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REVIEW

Functions of vitamin D in bone

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Abstract
Vitamin D, synthesized in the skin or absorbed from the diet, undergoes multi-step enzymatic conversion to its active form, 1,25-dihydroxy vitamin D [1,25$(OH)_2$D], followed by interaction with the vitamin D receptor (VDR), to modulate target gene expression. Loss-of function mutations in the genes encoding the enzymes regulating these processes, or in the VDR, result in human diseases, which have demonstrated the paramount role of 1,25$(OH)_2$D in mineral and skeletal homeostasis. Mouse genetics has been used to create disease phenocopies which have produced considerable insight into the mechanisms of 1,25$(OH)_2$D regulation of mineral and skeletal metabolism. Hypophosphatemia resulting from 1,25$(OH)_2$D deficiency or resistance can inhibit apoptosis in hypertrophic chondrocytes leading to abnormal development of the cartilaginous growth plate in rickets. Decreased 1,25$(OH)_2$D may also cause decreased vascular invasion associated with reduced chondroclast and osteoclast activity and thereby contribute to growth plate abnormalities. Reduced 1,25$(OH)_2$D-mediated intestinal and renal calcium transport can reduce calcium availability, increase parathyroid hormone secretion and phosphaturia, and impair mineral availability for normal matrix mineralization, resulting in reduced growth plate mineralization and osteomalacia. 1,25$(OH)_2$D may exert an anabolic effect in bone, apparently via the VDR in mature osteoblasts, by increasing osteoblast activity and reducing osteoclast activity. High ambient levels of exogenous 1,25$(OH)_2$D, or of elevated endogenous 1,25$(OH)_2$D in the presence of reduced calcium balance, can enhance bone resorption, and apparently prevent mineral deposition in bone. These actions demonstrate the critical role of vitamin D in regulating skeletal homeostasis both indirectly and directly via the 1,25$(OH)_2$D/VDR system.

Keywords Vitamin D · Mineral homeostasis · Cartilaginous growth plate · Bone formation · Bone resorption

Introduction

Vitamin D_3 (cholecalciferol) may be synthesized endogenously in the skin via ultraviolet irradiation of a precursor, 7-dehydrocholesterol, which can be photoconverted to previtamin D_3 and then to vitamin D_3, which enters the bloodstream. Alternatively, vitamin D may be absorbed from the diet, in the gut, as vitamin D_3 or as vitamin D_2 (ergocalciferol) and can then enter the bloodstream. Vitamin D (D_2 or D_3), from either endogenous or exogenous sources, circulates in the blood bound to vitamin D binding protein (DBP) and is converted in the liver, mainly via, a cytochrome P450 mixed-function oxidase (CYP) with 25-hydroxylase activity, termed CYP2R1, to 25-hydroxyvitamin D (25OHD). 25OHD, bound to DBP, is then transported to the kidney where it can be 1alpha-hydroxylated by CYP27B1, a mitochondrial CYP with 1-hydroxylase (1OHase) activity, to produce the bioactive hormonal moiety, 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. Alternatively, 25OHD may be 24-hydroxylated in the kidney by CYP24A1, a 24-hydroxylase (24OHase), to 24,25-dihydroxyvitamin D [24,25$(OH)_2$D], which is the first step in the eventual degradation of vitamin D to calcitroic acid. 1,25$(OH)_2$D may also be converted to 1,24,25 by the 24OHase (Bikle et al. 2014). Both the renal 1OHase and the renal 24OHase are tightly regulated by hormones and ions. Extra-renal 1OHase and 24OHase activity have also been reported in a number of tissues; however, the most important site of synthesis and

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degradation of the active form of vitamin D, $1,25(OH)_2D$ is the kidney.

The biological action of $1,25(OH)_2D$ is mediated by the vitamin D receptor (VDR), a member of the steroid receptor family (Pike and Christakos 2017).The VDR contains a DNA-binding domain (DBD) and a ligand-binding domain (LBD). $1,25(OH)_2D$ binding to VDR induces a conformational change that facilitates interaction with the retinoid X receptor (RXR) and coregulatory complexes, and binding to specific DNA sequences [vitamin D response elements (VDREs)], thus resulting in the transcription of target genes. The actions of $1,25(OH)_2D$ involve regulation of gene activity at a range of locations, often many kilobases from the transcription start site of target genes.

Loss-of-function mutations in the VDR (Malloy et al. 1999, 2006) or overexpression of a protein that binds to the VDRE and prevents interaction with the VDR (Chen et al. 2003) are both characterized by resistance to the action of $1,25(OH)_2D$, and result in the recessive disorders Hereditary Vitamin D-Resistant Rickets (HVDRR) A and B [also called Vitamin D-Dependent Rickets, type 2A (VDDR2A) and VDDR2B, respectively]. A loss-of-function mutation in CYP27B1 is characterized by the inability to synthesize $1,25(OH)_2D$, and results in the autosomal recessive disease Pseudodeficiency Rickets [also called Vitamin D-Dependent Rickets, type 1A(VDDR1A)] (Fu et al. 1997). Loss-of-function mutations in CYP2R1, characterized by decreased ability to synthesize 25OHD (Thacher et al. 2015), cause the recessive disorder Vitamin D-Dependent Rickets, type 1B (VDDR1B). Loss-of-function mutations of CYP24A1, characterized by impaired clearance of $1,25(OH)_2D$ (Schlingmann et al. 2011; Streeten et al. 2011), result in the disorder idiopathic infantile hypercalcemia (IIH). Targeted gene deletion in mice has produced phenocopies of these disorders (Yoshizawa et al. 1997; Li et al. 1998; Panda et al. 2001; Dardenne et al. 2001, St-Arnaud et al. 2000; Zhu et al. 2013) and shed considerable insight into vitamin D biology. What is striking about all these disorders is that they are all characterized most strikingly by abnormalities in mineral and skeletal homeostasis. Thus, although vitamin D has been implicated in many pleiotropic biological effects, these genetic disorders emphasize the importance of the mineral and bone actions of vitamin D irrespective of any other biological consequences.

