Intestinal Regulation of Calcium: Vitamin D and Bone Physiology

Sylvia Christakos, Vaishali Veldurthy, Nishant Patel, and Ran Wei

Introduction

Calcium, an essential ion for numerous physiological processes, is a major constituent of bone [1]. The intestine is the only source of new calcium. Absorption of dietary calcium, a process dependent on vitamin D, is essential for calcium 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 [2–4]. Nearly a century ago, McCollum et al. identified vitamin D as the factor that cured rickets [5]. Solar or UVB irradiation is needed to convert 7-dehydrocholesterol in the skin to pre-vitamin D3 that ultimately is converted to vitamin D_3 (cholecalciferol) by thermo-isomerization [3, 6]. Since the synthesis of vitamin D in the skin depends on the intensity of ultraviolet irradiation, geographical location and season play an important role in contributing to vitamin D sufficiency in man [3, 7]. Although cutaneous production of vitamin D remains an important source, the fortification of foods (including fortification of dairy products) largely contributed to the marked decrease in the incidence of vitamin D-dependent rickets in the Western world by the mid-twentieth century [5]. However, vitamin D deficiency in children and adults is still prevalent worldwide due, in part, to lack of exposure to sunlight and low vitamin D intake [3]. In this chapter the vitamin D endocrine system as well as the mechanisms by which 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) acts to maintain calcium homeostasis will be briefly reviewed followed by an emphasis on the intestinal actions of $1,25(OH)_2D_3$ and effects of bone.

S. Christakos (✉) • V. Veldurthy • N. Patel • R. Wei
Department 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: christak@njms.rutgers.edu

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The Vitamin D Endocrine System: Bioactivation and Mechanism of Action

Vitamin D, which is taken in the diet or which is synthesized in the skin by UV irradiation, must be metabolized to its active form in order to regulate calcium homeostasis. Vitamin D is transported by vitamin D binding protein (DBP, which binds and transports vitamin D and its metabolites in the serum) to the liver where 25-hydroxyvitamin D_3 ($25(OH)D_3$), the major circulating form of vitamin D and an important biomarker for vitamin D status, is generated [6, 8, 9]. It has been suggested that the cytochrome P450 (CYP) enzyme CYP2R1 is the key enzyme involved in the conversion of vitamin D to $25(OH)D_3$ [10]. Patients with mutations in CYP2R1 are deficient in $25(OH)D_3$ and develop vitamin D-dependent rickets [11]. Studies in *Cyp2r1* null mice which show that levels of $25(OH)D_3$ are diminished but not eliminated suggest the presence of additional vitamin D 25-hydroxylases is yet to be identified [12]. In the proximal renal tubule, CYP27B1 ($25(OH)D_3$ 1α hydroxylase) converts $25(OH)D_3$ to 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the hormonally active form of vitamin D which is responsible for the biological actions of vitamin D [6, 8, 9]. Mutations in CYP27B1 cause vitamin D-dependent rickets type 1 (VDDR1), characterized by hypocalcemia, hypophosphatemia, decreased mineralization, and low circulating $1,25(OH)_2D_3$ levels [13]. With regard to the regulation of vitamin D metabolism, parathyroid hormone (PTH), whose synthesis is increased in response to hypocalcemia, induces CYP27B1 and is the major stimulator of $1,25(OH)_2D_3$ production [8, 9]. $1,25(OH)_2D_3$ and FGF23 (which promotes phosphate excretion) and its co-receptor α klotho negatively regulate CYP27B1. As an autoregulatory mechanism, $1,25(OH)_2D_3$ induces CYP24A1 (25-hdyroxyvitamin D_3 24-hydroxylase), the enzyme that accelerates the catabolism of $1,25(OH)_2D_3$ preventing hypercalcemia resulting from high circulating $1,25(OH)_2D_3$ (see ref. [9] for review of the regulation of vitamin D metabolism). Thus, vitamin D, FGF23/α klotho, and serum calcium and phosphate act together to regulate calcium homeostasis.

The actions of $1,25(OH)_2D_3$, similar to other steroid hormones, are mediated by the vitamin D receptor (VDR). $1,25(OH)_2D_3$-occupied VDR heterodimerizes with the retinoid X receptor and together with chromatin active co-regulatory proteins interacts with vitamin D response elements in and around target genes resulting in the induction or suppression of gene expression [14].

