REVIEW ARTICLE

Vitamin D, calcium homeostasis and aging

Vaishali Veldurthy, Ran Wei, Leyla Oz, Puneet Dhawan, Yong Heui Jeon and Sylvia Christakos

Osteoporosis is characterized by low bone mass and microarchitecture deterioration of bone tissue, leading to enhanced bone fragility and consequent increase in fracture risk. Evidence is accumulating for an important role of calcium deficiency as the process of aging is associated with disturbed calcium balance. Vitamin D is the principal factor that maintains calcium homeostasis. Increasing evidence indicates that the reason for disturbed calcium balance with age is inadequate vitamin D levels in the elderly. In this article, an overview of our current understanding of vitamin D, its metabolism, and mechanisms involved in vitamin D-mediated maintenance of calcium homeostasis is presented. In addition, mechanisms involved in age-related dysregulation of $1,25(OH)_2D_3$ action, recommended daily doses of vitamin D and calcium, and the use of vitamin D analogs for the treatment of osteoporosis (which remains controversial) are reviewed. Elucidation of the molecular pathways of vitamin D action and modifications that occur with aging will be an active area of future research that has the potential to reveal new therapeutic strategies to maintain calcium balance.

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INTRODUCTION

Calcium is the fifth most abundant element in the human body and is essential for life.[1] It has a key role in many physiological processes including skeletal mineralization, muscle contraction, nerve impulse transmission, blood clotting, and hormone secretion. More than 99% of calcium in the body is stored in the skeleton as hydroxyapatite, which provides skeletal strength and is a source of calcium for the multiple calcium-mediated functions as well as for the maintenance of serum calcium within the normal range ($8–10\,mg\cdot dL^{-1}$). Less than 1% of calcium is located in the blood, soft tissues, and extracellular fluid. Serum calcium is either protein-bound (~40%), notably by albumin, bound as a complex to small anions (for example, phosphate or citrate; ~9%) or in the free or ionized state (~51%).[1] It is the ionized calcium that is available to enter cells and result in the activation of essential physiological processes. Calcium is only available to the body through dietary intake. In the elderly there is inadequate intestinal absorption of calcium combined with an age-related hormonal decline, which results in adverse effects on bone health.[2–3] 1,25-Dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], the hormonally active form of vitamin D, is the major controlling hormone of intestinal calcium absorption.[4] Calcium homeostasis is also regulated by parathyroid hormone and ionized calcium.[1,5] This review will focus on mechanisms involved in vitamin D regulation of calcium homeostasis, changes that occur with aging and current recommendations to address deficiencies.

VITAMIN D, METABOLISM, AND MAINTENANCE OF CALCIUM HOMEOSTASIS

Vitamin D is derived from the diet from fortified dairy products and fish oils or is synthesized in the skin from 7-dehydrocholesterol by ultraviolet irradiation.[6–7] Vitamin D is transported in the blood by vitamin D-binding protein (DBP). A series of hydroxylations, the first one at the 25th carbon (C-25) and the second at carbon 1 (C-1), are needed to produce the active form of vitamin D, $1,25(OH)_2D_3$. 25-Hydroxylation of vitamin D in the liver results in the formation of 25-hydroxyvitamin D [$25(OH)D_3$], the major circulating form of vitamin D and the most reliable index of vitamin D status.[6–7] CYP2R1 is now considered the key enzyme responsible for the conversion of vitamin D to $25(OH)D_3$.[8–9] Studies in CYP2R1 null mice, indicating

Department of Microbiology, Biochemistry and Molecular Genetics, Rutgers, The State University of New Jersey, New Jersey Medical School, Newark, NJ 07103, USA

Correspondence: Sylvia Christakos (christak@njms.rutgers.edu)