Vitamin D and mineral homeostasis

A primary function of vitamin D, in the postnatal state, is to maintain calcium homeostasis by increasing intestinal absorption of calcium. This is especially critical in hypocalcemic situations (Fig. 1). Although much of this action occurs in the duodenum, distal segments of the intestine, in addition to the duodenum, appear to be targets of vitamin D action. Thus, when dietary calcium is low, $1,25(OH)_2D$ stimulates transcellular intestinal calcium transport by increasing the expression of the apical membrane calcium channel TRPV6 (transient receptor potential vanilloid 6) and calcium-binding protein calbindin-D9k. The extrusion of calcium across the basolateral membrane is mediated by PMCA1b (plasma membrane calcium ATPase 1b). Other calcium transporters may also be involved. When calcium intake is high, paracellular calcium transport predominates, but this pathway may also be regulated by $1,25(OH)_2D$. Thus, the paracellular-associated proteins including claudin-2 and claudin-12, which are transmembrane components of tight junctions,cadherin-17, a cell adhesion protein, and aquaporin 8, a tight junction channel, can also be regulated by $1,25(OH)_2D$ (Fig. 1).

In the kidney, distal tubular calcium re-absorption involves an active transcellular mechanism in which calcium enters the distal tubule cell through TRPV5, calcium is transferred in the cytoplasm by binding to calbindin-D9k and calbindin-D28k, and is extruded on the apical surface by NCX1 (the sodium/calcium exchanger 1) and plasma membrane calcium pump 1b. The $1,25(OH)_2D$/VDR system is known to enhance gene expression of several calcium

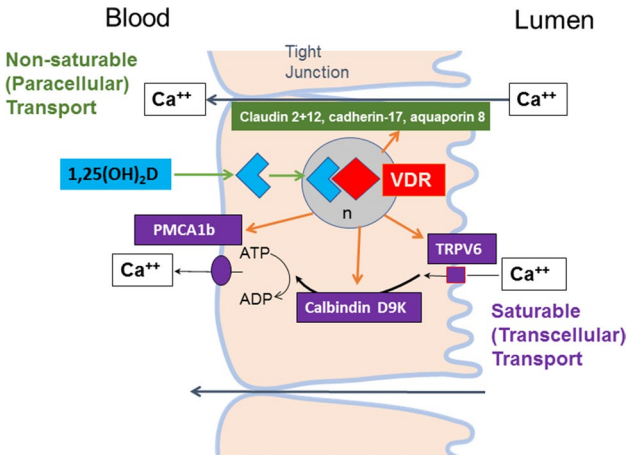

Fig. 1 $1,25(OH)_2D$/VDR-mediated calcium (Ca^{++}) transport in intestinal epithelium. $1,25(OH)_2D$ can stimulate saturable (transcellular) transport in the proximal small intestine. $1,25(OH)_2D$ enters the cell, binds to VDR in the nucleus (n), and increases gene transcription of the apical membrane Ca^{++} channel TRPV6, resulting in increased Ca^{++} uptake from the intestinal lumen into the cell. The $1,25(OH)_2D$/VDR system increases gene expression of calbindin 9 k, which can then shuttle Ca^{++} from the apical membrane to the basolateral membrane. The $1.25(OH)_2D$/VDR system can also upregulate the basolateral plasma membrane protein PMCA1b, facilitating Ca^{++} extrusion from the intestinal cell into the blood via an ATP-dependent process. $1,25(OH)_2D$/VDR signaling can also increase gene expression of the tight junction proteins claudin 2 and 12, cadherin-17.and aquaporin 8, and in this way facilitate passive, diffusional, and paracellular Ca^{++} fluxes across the intestine, particularly in the jejunum and ileum

transporters including Trpv5, calbindin-D9k, calbindin-D28k, and likely, NCX1 (Van Abel et al. 2003; Song et al. 2003).

The increased calcium absorbed from the intestine and reabsorbed from the kidney can facilitate skeletal mineralization, but can also activate the calcium sensing receptor in the parathyroid gland and inhibit release of parathyroid hormone (PTH), which is elevated in the presence of hypocalcemia, and results in secondary hyperparathyroidism. The elevated PTH can stimulate the renal 1(OH)ase to increase $1,25(OH)_2D$ when hypocalcemia prevails, whereas reduction of PTH after $1,25(OH)_2D$-mediated increases in extracellular calcium, subsequently decreases $1,25(OH)_2D$, achieving a new steady state (Fig. 2).

