CHAPTER TWO

Vitamin D and bone health: What vitamin D can and cannot do

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Abstract

Historically vitamin D deficiency had devastating consequences for children causing rickets resulting in severe bone deformities often leading to death. The mystery of the cause of rickets finally came to light when it was observed that cod liver oil and sunlight could prevent and cure rickets. The first vitamin D to be discovered was vitamin D_2 from ergosterol in ultraviolet irradiated yeast. Vitamin D_3 was discovered from UV exposure to the skin. Investigations revealed the two major functions of vitamin D were to increase intestinal calcium and phosphate absorption and mobilize calcium from the skeleton to maintain calcium and phosphorus homeostasis. Later studies demonstrated that vitamin D does not have an active role in bone mineralization. Vitamin D deficiency results in secondary hyperparathyroidism increasing bone resorption. As a result, this decreases bone mineral content and compromises the architectural integrity increasing risk for fracture. Vitamin D deficiency has also been shown to enhance aging of the bone causing cracks and enhancing bone fractures. Vitamin D deficiency also causes osteomalacia. Therefore, vitamin D sufficiency is extremely important to maximize bone health throughout life. It helps to prevent bone loss, but it cannot restore bone loss due to increased bone resorption that can occur under a variety of circumstances including menopause. The Endocrine Society Guidelines recommends for all ages that adequate

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vitamin D obtained from the sun, foods and supplements is necessary in order to maintain a circulating concentration of 25-hydroxyvitamin D of at least 30 ng/mL for maximum bone health.

1. Historical perspective

Rickets is a devastating crippling bone deforming disorder that affects children. There is some evidence that this bone deforming disorder was recognized as early as 900 BC by Homer (Pettifor Thandrayen & Thacher, 2024). Rickets was a common occurrence in London in the mid-16th century when Daniel Whistler in 1645 and Francis Glisson in 1650 published their observations and concluded that this distinct disorder that caused a variety of skeletal deformities including enlargement of the joints of the rib cage (costochondral junctions, rachitic rosary) and long bones including wrists and knees, curvature of the legs and spine, short stature, severe muscle weakness that often led to their early demise (Holick, 2006, 2023; Wacker & Holick, 2013). When the industrial revolution began around 1760 there was a dramatic transition from small cottage industries that produced goods often by hand in small factories using steam and waterpower to large factories using coal burning as the major energy source to mass-produce goods. Buildings were built in close proximity and the coal burning factories belched out a pall of black smoke that ultimately was recognized as the major cause of childhood rickets. The first insight into this association was made by Sniadecki in 1822. He recognized that children living in the inner city of Warsaw was plagued with rickets whereas children living in the farming communities outside of Warsaw had little evidence for it. He concluded that it was strong and obvious that sun exposure was important for the cure of rickets and the frequent occurrence of the disease in densely populated towns where the streets are narrow and poorly lit (Mozolowski, 1939; Wacker & Holick, 2013). Palm (1890) appreciated that rickets was common in relatively well-off London whereas he received reports from his colleagues in Asia informing him that rickets was uncommon. He not only concluded that the likely difference is that in London children were not afforded an opportunity to be exposed to any sunlight. He urged sunbathing as a means of preventing rickets. It was Huldschinsky (1919), (Huldschinsky, 1928) who remarkably decided to expose rachitic children to a mercury arc lamp and demonstrated a dramatic radiologic improvement in rickets with marked increased mineralization of the wrists based on x-ray evaluation after only 6 weeks. (Fig. 1)

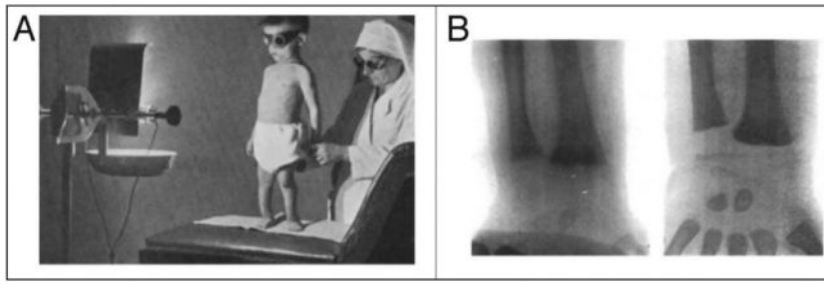

Fig. 1 UV radiation therapy for rickets. (A) Photograph from the 1920s of a child with rickets being exposed to artificial UV radiation. (B) Radiographs demonstrating florid rickets of the hand and wrist (left). The same hand and wrist taken after a course of treatment with 1-hour UV radiation 2 times/week for 6 week showing mineralization of the carpal bones and epiphyseal plates (right). Holick, copyright 2006. Reproduced with permission.

He also followed children at the same time not receiving his ultraviolet radiation (UVR) therapy and saw no improvement in their x-rays. He also cleverly concluded that exposure to UVR was producing a substance in the skin that could affect the entire skeleton when he demonstrated that exposure of an arm of a child with rickets resulted in the same radiologic benefit in the other arm. This observation was quickly followed by Hess & Unger (1921) who reported that children in New York City that were exposed to sunlight on the roof of their hospital showed dramatic improvement in their rickets. They also observed that children of color were at higher risk for rickets and concluded that melanin in the skin was reducing the effectiveness of sunlight in preventing and curing rickets thereby requiring longer exposure times to have the same effect on treating and curing rickets compared to fair skinned children (Hess 1929). Chick & Roscoe (1926), Pettifor et al. (2024) and Hess (1936) appreciated that rickets was much more common especially at the end of winter. Hess & Lundagen (1922) also noted that at the end of winter the serum phosphate levels were at their lowest and concluded that this was an important biochemical marker for rickets. It was also observed that cod liver oil could cure rickets in rats (Mellanby, 1976; Mellanby, 1918). The observation that aerated cod liver oil that was heated, which destroyed the vitamin A, resulted in the realization that it was not vitamin A that had antirachitic activity which it was thought to have at the time but a new vitamin that McCollum (McCollum, Simmonds, Becker, & Shipley, 1922) named this new nutrient as vitamin D. There was confusion as to whether sunlight or