Vitamin D and Intestinal Calcium Absorption

The principal function of $1,25(OH)_2D_3$ in the maintenance of calcium homeostasis is to increase calcium absorption from the intestine. This conclusion was made from studies in VDR null mice which showed that rickets, osteomalacia, hypocalcemia,

and hyperparathyroidism were prevented when VDR null mice were fed a rescue diet which included high calcium (2%), indicating that the skeletal abnormalities of VDR ablation are primarily the result of impaired intestinal calcium absorption (and resultant hyperparathyroidism and hypophosphatemia) [15, 16]. The abnormalities in the VDR null mice were reported to develop only after weaning [17] consistent with previous studies showing that intestinal VDR and the calcium binding protein calbindin-D_{9k} are induced at weaning, the time of onset of active intestinal calcium absorption [18, 19]. Although there is an increase in PTH and in the number of osteoblasts, osteoclast number is not increased in VDR null mice, suggesting that $1,25(OH)_2D_3$/VDR is needed for PTH-induced osteoclastogenesis [15]. Similar to VDR null mice, serum calcium and PTH were normalized in CYP27B1 null mice and in CYP27B1/VDR double null mice fed the high calcium rescue diet, confirming the importance of both $1,25(OH)_2D_3$ and VDR in intestinal calcium absorption [20, 21]. In addition, in CYP27B1 null mice, in spite of markedly elevated PTH levels, osteoclast numbers were also not increased above levels in normal wild-type (WT) mice, indicating that both VDR and $1,25(OH)_2D_3$ are necessary for PTH-mediated production of osteoclasts [21].

Direct evidence for the critical role of $1,25(OH)_2D_3$-mediated intestinal calcium absorption in bone homeostasis was noted in studies in transgenic mice with VDR expression limited to the intestine [22]. Transgenic expression of VDR in the intestine of VDR null mice restored calcium homeostasis and prevented the rachitic phenotype of the VDR null mice [22]. Thus, intestinal VDR is essential for controlling bone formation. In addition, when VDR is deleted specifically from the intestine (Vdr^{int-}), there is a decrease in intestinal calcium absorption, an inhibition of bone mineralization, and an increase in bone fractures in the Vdr^{int-}mice [23]. In these mice serum calcium is normal indicating that in the absence of VDR-mediated intestinal calcium absorption normal serum calcium will be maintained at the expense of skeletal integrity.

When there is an increased need for calcium (under low dietary calcium conditions, during growth, pregnancy, or lactation), the synthesis of $1,25(OH)_2D_3$ is increased and $1,25(OH)_2D_3$ acts at the intestine to increase active calcium absorption [6, 8]. The major defect from the loss of VDR is decreased intestinal calcium absorption resulting in decreased bone mineralization [15, 16]. If normal serum calcium cannot be maintained by intestinal calcium absorption, then $1,25(OH)_2D_3$ acts together with PTH to stimulate osteoclastogenesis resulting in the removal of calcium from the bone and to increase calcium reabsorption from the distal tubules of the kidney [6, 8].

Mechanisms Involved in $1,25(OH)_2D_3$ Regulation of Intestinal Calcium Absorption