2

significantly reduced levels of $25(OH)D_3$ in these mice, have confirmed the role of CYP2R1 in the hydroxylation of vitamin D at C-25.[10] However, synthesis of low levels of $25(OH)D_3$ in these mice suggests that other 25-hydroxylases, yet to be identified, are also involved in the conversion of vitamin D to $25(OH)D_3$. After its synthesis in the liver, $25(OH)D_3$ is transported by DBP to the kidney where it is internalized by megalin, a transmembrane protein that acts as a surface receptor for DBP.[11–12] In the proximal renal tubule, $25(OH)D_3$ is hydroxylated by $25(OH)D_3$ 1α hydroxylase (CYP27B1) resulting in the formation of $1,25(OH)_2D_3$, which is responsible for the biological actions of vitamin D. In humans, mutations resulting in nonfunctional or deleted CYP27B1 cause vitamin D dependency rickets type 1 (characterized by hypocalcemia, hyperparathyroidism, and decreased bone mineralization), indicating the importance of CYP27B1 for the maintenance of calcium homeostasis.[13] 25-Hydroxyvitamin D3 24hydroxylase (CYP24A1) is the enzyme responsible for the catabolism of $1,25(OH)_2D$.[14–15] Direct evidence for a role of CYP24A1 in $1,25(OH)_2D_3$ catabolism was provided by studies in CYP24A1 null mice. The survival rate of homozygous mutants is ~50%. CYP24A1 null mice that survive are unable to clear exogenously administered $1,25(OH)_2D_3$.[16] In humans, inactivating mutations in CYP24A1 have been reported to have a causal role in certain patients with idiopathic infantile hypercalcemia, providing further evidence for the role of CYP24A1 in $1,25(OH)_2D_3$ catabolism.[17] Elevated parathyroid hormone (PTH) resulting from hypocalcemia induces $1,25(OH)_2D_3$ synthesis in the kidney and inhibits CYP24A1. $1,25(OH)_2D_3$ in turn acts to suppress PTH production at the parathyroid gland and to negatively regulate CYP27B1, thus regulating its own production.[18–19] $1,25(OH)_2D_3$ can also do so by inducing CYP24A1, thus completing an auto-regulatory feedback loop and maintaining a stringent control mechanism.[14–15,19] FGF23, a phosphaturic factor that promotes renal phosphate excretion, also regulates vitamin D metabolism. αKlotho is a co-receptor for FGF23. Together, FGF23 and klotho suppress CYP27B1 and induce CYP24A1, resulting in a reduction in $1,25(OH)_2D_3$ levels.[20]

The genomic actions of $1,25(OH)_2D_3$ are mediated by the vitamin D receptor (VDR). $1,25(OH)_2D_3$-occupied VDR heterodimerizes with the retinoid X receptor and together with co-regulatory proteins interacts with vitamin D response elements in and around target genes and mediates their transcription.[21–22]

The principal function of $1,25(OH)_2D_3$ in the maintenance of calcium homeostasis is to increase calcium absorption from the intestine (Figure 1). VDR is expressed in all segments of the small and large intestine and active $1,25(OH)_2D_3$ calcium absorption has been reported in the distal as well as the proximal intestine.[4] Rickets and osteomalacia
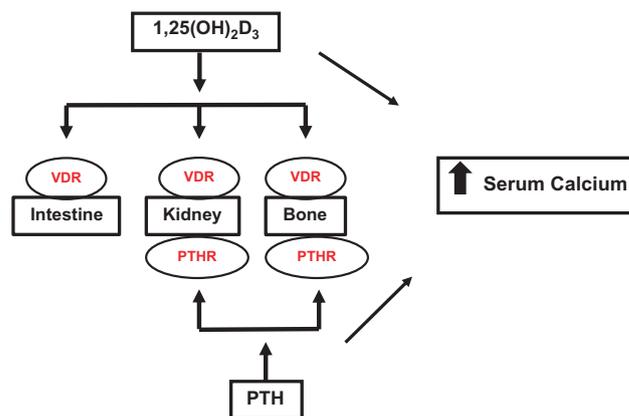

Figure 1. When serum calcium is low, $1,25(OH)_2D_3$ and parathyroid hormone (PTH) act to maintain calcium homeostasis. $1,25(OH)_2D_3$—the active form of vitamin D and the ligand for the vitamin D receptor (VDR)—acts to increase calcium absorption from the intestine. If normal calcium is unable to be maintained by intestinal calcium absorption, then $1,25(OH)_2D_3$ and PTH, together acting via their receptors, release calcium from the bone stores and increase reabsorption of calcium from the distal tubule of the kidney.

are prevented when VDR null mice are fed a diet high in calcium and lactose, indicating that $1,25(OH)_2D_3$ and VDR have a critical role in bone mineralization by regulating intestinal calcium absorption.[23–24] $1,25(OH)_2D_3$ has been reported to regulate every step of the intestinal transcellular calcium transport process. It induces the expression of the apical membrane calcium channel TRPV6, the calcium-binding protein calbindin-D_{9k} (it has been suggested that calbindin facilitates, in part, translocation of calcium through the enterocyte and buffers calcium preventing toxic levels of calcium from accumulating in the cell), and the plasma membrane CaATPase, PMCA1b. Thereby, $1,25(OH)_2D_3$ exerts its control in the intestine on calcium entry, calcium binding, and basolateral extrusion of calcium.[4]

Although the expression of calbindin-D_{9k} and TRPV6 is regulated by $1,25(OH)_2D_3$, calbindin-D_{9k} or TRPV6 null mice actively transport calcium similar to wild-type mice in response to $1,25(OH)_2D_3$, suggesting that other calcium channels or binding proteins can contribute to the calcium transport process in their absence as a compensatory mechanism.[25] However, increased bone turnover and impaired bone mineralization have been observed in TRPV6 null mice that are maintained on a low-calcium diet.[26] Moreover, overexpression of TRPV6 in the mouse intestine results in hypercalciuria, hypercalcemia, and soft tissue calcification, indicating a significant role for TRPV6 in intestinal calcium absorption.[27] In addition, our studies using calbindin-D_{9k}/TRPV6 double knockout mice revealed that when both genes are absent calcium absorption in response to low dietary calcium is least efficient, suggesting

that calbindin-D_{9k} and TRPV6 can act together in certain aspects of the active transcellular calcium transport process.[25]

