcium absorption is insufficient to maintain calcium homeostasis $1,25(OH)_2D_3$ acts together with PTH to enhance loss of calcium from bone and to increase calcium reabsorption from the kidney. $1,25(OH)_2D_3$ has effects on other tissues and cell types including keratinocytes, immune cells and cancer cells.

null mice show no difference in phenotype (normal serum calcium and bone mass) and no difference in $1,25(OH)_2D_3$ mediated active intestinal calcium absorption compared to wild type mice, thus challenging the traditional model of vitamin D mediated intestinal calcium absorption [41–43]. Thus active calcium transport can occur in the absence of these proteins suggesting compensation by other proteins yet to be identified. These findings also suggest the complexity of VDR mediated transepithelial uptake of calcium which may involve a network of multiple components involved in active calcium transport [44]. It should be noted, however, that under conditions of low dietary calcium in the TRPV6 KO mouse and in the TRPV6/calbinind-D_{9k} double null mice calcium absorption is reduced compared to single null mice and WT mice [41]. Thus TRPV6 and calbindin may act together in certain aspects of the absorptive process (for example calbindin may modulate calcium influx mediated by TRPV6). It is also possible that a principal function of calbindin is not facilitation of calcium movement through the cytosol but calcium buffering, preventing toxic levels of calcium from accumulating within the enterocyte during calcium absorption. Although the findings in the null mice indicate that other apical membrane calcium transporters may compensate for the loss of TRPV6, transgenic expression of TRPV6 in the intestine of VDR null mice results in enhanced calcium absorption and prevention of the hypocalcemia and osteomalacia observed in the VDR null mice [45]. These findings suggest that TRPV6 can mediate intestinal calcium absorption and that an inability to transport calcium into the enterocyte may be a primary defect in VDR dependent rickets. In addition to TRPV6, the in vivo physiological importance of the plasma membrane calcium pump (PMCA) in vitamin D mediated intestinal calcium absorption has recently been noted. Specific intestinal deletion in mice of PMCA1 (the major calcium pump of the intestine) resulted in decreased bone mineral density and impaired intestinal calcium absorption in response to $1,25(OH)_2D_3$ [46].

Most research on intestinal calcium absorption has utilized the duodenum. However, it is the distal intestine where most of the ingested calcium is absorbed [47]. Although it has been suggested that calcium absorption in the distal intestine is due to vitamin D independent passive diffusion [47], VDR and the traditional transcellular mediators are expressed in both proximal and distal intestine [38]. In addition, active $1,25(OH)_2D_3$ regulated calcium absorption occurs in the rat and human colon [48,49]. Studies in humans show that total calcium absorption is higher when the colon is preserved after small bowel resection [50]. To test directly the role of $1,25(OH)_2D_3$ and the VDR in the distal intestine, recent findings from the Christakos lab indicated that transgenic expression of VDR only in distal ileum, cecum and colon of VDR null mice, at levels equivalent to WT, completely rescued the bone and serum defects associated with VDR deletion [51]. The induction of TRPV6 mRNA and calbindin-D_{9k} in the distal intestine of the transgenic mice suggest that active transport is involved in the rescue of rickets of the VDR null mice expressing VDR in the distal intestine and that the distal intestine contributes to maintenance of whole body calcium and bone homeostasis.

4.1.1. Clinical relevance

Reduction of intestinal calcium absorption with advancing age has been reported to be a significant risk factor for fracture in the elderly [52]. Bariatric surgery with the by-pass of the upper intestine is associated with calcium malabsorption and bone loss [53]. Understanding the mechanisms involved in VDR mediated active calcium transport in the distal intestine may suggest new strategies to prevent bone loss caused by disrupted calcium metabolism due to bariatric surgery, small bowel resection or aging.

4.2. Effects of $1,25(OH)_2D_3$ on bone

Although a primary antirachitic effect of $1,25(OH)_2D_3$ is indirect, providing calcium to bone as a result of enhanced intestinal calcium absorption, $1,25(OH)_2D_3$ can stimulate osteoclastogenesis by upregulating RANKL (receptor activator of nuclear kB ligand) in osteoblastic cells. RANKL mediates osteoclast formation by mediating direct interactions between osteoblast/stromal cells and osteoclast precursors [54]. During a negative calcium balance $1,25(OH)_2D_3$ together with PTH (which also induces RANKL in osteoblasts) result in enhanced osteoclast formation and loss of calcium from bone [9,10,55]. Enhancer regions significantly

upstream of the RANKL start site mediate the regulation of RANKL by PTH and $1,25(OH)_2D_3$ [56,57]. In addition to osteoblasts, osteocytes have also been shown to be a major source of RANKL [58]. $1,25(OH)_2D_3$ also reduces matrix mineralization by increasing the expression of mineralization inhibitors, including osteopontin, in osteoblasts [59]. Thus when $1,25(OH)_2D_3$ regulation of intestinal calcium absorption is insufficient to maintain calcium homeostasis, maintenance of blood calcium levels is prioritized at the expense of skeletal integrity. It should be noted however, that the role of $1,25(OH)_2D_3$ in bone under conditions of positive calcium balance, although not clearly defined, has been suggested to involve osteoanabolic effects of $1,25(OH)_2D_3$, for example upregulation of LRP5 (low density lipoprotein receptor related 5), a mediator of bone mineral density and formation as part of the Wnt signaling mechanism [60].

4.2.1. Clinical relevance

These findings suggest that a combination of calcium and vitamin D is needed for treatment or prevention of osteoporosis since vitamin D alone may negatively affect bone when calcium homeostasis cannot be maintained by $1,25(OH)_2D_3$ mediated intestinal calcium absorption.

4.3. The role of $1,25(OH)_2D_3$ in the kidney: decreased $1,25(OH)_2D_3$ or increased FGF23 as an initial event in chronic kidney disease?

