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Vitamin D and aging: old concepts and new insights

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Abstract

Aging is a complex biological process driven by a selective class of molecules and pathways that affect overall deterioration of physiological functions to increase the risk of age-related diseases. A role of vitamin D in mammalian aging is well documented. Since vitamin D has an essential role in bone formation and mineralization, its deficiency results in impaired bone mineralization, such as rickets in children, osteomalacia in adults and osteoporosis in the aged population. Vitamin D replacement therapy therefore is one of the most commonly prescribed treatments for the elderly. Recent studies using genetically altered mouse models, such as in *Fgf-23*$^{-/-}$ and *klotho* mutant mice, that exhibit altered mineral ion metabolism due to high vitamin D activities showed features of premature aging that include atherosclerosis, emphysema, osteopenia/osteoporosis, hypogonadism, soft tissue calcifications and generalized atrophy of organs; the pathologic effects of vitamin D in these mouse models are obvious, as diminution or genetic ablation of the vitamin D pathway ameliorated most of the above-mentioned phenotypes, by reversing mineral ion metabolism, and the resultant effect being prolonged survival of the mutant mice. These in vivo mouse studies, although subject to further molecular characterization, add new insights into the role of vitamin D in aging.

Keywords: Vitamin D; Aging; Fgf-23; Klotho; Calcification; Survival

1. Introduction

Vitamin D is a circulating hormone and is involved in the regulation of various essential physiological functions that include skeletogenesis and maintenance of mineral ion homeostasis. The synthesis of vitamin D is molecularly well defined, a process that begins in the skin and continues further in the liver and kidney to produce biologically active 1,25 dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]. The bioactive metabolite, 1,25$(OH)_2D_3$, is produced by two sequential hydroxylations by the enzyme 25 hydroxylase (CYP27A1) in the liver to 25 hydroxyvitamin D [25$(OH)_2$D] and is further hydroxylated in the kidney by the enzyme 1α hydroxylase [1α(OH)ase; CYP27B1] to 1,25$(OH)_2D_3$. When the amount of 1,25$(OH)_2D_3$ is sufficient, it is further processed by the 24-hydroxylase (CYP24) to 24,25$(OH)_2$D in the kidney and consequently catabolized; similar catabolism can also take place in the liver.

The functionally active metabolite, 1,25$(OH)_2D_3$, is believed to exert its main effects by interacting with the high-affinity vitamin D receptor (VDR), which is a ligand-dependent transcription factor [1]. VDR is a member of the nuclear receptor superfamily that forms a heterodimer with the retinoid receptor to regulate gene transcription by binding to vitamin D responsive elements (VDREs) in the promoter region of target genes. Apart from these classical genomic functions of VDR, 1,25$(OH)_2D_3$ could also exert its bioactivities through a rapid response system; such responses are too quick to be explained by the conventional nuclear VDR signaling system. Rapid responses are believed to be mediated by separate sets of receptors located near or associated with the plasma membrane or its caveolae components [1].

In addition to the classic target organs, including intestine, bone, kidney and parathyroid gland, the receptors for vitamin D are also present in tissues and organs that are not directly involved in the regulation of calcium homeostasis, suggesting a possibility of a broad range of functions of vitamin D, as proposed in hypertension, immunoregulation, embryogenesis and tumorigenesis [2]. Numerous factors, including parathyroid hormone (PTH), affect the

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biosynthesis of vitamin D by stimulating the activity of the 1α(OH)ase, possibly by acting on the promoter region of the enzyme [3]; hypocalcemia and hypophosphatemia also exert similar stimulatory effects on the enzyme [4]. Furthermore, $1,25(OH)_2D_3$ and fibroblast growth factor 23 (FGF-23) can affect the vitamin D homeostasis, by negatively regulating the synthesis of 1α(OH)ase [5,6].

Since molecular mechanisms of multi-stage and multi-organ synthesis of vitamin D are well characterized, the effects of its altered regulation and clinical significance are also well studied and defined, as evident from the more than 40,000 scientific publications on this topic; the scientific interest and importance are also reflected from the progressively increased numbers of editorials and review articles published in the past 25 years (Fig. 1). There are many published studies and reviews that describe various clinical aspects of vitamin D, with the recommendations of how to prevent hypovitaminosis D in various age groups; some of these recommendations appear to be much generalized and mostly targeted towards subsets of populations, but may not be useful at the individual level. For instance, treatment of vitamin D and calcium supplementation might not be equally advisable to an elderly patient with the risk of developing cardiovascular calcification, as opposed to an elderly patient who has a healthy heart with unhealthy bone. Moreover, random and uncontrolled use of vitamin D supplements, without taking other necessary minerals, might not always give the desired beneficial effects, a possibility that is evident from the ineffectiveness or partial effectiveness of even controlled vitamin-D therapy in numerous large-scale clinical studies [7–9].