It has been suggested that $1,25(OH)_2D$ can also stimulate active phosphate absorption in the intestine; however, the mechanism involved is uncertain. In the kidney, elevated PTH can stimulate the internalization and lysosomal degradation of the sodium–phosphate cotransporters NPT2a and NPT2c in the proximal tubules (Bacic et al. 2006), thereby reducing proximal tubular phosphate re-absorption. Increased $1,25(OH)_2D$ can, therefore, indirectly enhance renal phosphate re-absorption by reducing the concentrations of circulating PTH. Nevertheless $1,25(OH)_2D$ can also enhance release of the phosphaturic factor, fibroblast growth factor (FGF)23 from osteocytes in bone (Fig. 2) (Nguyen-Yamamoto et al. 2017), which can decrease NPT2a and NPT2c in the proximal tubules and increase expression of αKlotho expression in the distal tubule (Forster et al. 2011), factors that facilitate renal phosphate loss, and this could negate the effects occurring via PTH.

Effects of reduced vitamin D status on the cartilaginous growth plate

Until after weaning, mice with targeted deletion of VDR, i.e., VDR−/− mice (which display vitamin D resistance), and with targeted deletion of 1α(OH)ase, i.e., 1α(OH)ase−/− mice (which manifest vitamin D deficiency), are normal in length and morphology and the mineral content of the growth plates and long bones are normal. However, after weaning, long bone growth and features of rickets such as expansion of the epiphyseal growth plate are observed, due to widening and disorganization of the hypertrophic zone with impaired apoptosis of hypertrophic chondrocytes. Low serum phosphate levels due to the vitamin D resistance or deficiency appear to decrease apoptosis of hypertrophic chondrocytes via the caspase-9-mediated mitochondrial pathway (Donohue and Demay 2002; Sabbagh et al. 2005)

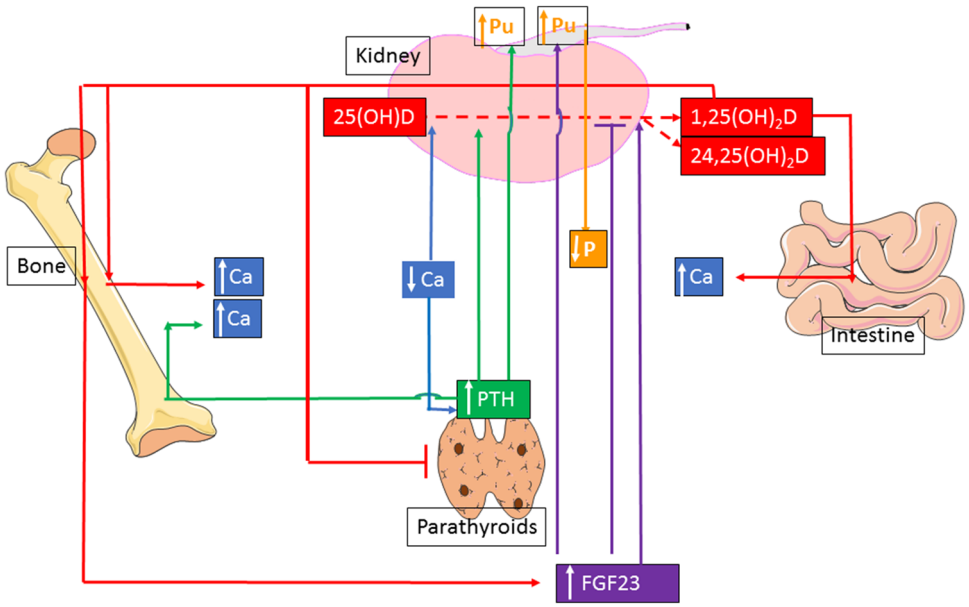

Fig. 2 Hormonal regulation of serum calcium (Ca) and phosphorus (P). A low serum calcium (↓Ca), due to negative calcium balance, can stimulate renal conversion of 25(OH)D to $1,25(OH)_2D$, and can stimulate the release of PTH from the parathyroid glands. The increased PTH (↑PTH) can further stimulate 25OHD conversion to $1,25(OH)_2D$ and can promote increased urine phosphorus (↑Pu), which can result in decreased serum phosphate (↓P). PTH and $1,25(OH)_2D$ can also produce bone resorption to elevate serum calcium (↑Ca). The elevated $1,25(OH)_2D$ can increase FGF23 release from bone, which can also enhance urine phosphorus and produce low serum phosphorus. The increased $1,25(OH)_2D$ can also enhance calcium absorption from intestine and elevate serum calcium. FGF23 will reduced conversion of 25OHD to $1,25(OH)_2D$ and increase $24,25(OH)_2D$, thus further reducing the elevated $1,25(OH)_2D$. The increased serum calcium will restore normocalcemia, thus reducing elevated $1,25(OH)_2D$, (which reduces increased FGF23), and inhibiting further PTH release

(Fig. 3), resulting in the widening and expansion of the growth plate. However, in addition, the production of chondroclasts and osteoclasts at the chondro-osseous junction may be defective due to decreased expression of receptor activator of nuclear factor κB ligand (RANKL) and due to decreased vascular endothelial growth factor (VEGF) and matrix metalloproteinase 9 leading to decreased vascular invasion; this leads to diminished removal of hypertrophic chondrocytes (Fig. 3). The enlargement of the cartilaginous growth plate, particularly the hypertrophic zone, may, therefore, be at least in part due directly to reduced activity of the $1,25(OH)_2D$/VDR system, resulting in altered cartilage growth plate remodeling (Lin et al. 2002).