During a negative calcium balance, when serum calcium cannot be maintained by intestinal calcium absorption, in addition to mobilizing calcium from the skeleton, $1,25(OH)_2D_3$ also acts together with PTH to increase calcium reabsorption from the distal tubule of the kidney. $1,25(OH)_2D_3$ regulates the active transcellular process at the distal tubule by inducing the expression of the apical calcium channel TRPV5 (which shows 73.4% sequence homology with TRPV6) and by inducing calbindin-D_{9k} (9000 Mr) in mouse kidney and calbindin-D_{28k} (28,000 Mr) in mouse, human and rat kidney [9,61]. $1,25(OH)_2D_3$ can also enhance the stimulatory effect of PTH on calcium reabsorption in part by increasing PTH receptor expression in the distal tubule [62]. Another major function of $1,25(OH)_2D_3$ in the kidney is the regulation of the vitamin D hydroxylases (inhibition of CYP27B1 and stimulation of CYP24A1 as an autoregulatory mechanism to prevent hypercalcemia). In addition to $1,25(OH)_2D_3$, calcium, PTH and FGF23 are also important regulators of $1,25(OH)_2D_3$ production (see Section 2; vitamin D metabolism and Fig. 1). In chronic kidney disease (CKD) there is a gradual decrease in the number of functional nephrons and alterations in renal vitamin D hydroxylases and vitamin D metabolites including low $1,25(OH)_2D_3$ [63]. This has been attributed to reduction in renal 1α-hydroxylase as renal mass is reduced as well as to the increase in FGF23 [63,64]. FGF23 is increased in CKD in order to maintain phosphate excretion per nephron but has been shown to be a major contributor to the decrease in $1,25(OH)_2D_3$ in early renal failure [64,65]. A decline in the FGF23 cofactor klotho has also been reported to be an early event in CKD, which may cause a compensatory increase in FGF23 [65].

4.3.1. Clinical relevance

Recent evidence indicates that FGF23 (and not decreased $1,25(OH)_2D_3$) is an initial event in CKD, a biomarker for CKD progression and a predictor of cardiovascular disease [64,66]. FGF23 would result in the decrease in $1,25(OH)_2D_3$, which would be followed by an increase in PTH [64]. Hyperphosphatemia is observed at the end stage of CKD when the kidney is incapable of excreting ingested phosphate [64] (Fig. 3). Recent findings suggest that a novel distal enhancer mediates the early rise in the expression of FGF23 in a diet induced CKD mouse model [67]. FGF23 antagonism combined with vitamin D supplementation and phosphate control has now been suggested as therapeutic treatment in CKD [66].

5. Non-classical actions of vitamin D

Although the essential role of vitamin D in calcium homeostasis is well documented, the possibility that $1,25(OH)_2D_3$ also affects numerous other physiological processes has been considered for decades due to the presence of VDR in numerous tissues and cells that are not involved in calcium regulation including pancreas, brain, skin, placenta, colon, breast and prostate cancer cells and immune cells [68,69]. In the innate immune system antimicrobial peptides provide protection against bacterial infection [70]. The human cathelicidin antimicrobial peptide has broad antibacterial activity against both Gram-positive and Gram negative bacteria [71,72]. $1,25(OH)_2D_3$ has been reported to induce the expression of the human cathelicidin antimicrobial peptide in monocytes, lung epithelial cells, intestinal epithelial cells, keratinocytes and trophoblasts of the placenta [72]. Recent studies have identified novel networks involved in vitamin D mediated regulation of cathelicidin [73]. Understanding the mechanisms involved in the regulation of cathelicidin may suggest new approaches to treat infection caused by antibiotic resistant pathogens. In addition to the innate immune system, $1,25(OH)_2D_3$ also affects adaptive immunity [reviewed in [9,72,74]]. $1,25(OH)_2D_3$ inhibits the production of inflammatory cytokines in T cells including IL-2, IFNγ, IL-12 and IL-17. $1,25(OH)_2D_3$ also suppresses antigen-presentation by dendritic cells and promotes activation of T regulatory cells which are involved in inhibition of inflammation [9,72,74]. Due to the effects of $1,25(OH)_2D_3$ on the adaptive

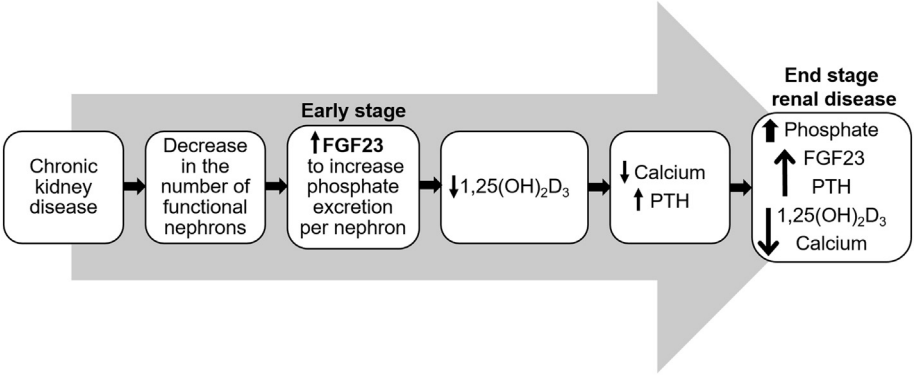

Fig. 3. Proposed model for changes in serum FGF23, $1,25(OH)_2D_3$, PTH, calcium and phosphate in chronic kidney disease (CKD). In CKD there is a gradual decrease in functional nephrons. FGF23 is increased to maintain phosphate excretion per nephron. A decline in the FGF23 receptor klotho (not shown) is also an early event in CKD which may cause a compensatory increase in FGF23. An increase in FGF23 results in a decrease in $1,25(OH)_2D_3$ followed by a decrease in serum calcium and an increase in PTH. Hyperphosphatemia is observed at end stage CKD when the kidney is incapable of excreting ingested phosphate. Elevated FGF23 has been suggested as an early biomarker for CKD and may contribute to acceleration of CKD progression [63–66].