cod liver oil had the same antirachitic properties until Powers (Powers et al 1921; Holick, 2006, 2023; Wacker & Holick, 2013; Pettifor et al., 2024) demonstrated that rachitic rats that received cod liver oil or UVR exposure had the same effect on resolving their rickets. These new revelations prompted Hess (Hess & Weinstock, 1924) and Steenbock (Haman & Steenbock, 1935) to irradiate foods with UVR and demonstrated that this process provided antirachitic activity. Steenbock patented the process for the UV irradiation of foods as a means of increasing their antirachitic properties. It was concluded that an inexpensive method to produce vitamin D/antirachitic activity was to irradiate yeast with UVR. As a result, milk was originally fortified with yeast exposed to UVR. This process provided the necessary antirachtic activity that was effective in preventing and treating rickets in children. Ultimately ergosterol recovered from yeast was irradiated and the irradiated product was directly added to milk to produce antirachitic activity (Haman & Steenbock, 1935; Holick, 2023; Wacker & Holick, 2013). The practice of fortification of milk with vitamin D to prevent rickets was quickly adopted in the United States and in England and other Northern European countries. The fortification of vitamin D became so popular that soap, soft drinks, shaving cream and even beer was fortified with vitamin D (Wacker & Holick, 2013). Parents were able to purchase a mercury arc lamp from their local pharmacy and used it several times a week exposing their children to UV radiation to prevent them from getting rickets (Holick, 2006, 2023; Wacker & Holick, 2013; Holick 2023). The popularity of fortifying a wide variety of foods and other products with vitamin D quickly came to a halt when there were several reports of infants in the early 1950s who not only had hypercalcemia but also had altered facial features, mild mental retardation and supravalvular aortic stenosis. This generated great hysteria, and The Royal College of Physicians and the British Pediatric Association were asked to investigate and concluded that the most likely cause was over fortification of milk with vitamin D (Vitamin D Toxicity 1964 Holick, 2015). This was based on literature that reported that when pregnant rodents were provided high doses of vitamin D their pups had many characteristics of the infants including altered facial features, supravalvular aortic stenosis and hypercalcemia. Since it was difficult to monitor the vitamin D content in fortified foods it was decided that the best way to prevent this from ever happening is to pass legislation forbidding the fortification of any food or personal use products with vitamin D. The concept was quickly adapted throughout Europe and most of the world except for the United States and Canada. Today this ban continues in many countries.

However, Sweden, Finland, India now permit some foods to be fortified with vitamin D (Holick, 2015, 2023). Is now recognized that the likely cause for infantile hypercalcemia in infants with elfin facies and supravalvular aortic stenosis was due to the rare genetic disorder, Williams syndrome (Holick, 2015, 2023; Pober, 2010). The hypercalcemia is due to the fact that those afflicted with Williams syndrome have a hypersensitivity to vitamin D.

2. Mechanisms for how vitamin D influences bone health

By 1940 the scourge of rickets had been essentially eliminated in industrialized countries as a result of the appreciation that adequate sun exposure, exposure to ultraviolet B radiation from an in-home device and or ingestion of foods with vitamin D provided enough of the vitamin to do its magic that is mineralize children's bones (Holick, 2023). It was also recognized that children with rickets had low concentrations of calcium and phosphate in their blood and that children receiving vitamin D demonstrated significant improvement of both their serum calcium and phosphate concentrations (Hess, 1936). One of the first insights as to how vitamin D improved rickets was the observation by Orr in 1923. He exposed vitamin D deficient rats to UVR and evaluated fecal excretion of calcium compared to rats that were untreated with UVR. He observed a decreased amount of calcium in the feces of rats exposed to UVR compared to rats that were not treated with UVR. They concluded that this was because of vitamin D increasing the efficiency of dietary calcium (Nicolaysen, 1937a, 1937b). By the early 1920s investigation into calcium and phosphate metabolism in children revealed that there was a change in phosphate metabolism that occurred earlier than calcium metabolism. It was thought that vitamin D may be acting primarily on phosphate metabolism. These early insightful observations were confirmed 10 years later by Nicolaysen et al (Nicolaysen, 1937a, 1937b). They demonstrated that vitamin D stimulated intestinal calcium and phosphate absorption in vitamin D deficient rats. It was also assumed at the time that vitamin D was also playing an important role in the mineralization of the skeleton. However, when vitamin D deficient rats were given a high calcium diet and injected with phosphate they were found to have normal bone mineralization. This was confusing so it was uncertain if vitamin D played any role directly in bone metabolism. In 1952 Carlsson (Carlsson, 1952)

reported that contrary to what would be expected of vitamin D's effect on bone metabolism that is enhancing calcium phosphate deposition into the skeleton, he observed the opposite. Rats on a low calcium diet receiving vitamin D raised their blood calcium concentrations (Carlsson, 1952). These results demonstrated that when there is inadequate calcium available from the diet vitamin D will go to the skeleton to increase calcium mobilization. Therefore, it was concluded that the function of vitamin D for bone metabolism was to increase intestinal calcium and phosphate absorption to raise and maintain serum concentrations of calcium and phosphate into a normal range that would permit the passive mineralization of the skeleton. These experiments however did not answer a fundamental question which was does vitamin D actively participate in the mineralization process once there is an adequate calcium phosphate product. We initially reported that Holzmann rats fed a vitamin D deficient diet containing lactose and high concentrations of calcium and phosphorus for 4 weeks maintained a normal serum calcium and normal serum phosphate despite undetectable 25-hydroxyvitamin D [25(OH)D] (Holtrop, Cox, Carnes, & Holick, 1986). This was not unexpected. However since these rats develop secondary hyperparathyroidism thereby increasing the renal production of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] from the extremely low concentrations of 25(OH)D. This is due to the fact that 1,25$(OH)_2$Dcirculates at 1000 times less concentration than 25(OH)D. Underwood and DeLuca (Underwood, Phelps, & DeLuca, 1984) raised rats on a vitamin D deficient diet for 5.5 weeks and then infused them with solutions of calcium and phosphate for 10 days to regain normal serum calcium and phosphate concentrations. These rats had normal widths of the epiphyseal plates and normal osteoid seams. However, the question remained since it is quite possible even at 5.5 weeks that there was circulating concentrations of 1,25$(OH)_2$D that could influence bone mineralization and chondrocyte maturation. Holtrop et al. (1986) fed Holzmann rats at one week of pregnancy a vitamin D deficient diet containing 20% lactose, 1% calcium and 0.4% phosphorus. The pups after weaning were maintained on a vitamin D deficient diet with varying concentrations of calcium and phosphorus with or without lactose whereas the control rats were fed a vitamin D deficient diet containing 0.44% calcium and 0.3% phosphorus and supplemented with 125 ng of vitamin D_3 orally 5 times a week or they were fed formula lab chow diet that was commercially available. The pups were sacrificed at 5, 6 or 7 wk of age. At 5 weeks of age blood was collected and it was observed that circulating concentrations of