Intestinal calcium absorption occurs by an active, saturable, transcellular mechanism or by a nonsaturable passive process which occurs through tight junctions and structures within intercellular spaces and requires a high luminal calcium concentration (>2–6 mM) [24]. $1,25(OH)_2D_3$ regulates the transcellular process by inducing TRPV6 (an apical membrane calcium channel), the calcium binding protein calbindin-D_{9k}, and the basolateral membrane calcium ATPase (PMCA1b) [8, 25, 26] (Fig. 1). Although it has been suggested that calbindin-D_{9k} mediates intracellular calcium diffusion, other studies suggest that a principal function of calbindin-D_{9k} is to buffer calcium preventing toxic levels from accumulating in the cell [27, 28]. When calcium is low, the $1,25(OH)_2D_3$-mediated transcellular calcium transport process is the predominant mechanism of calcium absorption [24]. Although TRPV6 and calbindin-D_{9k} are induced by $1,25(OH)_2D_3$, TRPV6 or calbindin-D_{9k} null mice have normal serum calcium and show no change in active intestinal calcium absorption compared to WT mice [29–31]. However, studies in TRPV6/calbindin-D_{9k} double null mice under conditions of low dietary calcium have shown that intestinal calcium absorption is least efficient in the absence of both proteins (compared to single null mice and WT mice), suggesting that TRPV6 and calbindin can act together in certain aspects of the absorptive process [29]. Findings in the single null mice suggest that in the absence of calbindin or TRPV6, there is compensation by other channels or proteins yet to be identified. Although other apical membrane calcium transporters may compensate for the loss of TRPV6, intestine-specific transgenic expression of TRPV6 has been shown to result in a marked increase in intestinal calcium absorption and bone density in VDR null mice, indicating a direct role for TRPV6 in the calcium absorptive process and that a primary defect in the VDR null mouse is low apical membrane calcium uptake [28].

The duodenum has been a focus of research related to $1,25(OH)_2D_3$ regulation of calcium absorption. However, it is the distal intestine where most of the ingested calcium is absorbed [24]. VDR, TRPV6, and calbindin-D_{9k} are expressed in all segments of the intestine and $1,25(OH)_2D_3$-regulated active calcium absorption occurs in the ileum, cecum, and colon [22, 32–36]. Recent studies have shown that transgenic expression of VDR specifically in the ileum, cecum, and colon can prevent abnormal calcium homeostasis and rickets in VDR null mice [37]. In addition, when VDR is deleted specifically from the distal region of the intestine, altered calcium metabolism is observed [38]. These findings indicate that the distal as well as the proximal segments of the intestine are important in vitamin D-mediated calcium homoeostasis and bone mineralization. Future studies related to mechanisms involved in $1,25(OH)_2D_3$-mediated regulation of calcium absorption in the distal intestine may suggest new strategies to increase the efficiency of calcium absorption in individuals at risk for bone loss including those with reduced calcium absorption due to small bowel resection or following menopause.

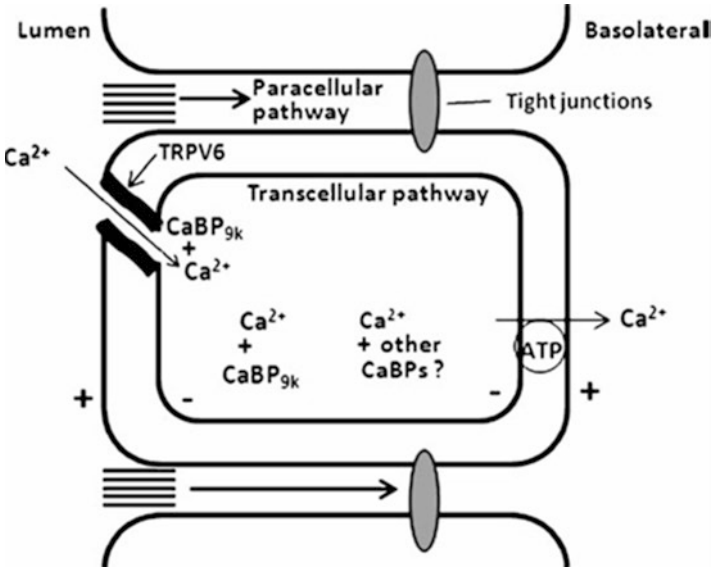

Fig. 1 Model of vitamin D-mediated intestinal calcium absorption. The transcellular pathway consists of influx through the apical calcium channel TRPV6, diffusion through the cytosol, and active extrusion at the basolateral membrane by the plasma membrane calcium ATPase (PMCA1b). Studies using TRPV6 and calbindin-D_{9k} ($CaBP_{9k}$) null mice indicate that our understanding of the vitamin D-mediated calcium transport process remains incomplete. In the absence of TRPV6 or calbindin-D_{9k} $1,25(OH)_2D_3$-mediated active transport still occurs, suggesting compensation by another channel or protein. Intracellular calcium transfer may involve calcium bound to calbindin as well as other calcium binding proteins. Calbindin as well as other calcium binding proteins may also act to prevent toxic levels of calcium from accumulating in the cell. It has been suggested that $1,25(OH)_2D_3$ can regulate the paracellular pathway by regulating tight junction proteins