immune system, it has been suggested that $1,25(OH)_2D_3$ may play an important role in the prevention or treatment of autoimmune diseases such a multiple sclerosis, inflammatory bowel disease and rheumatoid arthritis [74]. In addition to its immunomodulatory effects a role for $1,25(OH)_2D_3$ on cancer prevention or treatment has been suggested due, at least in part, to the effect of $1,25(OH)_2D_3$ on inhibition of cancer cell growth [9,75] Studies in mouse models have shown inhibition of mammary, prostate, colon and skin cancer by $1,25(OH)_2D_3$/VDR [75]. It has also been reported in clinical studies that a large number of diseases are associated with low $25(OH)D_3$ levels [9]. It should be noted however that, at this time, only a limited number of randomized controlled clinical trials (RCTs) have convincingly shown benefit from vitamin D supplementation [9,74,76]. One major exception to this is psoriasis, a chronic autoimmune inflammatory skin disease, involving hyperproliferation of keratinocytes [77]. Topical application of $1,25(OH)_2D_3$ and its analogs (calcipotriol, tacalcitol and maxacalcitol), which have been shown to increase differentiation and decrease proliferation of keratinocytes, and also have anti-inflammatory properties, are currently approved for use to treat psoriatic skin lesions [9,78]. Although, for most diseases, large scale clinical trials to determine effects of vitamin D on extraskeletal health are not yet available, compelling evidence in the laboratory indicates beneficial effects of $1,25(OH)_2D_3$ beyond bone and suggest that analogs of $1,25(OH)_2D_3$ may have a role together with traditional therapies to treat specific diseases including autoimmune diseases and some cancers at least in vitamin D deficient individuals [9,74,76,78].

6. Vitamin D sufficiency

6.1. 25(OH)D: total vs. free 25(OH)D

25(OH)D is the principal yardstick by which vitamin D sufficiency is determined. There are several reasons for this. First, 25(OH)D is the vitamin D metabolite in highest concentration in the blood, so is easiest to measure. Second, nearly all 25(OH)D is in the blood, unlike vitamin D itself, which is stored in tissues such as fat. Third, the conversion of vitamin D to 25(OH)D follows first order kinetics, meaning that the level of 25(OH)D is linearly correlated to the levels of vitamin D, although there is individual variation in the relationship between vitamin D intake and 25(OH)D levels achieved. The 25-hydroxylases are little regulated under most circumstances, unlike CYP27B1, so the conversion of vitamin D to 25(OH)D is not influenced by changes in hormones such as PTH, FGF23, or feedback regulation by 25(OH)D or $1,25(OH)_2D$. Finally, 25(OH)D has a half-life in blood measured in weeks, unlike hours for $1,25(OH)_2D$. That said, total 25(OH)D may not be the best measurement of vitamin D status. 25(OH)D, like other vitamin D metabolites, is carried in blood by two major proteins, vitamin D binding protein (DBP) and albumin. DBP binds approximately 85% of total 25(OH)D, albumin 15%, with approximately 0.03% free [79]. Except for tissues expressing the megalin/cubilin complex, it is the free fraction that enters the cells (the free hormone hypothesis).This is similar to that for the thyroid and steroid hormones [80,81], which like vitamin D metabolites are transported in blood bound to their respective binding proteins. Although an earlier monoclonal assay reported marked differences in DBP levels between racial groups, this appears to be due to a decreased ability to measure the Gc1f allele of DBP found most commonly in individuals of African descent, and has not been seen with other polyclonal assays or mass spectroscopy [82]. There are a number of drugs and cytokines (dexamethasone and IL-6 for example) as well as clinical conditions (for example liver disease, nephrotic syndrome, primary hyperparathyroidism, acute trauma) that contribute to changes in DBP levels that alter the ratio of total to free 25(OH)D [83] suggesting that determination of the free level of 25(OH)D might be a better representation of vitamin D status than the total level, but this remains controversial [84].

6.2. Determination of 25(OH)D levels

The data used for determining recommended levels of 25OHD come primarily from association studies correlating 25(OH)D levels to disease incidence/prevalence, but with a growing number of randomized controlled clinical trials (RCTs) evaluating whether vitamin D supplementation reduces the incidence/prevalence of a given marker or disease. The most consistent data, and the data used in establishing recommendations either for optimal levels of circulating 25(OH)D levels or levels of dietary supplements, comes from studies involving the musculoskeletal system. To state the obvious, measurement of 25(OH)D is required in these studies, but unfortunately the assays that have been used vary [85]. Most of these assays involve antibodies that may differ between laboratories with variable ability to detect $25(OH)D_2$ as well as $25(OH)D_3$ and sensitivity to interfering substances [82]. As more laboratories implement LC/MS to measure vitamin D metabolites, many of the problems with immunometric assays will be solved. Meanwhile efforts are being made to harmonize the data from different laboratories in different countries [86], but recommendations based on earlier non harmonized data persist. Moreover, different groups select different studies or weight them differently when establishing their recommendations. Finally, it is not clear that vitamin D levels that are optimal for Caucasians are the same as those for Black Africans or Asians. Not surprisingly current guidelines vary, but efforts to gain consensus are being made especially as new data from RCTs based on better assays are being generated [87].

6.3. Evaluation of vitamin D deficiency and sufficiency and recommendations for achieving vitamin D sufficiency

Terms to consider are estimated average requirement (EAR)—the median level at which 50% of the population would be sufficient—and the required daily requirement (RDA)—the level that meets the needs of 97.5% of the population. Other countries use different terms such as adequate intake (AI), reference nutrient intake (RNI), and recommended intake (RI). The recommended levels assume no additional contribution from epidermal production of vitamin D. Thirty three nominated experts in pediatric endocrinology, pediatrics, nutrition, epidemiology, public health, and health economics from around the world recently published their consensus recommendations on the prevention and management of nutritional rickets [32]. They defined vitamin D sufficiency as 25(OH)D levels above 50 nM (20 ng/ml), insufficiency between 30 and 50 nM, and deficiency below 30 nM (12 ng/ml). Moreover, they noted that the consequences with respect to the development of rickets/osteomalacia were also dependent on calcium intake. They defined calcium sufficiency as ≫500 mg/d, insufficiency as 300–500 mg/d, and insufficiency as ≪300 mg/d. It was individuals with the combination of insufficient or deficient levels of either calcium or vitamin D and deficient levels of the other that were most at risk for rickets/osteomalacia. Deficiency of vitamin D with a normal calcium intake might lead to biochemical abnormalities but not rickets/osteomalacia according to their analysis. They recommended 400 iu vitamin D/d for infants up to 1 year and followed the Institute of Medicine (IOM) (now known as the National Academy of Medicine (NAM)) recommendations for older children and adults (see below) (also Table 1). They further recommended 200 mg calcium/d for infants up to 6mo, and 260 mg/d from 6 to 12 mo. Older children were recommended to consume at least 500 mg calcium/d. Other than the recommendation by NAM for adults to ingest 700–1300 mg calcium/d [88], most guidelines are silent with respect to calcium. Pilz et al. [89] recently tabulated dietary recommendations for vitamin D from a number of countries including those from the USA and Canada (Institute of Medicine, NAM); Europe (European Food Safety Authority, EFSA), Germany, Austria, Switzerland (DACH), United Kingdom (Scientific Advisory Committee on Nutrition (SACN)), and the Nordic countries (NORDEN). What was listed was the RDA (NAM), Adequate Intake (EFSA, DACH), Reference

Table 1
Recommendations for vitamin D supplementation by age.