25(OH)D was < 3 ng/mL and $1,25(OH)_2D$ < 10 pg/mL whereas pups that were supplemented with vitamin D had circulating concentrations of $1,25(OH)_2D$ of 183 pg/mL. The serum calcium and phosphate levels were normal in both groups. Their bones were collected and stained with von Kossa staining. A histologic evaluation revealed two important observations. The first is that the vitamin D deficient pups on the high calcium, high phosphorus lactose diet demonstrated that the trabeculae at the growth plate had normal mineralization but that the epiphyseal plates were somewhat widened. These pups had normal serum calcium but low serum phosphorus concentrations. The second important observation was that pups that received higher dietary phosphate demonstrated that the serum phosphate rose almost to normal, and the width of the epiphyseal plates appeared normal with normal mineralization of the trabeculae. To be certain that parathyroid hormone was also not having a direct effect on bone mineralization a similar study was done in pups that at 4 weeks had thyroparathyroid surgery. Histologic evaluation of their long bones revealed that neither parathyroid hormone (PTH) nor calcitonin influenced bone mineralization or chondrocyte maturation. The authors concluded that maintaining vitamin D deficient rats on a diet that maintained normal serum concentrations of calcium and phosphorus in the absence of calcium regulating hormones including PTH, calcitonin and $1,25(OH)_2D$ prevented the development of rickets including decreased chondrocyte maturation leading to widened epiphyseal plates and normal mineralization (Holtrop et al., 1986).

3. Vitamin D and bone biology

Bone is a dynamic tissue that is constantly remodeling. An alteration in bone remodeling such as vitamin D deficiency can lead to osteomalacia, osteosclerosis and osteoporosis (Nakamichi, Takahashi & Udagawa, 2024). It is well recognized that vitamin D has no influence directly on calcium and phosphate metabolism. Vitamin D (represents vitamin D_2 and/or vitamin D_3) must undergo sequential hydroxylations first in the liver to 25(OH)D and then in the kidneys to $1,25(OH)_2D$ (Fig. 2). $1,25(OH)_2D$ interacts with its nuclear receptor in calcium regulating tissues including intestine, bone, kidneys, and parathyroid glands (Charoenngam, Shirvani, & Holick, 2019a; Holick, 2007). $1,25(OH)_2D$ interacts with the vitamin D receptor in the small intestine resulting in an increase in calcium and phosphate absorption. The maintenance of a normal serum calcium and phosphate (calciumXphosphate product) results in the crystallization and

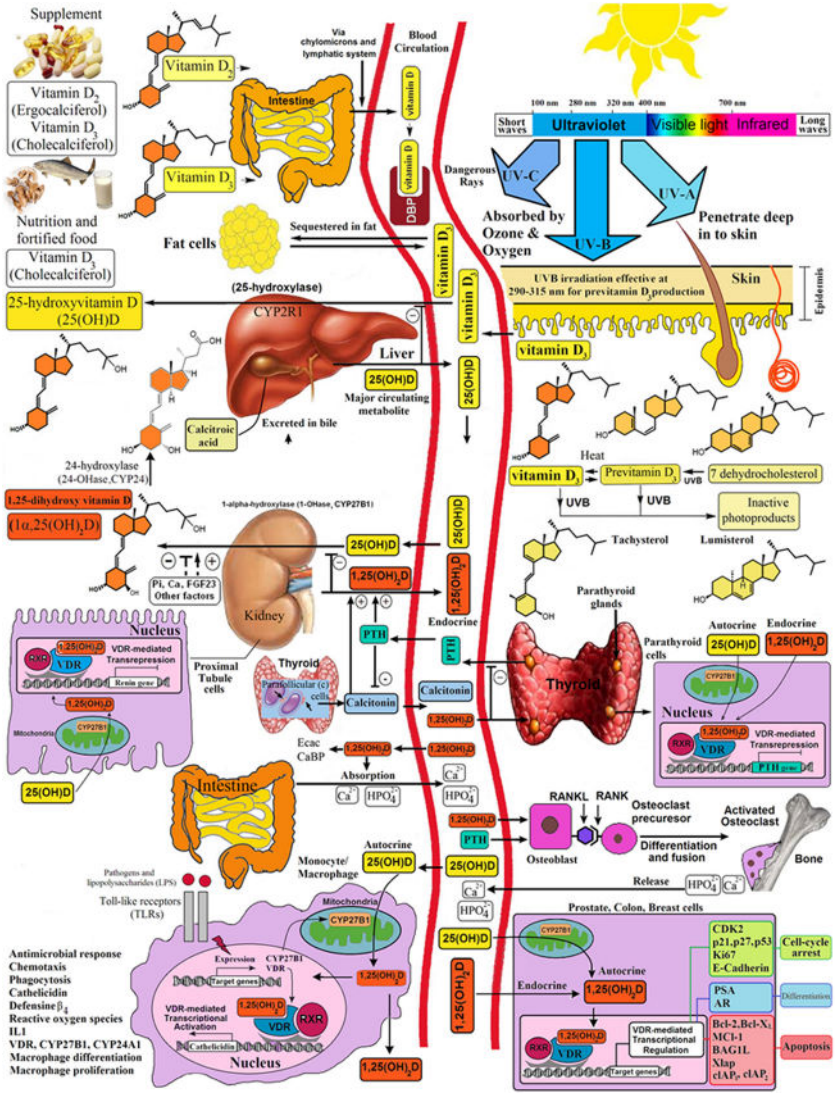

Fig. 2 Schematic representation of the synthesis and metabolism of vitamin D for skeletal and nonskeletal function. *1-OHase*, 25-hydroxyvitamin D-1α-hydroxylase; *24-OHase*, 25-hydroxyvitamin D-24-hydroxylase; *25(OH)D*, 25-hydroxyvitamin D; *1,25(OH)2D*, 1,25-dihydroxyvitamin D; *CaBP*, calcium-binding protein; *CYP27B1*, Cytochrome P450–27B1; *DBP*, vitamin D–binding protein; *ECaC*, epithelial calcium channel; *FGF-23*, fibroblast growth factor-23; *PTH*, parathyroid hormone; *RANK*, receptor activator of the NF-kB; *RANKL*, receptor activator of the NF-kB ligand; *RXR,* retinoic acid receptor; *TLR2/1*, Toll-like receptor 2/1; *VDR,* vitamin D receptor; *vitamin D,* vitamin D_2 or v–itamin D_3. Copyright Holick 2013, reproduced with permission.