Effect of reduced vitamin D status on mineralization

Mineralization of bone is a well-regulated multiphasic process (van Driel et al. 2017) Initially, calcium is taken up, via calcium-binding acidic phospholipids and calcium-binding proteins (Anderson 1995, 2003) by nano-sized extracellular matrix vesicles (EVs) that bud from the cell membrane of osteoblasts. The phosphate concentration inside the vesicles is regulated by phosphohydrolases such as alkaline phosphatase that use inorganic pyrophosphate as substrate. When the solubility product of calcium and phosphate is exceeded, hydroxyapatite (HA) mineral deposits are formed inside the extracellular vesicles. Subsequently, the preformed HA crystals are deposited (Anderson 2003) outside these vesicles and propagate in the extracellular matrix with a resulting accumulation of mineral (Ali et al. 1970; Anderson 1995).

A large number of other proteins may also contribute to mineralization (Thouverey et al. 2011; Xiao et al. 2007; Staines et al. 2014) either by promoting or inhibiting this process. Thus, in addition to alkaline phosphatase, bone-specific phosphatase PHOSPHO1 may augment mineralization by accumulating phosphate within the extra-cellular vesicles (Roberts et al. 2007; Stewart et al. 2006; Yadav et al. 2011). Important inhibitors are pyrophosphatase phosphodiesterase 1 (NPP1) and ankylosis protein (ANK). NPP1 generates pyrophosphate (PPi) and the transmembrane protein ANK allows PPi to pass through the plasma membrane to the extracellular matrix, therefore, inhibiting HA formation in the extracellular vesicles (Kim et al. 2010; Millan 2013). Osteopontin (Opn) is a highly phosphorylated extra-cellular matrix sialoprotein that has strong mineral-binding capacities and that may also inhibit mineralization. Nevertheless, the effects of vitamin D deficiency on reduced mineralization of the cartilage in the growth plate in rickets and the reduced mineralization of the bone matrix in osteomalacia may be indirect. In 1α(OH)ase−/− (Panda et al. 2004; Dardenne et al. 2003) and VDR−/− mice (Amling et al. 1999) maintained on a normal diet, hypocalcemia, hypophosphatemia, and secondary hyperparathyroidism are observed after weaning with typical histological features of rickets and osteomalacia, including, inadequate mineralization of cartilage, primary spongiosa, and cortical bone, and an increase in unmineralized osteoid in both trabecular and cortical bone. Treating these animals with a high calcium, high phosphate, high lactose "rescue" diet (lactose enhances intestinal absorption of these minerals) restored serum mineral and PTH levels to normal, and the growth plate mineralization was improved and osteoid was reduced. Similarly,

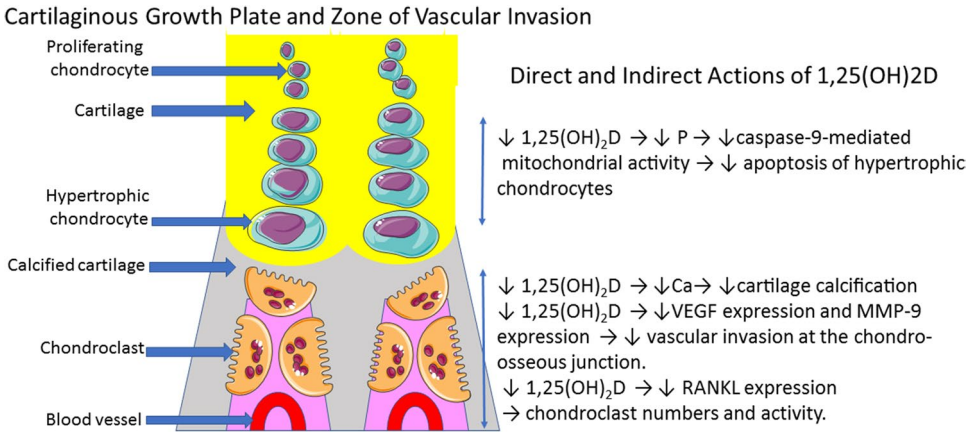

Fig. 3 Direct and indirect actions of $1,25(OH)_2D$ on the growth plate. Reduced (↓) $1,25(OH)_2D$ can result in diminished extracellular fluid phosphorus which can decrease apoptosis of hypertrophic chondrocytes via reducing activity of the caspase-mediated mitochondrial pathway. Reduced $1,25(OH)_2D$ can also reduce availability of calcium and diminish calcification of cartilage. Decreased $1,25(OH)_2D$ can result in decreased VEGF and decrease vascular invasion and also decrease RANKL resulting in reduced number and activity of chondroclasts/osteoclasts. These mechanisms combine to produce the rachitic growth plate changes of decreased $1,25(OH)_2D$ or resistance to $1,25(OH)_2D$

in humans, improvement of rickets and osteomalacia can be obtained even in patients with HVDRR after administering calcium intravenously (al-Aqeel et al. 1993). Therefore, the rachitic and osteomalacic changes appear to be due to an indirect effect of the $1,25(OH)_2D$/VDR system, mediated by reduced extracellular mineral levels because of the absence of $1,25(OH)_2D$ or VDR.

Osteomalacia has been reported in animal models where pronounced increases of $1,25(OH)_2D$ have been induced, including mice with deletion of VDR from the intestine of mice which are profoundly hypocalcemic, (Lieben et al. 2012) and mice with deletion of the 24OHase enzyme which is responsible for $1,25(OH)_2D$ clearance, and which are hypercalcemic (St-Arnaud et al. 2000). The very elevated $1,25(OH)_2D$ levels in the former model were associated with increased expression of ectonucleotide pyrophosphatase phosphodiesterase (Ennp)1 and Ennp3, which generate PPi from trinucleotides, and by increased levels of progressive ankylosis (Ank), which enhances the transport of PPi (Kim et al. 2010), thus increasing PPi levels and inhibiting mineralization (Millan 2013). $1,25(OH)_2D_3$ also increased expression of osteopontin, another potent mineralization inhibitor. It was suggested that the $1,25(OH)_2D_3$-mediated inhibition of bone matrix mineralization may contribute to preserving normal serum calcium levels (Lieben et al. 2012) in situations of severe hypocalcemia.