	Age	Dose	Goal
National Academy of Medicine	≪1 yr	400 iu	20 ng/ml
	1–70 yr	600 iu	20 ng/ml
	≫70 yr	800 iu	20 ng/ml
Endocrine Society	≪1 yr	400–1000 iu	30 ng/ml
	1–18 yr	600–1000 iu	30 ng/ml
	≫18 yr	1500–2000 iu	30 ng/ml

The National Academy of Medicine (previously known as the Institute of Medicine) recommendations are for the population at large, whereas the Endocrine Society is more directed at patient populations.

Nutrition Intake (SACN), or Recommended Intake (NORDEN). The NAM recommended 600 iu from years 1–70 and 800 iu above 70; the EFSA recommended 400 iu/d for infants 7–12 mo and 600 iu/d for all older age groups; DACH recommended 400 iu/d for infants and 800 iu/d for all older age groups; SACN and NORDEN recommended 400 iu/d for all ages over 1 yr, with SACN recommending slightly lower (340–400 iu) for infants. The NAM considered a 25(OH)D serum level of 50 nM (20 ng/ml) to suffice for 97.5% of the population [88], whereas the Endocrine Society guidelines recommend 75 nM (30 ng/ml), considering levels between 50 and 75 nM as insufficient and ≪50 nM as deficient [90]. Although the Endocrine Society recommendations for children and adults are comparable to other groups (400 iu/d for infants, 600 iu/d from ages 1–70, 800 iu/d for those over 70), the Endocrine Society suggests that doses up to 2000 iu vitamin D/d may be necessary to achieve and maintain a serum 25OHD level of 75 nM (30 ng/ml) (Table 1).

As noted these recommendations are based primarily on data from studies of the musculoskeletal system. 400 iu vitamin D sufficed to prevent rickets in a study in Turkey where deficiency was extensive [91]. Other studies in adult populations demonstrate elevated PTH levels and reduced bone mineral density at 25(OH)D levels below 50 nM [92,93] and increased osteoid suggestive of osteomalacia in hip fracture patients with 25(OH)D levels below 30 nM [33,94]. At these levels vitamin D supplementation shows clear benefit [95]. Vitamin D supplementation works better when used in conjunction with calcium [96–98] especially in the elderly and vitamin D deficient. Although not all studies have demonstrated protection against fracture, several have when elderly vitamin D deficient populations are studied [98,99], but not when vitamin D sufficient populations are studied [100]. Moreover, the amount and administration schedule also matter. Use of very high doses of vitamin D (i.e. 300,000–500,000 iu given annually) seems to increase fracture risk [101,102]. Similar conclusions can be reached for falls. Daily doses of 700–1000 iu vitamin D reduce the risk of falls in the elderly especially those with vitamin D insufficiency [103,104], but higher doses intermittently administered appear to increase the risk of falling [101,105]. The reduction in falls likely related to improvement in muscle function is seen best in the most frail with lowest 25(OH)D levels [106]. Although vitamin D supplementation has been shown to reduce overall mortality and cancer mortality, with the exception of upper respiratory illnesses and asthma, most diseases which have shown an association with low 25(OH)D levels have not yet shown consistent benefit with vitamin D supplementation [107,108]. However, most of these studies have not discriminated between participants with sufficient vs insufficient 25(OH)D levels, so the jury is still out.

In conclusion, recommended levels of vitamin D intake and optimal levels of circulating 25(OH)D levels vary somewhat from country to country, and what is optimal for one group may differ for another group. Moreover, assays measuring 25(OH)D vary although with the development of mass spectrometry replacing immunoassays much of this variation can be eliminated. However, the question of whether free 25(OH)D measurements offer a better assessment of vitamin D status remains to be settled. Furthermore, recommended levels based on studies of the musculoskeletal system may or may not be appropriate for other vitamin D impacted conditions such as immune function, cancer prevention, cardiovascular health, neurologic function. Only further investigation will settle these issues.

7. Future directions

Tight regulation of vitamin D metabolism is critical to the maintenance of normal calcium homeostasis and bone health. However, we are only beginning to understand the molecular mechanisms involved in the control of the expression of the vitamin D hydroxylases. With the technologies now available new insight will be obtained related to the regulatory regions including individual enhancers as well as transcription factor complexes and histone modifications that are involved in $1,25(OH)_2D_3$ synthesis and catabolism. Understanding the mechanisms involved is critical to understanding dysregulation of $1,25(OH)_2D_3$ production that occurs for example in chronic kidney disease and with age related bone loss. New targets of $1,25(OH)_2D_3$ will be identified which will provide a better understanding of $1,25(OH)_2D_3$ actions in different regions of the intestine as well as in multiple other target tissues. New data from RCTs based on better assays of $25(OH)D_3$ will result in less variability and a gain in consensus for optimal vitamin D levels in different groups. In addition, further large scale clinical trials that discriminate between participants with sufficient and insufficient vitamin D levels will be needed to determine the suggested impact of vitamin D on immune function, cancer prevention and other diseases. These future studies will result in a new dimension in our understanding of the impact of the vitamin D endocrine system on skeletal health and on extraskeletal biological responses.

Funding

The work of the authors cited in this article was supported in part by National Institutes of Health Grants DK38961, AG044552 and DK112365 (to S.C.) and AR055924 (to D.D.B.) and I01 BX003814 (VA) (to D.D.B.).