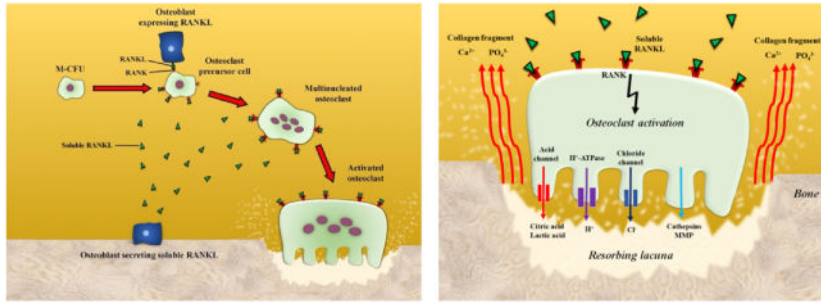

Fig. 3 Left hand panel. In vitamin D-deficient bone, increased parathyroid hormone induces the osteoblast to express receptor activator of NF-kB ligand (RANKL) on their cell surface and to secrete soluble RANKL into the extracellular matrix. Both surface RANKL and soluble RANKL interact with receptor activator of NF-kB on the surface of osteoclast precursor cells which are differentiated from macrophage-colony forming unit (M-CFU). RANK-RANKL interaction leads to the differentiation of osteoclast precursor cells into multinucleated osteoclasts which are then activated to exert its bone resorbing activity. **Right hand panel.** Activation of receptor activator NF-kB (RANK) by receptor activator NF-kB ligand (RANKL) leads to osteoclast differentiation and osteoclast activation. It promotes bone resorbing activity of the osteoclast in the lacuna by inducing its secretion of acids including citric acid, lactic acid, hydrochloric acid, and enzymes including cathepsins and matrix metalloproteinase (MMP), leading to a release in collagen fragment, calcium and phosphate from the bone into the extracellular matrix. Copyright Holick 2019, reproduced with permission.

deposition of calcium hydroxyapatite into the newly laid down collagen matrix by osteoblasts. In osteoblasts 1,25$(OH)_2$D interacts with the vitamin D receptor resulting in the production of receptor activator of NF kappa B (RANKL). This ligand either on the surface of the osteoblast or released locally interacts with the NF kappa B receptor (RANK) on monocytes inducing them to amalgamate and mature into a fully functioning osteoclast (Fig. 3). PTH initiates the same process. As a result of vitamin D deficiency and/or calcium deficiency results in increased number and activity of osteoclasts in the skeleton (Fig. 2). The osteoclasts release hydrochloric acid to dissolve the calcium hydroxyapatite and the matrix which can no longer be re-mineralized is removed by proteases including cathepsin K (Fig. 3). As a result of a loss of mineral and matrix there is a decrease in bone mineral content thereby increasing risk for fracture (Dawson-Hughes, 1997; Chapuy et al., 1992; Trivedi et al., 2003; Kazemian et al., 2023). 1,25$(OH)_2$D does help control osteoclastogenesis by inducing the osteoblasts to produce a soluble peptide, osteoprotegerin, that binds to RANK on monocytes thereby decreasing the ability of

RANKL to induce them to become osteoclasts (Holick, 2007; Charoenngam, Shirvani, & Holick, 2019a). 1,25$(OH)_2$D has no influence on the production of collagen matrix produced by osteoblasts. 1,25$(OH)_2$D also influences phosphate metabolism by increasing the production of fibroblast growth factor 23 in osteocytes which results in an increase loss of phosphate into the urine (Nakamichi, Takahashi & Udagawa, 2024). Although this may appear to be counterproductive it is in fact the careful monitoring of serum phosphate by osteocytes to keep it in a normal range that is very important for many metabolic functions including the generation of ATP. If the serum phosphate becomes elevated this will lead to soft tissue calcification and nephrocalcinosis. Thus, the interplay of PTH and 1,25$(OH)_2$D delicately balance calcium and phosphate metabolism to maintain healthy normal serum concentrations of both calcium and phosphate.

4. Definition of vitamin D deficiency for bone health

There remains controversy as to what the circulating concentration of 25(OH)D should be for maintaining maximum bone health. The Institute of Medicine recommended that a 25(OH)D of 20 ng/mL was adequate to maintain maximum bone health (Institute of Medicine Committee to Review Dietary Reference Intakes for Vitamin D). However, the Endocrine Society Guidelines on Vitamin D after a careful review of the literature concluded that vitamin D deficiency and insufficiency for bone health should be less than 20 and 21–29 ng/mL respectively (Holick et al., 2011). This recommendation was based on a number of studies. Several studies have reported that PTH was inversely related to serum 25(OH)D concentrations and therefore has been recognized as a good surrogate for vitamin D deficiency (Charoenngam et al., 2019a; Holick, 2007). A study of 1536 community dwelling women not only revealed this indirect relationship but also reported that women with a serum concentration of 25(OH)D of between 20 and 24 ng/mL had a 3 times higher risk of having secondary hyperparathyroidism compared to women with a 25(OH)D of 30–34 ng/mL (Holick et al., 2005). This observations confirmed what had been previously reported (Chapuy et al., 1996). Secondary hyperparathyroidism results in increased osteoclastic activity thereby decreasing bone mineral density and increasing risk for fracture (Fig. 5) (Charoenngam et al., 2019a; Hofbauer & Hamann, 2013; Holick et al., 2011; Holick, 2007). A double-blind placebo-controlled trial evaluating