In addition to regulation by vitamin D of the active transcellular process, early studies suggested that the passive, nonsaturable process of intestinal calcium absorption can also be enhanced by vitamin D [39]. More recent studies have shown that $1,25(OH)_2D_3$ can regulate intestinal proteins that are involved in tight junctions or cell adhesion. Claudin-2 and claudin-12 are induced and cadherin-17 is inhibited by $1,25(OH)_2D_3$ [40, 41]. These findings suggest that $1,25(OH)_2D_3$, by regulating these proteins, can facilitate calcium absorption through the paracellular path (Fig. 1). The identification of multiple mechanisms and novel vitamin D targets involved in $1,25(OH)_2D_3$-mediated calcium absorption in different segments of the intestine is needed in order to identify new approaches to maximize calcium absorption and minimize bone loss.

Vitamin D: Direct Effects on Bone

Although the primary role of the vitamin D endocrine system on bone is indirect (providing calcium to bone by stimulating intestinal calcium absorption), direct effects of $1,25(OH)_2D_3$ on bone cells have also been demonstrated (see [42] for review). As indicated above in studies in VDR and CYP27B1 null mice, VDR and $1,25(OH)_2D_3$ are needed for PTH-mediated osteoclastogenesis [15, 21]. Osteoclastogenesis mediated by $1,25(OH)_2D_3$ as well as by PTH involves upregulation of receptor activator of nuclear kB ligand (RANKL) in osteoblastic cells and requires cell to cell contact between osteoblasts and osteoclast precursors [43]. In addition $1,25(OH)_2D_3$ can also stimulate the production in osteoblasts of the calcium binding proteins osteocalcin and osteopontin (OPN) [8]. OPN has been reported to inhibit bone matrix mineralization [44]. Thus, during a negative calcium balance, $1,25(OH)_2D_3$ action can promote increased bone resorption and reduced bone matrix mineralization in order to maintain normal serum calcium levels [23]. $1,25(OH)_2D_3$ has also been shown to induce LRP5 (low density lipoprotein receptor-related 5) which facilitates β catenin activation and exerts an anabolic effect on bone formation [45]. These findings indicate that the effects of $1,25(OH)_2D_3$ on osteogenic cells are complex and can result in either bone resorption or formation.

Vitamin D: The Kidney and Calcium Homeostasis

Vitamin D-mediated calcium homeostasis is regulated by an integrated system involving not only the intestine and bone but also the kidney. Although most of the filtered calcium is reabsorbed by a passive, paracellular path in the proximal renal tubule that is independent of $1,25(OH)_2D_3$, 10–15% of the filtered calcium is reabsorbed in the distal convoluted tubule and connecting tubule and is regulated by PTH and $1,25(OH)_2D_3$ [46]. Similar to studies in the intestine, $1,25(OH)_2D_3$ regulates an active, transcellular process in the distal portion of the nephron by inducing the apical calcium channel TRPV5 (which shares 75% sequence homology with TRPV6) and by inducing the calbindins [both calbindin-D_{9k} (9,000 M_r) and calbindin-D_{28k} (28,000 M_r) are present in the mouse kidney and only calbindin-D_{28k} is present in rat and human kidney] [8]. Calcium is extruded via PMCA1b and the Na^+Ca^{++} exchanger [8]. It has been shown that calbindin-D_{28k} binds to TRPV5 and modulates calcium influx [47]. PTH has been reported to activate TRPV5 via protein kinase A phosphorylation [48]. The kidney is also the major site of production of $1,25(OH)_2D_3$ and its regulation [9]. Thus, the kidney, by regulating transport processes and as a major site of synthesis of $1,25(OH)_2D_3$, plays an essential role in the maintenance of calcium homeostasis.