Author contributions

All authors have participated in manuscript preparation and have approved the final article.

Declaration of Competing Interest

None.

References

[1] McCollum EV, Pitz W, Simmonds N, Becker JE, Shipley PG, Bunting RW. The effect of additions of fluorine to the diet of the rat on the quality of the teeth. 1925. Studies on experimental rickets. XXI. An experimental demonstration of the existence of a vitamin which promotes calcium deposition. 1922. The effect of additions of fluorine to the diet of the rat on the quality of the teeth. 1925. J Biol Chem 2002;277:E8.
[2] Holick MF, MacLaughlin JA, Clark MB, Holick SA, Potts Jr JT, Anderson RR, et al. Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science 1980;210:203–5.
[3] Zhu JG, Ochalek JT, Kaufmann M, Jones G, Deluca HF. CYP2R1 is a major, but not exclusive, contributor to 25-hydroxyvitamin D production in vivo. Proc Natl Acad Sci U S A 2013;110:15650–5.
[4] Cheng JB, Motola DL, Mangelsdorf DJ, Russell DW. De-orphanization of cytochrome P450 2R1: a microsomal vitamin D 25-hydroxilase. J Biol Chem 2003;278:38084–93.
[5] Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci U S A 2004;101:7711–5.
[6] Thacher TD, Fischer PR, Singh RJ, Roizen J, Levine MA. CYP2R1 mutations impair generation of 25-hydroxyvitamin D and cause an atypical form of vitamin D deficiency. J Clin Endocrinol Metab 2015;100:E1005–13.
[7] Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. J Lipid Res 2014;55:13–31.