calcium and vitamin D supplementation demonstrated benefit to bone mineral density (Dawson-Hughes, Harris, Krall, & Dallal, 1997). A meta-analysis of double-blind randomized controlled trials with oral vitamin D supplement considered a threshold of 30 ng/mL for the prevention of hip and nonvertebral fractures (DIPART Vitamin D Individual Patient Analysis of Randomized Trials Group, 2010). The study by Priemel et al. (2010) provided additional evidence that a serum 25(OH)D of less than 30 ng/mL is associated with vitamin D deficiency osteomalacia. They reported bone histomorphometry on 675 iliac crest biopsies from male and female individuals who died in some type of accident. The age distribution ranged from 20–90+ years. The autopsy allows the exclusion of anyone who presented with diseases known to affect the skeleton. They related the histomorphometric analyses to osteoid volume and osteoid surface with a blood concentration of 25(OH)D. They chose the most conservative threshold to a pathologic mineralization status, that is osteomalacia. They found no pathologic accumulation of osteoid in any adult with a circulating 25(OH)D above 30 ng/mL. It is also important to recognize that 21% (6 of 28) of otherwise healthy men and women who had a serum 25(OH)D of 21–29 ng/mL had evidence for pathologic osteoid consistent with vitamin D deficiency metabolic bone disease.

4.1 Clinical trials evaluating vitamin D status with osteoporosis and fracture risk

There continues to be debate as to whether maintaining an adequate vitamin D status with a 25(OH)D of at least 30 ng/mL is necessary to reduce risk of osteoporosis and fractures. A study evaluating intestinal calcium absorption and relating it to circulating concentrations of 25(OH)D observed that maximum calcium absorption was observed when 25(OH)D was 32 ng/mL (Heaney et al., 2003; Holick et al., 2011). Therefore, when maximum calcium absorption is compromised in adults on a low calcium diet the ionized calcium temporarily decreases resulting in an increase in circulating concentrations of PTH. The increase in PTH concentrations can be subtle and remain in the normal range. However, the increased PTH will conserve calcium by increasing tubular reabsorption of calcium in the kidneys and increase osteoclast activity to remove calcium and matrix from the skeleton. This inevitably leads to a decrease in BMD thereby increasing risk for fracture (Fig. 4) (Charoenngam et al., 2019a). Continued vitamin D deficiency results in increased production of PTH ultimately leading to secondary hyperparathyroidism. The elevated

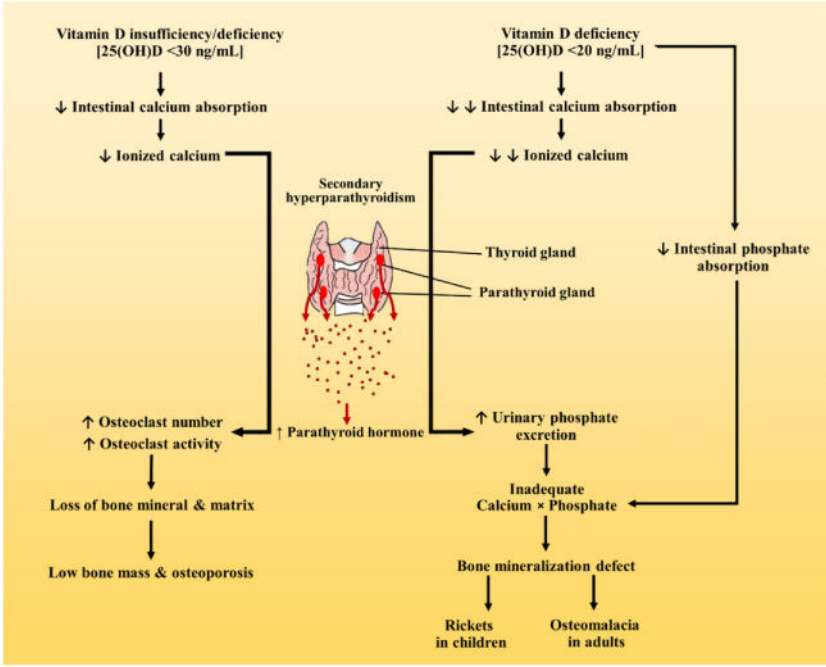

Fig. 4 When serum 25-hydroxyvitamin D (25(OH)D) is less than 30 ng/mL, there is a significant decrease in intestinal calcium and phosphate absorption. This causes a decrease in serum ionized calcium concentration and subsequent secondary hyperparathyroidism. Elevated parathyroid hormone (PTH) induces differentiation of preosteoclast into mature osteoclast leading to increased osteoclast activity. This results in increased bone resorption, loss of bone mineral and matrix, and subsequent low bone mass and osteoporosis. In addition, PTH displays a phosphaturic effect leading to an increase in urinary phosphate excretion. Urinary phosphate loss and decreased intestinal phosphate absorption due to vitamin D deficiency [25(OH)D <20 ng/mL] contribute to inadequate calcium-phosphate product, thereby resulting in defective bone mineralization and development of rickets and osteomalacia. Copyright Holick 2019, reproduced with permission.

PTH concentration not only increases removal of calcium and matrix from the skeleton, but it also causes phosphaturia. When the serum phosphate decreases below the mineralization threshold for the calciumXphosphate product, this will result in the newly laid down osteoid being unable to be mineralized. This can lead to rickets in children and osteomalacia in adults (Fig. 4). The combination of increased skeletal calcium and bone matrix removal by increased numbers of osteoclasts in combination with the inability to mineralize newly laid down collagen matrix can inevitably