Effect of the $1,25(OH)_2D$/VDR system on bone turnover

Bone undergoes continuous turnover or remodeling throughout life, with osteoblastic bone formation building bone (an anabolic effect) and osteoclastic bone resorption removing bone (a catabolic effect). These actions are generally coupled, although not necessarily balanced. Cells of the osteoblast lineage are derived from mesenchymal stem cells in the bone marrow and differentiate through osteoblasts through to mature osteoblasts and osteocytes. Osteoclasts are derived from hematogenous precursors and result from fusion of mononuclear cells of the monocyte/macrophage lineage.

Effect of the $1,25(OH)_2D$/VDR system on bone formation

In both 1α(OH)ase−/− mice and VDR−/− mice that were hypocalcemic, hypophosphatemic, and exhibited secondary hyperparathryoidism, the numbers of osteoblasts, but not osteoclasts lining bone surfaces were increased and trabecular bone in the primary spongiosa was augmented, although the increased bone volume is largely due to increased unmineralized osteoid (Li et al. 1997; Panda et al. 2004; Dardenne et al. 2001). This suggested an uncoupling of bone turnover in the presence of a defective $1,25(OH)_2D$/VDR system, even though PTH, which can augment osteoclasts, was elevated. When the 1α(OH)ase−/− mice were then treated with a rescue diet, and serum calcium, phosphorus, and PTH were normalized, the amounts of mineralized bone increased but were below the levels seen in wild-type mice. In contrast, treatment of the 1(OH)ase−/− mice with $1,25(OH)_2D_3$ restored normal mineral and PTH levels and completely normalized their bones. Consequently, in these studies, normalizing calcium, phosphorus, and PTH appeared to unmask a requirement for the $1,25(OH)_2D$/VDR system for normal bone formation. In other studies using a model of 1α(OH)ase−/− mice, it was also concluded that the rescue diet did not appear as effective as $1,25(OH)_2D_3$ replacement therapy, since bone growth remained impaired (Dardenne et al. 2003; Panda et al. 2004); this was completely reversed by treatment with $1,25(OH)_2D_3$. Furthermore, $1,25(OH)_2D$ administered to young, genetically modified mice with deficiency in both $1,25(OH)_2D$ and PTH, promoted endochondral bone growth independent of PTH (Xue et al. 2005). Nevertheless, a rescue diet, which normalized calcium, phosphorus, and PTH, has been reported to completely normalize bones of Vdr−/− mice at 10 weeks relative to wild-type littermates fed regular chow (Amling et al. 1999); the source of the discrepant results in these models is unclear.

In humans with HVDRR, administration of calcium alone has been reported to normalize bone; however, treated HVDRR patients frequently exhibit residual low normal or low calcium and high normal or elevated serum PTH even after calcium administration (Tiosano et al. 2011). The extent to which remaining increases in circulating PTH contribute to the apparent normalization of bone mass remains uncertain.

A direct anabolic effect of the $1,25(OH)_2D$ system in bone is further supported by studies in a transgenic mouse model in which the VDR was expressed in late osteoblasts/early osteocytes (Gardiner et al. 2000). A major increase in cortical bone was observed associated with an increase in periosteal bone formation and a reduction in bone resorption. Trabecular bone was also increased (Triliana et al. 2016). Therefore, VDR activity in more mature osteoblasts appears to be both anabolic and anticatabolic. The VDR-induced anti-resorptive effect appears to be mediated by decreasing the ratio of the osteoclast-stimulating cytokine, receptor activator of nuclear factor kappaB ligand (RANKL) to its naturally occurring soluble decoy receptor termed osteoprotegerin (OPG). In contrast, the anabolic effect may be the result of increased expression of lipoprotein receptor-related protein (LRP)-5, a co-receptor in wingless (Wnt) signaling, a pathway known to mediate anabolic effects in osteoblasts