Vitamin D: The Intestine and Bone Health

In aging intestinal calcium absorption declines which results in secondary hyperparathyroidism and increased fracture risk [49]. Decreased intestinal calcium absorption with age has been shown to correlate with decreased expression of TRPV6 and calbindin-D_{9k}, the two major targets of $1,25(OH)_2D_3$ in the intestine [50, 51] (Fig. 2). Increasing evidence indicates that the reason for disturbed calcium balance with age is that vitamin D status is often inadequate in the elderly [52]. With age there is a decline in the ability of the kidney to synthesize $1,25(OH)_2D_3$ and an increase in CYP24A1 which would result in enhanced catabolism of $1,25(OH)_2D_3$ [53–55] (Fig. 2). Intestinal resistance to $1,25(OH)_2D_3$ with age has also been reported [56–58]. It has been suggested that this resistance is due to a decrease in the content of intestinal VDR with age [57]. However, this has been a matter of debate [58]. It is possible that the resistance to $1,25(OH)_2D_3$ may also be due to altered recruitment by $1,25(OH)_2D_3$ of VDR and VDR coactivators to intestinal vitamin D target genes and/or to epigenetic changes. To reduce fracture risk, a combination of calcium and vitamin D supplementation has been recommended [59]. The current recommended daily doses for vitamin D-sufficient individuals are 800 IU calcium and 1,000 mg calcium [60].

In addition to effects of aging, gastric bypass surgery has also been reported to result in calcium malabsorption and decreased bone mineral density in patients [61–63]. Animal studies have also noted gastric bypass-associated bone resorption which is due to several factors including vitamin D and calcium malabsorption and acid/base dysregulation [64, 65]. Patients with inflammatory bowel disease are also at risk for bone disease due in part to impaired intestinal calcium absorption as well as to proinflammatory cytokines which are involved in the intestinal immune response but can also enhance bone resorption [66]. Future studies related to mechanisms involved in VDR-mediated activation of intestinal calcium absorption may suggest new mechanisms to compensate for calcium malabsorption in order to minimize bone loss due to aging, bariatric surgery, or inflammatory bowel disease.

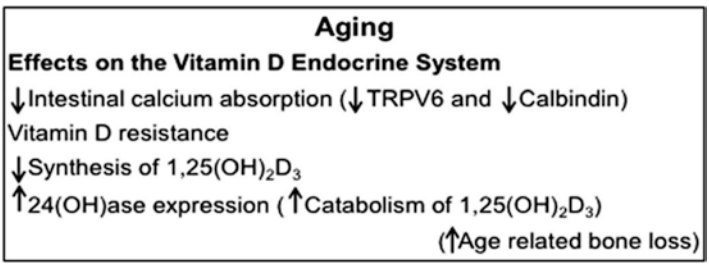

Fig. 2 Age-related effects on the vitamin D endocrine system

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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): S23–30.
2. Bouillon R, Bischoff-Ferrari H, Willett W. Vitamin D and health: perspectives from mice and man. J Bone Miner Res. 2008;23(7):974–9.
3. Holick MF. Resurrection of vitamin D deficiency and rickets. J Clin Invest. 2006;116(8):2062–72.
4. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: consequences for bone loss and fractures and therapeutic implications. Endocr Rev. 2001;22(4):477–501.
5. Deluca HF. History of the discovery of vitamin D and its active metabolites. Bonekey Rep. 2014;3:479.
6. DeLuca HF. Evolution of our understanding of vitamin D. Nutr Rev. 2008;66(10 Suppl 2):S73–87.
7. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. J Clin Endocrinol Metab. 1988;67(2):373–8.
8. Christakos S, et al. Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. Physiol Rev. 2016;96(1):365–408.
9. Christakos S, et al. Vitamin D: metabolism. Endocrinol Metab Clin N Am. 2010;39(2):243–53. table of contents
10. Cheng JB, et al. Genetic evidence that the human CYP2R1 enzyme is a key vitamin D 25-hydroxylase. Proc Natl Acad Sci U S A. 2004;101(20):7711–5.
11. Thacher TD, et al. CYP2R1 mutations impair generation of 25-hydroxyvitamin D and cause an atypical form of vitamin D deficiency. J Clin Endocrinol Metab. 2015;100(7):E1005–13.
12. Zhu JG, et al. CYP2R1 is a major, but not exclusive, contributor to 25-hydroxyvitamin D production in vivo. Proc Natl Acad Sci U S A. 2013;110(39):15650–5.
13. Kitanaka S, 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(10):653–61.
14. Pike JW, Meyer MB. Fundamentals of vitamin D hormone-regulated gene expression. J Steroid Biochem Mol Biol. 2014;144(Pt A):5–11.
15. Amling M, 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(11):4982–7.
16. Masuyama R, 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(7):1217–26.
17. Yoshizawa T, et al. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat Genet. 1997;16(4):391–6.
18. Huang YC, et al. Effect of hormones and development on the expression of the rat 1,25-dihydroxyvitamin D3 receptor gene. Comparison with calbindin gene expression. J Biol Chem. 1989;264(29):17454–61.
19. Halloran BP, DeLuca HF. Calcium transport in small intestine during early development: role of vitamin D. Am J Phys. 1980;239(6):G473–9.
20. Dardenne O, et al. Correction of the abnormal mineral ion homeostasis with a high-calcium, high-phosphorus, high-lactose diet rescues the PDDR phenotype of mice deficient for the 25-hydroxyvitamin D-1alpha-hydroxylase (CYP27B1). Bone. 2003;32(4):332–40.