[8] Bikle DD. Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol 2014;21:319–29.
[9] Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. Physiol Rev 2016; 96:365–408.
[10] Pike JW, Christakos S. Biology and mechanisms of action of the vitamin D hormone. Endocrinol Metab Clin N Am 2017;46:815–43.
[11] Jones G, Kottler ML, Schlingmann KP. Genetic diseases of vitamin D metabolizing enzymes. Endocrinol Metab Clin N Am 2017;46:1095–117.
[12] Canaff L, Hendy GN. Human calcium-sensing receptor gene. Vitamin D response elements in promoters P1 and P2 confer transcriptional responsiveness to 1,25-dihydroxyvitamin D. J Biol Chem 2002;277:30337–50.
[13] Demay MB, Kiernan MS, DeLuca HF, Kronenberg HM. Sequences in the human parathyroid hormone gene that bind the 1,25-dihydroxyvitamin D3 receptor and mediate transcriptional repression in response to 1,25-dihydroxyvitamin D3. Proc Natl Acad Sci U S A 1992;89:8097–101.
[14] Quarles LD. Skeletal secretion of FGF-23 regulates phosphate and vitamin D metabolism. Nat Rev Endocrinol 2012;8:276–86.
[15] Hu MC, Shiizaki K, Kuro-o M, Moe OW. Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. Annu Rev Physiol 2013;75:503–33.
[16] Bikle DD, Patzek S, Wang Y. Physiologic and pathophysiologic roles of extra renal CYP27b1: case report and review. Bone Rep 2018;8:255–67.
[17] Tsai KS, Heath 3rd H, Kumar R, Riggs BL. Impaired vitamin D metabolism with aging in women. Possible role in pathogenesis of senile osteoporosis. J Clin Invest 1984;73:1668–72.
[18] Armbrecht HJ, Zenser TV, Davis BB. Effect of age on the conversion of 25-hydroxyvitamin D3 to 1,25-dihydroxyvitamin D3 by kidney of rat. J Clin Invest 1980;66:1118–23.
[19] Matkovits T, Christakos S. Variable in vivo regulation of rat vitamin D-dependent genes (osteopontin, Ca,Mg-adenosine triphosphatase, and 25-hydroxyvitamin D3 24-hydroxylase): implications for differing mechanisms of regulation and involvement of multiple factors. Endocrinology 1995;136:3971–82.
[20] Johnson JA, Beckman MJ, Pansini-Porta A, Christakos S, Bruns ME, Beitz DC, et al. Age and gender effects on 1,25-dihydroxyvitamin D3-regulated gene expression. Exp Gerontol 1995;30:631–43.
[21] Molin A, Wiedemann A, Demers N, Kaufmann M, Do Cao J, Mainard L, et al. Vitamin D-dependent rickets type 1B (25-hydroxylase deficiency): a rare condition or a misdiagnosed condition? J Bone Miner Res 2017;32:1893–9.
[22] Fraser D, Kooh SW, Kind HP, Holick MF, Tanaka Y, DeLuca HF. Pathogenesis of hereditary vitamin-D-dependent rickets. An inborn error of vitamin D metabolism involving defective conversion of 25-hydroxyvitamin D to 1 alpha,25-dihydroxyvitamin D. N Engl J Med 1973;289:817–22.
[23] Schlingmann KP, Kaufmann M, Weber S, Irwin A, Goos C, John U, et al. Mutations in CYP24A1 and idiopathic infantile hypercalcemia. N Engl J Med 2011; 365:410–21.
[24] Dinour D, Beckerman P, Ganon L, Tordjman K, Eisenstein Z, Holtzman EJ. Loss-of-function mutations of CYP24A1, the vitamin D 24-hydroxylase gene, cause long-standing hypercalciuric nephrolithiasis and nephrocalcinosis. J Urol 2013;190: 552–7.
[25] Tebben PJ, Milliner DS, Horst RL, Harris PC, Singh RJ, Wu Y, et al. Hypercalcemia, hypercalciuria, and elevated calcitriol concentrations with autosomal dominant transmission due to CYP24A1 mutations: effects of ketoconazole therapy. J Clin Endocrinol Metab 2012;97:E423–7.
[26] Roizen JD, Li D, O'Lear L, Javaid MK, Shaw NJ, Ebeling PR, et al. CYP3A4 mutation causes vitamin D-dependent rickets type 3. J Clin Invest 2018;128:1913–8.
[27] Orlov I, Rochel N, Moras D, Klaholz BP. Structure of the full human RXR/VDR nuclear receptor heterodimer complex with its DR3 target DNA. EMBO J 2012;31: 291–300.
[28] Seth-Vollenweider T, Joshi S, Dhawan P, Sif S, Christakos S. Novel mechanism of negative regulation of 1,25-dihydroxyvitamin D3-induced 25-hydroxyvitamin D3 24-hydroxylase (Cyp24a1) Transcription: epigenetic modification involving cross-talk between protein-arginine methyltransferase 5 and the SWI/SNF complex. J Biol Chem 2014;289:33958–70.
[29] Malloy PJ, Tasic V, Taha D, Tutunculer F, Ying GS, Yin LK, et al. Vitamin D receptor mutations in patients with hereditary 1,25-dihydroxyvitamin D-resistant rickets. Mol Genet Metab 2014;111:33–40.
[30] Pike JW, Meyer MB, Benkusky NA, Lee SM, St John H, Carlson A, et al. Genomic determinants of vitamin D-regulated gene expression. Vitam Horm 2016;100:21–44.
[31] Pike JW, Meyer MB, St John HC, Benkusky NA. Epigenetic histone modifications and master regulators as determinants of context dependent nuclear receptor activity in bone cells. Bone 2015;81:757–64.
[32] Munns CF, Shaw N, Kiely M, Specker BL, Thacher TD, Ozono K, et al. Global consensus recommendations on prevention and management of nutritional rickets. J Clin Endocrinol Metab 2016;101:394–415.
[33] Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev 2001;22:477–501.
[34] Christakos S, Veldurthy V, Patel N, Wei R. Intestinal regulation of calcium: vitamin D and bone physiology. Adv Exp Med Biol 2017;1033:3–12.
[35] Amling M, Priemel M, Holzmann T, Chapin K, Rueger JM, Baron R, et al. Rescue of the skeletal phenotype of vitamin D receptor-ablated mice in the setting of normal mineral ion homeostasis: formal histomorphometric and biomechanical analyses. Endocrinology 1999;140:4982–7.
[36] Masuyama R, Nakaya Y, Katsumata S, Kajita Y, Uehara M, Tanaka S, et al. Dietary calcium and phosphorus ratio regulates bone mineralization and turnover in vitamin D receptor knockout mice by affecting intestinal calcium and phosphorus absorption. J Bone Miner Res 2003;18:1217–26.