increased risk for skeletal architectural compromise increasing risk for fracture (Charoenngam et al., 2019a). This concept is supported Busse et al (Busse et al., 2013). Who reported that vitamin D deficiency induces early signs of aging in human bone thereby increasing risk for fracture. They reported an increase in osteoid-covered surfaces in vitamin D deficient bone that they stated hampers remodeling of the remaining mineralized bone tissue. Using spatially resolved synchrotron bone mineral density distribution analyses and spectroscopic techniques, they reported that the bone tissue within the osteoid frame has a higher mineral content with mature collagen and mineral constituents, which are characteristic of aged tissue. They then performed in situ fracture mechanics measurements and synchrotron radiation micro–computed tomography of the crack path. They observed that vitamin D deficiency increases both the initiation and propagation of cracks by 22%–31%. Therefore, they concluded that whereas vitamin D deficiency decreases bone mass there are other alterations suggesting that the remaining mineralized bone was at increased risk for fracture. These observations help explain studies that have demonstrated that vitamin D and calcium supplementation can increase or maintain bone mineral density (Dawson-Hughes et al., 1997). Chapuy et al. (1992) reported a double-blind placebo-controlled trial where 3270 elderly women were either given 800 IUs vitamin D_3 and 1200 mg of calcium daily for 3 years or a placebo. They reported the reduction of the risk of nonvertebral fractures by 32% and risk of hip fracture by 43% compared to the control group. A meta-analysis by Bischoff-Ferrari et al. (2005) reported on 5 randomized controlled trials (n = 9294) for hip fracture and 7 randomized controlled trial for nonvertebral fracture risk (n = 9820) that used vitamin D_3 supplementation. They concluded that vitamin D supplementation (700-800 IUs/d) reduced relative risk of hip fracture by 26% and nonvertebral fractures by 23%. There was no significant benefit for persons taking 400 IUs daily. A follow-up meta-analysis in 2009 by Bischoff Ferrari et al. confirmed that the higher dose vitamin D reduced nonvertebral fractures in community dwelling individuals by 29% and institutionalized older individual by 15%. They further reported this effect was independent of additional calcium supplementation (Bischoff Ferrari et al., 2009). However, several large clinical trials and meta-analyses have reported little or no benefit of vitamin D supplementation in prevention of fractures (Avenell, Mak, & O'Connell, 2014; DIPART, 2010). Recently LeBoff et al. (2022) conducted an ancillary trial of the Vitamin D and Omega-3 Trial (VITAL) evaluating the effect of either ingesting 2000 IUs daily of vitamin D_3 and n-3 fatty acids or both or placebo in men 50 years of age or older and

women 55 years of age or older to determine if these interventions reduced risk of cardiovascular disease and cancer. The authors noted that the participants were not recruited based on vitamin D deficiency, low bone mass or osteoporosis. Incident fractures were reported by participants annually. It was observed that when they compared the group receiving supplemental vitamin D_3 compared to the placebo group there was no significant effect on total fractures. The conclusion was that vitamin D supplementation did not result in a significantly lower risk of fractures compared to the placebo group. This ancillary study received the attention of the press with a follow-up editorial suggesting that the study is the death knell "final verdict for vitamin D supplementation (Cummings & Rosen, 2022). However, it is worthwhile remembering the design of the study which was they did not recruit for vitamin D deficiency or BMD. The VITAL study permitted participants to take up to 800 IUs vitamin D daily. This ancillary study reported that 42.6% of the study subjects took a vitamin D supplement that was limited to 800 IUs daily. Supplement manufacturers typically put in at least 20% and up to 50% more ingredient to maintain shelf life. Therefore, it was possible that the study subjects taking vitamin D supplementation up to 800 IUs daily could have been taking as much as 1200 IUs daily. This could explain why the mean baseline concentration of 25(OH)D in the 16,757 participants was 30.7 ± 10 ng/mL. Therefore 64.7% of the subjects recruited in this study were vitamin D sufficient at the beginning of the trial based on the Endocrine Practice Guidelines on Vitamin D. Only 12.9% of the study subjects had a baseline 25(OH)D concentration of less than 20 ng/mL and 2.4% were found to have a 25(OH)D of less than 12 ng/mL. This translates into 87.1% of the participants at baseline having a 25(OH)D concentration that is considered for maximum bone health and only 2.4% of the participating subjects were vitamin D deficient by the Institute of Medicine's definitions of vitamin D sufficiency and deficiency respectively (Institute of Medicine Committee to Review Dietary Reference Intakes for Vitamin D). Using the definition of vitamin D deficiency by the Institute of Medicine and evaluation of the number of fractures that occurred in the placebo group was 8 compared to 7 in the vitamin D group. Clearly this was not a placebo-controlled trial. It is worthwhile to appreciate that a circulating concentration of 25(OH)D <30 ng/mL causes aging of the bone mineral increasing risk for cracks and fractures, pathologic evidence of osteomalacia on bone biopsy and increasing concentrations of PTH which increases osteoclast number and activity. These multiple insults on the skeleton compromise its architectural integrity thereby reducing bone mineral density, preventing newly laid down bone matrix from being mineralized thereby increasing risk for

fracture. Studies (Chapuy et al., 1992; Dawson-Hughes et al., 1997; Trivedi et al., 2003; Busse et al., 2013; Hofbauer & Hamann, 2013; Priemel et al., 2010; Kazemian et al. 2023; Bischoff-Ferrari et al., 2005).

5. Treatment approaches for vitamin D deficiency/ insufficiency

There are two forms of vitamin D. Vitamin D_2 was generated from ergosterol in yeast in the 1930s and was found to be effective in treating and preventing rickets. Vitamin D_2 was also used to fortify milk to prevent rickets and was used as a supplement to treat and prevent vitamin D deficiency. Vitamin D_2 was the main vitamin D used to fortify foods and was available as a supplement and pharmaceutical up until about 2004 (Holick, 2023). Vitamin D_3 is produced in the skin as a result of being exposed to sunlight and is found in cod liver oil and oily fish (Fig. 2) (Holick, 2007). Vitamin D_3 has been recovered from oily fish and livers from cod and other fish and was used as a supplement. Advances in chemistry resulted in the production of large and inexpensive quantities of vitamin D_3 from cholesterol obtained from sheep's wool. A study in 2004 changed the landscape for vitamin D fortification and supplementation. Armas et al (Armas, Hollis, & Heaney, 2004). reported that healthy adults who received a pharmaceutical 50,000 IUs vitamin D_2 was approximately two thirds less effective in raising circulating concentrations of 25(OH)D compared to healthy adults who took 10 tablets of 5000 IUs vitamin D_3. They also found that a single dose of 50,000 IUs vitamin D_2 significantly decreased total 25(OH)D below baseline at 20 and 28 days when corrected for seasonal rise. These results suggested that vitamin D_2 was not only much less effective than vitamin D_3 in raising circulating concentrations of 25(OH)D and that taking vitamin D_2 would accelerate the decline of total 25(OH)D thereby causing vitamin D deficiency. As a result of this observation vitamin D_2 was replaced with vitamin D_3 in food fortification and supplements. To evaluate the physiologic significance of this report and other observations (Martineau et al., 2019) that have suggested that vitamin D_2 is less effective in maintaining vitamin D status, studies were conducted evaluating physiologic doses of vitamin D_2 and vitamin D_3 on total circulating concentrations of 25(OH)D (Holick et al., 2008a, 2008b; Keegan, Lu, Bogusz, Williams, & Holick, 2013) as well as total circulating concentrations of total 1,25$(OH)_2$D (Biancuzzo, Clarke, Reitz, Travison, & Holick, 2013). These studies found that ingesting 1000 or 2000 IUs of vitamin D_2 or vitamin D_3 for 12 weeks resulted in the rise in total circulating concentrations of 25(OH)