21. Panda DK, et al. Inactivation of the 25-hydroxyvitamin D 1alpha-hydroxylase and vitamin D receptor demonstrates independent and interdependent effects of calcium and vitamin D on skeletal and mineral homeostasis. J Biol Chem. 2004;279(16):16754–66.
22. Xue Y, Fleet JC. Intestinal vitamin D receptor is required for normal calcium and bone metabolism in mice. Gastroenterology. 2009;136(4):1317–27. e1-2
23. Lieben L, 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(5):1803–15.
24. Wasserman RH. Vitamin D and the dual processes of intestinal calcium absorption. J Nutr. 2004;134(11):3137–9.
25. Christakos S. Recent advances in our understanding of 1,25-dihydroxyvitamin D(3) regulation of intestinal calcium absorption. Arch Biochem Biophys. 2012;523(1):73–6.
26. Christakos S, et al. Vitamin D endocrine system and the intestine. Bonekey Rep. 2014;3:496.
27. Schroder B, et al. Role of calbindin-D9k in buffering cytosolic free Ca2+ ions in pig duodenal enterocytes. J Physiol. 1996;492(Pt 3):715–22.
28. Cui M, 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(10):2097–107.
29. Benn BS, et al. Active intestinal calcium transport in the absence of transient receptor potential vanilloid type 6 and calbindin-D9k. Endocrinology. 2008;149(6):3196–205.
30. Kutuzova GD, 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(50):19655–9.
31. Akhter S, et al. Calbindin D9k is not required for 1,25-dihydroxyvitamin D3-mediated Ca2+ absorption in small intestine. Arch Biochem Biophys. 2007;460(2):227–32.
32. Hirst MA, Feldman D. 1,25-Dihydroxyvitamin D3 receptors in mouse colon. J Steroid Biochem. 1981;14(4):315–9.
33. Stumpf WE, et al. Target cells for 1,25-dihydroxyvitamin D3 in intestinal tract, stomach, kidney, skin, pituitary, and parathyroid. Science. 1979;206(4423):1188–90.
34. Lee DB, et al. Intestinal and metabolic effect of 1,25-dihydroxyvitamin D3 in normal adult rat. Am J Phys. 1981;240(1):G90–6.
35. Favus MJ, Angeid-Backman E. Effects of 1,25(OH)2D3 and calcium channel blockers on cecal calcium transport in the rat. Am J Phys. 1985;248(6 Pt 1):G676–81.
36. Favus MJ, et al. Effects of diet calcium and 1,25-dihydroxyvitamin D3 on colon calcium active transport. Am J Phys. 1980;238(2):G75–8.
37. Christakos S, et al. Vitamin D biology revealed through the study of knockout and transgenic mouse models. Annu Rev Nutr. 2013;33:71–85.
38. Reyes-Fernandez PC, Fleet JC. Compensatory changes in calcium metabolism accompany the loss of vitamin D receptor (VDR) from the distal intestine and kidney of mice. J Bone Miner Res. 2016;31(1):143–51.
39. Wasserman RH, Kallfelz FA. Vitamin D3 and unidirectional calcium fluxes across the rachitic chick duodenum. Am J Phys. 1962;203:221–4.
40. Fujita H, et al. Tight junction proteins claudin-2 and -12 are critical for vitamin D-dependent Ca2+ absorption between enterocytes. Mol Biol Cell. 2008;19(5):1912–21.
41. Kutuzova GD, Deluca HF. Gene expression profiles in rat intestine identify pathways for 1,25-dihydroxyvitamin D(3) stimulated calcium absorption and clarify its immunomodulatory properties. Arch Biochem Biophys. 2004;432(2):152–66.
42. Bikle DD. Vitamin D and bone. Curr Osteoporos Rep. 2012;10(2):151–9.
43. Yasuda H, 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(7):3597–602.
44. Prince CW, Butler WT. 1,25-Dihydroxyvitamin D3 regulates the biosynthesis of osteopontin, a bone-derived cell attachment protein, in clonal osteoblast-like osteosarcoma cells. Coll Relat Res. 1987;7(4):305–13.