2. Hypovitaminosis D and aging

Low levels of vitamin D, which range from hypovitaminosis, insufficiency to deficiency, are clinically estimated from measuring the plasma levels of $25(OH)_2D$ (a biologically inactive circulating storage form of vitamin D); such values are not always representative; for instance, plasma $25(OH)_2D$ levels are influenced by ecological factors (ranging from season, weather condition to latitude), lifestyle (ranging from clothing patterns to dietary habits) and individual factors (ranging from race, pigmentation to age); moreover, biochemical assays have wide variability and sensitivities depending on the method used to detect the level of $25(OH)_2D$. The plasma levels of $25(OH)_2D$ in a certain time point often reflect the recent events (season, holiday, outdoor activities, etc.), and a careful assessment is needed to use these values to determine previous vitamin D levels and to predict the future status of vitamin D in an individual [10]; of relevance, the level of the active metabolite $1,25(OH)_2D_3$ is not a suitable measurement as well and could even be misleading, as its levels are either normal or elevated in mild to moderate osteomalacia, possibly due to secondary hyperparathyroidism [11]. Moreover, most of the peripheral tissues are able to convert circulating $25(OH)_2D$ to the active metabolite $1,25(OH)_2D_3$ to cover their local requirements and might not be reflected from its serum levels [12]. Despite limitations, plasma levels of $25(OH)_2D$ (half-life in circulation is approximately 2–3 weeks) are at present used as a tool to assess the amount of vitamin D, particularly in the elderly populations. In general, hypovitaminosis D is referred to when $25(OH)_2D$ levels are ranging from 50 to 100 nmol/L; the levels between 50 and 25 nmol/L are considered as vitamin D insufficiency, while the levels below 25 nmol/L indicate vitamin D deficiency [11]. It is, however, worthwhile to mention that precisely defining hypovitaminosis, insufficiency and deficiency groups is not always straightforward; as we mentioned above, the values of $25(OH)_2D$ could be influenced by the secondary effects of various serum parameters of a particular individual.

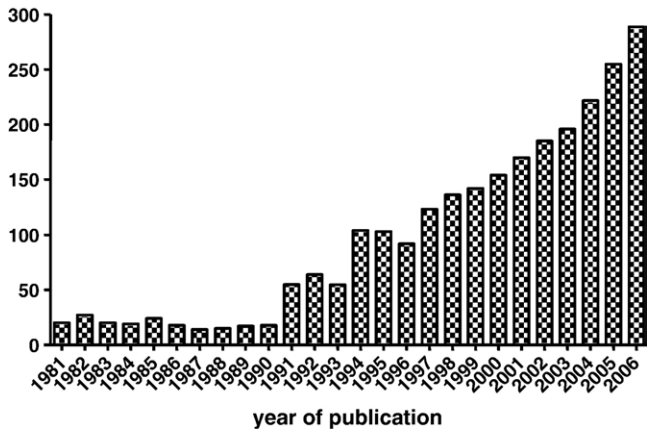

Fig. 1. Numbers of review articles and editorials published on vitamin D each year during the last 25 years. Note a significant increase in numbers of publications from 1990 onwards, reflecting the significance and importance of the topic. The data are collected from Science Citation Index (SCI), ISI Web of Knowledge.

The elderly population is particularly at risk of developing clinical complications related to low vitamin D levels, as their ability to generate the precursor of vitamin D in the skin is reduced with the advancement of age, which is further aggravated by the change in lifestyle that reduces outdoor physical activities and by immobility [13–16]. Moreover, the messages and widely spread information of adverse effect of chronic unprotected sunlight exposure, at times, lead to overprotection, either by avoiding sunlight or by using sun protective agents to reduce exposure, which further contribute to inadequate vitamin D levels. Vitamin D supplementation therefore is one of the most commonly prescribed treatments for the elderly. Of relevance, in the elderly population chronic vitamin D deficiency leads to osteoporosis or gradual loss of bone that results in impaired structural integrity of trabecular bones, with thinner and more porous cortical bones, and thereby making the bones weaker and more likely to fracture. A World Health Organization committee has defined osteoporosis based on the bone density; with the use of standardized bone density