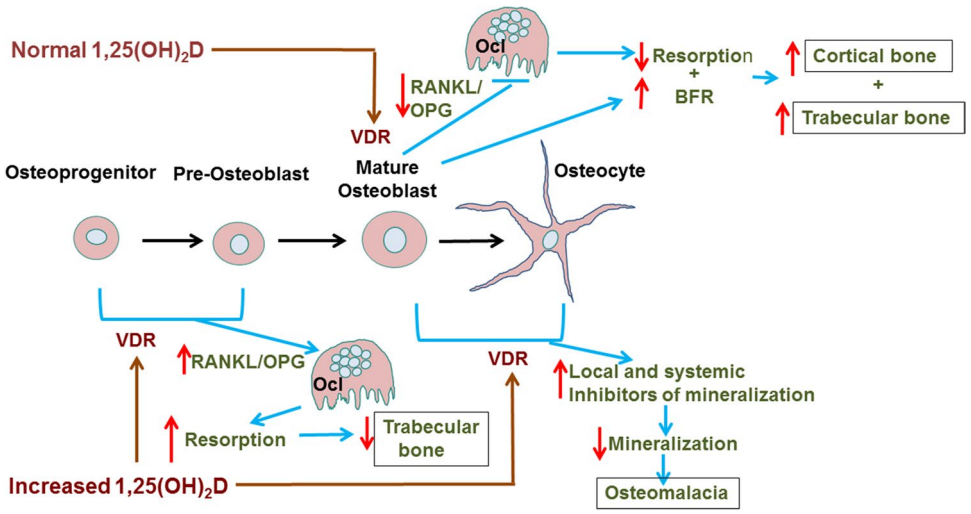

Fig. 4 Tentative scheme of direct actions of $1,25(OH)_2D$/VDR on bone. Normal levels of $1,25(OH)_2D$ act via the VDR in mature osteoblasts to decrease the ratio of RANKL/OPG and reduce osteoclastic bone resorption. As well, $1,25(OH)_2D$ action via the VDR in mature osteoblasts increases the bone formation rate (BFR). The net result is increased cortical and trabecular bone. Increased levels of $1,25(OH)_2D$ acting via the VDR in less mature osteoblasts may increase RANKL/OPG, stimulate osteoclastic bone resorption, and reduce trabecular bone. The action of high levels of $1,25(OH)_2D$ in mature osteoblasts and osteocytes can increase local and systemic inhibitors of osseous mineralization and decrease mineralization of bone leading to osteomalacia

(Fretz et al. 2006). Consequently, the $1,25(OH)_2D$/VDR system may exert anabolic effects in bone via its actions in late osteoblastic and osteocytic cells (Fig. 4).

Effect of vitamin D on bone resorption

$1,25(OH)_2D$ is a potent stimulator of osteoclastogenesis in vitro, and administration of high doses of $1,25(OH)_2D$ can bind the VDR in osteoblasts and increase expression and release of RANKL from osteoblasts (Kitazawa et al. 2008; Kim et al. 2006) and thus increase the ratio of RANKL to OPG. RANKL can bind to its cognate receptor RANK in osteoclasts, and increase osteoclast formation and action (Boyle et al. 2003). Increased endogenous $1,25(OH)_2D$ in the presence of hypocalcemia may also increase osteoclastogenesis to restore normocalcemia (Lieben et al. 2012). Studies of conditional deletion of the VDR from the osteoblast lineage suggest that early osteoblastic cells may mediate an increase in bone resorption induced by $1,25(OH)_2D$ (Yamamoto et al. 2013) (Fig. 4).

The effects of vitamin D on bone turnover may, therefore, depend on the state of calcium balance of the organism (Christakos et al. 2016) but may also depend on the stage of osteoblast differentiation which may determine the $1,25(OH)_2D$ response. Thus, there may be a differential impact of $1,25(OH)_2D$ on the transcriptomes of preosteoblastic and differentiated osteoblastic cells (St John et al. 2014) reflecting the dynamic changes occurring to the genome when cells differentiate. Furthermore, the relative numbers of less differentiated and more mature cells of the osteoblast lineage may change during growth and aging (Kim et al. 2017; Farr et al. 2016) and alter the nature of the action of $1,25(OH)_2D$ at different stages of the life cycle. Nevertheless, the resorptive action of $1,25(OH)_2D$ appears to be the most commonly occurring mechanism influencing skeletal economy.

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References

al-Aqeel A, Ozand P, Sobki S, Sewairi W, Marx S (1993) The combined use of intravenous and oral calcium for the treatment of vitamin D dependent rickets type II (VDDRII). Clin Endocrinol (Oxf) 39:229–237

Ali SY, Sajdera SW, Anderson HC (1970) Isolation and characterization of calcifying matrix vesicles from epiphyseal cartilage. Proc Natl Acad Sci USA 67:1513–1520

Amling M, Priemel M, Holzmann T, Chapin K, Rueger JM, Baron R, Demay MB (1999) 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 140:4982–4987

Anderson HC (1995) Molecular biology of matrix vesicles. Clin Orthop Relat Res 314:266–280

Anderson HC (2003) Matrix vesicles and calcification. Curr Rheumatol Rep 5:222–226

Bacic D, Lehir M, Biber J, Kaissling B, Murer H, Wagner CA (2006) The renal Na/phosphate cotransporter NaPi-IIa is internalized via the receptor-mediated endocytic route in response to parathyroid hormone. Kidney Int 69:495–503

Bikle DD (2014) Vitamin D metabolism, mechanism of action, and clinical applications. Chem Biol 21:319–329

Boyle WJ, Simonet WS, Lacey DL (2003) Osteoclast differentiation and activation. Nature 423:337–3429

Chen H, Hewison M, Hu B, Adams JS (2003) Heterogeneous nuclear ribonucleoprotein (hnRNP) binding to hormone response elements: a cause of vitamin D resistance. Proc Natl Acad Sci USA 100:6109–6114

Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G (2016) Vitamin D: metabolism, molecular mechanism of action, and pleiotropic effects. Physiol Rev 96:365–408

Dardenne O, Prud'homme J, Arabian A, Glorieux FH, St-Arnaud R (2001) Targeted inactivation of the 25-hydroxyvitamin D(3)-1(alpha)-hydroxylase gene (CYP27B1) creates an animal model of pseudovitamin D-deficiency rickets. Endocrinology 142:3135–3141

Dardenne O, Prudhomme J, Hacking SA, Glorieux FH (2003) 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 32:332–340

Donohue MM, Demay MB (2002) Rickets in VDR null mice is secondary to decreased apoptosis of hypertrophic chondrocytes. Endocrinology 143:3691–3694