45. Fretz JA, et al. 1,25-Dihydroxyvitamin D3 regulates the expression of low-density lipoprotein receptor-related protein 5 via deoxyribonucleic acid sequence elements located downstream of the start site of transcription. Mol Endocrinol. 2006;20(9):2215–30.
46. Boros S, Bindels RJ, Hoenderop JG. Active Ca(2+) reabsorption in the connecting tubule. Pflugers Arch. 2009;458(1):99–109.
47. Lambers TT, et al. Calbindin-D28K dynamically controls TRPV5-mediated Ca2+ transport. EMBO J. 2006;25(13):2978–88.
48. de Groot T, et al. Parathyroid hormone activates TRPV5 via PKA-dependent phosphorylation. J Am Soc Nephrol. 2009;20(8):1693–704.
49. Ensrud KE, 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(5):345–53.
50. van Abel M, et al. Age-dependent alterations in Ca2+ homeostasis: role of TRPV5 and TRPV6. Am J Physiol Ren Physiol. 2006;291(6):F1177–83.
51. 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(1):51–8.
52. Cauley JA, et al. Serum 25-hydroxyvitamin D concentrations and risk for hip fractures. Ann Intern Med. 2008;149(4):242–50.
53. 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(5):1118–23.
54. 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(9):3971–82.
55. Johnson JA, et al. Age and gender effects on 1,25-dihydroxyvitamin D3-regulated gene expression. Exp Gerontol. 1995;30(6):631–43.
56. Francis RM, et al. Calcium malabsorption in elderly women with vertebral fractures: evidence for resistance to the action of vitamin D metabolites on the bowel. Clin Sci (Lond). 1984;66(1):103–7.
57. Ebeling PR, 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(1):176–82.
58. Wood RJ, et al. Intestinal calcium absorption in the aged rat: evidence of intestinal resistance to 1,25(OH)2 vitamin D. Endocrinology. 1998;139(9):3843–8.
59. Ross AC, 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(1):53–8.
60. Gallagher JC. Vitamin D and aging. Endocrinol Metab Clin N Am. 2013;42(2):319–32.
61. Casagrande DS, et al. Changes in bone mineral density in women following 1-year gastric bypass surgery. Obes Surg. 2012;22(8):1287–92.
62. Fleischer J, et al. The decline in hip bone density after gastric bypass surgery is associated with extent of weight loss. J Clin Endocrinol Metab. 2008;93(10):3735–40.
63. Schafer AL, et al. Intestinal calcium absorption decreases dramatically after gastric bypass surgery despite optimization of vitamin D status. J Bone Miner Res. 2015;30(8):1377–85.
64. Abegg K, et al. Roux-en-Y gastric bypass surgery reduces bone mineral density and induces metabolic acidosis in rats. Am J Phys Regul Integr Comp Phys. 2013;305(9):R999–R1009.
65. Canales BK, et al. Gastric bypass in obese rats causes bone loss, vitamin D deficiency, metabolic acidosis, and elevated peptide YY. Surg Obes Relat Dis. 2014;10(5):878–84.
66. Bernstein CN, Leslie WD. The pathophysiology of bone disease in gastrointestinal disease. Eur J Gastroenterol Hepatol. 2003;15(8):857–64.