If normal serum calcium cannot be maintained by intestinal calcium absorption, then $1,25(OH)_2D_3$ acts together with PTH to increase calcium reabsorption from the renal distal tubule and to remove calcium from bone (Figure 1). In the distal tubule of the kidney, similar to the intestine, $1,25(OH)_2D_3$ regulates the transcellular transport process by inducing an epithelial calcium channel TRPV5 (75% sequence homology with TRPV6), which facilitates apical calcium entry, and by inducing the calbindins (calbindin-D_{9k} and calbindin-D_{28k} are both present in mouse kidney; only calbindin-D_{28k} is present in rat and human kidney).[28–29] Extrusion of calcium at the distal tubule is via PMCA1b and the Na^+/Ca^{2+} exchanger. Although it has been a matter of debate, studies in Cyp27b1 null mice have shown that the Na^+/Ca^{2+} exchanger is decreased, suggesting regulation of the Na^+/Ca^{2+} exchanger as well as the calbindins and TRPV5 by $1,25(OH)_2D_3$.[30] The importance of TRPV5 in renal calcium reabsorption was noted in studies in TRPV5 null mice. TRPV5 null mice display severe hypercalciuria and significant changes in the bone structure.[31] In bone, both PTH and $1,25(OH)_2D_3$ stimulate osteoclastogenesis.[22] Osteoclastic bone resorption results in the release of calcium from bone to maintain calcium homeostasis.

VITAMIN D AND AGING

During the aging process, changes occur in many factors involved in the regulation of calcium homeostasis. In both animals and humans there is a decline in intestinal calcium absorption with age, resulting in secondary hyperparathyroidism and bone loss.[2–3,32] This decrease in calcium absorption correlates with decreased expression of intestinal TRPV6 and calbindin-D_{9k}.[33–34] We and others have noted that renal CYP24A1, which limits the amount of $1,25(OH)_2D_3$ by accelerating the catabolism of $1,25(OH)_2D_3$, increases with age.[35–36] In addition, with age there is a defect in 1 α hydroxylation.[37] Thus, the combined effect of a decline in intestinal calcium absorption, a decline in the ability of the kidney to synthesize $1,25(OH)_2D_3$, and an increase in catabolism of $1,25(OH)_2D_3$ by CYP24A1

contribute to age-related bone loss (Figure 2). It has been suggested that intestinal calcium malabsorption is due to reduction in circulating levels of $1,25(OH)_2D_3$ as well as intestinal resistance to $1,25(OH)_2D_3$.[38] The contribution of VDR to calcium absorption in the aging intestine is controversial. There have been studies that support a reduction in intestinal VDR content with age in humans and animals.[39–40] However, others have reported no change in intestinal VDR number with aging in humans and animals.[41–42] It is possible that the age-related resistance of the intestine to $1,25(OH)_2D_3$ and decreased expression of vitamin D target genes (for example, TRPV6) may be due, at least in part, to altered recruitment by $1,25(OH)_2D_3$ of VDR and VDR co-activators and epigenetic changes.

In addition to the intestine, there are age-related changes in the kidney that affect calcium homeostasis. With age, there is a decline in kidney function and a gradual decrease in the glomerular filtration rate, which is associated with progressive structural deterioration of the kidney.[43] Senescence affects vitamin D metabolism as indicated above. The age-related decrease in glomerular filtration rate has been reported to correlate with decreased serum $1,25(OH)_2D_3$.[44] Recent studies have suggested that increased FGF23 may be the initial event leading to the suppression of $1,25(OH)_2D_3$ synthesis that is associated with functional deterioration of the kidney.[45] Although PTH is elevated with age, renal production of $1,25(OH)_2D_3$ in response to PTH declines with age.[46] Coincident with decline in PTH-stimulated renal production of $1,25(OH)_2D_3$, there is also an age-related decrease in renal VDR and TRPV5 expression with age, which is accompanied by lower calcium renal reabsorption efficacy.[33] Aging is also associated with a decrease in the intrinsic capacity of the kidney to reabsorb phosphate, which has been reported to be independent of PTH.[47]

VITAMIN D AND BONE HEALTH

Osteoporosis is a systemic skeletal disease characterized by decreased bone strength and increased risk of fractures. Although osteoporosis affects both aging men and women, it is more frequently observed in postmenopausal women.[48] The National Osteoporosis Foundation estimates that one in every two women and one in every five men over 50 will experience osteoporosis-related fractures during their lifetime.[49] The loss of estrogen in menopause leads to a decline in bone mineral density (BMD).[50] It has been reported that not only in women but also in men there is an association between low estradiol levels and increased fracture.[50–51] Thus, low estradiol is a key factor predicting bone loss in older adults.[50–51]