D that were not statistically significantly different. It was also observed that in the study of healthy vitamin D deficient/insufficient adults taking 1000 IUs of vitamin D_2 or vitamin D_3 that the total $1,25(OH)_2D$ was also not significantly different between the 2 groups (Biancuzzo et al., 2013). The group that received vitamin D_2 showed the expected decrease in $25(OH)D_3$ since the liver is exposed to vitamin D_2 and thus is converting it to its 25(OH)D metabolite.

In the United States the only pharmaceutical form of vitamin D is vitamin D_2 and is available in a 50,000 IU capsule (Holick, 2007). This is because vitamin D_2 predated the FDA and when vitamin D_3 became available it was never evaluated by the FDA. Vitamin D_3 however is routinely available in supplements that can be purchased over the counter in doses of 1000, 2000, 5000 and 10,000 IUs. A 50,000 IU vitamin D_3 supplement is available as a prescription medication/supplement (Holick, 2007). The Endocrine Society Guidelines on Vitamin D provide guidance for how to treat and prevent vitamin D deficiency in all age groups and in adults with a BMI of $<30\ kg/m^2$ (Holick et al., 2011). For adults who are vitamin D deficient and have a normal weight an effective treatment strategy is to give them 50,000 IUs of vitamin D_2 or vitamin D_3 once a week for 8 weeks. This is equivalent to approximately 6600 IUs daily. This treatment will substantially increase circulating concentrations of 25(OH)D into the range of 30 ng/mL (Malabanan, Veronikis, & Holick, 1998; Pietras, Obayan, Cai, & Holick, 2009). To maintain vitamin D sufficiency 50,000 IUs of vitamin D_2 or vitamin D_3 once every 2 weeks is effective (Holick et al., 2011; Pietras et al., 2009). For toddlers and children 50,000 IUs vitamin D_2 or vitamin D_3 once a week for 6 weeks corrects vitamin D deficiency. Alternatively, 2000 IUs of vitamin D_2 or vitamin D_3 is equally effective (Holick et al., 2011). Obese adults require 2–3 times more vitamin D to satisfy their requirement (Ekwaru, Zwicker, Holick, Giovannucci, & Veugelers, 2014; Holick et al., 2011). Table 1 provides vitamin D intakes recommended by the Endocrine Practice Guidelines. To maintain circulating concentrations of 25(OH)D above 30 ng/mL it is reasonable to follow the recommendations of the Endocrine Practice Guidelines.

6. Vitamin D intoxication

There has always been great concern by health care professionals that vitamin D is one of the most toxic fat-soluble vitamins and needs to be very

Table 1 The Endocrine Society Practice Guidelines for vitamin D intake in individuals who are at risk for vitamin D deficiency and dosage of vitamin therapy treatment for patients with vitamin D deficiency.

Age group	For individuals at risk for vitamin D deficiency		Treatment for patients with vitamin D deficiency
	Daily requirement	Upper limit	
0–1 years	400–1000 IU	2000 IU	• 2000 IU/d or 50,000 IU/wk of vitaminD_2 or D_3 for at least 6 wk to achieve serum 25(OH)D >30 ng/mL • maintenance therapy of 400–1000 IU/d
1–18 years	600–1000 IU	4000 IU	• 2000 IU/d or 50,000 IU/wk of vitaminD_2 or D_3 for at least 6 wk to achieve serum 25(OH)D >30 ng/mL • maintenance therapy of 600–1000 IU/d
>18 years	1500–2000 IU	10,000 IU	• 6000 IU/d or 50,000 IU/wk of vitaminD_2 or D_3 for 8 wk to achieve serum 25(OH)D >30 ng/mL • maintenance therapy of 1500–2000 IU/d

Adapted from Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. *The Journal of Clinical Endocrinology and Metabolism, 96* (7), 1911–1930 (2011).

carefully monitored for intake. This concept originated in Great Britain in the early 1950s where several cases of infants with facial abnormalities, supravalvular aortic stenosis, mild mental retardation and hypercalcemia were reported (Vitamin D Toxicity 1964; Samual 1964; Holick, 2015; Holick, 2023; Palermo & Holick, 2014; Stapleton, MacDonald, & Lightwood, 1957; Wacker & Holick, 2013). There are several studies demonstrating that ingestion of vitamin D up to 20,000 IUs daily for years was not associated with any untoward side effects related to vitamin D toxicity including hypercalcemia, hypophosphatemia, soft tissue calcifications and nephrocalcinosis (Ekwaru et al., 2014). The Endocrine Practice Guidelines provides