Farr JN, Fraser DG, Wang H, Jaehn K, Ogrodnik MB, Weivoda MM, Drake MT, Tchkonia T, LeBrasseur NK, Kirkland JL, Bonewald LF, Pignolo RJ, Monroe DG, Khosla S (2016) Identification of senescent cells in the bone microenvironment. J Bone Miner Res 31:1920–1929

Forster RE, Jurutka PW, Hsieh JC, Haussler CA, Lowmiller CL, Kaneko I, Haussler MR, Kerr Whitfield G (2011) Vitamin D receptor controls expression of the anti-aging klotho gene in mouse and human renal cells. Biochem Biophys Res Commun 414:557–562

Fretz JA, Zella LA, Kim S, Shevde NK, Pike JW (2006) 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 20:2215–2230

Fu GK, Lin D, Zhang MY, Bikle DD, Shackleton CH, Miller WL, Portale AA (1997) Cloning of human 25-hydroxyvitamin D-1 alpha-hydroxylase and mutations causing vitamin D-dependent rickets type 1. Mol Endocrinol 11:1961–1970

Gardiner EM, Baldock PA, Thomas GP, Sims NA, Henderson NK, Hollis B, White CP, Sunn KL, Morrison NA, Walsh WR, Eisman JA (2000) Increased formation and decreased resorption of bone in mice with elevated vitamin D receptor in mature cells of the osteoblastic lineage. FASEB J 14:1908–1916

Kim S, Yamazaki S, Zella LA, Shevde NK, Pike JW (2006) Activation of receptor activator of NF-kappaB ligand gene expression by 1,25-dihydroxyvitamin D3 is mediated through multiple long-range enhancers. Mol Cell Biol 26:6469–6486

Kim HJ, Minashima T, McCarthy EF, Winkles JA, Kirsch T (2010) Progressive ankylosis protein (ANK) in osteoblasts and osteoclasts controls bone formation and bone remodeling. J Bone Miner Res 25:1771e1783

Kim HN, Chang J, Shao L, Han L, Iyer S, Manolagas SC, O'Brien CA, Jilka RL, Zhou D, Almeida M (2017) DNA damage and senescence in osteoprogenitors expressing Osx1 may cause their decrease with age. Aging Cell 16:693–703

Kitazawa R, Mori K, Yamaguchi A, Kondo T, Kitazawa S (2008) Modulation of mouse RANKL gene xpression by Runx2 and vitamin D3. J Cell Biochem 105:1289–1297

Li YC, Pirro AE, Amling M, Delling G, Baron R, Bronson R, Demay MB (1997) Targeted ablation of the vitamin D receptor: an animal model of vitamin D-dependent rickets type II with alopecia. Proc Natl Acad Sci USA 94:9831–9835

Li YC, Amling M, Pirro AE, Priemel M, Meuse J, Baron R, Delling G, Demay MB (1998) Normalization of mineral ion homeostasis by dietary means prevents hyperparathyroidism, rickets, and osteomalacia, but not alopecia in vitamin D receptor-ablated mice. Endocrinology 139:4391–4396

Lieben L, Masuyama R, Torrekens S, Van Looveren R, Schrooten J, Baatsen P, Lafage-Proust MH, Dresselaers T, Feng JQ, Bonewald LF, Meyer MB, Pike JW, Bouillon R, Carmeliet G (2012) Normocalcemia is maintained in mice under conditions of calcium ma-labsorption by vitamin D-induced inhibition of bone mineralization. J Clin Investig 122:1803–1815

Lin R, Amizuka N, Sasaki T, Aarts MM, Ozawa H, Goltzman D, Henderson JE, White JH (2002) 1Alpha,25-dihydroxyvitamin D3 promotes vascularization of the chondro-osseous junction by stimulating expression of vascular endothelial growth factor and matrix metalloproteinase 9. J Bone Miner Res 17:1604–1612

Malloy PJ, Pike JW, Feldman D (1999) The vitamin D receptor and the syndrome of hereditary 1,25-dihydroxyvitamin D-resistant rickets. Endocr Rev 20:156–188

Malloy PJ, Wang J, Peng L, Nayak S, Sisk JM, Thompson CC, Feldman D (2006) A unique insertion/duplication in the VDR gene that truncates the VDR causing hereditary 1,25-dihydroxyvitamin D-resistant rickets without alopecia. Arch Biochem Biophys 460:285–292

Millan JL (2013) The role of phosphatases in the initiation of skeletal mineralization. Calcif Tissue Int 93:299–306

Nguyen-Yamamoto L, Karaplis AC, St-Arnaud R, Goltzman D (2017) Fibroblast growth factor 23 regulation by systemic and local osteoblast-synthesized 1,25-dihydroxyvitamin D. J Am Soc Nephrol 28:586–597

Panda DK, Miao D, Tremblay ML, Sirois J, Farookhi R, Hendy GN, Goltzman D (2001) Targeted ablation of the 25-hydroxyvitamin D 1alpha-hydroxylase enzyme: evidence for skeletal, reproductive, and immune dysfunction. Proc Natl Acad Sci USA 98:7498–7503

Panda DK, Miao D, Bolivar I, Li J, Huo R, Hendy GN, Goltzman D (2004) Inactivation of the 25-hydroxyvitamin D 1α-hydroxylase and vitamin D receptor demonstrates independent and interdependent effects of calcium and vitamin D on skeletal and mineral homeostasis. J Biol Chem 279:16754–16766