In addition to low estradiol, low serum $25(OH)D_3$ is also associated with adverse skeletal outcomes.[52] The Institute

Aging
Effects on the Vitamin D Endocrine System
↓Intestinal calcium absorption (↓TRPV6 and ↓Calbindin)
Vitamin D resistance
↓Synthesis of $1,25(OH)_2D_3$
↑24(OH)ase expression (↑Catabolism of $1,25(OH)_2D_3$)
(↑Age related bone loss)
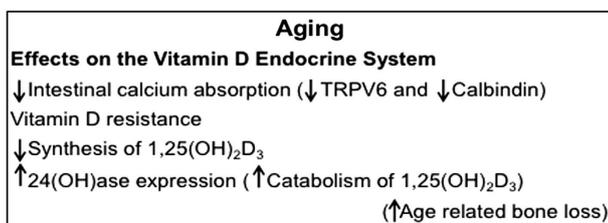
Figure 2. Age-related effects on the vitamin D endocrine system.

4

of Medicine considers a 25(OH)D level of 20 ng·mL^{-1} sufficient for the general population without underlying disease-related conditions.[53] Risk factors for vitamin D deficiency include older age, inadequate exposure to sunlight, dark skin tone, and obesity.[54] Vitamin D deficiency, which is common among the elderly, causes secondary hyperparathyroidism that can result in decreased bone density and increased risk of fracture. In a randomized, placebo-controlled trial of postmenopausal white women with 25(OH)D levels of 20 ng·mL^{-1} or less, Gallagher et al.[55] reported that a vitamin D dose of 800 IU per day (in conjunction with sufficient calcium intake; 1 200–1 400 mg) increased 25(OH)D levels greater than 20 ng·mL^{-1} in 97.5% of the women. This level, as indicated by the Institute of Medicine, is associated with reduced fracture risk. It should be noted, however, that some studies have suggested that a threshold of 30 ng·mL^{-1} is preferable to maintain skeletal health.[56] Some individuals, however, do not respond to vitamin D supplementation with an increase in 25(OH)D. The factors controlling this lack of response are unknown. It has recently been shown that DNA methylation levels of CYP2R1 and CYP24A1 are higher in non-responders, suggesting that the DNA methylation levels of these enzymes involved in vitamin D metabolism may predict which patients will not respond to vitamin D.[57] The current standard recommended daily doses of vitamin D and calcium are 800 IU and 1 000 mg, respectively, for vitamin D-sufficient individuals.[58] Pharmacological treatment for osteoporosis includes bisphosphonates, denosumab (monoclonal antibody against RANKL), and PTH peptides.[59] A combination of alendronate (a bisphosphonate; 70 mg) and 5 600 IU vitamin D3 administered weekly was found to be effective (increased BMD after 12 months) in treating osteoporotic postmenopausal women who had 25(OH)D levels between 8 and 20 ng·mL^{-1}, suggesting that correcting vitamin D deficiency may optimize the treatment of osteoporosis.[60]

VITAMIN D ANALOGS AND TREATMENT OF AGE-RELATED OSTEOPOROSIS

Besides pharmacological intervention with bisphosphonates, RANKL inhibitor (antiresorptive compounds), and PTH peptides (anabolic drug, teriparatide), vitamin D analogs have also been studied for possible osteoporosis treatment. However, their therapeutic efficacy in osteoporosis treatment remains controversial. Alphacalcidol (1αOHD$_3$), which is metabolized to 1,25(OH)$_2$D$_3$ in the liver, has been reported to inhibit bone resorption to increase BMD and to reduce vertebral and non-vertebral fractures.[61–65] Although it is a less effective antiresorptive agent compared with bisphosphonates, it has been suggested that alfacalcidol is superior to vitamin D plus

calcium in increasing lumbar BMD. It was reported that serum calcium was not significantly different between the vitamin D plus calcium group and the alfacalcidol group, suggesting similar safety characteristics.[66]

Eldecalcitol, 1α25(OH)$_2$-2b-(3-hydroxypropyloxy) vitamin D$_3$ (ED71), which has been approved for treatment of osteoporosis in Japan, is 1,25(OH)$_2$D$_3$ with a hydroxypropyloxy group at the carbon 2β position. Eldecalcitol has a lower affinity than 1,25(OH)$_2$D$_3$ for VDR but a 2.7-fold greater affinity for the DBP.[67] Eldecalcitol has a longer half-life than 1,25(OH)$_2$D$_3$. It has been suggested that tight binding of eldecalcitol to DBP can explain the longer half-life of eldecalcitol.[68] Eldecalcitol has also shown resistance to metabolic degradation via 24 hydroxylation, which may also contribute to its longer half-life and efficacy.[69] In silico modeling has shown that eldecalcitol does not fit in the active site of CYP24A1 because of the 3-HP group, suggesting a mechanism for its poor metabolic clearance by CYP24A1.[70] Studies in mice indicated that daily administration of eldecalcitol increased BMD, at least in part, by suppressing RANKL expression in trabecular bone.[71] Eldecalcitol has also been reported to reduce the number of osteoclasts while also stimulating focal bone formation in ovariectomized cynomolgus monkeys.[72] In a randomized double-blind study over 3 years in osteoporotic patients in comparison with alfacalcitol, eldecalcitol was more potent in increasing hip and lumbar BMD and reducing vertebral and wrist fractures. Urinary calcium was increased with treatment with both alfacalcitol and eldecalcitol. Eldecalcitol recipients had a greater increase in serum calcium compared with alfacalcitol recipients.[73–74] It has also been reported that combination treatment of alendronate and eldecalcitol is more effective in reducing bone turnover markers and increasing femoral neck BMD than alendronate, vitamin D plus calcium treatment in Japanese patients with primary osteoporosis.[75] However, close monitoring of blood and urinary calcium is recommended for all patients treated with eldecalcitol.[73]