[37] Al-Aqeel A, Ozand P, Sobki S, Sewairi W, Marx S. The combined use of intravenous and oral calcium for the treatment of vitamin D dependent rickets type II (VDDRII). Clin Endocrinol 1993;39:229–37.
[38] Xue Y, Fleet JC. Intestinal vitamin D receptor is required for normal calcium and bone metabolism in mice. Gastroenterology 2009;136 [1317-1327, e1311-1312].
[39] DeLuca HF. Evolution of our understanding of vitamin D. Nutr Rev 2008;66:S73–87.
[40] Christakos S, Lieben L, Masuyama R, Carmeliet G. Vitamin D endocrine system and the intestine. BoneKEy reports 2014;3:496.
[41] Benn BS, Ajibade D, Porta A, Dhawan P, Hediger M, Peng JB, et al. Active intestinal calcium transport in the absence of transient receptor potential vanilloid type 6 and calbindin-D9k. Endocrinology 2008;149:3196–205.
[42] Akhter S, Kutuzova GD, Christakos S, DeLuca HF. Calbindin D9k is not required for 1,25-dihydroxyvitamin D3-mediated Ca2+ absorption in small intestine. Arch Biochem Biophys 2007;460:227–32.
[43] Kutuzova GD, Sundersingh F, Vaughan J, Tadi BP, Ansay SE, Christakos S, et al. TRPV6 is not required for 1alpha,25-dihydroxyvitamin D3-induced intestinal calcium absorption in vivo. Proc Natl Acad Sci U S A 2008;105:19655–9.
[44] Lee SM, Riley EM, Meyer MB, Benkusky NA, Plum LA, DeLuca HF, et al. 1,25-Dihydroxyvitamin D3 controls a cohort of vitamin D receptor target genes in the proximal intestine that is enriched for calcium-regulating components. J Biol Chem 2015;290:18199–215.
[45] Cui M, Li Q, Johnson R, Fleet JC. Villin promoter-mediated transgenic expression of transient receptor potential cation channel, subfamily V, member 6 (TRPV6) increases intestinal calcium absorption in wild-type and vitamin D receptor knockout mice. J Bone Miner Res 2012;27:2097–107.
[46] Ryan ZC, Craig TA, Filoteo AG, Westendorf JJ, Cartwright EJ, Neyses L, et al. Deletion of the intestinal plasma membrane calcium pump, isoform 1, Atp2b1, in mice is associated with decreased bone mineral density and impaired responsiveness to 1, 25-dihydroxyvitamin D3. Biochem Biophys Res Commun 2015;467:152–6.
[47] Wasserman RH. Vitamin D and the dual processes of intestinal calcium absorption. J Nutr 2004;134:3137–9.
[48] Favus MJ, Langman CB. Effects of 1,25-dihydroxyvitamin D3 on colonic calcium transport in vitamin D-deficient and normal rats. Am J Phys 1984;246:G268–73.
[49] Barger-Lux MJ, Heaney RP, Recker RR. Time course of calcium absorption in humans: evidence for a colonic component. Calcif Tissue Int 1989;44:308–11.
[50] Hylander E, Ladefoged K, Jarnum S. Calcium absorption after intestinal resection. The importance of a preserved colon. Scand J Gastroenterol 1990;25:705–10.
[51] Dhawan P, Veldurthy V, Yehia G, Hsaio C, Porta A, Kim KI, et al. Transgenic expression of the vitamin D receptor restricted to the ileum, cecum, and colon of vitamin D receptor knockout mice rescues vitamin D receptor-dependent rickets. Endocrinology 2017;158:3792–804.
[52] Ensrud KE, Duong T, Cauley JA, Heaney RP, Wolf RL, Harris E, et al. Low fractional calcium absorption increases the risk for hip fracture in women with low calcium intake. Study of Osteoporotic Fractures Research Group. Ann Intern Med 2000; 132:345–53.
[53] Casagrande DS, Repetto G, Mottin CC, Shah J, Pietrobon R, Worni M, et al. Changes in bone mineral density in women following 1-year gastric bypass surgery. Obes Surg 2012;22:1287–92.
[54] Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, et al. Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL. Proc Natl Acad Sci U S A 1998;95:3597–602.
[55] Maes C, Kronenberg HM. Bone development and remodeling. In: Jameson JL, DeGroot L, editors. Endocrinology: adult and pediatric. Elsevier: New York; 2015. p. 1038–62.
[56] Nerenz RD, Martowicz ML, Pike JW. An enhancer 20 kilobases upstream of the human receptor activator of nuclear factor-kappaB ligand gene mediates dominant activation by 1,25-dihydroxyvitamin D3. Mol Endocrinol 2008;22: 1044–56.
[57] Onal M, St John HC, Danielson AL, Markert JW, Riley EM, Pike JW. Unique distal enhancers linked to the mouse Tnfsf11 gene direct tissue-specific and inflammation-induced expression of RANKL. Endocrinology 2016;157:482–96.
[58] Xiong J, O'Brien CA. Osteocyte RANKL: new insights into the control of bone remodeling. J Bone Miner Res 2012;27:499–505.
[59] Lieben L, Masuyama R, Torrekens S, Van Looveren R, Schrooten J, Baatsen P, et al. Normocalcemia is maintained in mice under conditions of calcium malabsorption by vitamin D-induced inhibition of bone mineralization. J Clin Invest 2012;122: 1803–15.
[60] Pike JW, Lee SM, Meyer MB. Regulation of gene expression by 1,25-dihydroxyvitamin D3 in bone cells: exploiting new approaches and defining new mechanisms. BoneKEy rep 2014;3:482.
[61] Ajibade D, Benn BS, Christakos S. Mechanism of action of 1,25-dihydroxyvitamin D3 in intestinal calcium absorption and renal calcium transport. In: Holick MF, editor. Nutrition and Health: vitamin D. New York: Springer Science; 2010. p. 175–87.
[62] Sneddon WB, Barry EL, Coutermarsh BA, Gesek FA, Liu F, Friedman PA. Regulation of renal parathyroid hormone receptor expression by 1, 25-dihydroxyvitamin D3 and retinoic acid. Cell Physiol Biochem 1998;8:261–77.
[63] Nigwekar SU, Tamez H, Thadhani RI. Vitamin D and chronic kidney disease-mineral bone disease (CKD-MBD). Bonekey Rep 2014;3:498.
[64] Kuro OM. Klotho and endocrine fibroblast growth factors: markers of chronic kidney disease progression and cardiovascular complications? Nephrol Dial Transplant 2019;34:15–21.
[65] Quarles LD. Role of FGF23 in vitamin D and phosphate metabolism: implications in chronic kidney disease. Exp Cell Res 2012;318:1040–8.