Pike JW, Christakos S (2017) Biology and mechanisms of action of the vitamin D hormone. Endocrinol Metab Clin N Am 46:815–843

Roberts S, Narisawa S, Harmey D, Millan JL, Farquharson C (2007) Functional involvement of PHOSPHO1 in matrix vesicle-mediated skeletal mineralization. J Bone Miner Res 22:617–627

Sabbagh Y, Carpenter TO, Demay MB (2005) Hypophosphatemia leads to rickets by impairing caspase-mediated apoptosis of hypertrophic chondrocytes. Proc Natl Acad Sci USA 102:9637–9642

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

Song Y, Peng X, Porta A, Takanaga H, Peng JB, Hediger MA, Fleet JC, Christakos S (2003) Calcium transporter 1 and epithelial calcium channel messenger ribonucleic acid are differentially regulated by 1,25 dihydroxyvitamin D3 in the intestine and kidney of mice. Endocrinology 144:3885–3894

St John HC1, Bishop KA, Meyer MB, Benkusky NA, Leng N, Kendziorski C, Bonewald LF, Pike JW (2014) The osteoblast to osteocyte transition: epigenetic changes and response to the vitamin D3 hormone. Mol Endocrinol 28:1150–1165

Staines KA, Zhu D, Farquharson C, MacRae VE (2014) Identification of novel regulators of osteoblast matrix mineralization by time series transcriptional profiling. J Bone Miner Metab 32:240–251

St-Arnaud R, Arabian A, Travers R, Barletta F, Raval-Pandya M, Chapin K, Depovere J, Mathieu C, Christakos S, Demay MB,

Glorieux FH (2000) 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 141:2658–2666

Stewart AJ, Roberts SJ, Seawright E, Davey MG, Fleming RH, Farquharson C (2006) The presence of PHOSPHO1 in matrix vesicles and its developmental expression prior to skeletal mineralization. Bone 39:1000–1007

Streeten EA, Zarbalian K, Damcott CM (2011) CYP24A1 mutations in idiopathic infantile hypercalcemia. N Engl J Med 365:1741–1742

Thacher TD, Fischer PR, Singh RJ, Roizen J, Levine MA (2015) CYP2R1 mutations impair generation of 25-hydroxyvitamin D and cause an atypical form of vitamin D deficiency. J Clin Endocrinol Metab 100:E1005–E1013

Thouverey C, Malinowska A, Balcerzak M, Strzelecka-Kiliszek A, Buchet R, Dadlez M, Pikula S (2011) Proteomic characterization of biogenesis and functions of matrix vesicles released from mineralizing human osteoblast-like cells. J Proteom 74:1123–1134

Tiosano D, Hadad S, Chen Z, Nemirovsky A, Gepstein V, Militianu D, Weisman Y, Abrams SA (2011) Calcium absorption, kinetics, bone density, and bone structure in patients with hereditary vitamin D-resistant rickets. J Clin Endocrinol Metab 96:3701–3709

Triliana R, Lam NN, Sawyer RK, Atkins GJ, Morris HA, Anderson PH (2016) Skeletal characterization of an osteoblast-specific vitamin D receptor transgenic (ObVDR-B6) mouse model. J Steroid Biochem Mol Biol 164:331–336

van Driel M, van Leeuwen JPTM. (2017) Vitamin D endocrinology of bone mineralization. Mol Cell Endocrinol 453:46–51

Van Abel M, Hoenderop JG, van der Kemp AW, van Leeuwen JP, Bindels RJ (2003) Regulation of the epithelial Ca^{2+} channels in small intestine as studied by quantitative mRNA detection. Am J Physiol Gastrointest Liver Physiol 285:G78–G85

Xiao Z, Camalier CE, Nagashima K, Chan KC, Lucas DA, de la Cruz MJ, Gignac M, Lockett S, Issaq HJ, Veenstra TD, Conrads TP, Beck GR Jr (2007) Analysis of the extracellular matrix vesicle proteome in mineralizing osteo-blasts. J Cell Physiol 210:325–335

Xue Y, Karaplis AC, Hendy GN, Goltzman D, Miao D (2005) Genetic models show that parathyroid hormone and 1,25-dihydroxyvitamin D3 play distinct and synergistic roles in postnatal mineral ion homeostasis and skeletal development. Hum Mol Genet 14:1515–1528

Yadav MC, Simao AM, Narisawa S, Huesa C, McKee MD, Farquharson C, Millan JL (2011) Loss of skeletal mineralization by the simultaneous ablation of PHOSPHO1 and alkaline phosphatase function: a unified model of the mechanisms of initiation of skeletal calcification. J Bone Miner Res 26:286–297

Yamamoto Y, Yoshizawa T, Fukuda T, Shirode-Fukuda Y, Yu T, Sekine K, Sato T, Kawano H, Aihara K, Nakamichi Y, Watanabe T, Shindo M, Inoue K, Inoue E, Tsuji N, Hoshino M, Karsenty G, Metzger D, Chambon P, Kato S, Imai Y (2013) Vitamin D receptor in osteoblasts is a negative regulator of bone mass control. Endocrinology 154:1008–1020

Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, Kawakami T, Alioka K, Sato H, Uchiyama Y, Masushige S, Fukamizu A, Matsumoto T, Kato S (1997) Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat Genet 16:391–396

Zhu JG, Ochalek JT, Kaufmann M, Jones G, Deluca HF (2013) CYP2R1 is a major, but not exclusive, contributor to 25-hydroxyvitamin D production in vivo. Proc Natl Acad Sci USA 110:15650–15655