2-Methylene-19-nor (20S)-1α25-dihydroxyvitamin D$_3$ (2MD) is a vitamin D analog, which was found to act as a bone anabolic agent. In ovariectomized rats, 2MD was reported to increase trabecular and cortical bone mass and to improve bone strength without hypercalcemia.[76–77] However, in a randomized, double-blind, placebo-controlled trial of osteopenic postmenopausal women, treatment with 2MD for 1 year did not change BMD.[78] It has been suggested that the difference between the rat and human data is because of less resorptive activity in the rat compared with humans. The resorptive effect of 2MD in humans may exceed its activity on bone formation.[78] However, 2MD has been shown to be 10 times more effective than 1α hydroxyvitamin D$_2$ (hectorol) or 19-nor-1α25-dihydroxyvitamin D$_2$ (Zemplar) in suppressing PTH

without affecting serum calcium.[79] Thus, 2MD may be a potent alternative to currently available compounds to suppress PTH in renal failure patients.

In summary, although some vitamin D analogs have been useful for treatment of osteoporosis, increased serum calcium remains a concern in countries where there is a greater normal intake of dietary calcium.

CONCLUSION AND FUTURE DIRECTIONS

Vitamin D deficiency is common among the elderly and can result in secondary hyperparathyroidism, decreased bone density, and increased risk of fracture. Correcting vitamin D deficiency is a reasonable approach to help maintain skeletal health and to optimize treatment of osteoporosis. Despite the importance of vitamin D in optimal calcium homeostasis and bone health, a detailed understanding of the mechanisms by which inadequate vitamin D contributes to osteoporosis are not yet known. Future studies using newer technologies, including those designed to provide genome-scale insights into the factors involved in regulating vitamin D genes as well as age-related changes in co-activator protein and epigenetic regulation of VDR function, will provide important insight into mechanisms involved in dysregulation of calcium homeostasis that occurs with aging. These molecular mechanistic studies will facilitate the development of drugs that selectively modulate vitamin D target genes with therapeutic potential to maintain calcium responsiveness during aging.

Competing interests

The authors declare no conflict of interest.

References

1 Peacock M. Calcium metabolism in health and disease. *Clin J Am Soc Nephrol* 2010; **5** (Suppl 1): S23–S30.

2 Morris HA, Need AG, Horowitz M *et al.* Calcium absorption in normal and osteoporotic postmenopausal women. *Calcif Tissue Int* 1991; **49**: 240–243.

3 Ensrud KE, Duong T, Cauley JA *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**: 345–353.

4 Christakos S. Recent advances in our understanding of 1,25-dihydroxyvitamin D(3) regulation of intestinal calcium absorption. *Arch Biochem Biophys* 2012; **523**: 73–76.

5 Brown EM. The calcium-sensing receptor: physiology, pathophysiology and CaR-based therapeutics. *Subcell Biochem* 2007; **45**: 139–167.

6 Plum LA, DeLuca HF. Vitamin D, disease and therapeutic opportunities. *Nat Rev Drug Discov* 2010; **9**: 941–955.

7 Bikle DD, Adams J, Christakos S. Vitamin D: production, metabolism and clinical requirements//Rosen C. Primer on Metabolic Bone Diseases. Hoboken: John Wiley and Sons, 2013: 235–245.

8 Zhu J, DeLuca HF. Vitamin D 25-hydroxylase - four decades of searching, are we there yet? *Arch Biochem Biophys* 2012; **523**: 30–36.

9 Cheng JB, Motola DL, Mangelsdorf DJ *et al.* De-orphanization of cytochrome P450 2R1: a microsomal vitamin D 25-hydroxylase. *J Biol Chem* 2003; **278**: 38084–38093.

10 Zhu JG, Ochalek JT, Kaufmann M *et al.* CYP2R1 is a major, but not exclusive, contributor to 25-hydroxyvitamin D production *in vivo. Proc Natl Acad Sci USA* 2013; **110**: 15650–15655.