[66] Lu X, Hu MC. Klotho/FGF23 axis in chronic kidney disease and cardiovascular disease. Kidney Dis (Basel) 2017;3:15–23.
[67] Onal M, Carlson AH, Thostenson JD, Benkusky NA, Meyer MB, Lee SM, et al. A novel distal enhancer mediates inflammation-, PTH-, and early onset murine kidney disease-induced expression of the mouse Fgf23 gene. JBMR Plus 2018;2:32–47.
[68] Wang Y, Zhu J, DeLuca HF. Where is the vitamin D receptor? Arch Biochem Biophys 2012;523:123–33.
[69] Christakos S, DeLuca HF. Minireview: vitamin D: is there a role in extraskeletal health? Endocrinology 2011;152:2930–6.
[70] Lai Y, Gallo RL. AMPed up immunity: how antimicrobial peptides have multiple roles in immune defense. Trends Immunol 2009;30:131–41.
[71] Vandamme D, Landuyt B, Luyten W, Schoofs L. A comprehensive summary of LL-37, the factotum human cathelicidin peptide. Cell Immunol 2012;280:22–35.
[72] Wei R, Christakos S. Mechanisms underlying the regulation of innate and adaptive immunity by vitamin D. Nutrients 2015;7:8251–60.
[73] Wei R, Dhawan P, Baiocchi RA, Kim KY, Christakos S. PU.1 and epigenetic signals modulate 1,25-dihydroxyvitamin D3 and C/EBPalpha regulation of the human cathelicidin antimicrobial peptide gene in lung epithelial cells. J Cell Physiol 2019;234:10345–59.
[74] Vanherwegen AS, Gysemans C, Mathieu C. Regulation of immune function by vitamin D and its use in diseases of immunity. Endocrinol Metab Clin N Am 2017;46:1061–94.
[75] Welsh J. Cellular and molecular effects of vitamin D on carcinogenesis. Arch Biochem Biophys 2012;523:107–14.
[76] Bikle DD. Extraskeletal actions of vitamin D. Ann N Y Acad Sci 2016;1376:29–52.
[77] Schon MP, Boehncke WH. Psoriasis. N Engl J Med 2005;352:1899–912.
[78] Leyssens C, Verlinden L, Verstuyf A. The future of vitamin D analogs. Front Physiol 2014;5:122.
[79] Bikle DD, Malmstroem S, Schwartz J. Current controversies: are free vitamin metabolite levels a more accurate assessment of vitamin D status than total levels? Endocrinol Metab Clin N Am 2017;46:901–18.
[80] Refetoff S. Thyroid hormone serum transport proteins. In: DeGroot LJ, Chrousos G, Dungan K, Feingold KR, Grossman A, Hershman JM, Koch C, Korbonits M, McLachlan R, New M, Purnell J, Rebar R, Singer F, Vinik A, editors. Endotex; 2000 South Dartmouth, MA.
[81] Siiteri PK, Murai JT, Hammond GL, Nisker JA, Raymoure WJ, Kuhn RW. The serum transport of steroid hormones. Recent Prog Horm Res 1982;38:457–510.
[82] Bikle DD. Vitamin D assays. Front Horm Res 2018;50:14–30.
[83] Bikle DD, Halloran BP, Gee E, Ryzen E, Haddad JG. Free 25-hydroxyvitamin D levels are normal in subjects with liver disease and reduced total 25-hydroxyvitamin D levels. J Clin Invest 1986;78:748–52.
[84] Schwartz JB, Gallagher JC, Jorde R, Berg V, Walsh J, Eastell R, et al. Determination of free 25(OH)D concentrations and their relationships to total 25(OH)D in multiple clinical populations. J Clin Endocrinol Metab 2018;103:3278–88.
[85] Fuleihan Gel H, Bouillon R, Clarke B, Chakhtoura M, Cooper C, McClung M, et al. Serum 25-Hydroxyvitamin D levels: variability, knowledge gaps, and the concept of a desirable range. J Bone Miner Res Off J Am Soc Bone Miner Res 2015;30:1119–33.
[86] Sempos CT, Heijboer AC, Bikle DD, Bollerslev J, Bouillon R, Brannon PM, et al. Vitamin D assays and the definition of hypovitaminosis D: results from the First International Conference on Controversies in Vitamin D. Br J Clin Pharmacol 2018;84:2194–207.
[87] Bouillon R, Van Schoor NM, Gielen E, Boonen S, Mathieu C, Vanderschueren D, et al. Optimal vitamin D status: a critical analysis on the basis of evidence-based medicine. J Clin Endocrinol Metab 2013;98:E1283–304.
[88] Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK, et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab 2011;96:53–8.
[89] Pilz S, Marz W, Cashman KD, Kiely ME, Whiting SJ, Holick MF, et al. Rationale and plan for vitamin D food fortification: a review and guidance paper. Front Endocrinol 2018;9:373.
[90] Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911–30.
[91] Hatun S, Ozkan B, Bereket A. Vitamin D deficiency and prevention: Turkish experience. Acta Paediatr 2011;100:1195–9.
[92] Lips P, Duong T, Oleksik A, Black D, Cummings S, Cox D, et al. A global study of vitamin D status and parathyroid function in postmenopausal women with osteoporosis: baseline data from the multiple outcomes of raloxifene evaluation clinical trial. J Clin Endocrinol Metab 2001;86:1212–21.
[93] Kuchuk NO, van Schoor NM, Pluijm SM, Chines A, Lips P. Vitamin D status, parathyroid function, bone turnover, and BMD in postmenopausal women with osteoporosis: global perspective. J Bone Miner Res Off J Am Soc Bone Miner Res 2009;24:693–701.
[94] Lips P, Netelenbos JC, Jongen MJ, van Ginkel FC, Althuis AL, van Schaik CL, et al. Histomorphometric profile and vitamin D status in patients with femoral neck fracture. Metab Bone Dis Relat Res 1982;4:85–93.
[95] Macdonald HM, Reid IR, Gamble GD, Fraser WD, Tang JC, Wood AD. 25-Hydroxyvitamin D threshold for the effects of vitamin D supplements on bone density: secondary analysis of a randomized controlled trial. J Bone Miner Res Off J Am Soc Bone Miner Res 2018;33:1464–9.
[96] Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, et al. Vitamin D3 and calcium to prevent hip fractures in elderly women. N Engl J Med 1992;327:1637–42.
[97] Dawson-Hughes B, Harris SS, Krall EA, Dallal GE. Effect of calcium and vitamin D supplementation on bone density in men and women 65 years of age or older. N Engl J Med 1997;337:670–6.
[98] Larsen ER, Mosekilde L, Foldspang A. Vitamin D and calcium supplementation prevents osteoporotic fractures in elderly community dwelling residents: a pragmatic population-based 3-year intervention study. J Bone Miner Res Off J Am Soc Bone Miner Res 2004;19:370–8.
[99] Chapuy MC, Pamphile R, Paris E, Kempf C, Schlichting M, Arnaud S, et al. Combined calcium and vitamin D3 supplementation in elderly women: confirmation of reversal of secondary hyperparathyroidism and hip fracture risk: the Decalyos II study. Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA 2002;13:257–64.
[100] Khaw KT, Stewart AW, Waayer D, Lawes CMM, Toop L, Camargo Jr CA, et al. Effect of monthly high-dose vitamin D supplementation on falls and non-vertebral fractures: secondary and post-hoc outcomes from the randomised, double-blind, placebo-controlled ViDA trial. Lancet Diabetes Endocrinol 2017;5:438–47.
[101] Sanders KM, Stuart AL, Williamson EJ, Simpson JA, Kotowicz MA, Young D, et al. Annual high-dose oral vitamin D and falls and fractures in older women: a randomized controlled trial. JAMA 2010;303:1815–22.
[102] Smith H, Anderson F, Raphael H, Maslin P, Crozier S, Cooper C. Effect of annual intramuscular vitamin D on fracture risk in elderly men and women–a population-based, randomized, double-blind, placebo-controlled trial. Rheumatology 2007;46:1852–7.
[103] Bischoff-Ferrari HA, Dawson-Hughes B, Staehelin HB, Orav JE, Stuck AE, Theiler R, et al. Fall prevention with supplemental and active forms of vitamin D: a meta-analysis of randomised controlled trials. BMJ 2009;339:b3692.
[104] Cameron ID, Gillespie LD, Robertson MC, Murray GR, Hill KD, Cumming RG, et al. Interventions for preventing falls in older people in care facilities and hospitals. Cochrane Database Syst Rev 2012(12):CD005465.
[105] Bischoff-Ferrari HA, Dawson-Hughes B, Orav EJ, Staehelin HB, Meyer OW, Theiler R, et al. Monthly high-dose vitamin D treatment for the prevention of functional decline: a randomized clinical trial. JAMA Intern Med 2016;176:175–83.
[106] Lagari V, Gomez-Marin O, Levis S. The role of vitamin D in improving physical performance in the elderly. J Bone Miner Res Off J Am Soc Bone Miner Res 2013;28:2194–201.
[107] Autier P, Mullie P, Macacu A, Dragomir M, Boniol M, Coppens K, et al. Effect of vitamin D supplementation on non-skeletal disorders: a systematic review of meta-analyses and randomised trials. Lancet Diabetes Endocrinol 2017;5:986–1004.
[108] Manson JE, Cook NR, Lee IM, Christen W, Bassuk SS, Mora S, et al. Vitamin D supplements and prevention of cancer and cardiovascular disease. N Engl J Med 2019;380:33–44.