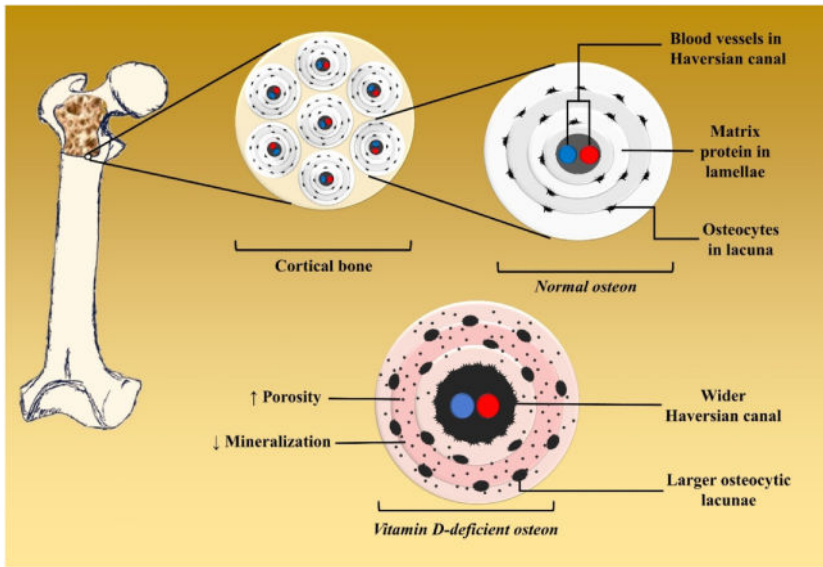

Fig. 5 Bone consists of two major components, including cortical bone which is a major determinant of bone strength and resistance to fracture, and trabecular bone which acts as bridges built inside the cortical bone to intensify bone strength. Cortical bone is a built of cylinders called osteons. Each osteon is composed of bone collagen matrix protein arranged concentrically in lamellae around a Haversian canal containing venous (blue) and arterial (red) blood vessels. Osteocytes and osteoclasts are located in lacunae between each lamellae. Vitamin D-deficient osteons displayed larger lacunae, wider Haversian canal due to the PTH induced increase in numbers and activity of osteoclasts thereby increasing the porosity. In addition, there is defect in osteoid mineralization (light pink area) compared with those of normal bone. Copyright Holick 2019, reproduced with permission.

guidance for the upper limit (UL) for vitamin D intake for all age groups (Table 1) (Holick et al., 2011). For adults that UL is 10,000 IUs daily. A study of healthy vitamin D deficient and insufficient adults who received either 600, 4000 or 10,000 IUs vitamin D_3 daily for 6 months revealed no evidence of vitamin D intoxication in the group that received 10,000 IUs daily. The study did observe a significant dose dependent increase in gene expression and activity in the immune system suggesting a potential benefit of ingesting increased amounts of vitamin D above the guidelines recommended by the Institute of Medicine and the Endocrine Society (Shirvani, Kalajian, & Song, 2019). The Guidelines also recommended that to maintain vitamin D sufficiency the circulating concentrations of 25(OH)D should be at least 30 and up to 100 ng/mL was considered safe (Holick et al., 2011).

Vitamin D intoxication is usually not observed until the circulating concentration of 25(OH)D is above 150 ng/mL (Dudenkov et al., 2015; Holick et al., 2011) There are a few exceptions where vitamin D sensitivity is substantially enhanced due to granulomatous disorders such as sarcoidosis which results in excessive production of 1,25(OH)D by activated macrophages resulting in hypercalciuria and hypercalcemia and by a 24 hydroxylase (CYP24R) deficiency resulting in the inability to catabolize 1,25(OH)D (Holick et al., 2011; Holick 2015; Schlingmann et al., 2011). Vitamin D intoxication is one of the rarest medical conditions caused by inadvertent or intentional ingestion of massive quantities of vitamin D in the range of hundreds of thousands to more than 1 million IUs of vitamin D daily for several months to several years (Holick, 2007; Koutkia, Chen & Holick, 2001).

7. Conclusion

Vitamin D throughout Evolution has played a fundamental role in not only maintaining calcium and phosphorus homeostasis but also for the development and maintenance of a vertebrate skeleton on terra firma. The major function of vitamin D is to maintain calcium and phosphate homeostasis for a multitude of metabolic functions and maintenance of neuromuscular activity. Vitamin D does not actively participate in either the production of skeletal collagen matrix or inducing the formation and deposition of the bone mineral calcium hydroxyapatite. By maintaining an adequate serum and extracellular calciumXphosphate product newly laid down skeletal collagen matrix is passively mineralized. Rather than actively mineralizing the bone collagen matrix vitamin D instead removes calcium from the skeleton to maintain calcium homeostasis when there is inadequate dietary calcium. $1,25(OH)_2D$ and secondary hyperparathyroidism caused by vitamin D deficiency/insufficiency increase osteoclastic number and enhance their activity in the skeleton resulting in the loss of mineral and matrix thereby literally punching a hole in the bone. The osteoclasts continue releasing hydrochloric acid and enzymes thereby continuing the erosion of the skeleton and compromising its architectural integrity (Figs. 4 and 5)

These concepts are important to understand when considering the use of vitamin D for treating osteoporosis. Osteoporosis is due to an imbalance in bone formation and bone resorption where bone resorption exceeds bone formation (Charoenngam et al., 2019a; Hofbauer & Hamann, 2013). This results in loss of mineral and matrix. Vitamin D cannot treat osteoporosis

since it cannot induce osteoblasts can neither produce bone collagen matrix nor induce collagen matrix mineralization. Many clinical trials have shown that treatment with vitamin D with or without drug intervention can lead to an increase in bone mineral density thereby suggesting that vitamin D is improving osteoporosis. The most likely explanation for these observations is that these patients were vitamin D deficient for a significant period of time and have unmineralized matrix in their skeleton. Correction of the vitamin D deficiency resulting in restoration of a normal calciumXphosphate product will result in mineralization of the matrix and therefore increase bone mineral density. Chronic vitamin D deficiency that results in an increase in PTH will inevitably lead to increased bone resorption thereby compromising the architectural structure of the skeleton that will result in increased risk for fracture. This is the reason why it is important to maintain vitamin D sufficiency throughout life to maximize skeletal health. For maximum bone health in children and adults a circulating concentration of 25(OH)D of at least 30 ng/mL. should beachieved and maintained (Holick et al., 2011; Heaney et al., 2011). Once the circulating concentration of 25(OH)D concentration is between 15–20 ng/mL it has been reported that for every 100 IUs of vitamin D ingested will result in an increase in circulating concentrations of 25(OH)D of approximately 0.6–1 ng/mL (Heaney et al., 2003). Therefore, it is reasonable to follow the Endocrine Practice Guidelines for vitamin D intake to achieve a serum concentration of 25(OH)D at least 30 ng/mL for maximum bone health (Charoenngam, Shirvani, & Holick, 2019b; Holick et al., 2011).

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Conflicts of interest

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