11 Chun RF, Peercy BE, Orwoll ES *et al.* Vitamin D and DBP: the free hormone hypothesis revisited. *J Steroid Biochem Mol Biol* 2014; **144**: 132–137.

12 Nykjaer A, Dragun D, Walther D *et al.* An endocytic pathway essential for renal uptake and activation of the steroid 25-(OH) vitamin D3. *Cell* 1999; **96**: 507–515.

13 Kitanaka S, Takeyama K, Murayama A *et al.* Inactivating mutations in the 25-hydroxyvitamin D3 1alpha-hydroxylase gene in patients with pseudovitamin D-deficiency rickets. *N Engl J Med* 1998; **338**: 653–661.

14 Jones G, Prosser DE, Kaufmann M. 25-Hydroxyvitamin D-24-hydroxylase (CYP24A1): its important role in the degradation of vitamin D. *Arch Biochem Biophys* 2012; **523**: 9–18.

15 Veldurthy V, Wei R, Campbell M *et al.* 25-Hydroxyvitamin D(3) 24-hydroxylase: a key regulator of 1,25(OH)(2)D(3) catabolism and calcium homeostasis. *Vitam Horm* 2016; **100**: 137–150.

16 St-Arnaud R, Arabian A, Travers R *et al.* Deficient mineralization of intramembranous bone in vitamin D-24-hydroxylase-ablated mice is due to elevated 1,25-dihydroxyvitamin D and not to the absence of 24,25-dihydroxyvitamin D. *Endocrinology* 2000; **141**: 2658–2666.

17 Schlingmann KP, Kaufmann M, Weber S *et al.* Mutations in CYP24A1 and idiopathic infantile hypercalcemia. *N Engl J Med* 2011; **365**: 410–421.

18 Henry HL. Regulation of vitamin D metabolism. *Best Pract Res Clin Endocrinol Metab* 2011; **25**: 531–541.

19 Brenza HL, DeLuca HF. Regulation of 25-hydroxyvitamin D3 1alpha-hydroxylase gene expression by parathyroid hormone and 1,25-dihydroxyvitamin D3. *Arch Biochem Biophys* 2000; **381**: 143–152.

20 Hu MC, Shiizaki K, Kuro-o M *et al.* Fibroblast growth factor 23 and Klotho: physiology and pathophysiology of an endocrine network of mineral metabolism. *Annu Rev Physiol* 2013; **75**: 503–533.

21 Pike JW, Meyer MB. Fundamentals of vitamin D hormone-regulated gene expression. *J Steroid Biochem Mol Biol* 2014; **144**: 5–11.

22 Christakos S, Dhawan P, Verstuyf A *et al.* Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. *Physiol Rev* 2016; **96**: 365–408.

23 Amling M, Priemel M, Holzmann T *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**: 4982–4987.

24 Li YC, Amling M, Pirro AE *et al.* Normalization of mineral ion homeostasis by dietary means prevents hyperparathyroidism, rickets, and osteomalacia, but not alopecia in vitamin D receptor-ablated mice. *Endocrinology* 1998; **139**: 4391–4396.

25 Benn BS, Ajibade D, Porta A *et al.* Active intestinal calcium transport in the absence of transient receptor potential vanilloid type 6 and calbindin-D9k. *Endocrinology* 2008; **149**: 3196–3205.

26 Lieben L, Benn BS, Ajibade D *et al.* Trpv6 mediates intestinal calcium absorption during calcium restriction and contributes to bone homeostasis. *Bone* 2010; **47**: 301–308.

27 Cui M, Li Q, Johnson R et al. 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: 2097–2107.

28 de Groot T, Bindels RJ, Hoenderop JG. TRPV5: an ingeniously controlled calcium channel. Kidney Int 2008; 74: 1241–1246.

29 Ajibade D, Benn BS, Christakos S. Mechanism of action of 1.25 dihydroxyvitamin D3 in intestinal calcium absorption and renal calcium transport//Holick MF. Vitamin D: Physiology, Molecular, Biological and Clinical Applications. Totowa: Humana Press, 2010: 175–187.

30 Hoenderop JG, Dardenne O, Van Abel M et al. Modulation of renal Ca2+ transport protein genes by dietary Ca2+ and 1,25-dihydroxyvitamin D3 in 25-hydroxyvitamin D3-1alpha-hydroxylase knockout mice. FASEB J 2002; 16: 1398–1406.

31 Hoenderop JG, van Leeuwen JP, van der Eerden BC et al. Renal Ca2+ wasting, hyperabsorption, and reduced bone thickness in mice lacking TRPV5. J Clin Invest 2003; 112: 1906–1914.

32 Armbrecht HJ, Zenser TV, Bruns ME et al. Effect of age on intestinal calcium absorption and adaptation to dietary calcium. Am J Physiol 1979; 236: E769–E774.

33 van Abel M, Huybers S, Hoenderop JG et al. Age-dependent alterations in Ca2+ homeostasis: role of TRPV5 and TRPV6. Am J Physiol Renal Physiol 2006; 291: F1177–F1183.

34 Brown AJ, Krits I, Armbrecht HJ. Effect of age, vitamin D, and calcium on the regulation of rat intestinal epithelial calcium channels. Arch Biochem Biophys 2005; 437: 51–58.

35 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: 3971–3982.

36 Johnson JA, Beckman MJ, Pansini-Porta A et al. Age and gender effects on 1,25-dihydroxyvitamin D3-regulated gene expression. Exp Gerontol 1995; 30: 631–643.

37 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: 1118–1123.

38 Pattanaungkul S, Riggs BL, Yergey AL et al. Relationship of intestinal calcium absorption to 1,25-dihydroxyvitamin D [1,25(OH)2D] levels in young versus elderly women: evidence for age-related intestinal resistance to 1,25(OH)2D action. J Clin Endocrinol Metab 2000; 85: 4023–4027.

39 Ebeling PR, Sandgren ME, DiMagno EP et al. Evidence of an age-related decrease in intestinal responsiveness to vitamin D: relationship between serum 1,25-dihydroxyvitamin D3 and intestinal vitamin D receptor concentrations in normal women. J Clin Endocrinol Metab 1992; 75: 176–182.

40 Horst RL, Goff JP, Reinhardt TA. Advancing age results in reduction of intestinal and bone 1,25-dihydroxyvitamin D receptor. Endocrinology 1990; 126: 1053–1057.

41 Kinyamu HK, Gallagher JC, Prahl JM et al. Association between intestinal vitamin D receptor, calcium absorption, and serum 1,25 dihydroxyvitamin D in normal young and elderly women. J Bone Miner Res 1997; 12: 922–928.

42 Wood RJ, Fleet JC, Cashman K et al. Intestinal calcium absorption in the aged rat: evidence of intestinal resistance to 1,25(OH)2 vitamin D. Endocrinology 1998; 139: 3843–3848.

43 Weinstein JR, Anderson S. The aging kidney: physiological changes. Adv Chronic Kidney Dis 2010; 17: 302–307.

44 Reichel H, Deibert B, Schmidt-Gayk H et al. Calcium metabolism in early chronic renal failure: implications for the pathogenesis of hyperparathyroidism. Nephrol Dial Transplant 1991; 6: 162–169.

45 Quarles LD. Role of FGF23 in vitamin D and phosphate metabolism: implications in chronic kidney disease. Exp Cell Res 2012; 318: 1040–1048.

46 Armbrecht HJ, Wongsurawat N, Zenser TV et al. Differential effects of parathyroid hormone on the renal 1,25-dihydroxyvitamin D3 and 24,25-dihydroxyvitamin D3 production of young and adult rats. Endocrinology 1982; 111: 1339–1344.

47 Mulroney SE, Woda C, Haramati A. Changes in renal phosphate reabsorption in the aged rat. Proc Soc Exp Biol Med 1998; 218: 62–67.

48 Raisz LG. Pathogenesis of osteoporosis: concepts, conflicts, and prospects. J Clin Invest 2005; 115: 3318–3325.

49 Cosman F, de Beur SJ, LeBoff MS et al. Clinician's guide to prevention and treatment of osteoporosis. Osteoporos Int 2014; 25: 2359–2381.

50 Riggs BL, Khosla S, Melton LJ 3rd. Sex steroids and the construction and conservation of the adult skeleton. Endocr Rev 2002; 23: 279–302.

51 Cauley JA. Estrogen and bone health in men and women. Steroids 2015; 99: 11–15.

52 Cauley JA, LaCroix AZ, Wu L et al. Serum 25 hydroxyvitamin D concentrations and the risk of hip fractures: the women's health initiative. Ann Intern Med 2008; 149: 242–250.

53 Ross AC, Manson JE, Abrams SA 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: 53–58.

54 Holick MF, Binkley NC, Bischoff-Ferrari HA et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011; 96: 1911–1930.

55 Gallagher JC, Sai A, Templin T 2nd et al. Dose response to vitamin D supplementation in postmenopausal women: a randomized trial. Ann Intern Med 2012; 156: 425–437.

56 Priemel M, von Domarus C, Klatte TO et al. Bone mineralization defects and vitamin D deficiency: histomorphometric analysis of iliac crest bone biopsies and circulating 25-hydroxyvitamin D in 675 patients. J Bone Miner Res 2010; 25: 305–312.

57 Zhou Y, Zhao LJ, Xu X et al. DNA methylation levels of CYP2R1 and CYP24A1 predict vitamin D response variation. J Steroid Biochem Mol Biol 2014; 144: 207–214.

58 Gallagher JC. Vitamin D and aging. Endocrinol Metab Clin North Am 2013; 42: 319–332.

59 Tella SH, Gallagher JC. Prevention and treatment of postmenopausal osteoporosis. J Steroid Biochem Mol Biol 2014; 142: 155–170.

60 Ralston SH, Binkley N, Boonen S et al. Randomized trial of alendronate plus vitamin D3 versus standard care in osteoporotic postmenopausal women with vitamin D insufficiency. Calcif Tissue Int 2011; 88: 485–494.

61 Weber K, Kaschig C, Erben RG. 1 Alpha-hydroxyvitamin D2 and 1 alpha-hydroxyvitamin D3 have anabolic effects on cortical bone, but induce intracortical remodeling at toxic doses in ovariectomized rats. Bone 2004; 35: 704–710.

62 Li M, Healy DR, Simmons HA et al. Alfacalcidol restores cancellous bone in ovariectomized rats. J Musculoskelet Neuronal Interact 2003; 3: 39–46.

63 Orimo H, Shiraki M, Hayashi Y et al. Effects of 1 alpha-hydroxyvitamin D3 on lumbar bone mineral density and vertebral fractures in patients with postmenopausal osteoporosis. Calcif Tissue Int 1994; 54: 370–376.

64 ShikariKushida M, Yamazaki K, Nagai K et al. H. Effects of 2 years' treatment of osteoporosis with 1 alpha-hydroxy vitamin D3 on bone mineral density and incidence of fracture: a placebo-controlled, double-blind prospective study. Endocr J 1996; 43: 211–220.

65 Hayashi Y, Fujita T, Inoue T. Decrease of vertebral fracture in osteoporotics by administration of 1α-hydroxy-vitamin D3. *J Bone Miner Metab* 1992; **10**: 50–54.

66 Nuti R, Bianchi G, Brandi ML *et al.* Superiority of alfacalcidol compared to vitamin D plus calcium in lumbar bone mineral density in postmenopausal osteoporosis. *Rheumatol Int* 2006; **26**: 445–453.

67 Kubodera N, Tsuji N, Uchiyama Y *et al.* A new active vitamin D analog, ED-71, causes increase in bone mass with preferential effects on bone in osteoporotic patients. *J Cell Biochem* 2003; **88**: 286–289.

68 Abe M, Tsuji N, Takahashi F *et al.* Overview of the clinical pharmacokinetics of eldecalcitol, a new active vitamin D3 derivative. *Jpn Pharmacol Ther* 2011; **39**: 261–274.

69 Ritter CS, Brown AJ. Suppression of PTH by the vitamin D analog eldecalcitol is modulated by its high affinity for the serum vitamin D-binding protein and resistance to metabolism. *J Cell Biochem* 2011; **112**: 1348–1352.

70 Kondo S, Takano T, Ono Y *et al.* Eldecalcitol reduces osteoporotic fractures by unique mechanisms. *J Steroid Biochem Mol Biol* 2015; **148**: 232–238.

71 Harada S, Mizoguchi T, Kobayashi Y *et al.* Daily administration of eldecalcitol (ED-71), an active vitamin D analog, increases bone mineral density by suppressing RANKL expression in mouse trabecular bone. *J Bone Miner Res* 2012; **27**: 461–473.

72 Saito M, Grynpas MD, Burr DB *et al.* Treatment with eldecalcitol positively affects mineralization, microdamage, and collagen crosslinks in primate bone. *Bone* 2015; **73**: 8–15.

73 Matsumoto T, Ito M, Hayashi Y *et al.* A new active vitamin D3 analog, eldecalcitol, prevents the risk of osteoporotic fractures--a randomized, active comparator, double-blind study. *Bone* 2011; **49**: 605–612.

74 Matsumoto T, Takano T, Saito H *et al.* Vitamin D analogs and bone: preclinical and clinical studies with eldecalcitol. *Bonekey Rep* 2014; **3**: 513.

75 Sakai A, Ito M, Tomomitsu T *et al.* Efficacy of combined treatment with alendronate (ALN) and eldecalcitol, a new active vitamin D analog, compared to that of concomitant ALN, vitamin D plus calcium treatment in Japanese patients with primary osteoporosis. *Osteoporos Int* 2015; **26**: 1193–1202.

76 Plum LA, Fitzpatrick LA, Ma X *et al.* 2MD, a new anabolic agent for osteoporosis treatment. *Osteoporos Int* 2006; **17**: 704–715.

77 Ke HZ, Qi H, Crawford DT *et al.* A new vitamin D analog, 2MD, restores trabecular and cortical bone mass and strength in ovariectomized rats with established osteopenia. *J Bone Miner Res* 2005; **20**: 1742–1755.

78 DeLuca HF, Bedale W, Binkley N *et al.* The vitamin D analogue 2MD increases bone turnover but not BMD in postmenopausal women with osteopenia: results of a 1-year phase 2 double-blind, placebo-controlled, randomized clinical trial. *J Bone Miner Res* 2011; **26**: 538–545.

79 Zella JB, Plum LA, Plowchalk DR *et al.* Novel, selective vitamin D analog suppresses parathyroid hormone in uremic animals and postmenopausal women. *Am J Nephrol* 2014; **39**: 476–483.