measurements of the total hip, a value greater than 833 mg/cm^2 is considered as normal bone, between 833 and 648 mg/cm^2 is considered as osteopenia, lower than 648 mg/cm^2 is considered as osteoporosis, and when there has been a fragility fracture, the condition is referred to as severe or established osteoporosis. Subsequently, further modifications have been suggested and adopted to clinically classify various stages of osteoporosis. As mentioned above, vitamin D supplementation is one of the most commonly prescribed treatments for the elderly, although the randomized clinical trials of vitamin D supplementation have provided differential effects to support the absolute clinical benefits of such randomized treatments. For instance, osteoporosis in menopausal woman due to estrogen loss and aging thereafter has independent mechanisms and cannot be generalized in terms of treatment.

There are numerous published articles that have described the utility of consuming vitamin D in the elderly individuals with differential effects. For instance, in a clinical trial involving individuals living in elderly apartments, who were given 400 IU of vitamin D_3 supplementation (without calcium supplementation) or placebo for an average period of 2 years, there were no differences in the risk of falls between the treated and control groups in a prospective monitoring over a period of 28 weeks [17]. The study by Dawson-Hughes et al. [18] also failed to detect reduction in hip fractures in vitamin D (cholecalciferol) and calcium-supplemented elderly patients, although the investigators did find less nonvertebral fracture [18]. Accordingly, in two other separate studies, despite relatively less fractures in the vitamin D recipients, no significant difference in mortality was found between vitamin D-treated and control subjects [7,8]. To investigate the effects of prophylaxis supplementation of vitamin D on fractures of the elderly people, a yearly intramuscular injection of ergocalciferol (150,000–300,000 IU) was given to elderly people (75 years or older) for 5 years. In the 341 vitamin D recipients, a total of 56 fractures occurred (16.4%), compared to the 100 fractures in 458 controls (21.8%); although apparently less in the vitamin D-treated group, no statistical difference, either in the occurrence of fracture or in total mortality, was found in vitamin D-treated or untreated individuals [8]. In a relatively recent study with 36,282 postmenopausal women, ages between 50 and 79 years, receiving calcium (daily dose of 1000 mg of calcium carbonate) and vitamin D supplements (daily dose of 400 IU of vitamin D_3) or placebo pills showed a modest benefit in preserving bone mass and only prevented hip fractures in certain age groups of women, but did not prevent other types of fractures or colorectal cancer [9]. During the study period, 374 women had hip fractures with a fracture rate of 14 per 10,000 cases per year in the supplemented group compared to 16 per 10,000 per year in the placebo group, although such reduction was not statistically significant; moreover, such vitamin D and calcium supplementation were also associated with an increased rate of kidney stone formation [9].

The generation of either *1α(OH)ase*$^{-/-}$ or *VDR*$^{-/-}$ mice has shed lights on in vivo effects of vitamin D deficiency. The ablation of the *VDR* gene resulted in severe hypophosphatemia, hypocalcemia and secondary hyperparathyroidism in the null mice, and the resultant effect being rickets and osteomalacia [19–21]. The active form of vitamin D, 1,25(OH)$_2$D$_3$, is mainly formed in the kidney by the enzymatic hydroxylation of the 1α(OH)ase. Ablation of the *1α(OH)ase* gene from mice showed essentially similar phenotypes as *VDR*$^{-/-}$ mice, which include rickets, osteomalacia, hyperparathyroidism, hypocalcemia, hypophosphatemia and growth retardation [19–21]. Interestingly, most of the altered serum parameters, and to some extent the skeletal phenotypes, could be rescued by feeding these animals with a high-calcium, high-phosphate and high-lactose diet [22,23]; these experimental studies suggest that restoration of mineral ion balance could significantly improve skeletal anomalies, even when vitamin D activities are absent, an information that has enormous applicability in relevant human diseases. Of relevance, extremely high circulating levels of 1,25(OH)$_2$D$_3$ in *Fgf-23* and *klotho* gene knockout mice [24,25] showed some of the skeletal features, as osteopenia and osteomalacia that could also be found during inadequate vitamin D activities. In clinical practice, mild to moderate vitamin D deficiency is, however, much more prevalent than hypervitaminosis D.

3. Hypervitaminosis D and aging

Hypervitaminosis D, at large, is thought to be more of a theoretic concern than reality. Such assumption is partly based on the belief that excessive sunlight exposure or food intake cannot produce enough 1,25(OH)$_2$D$_3$ to induce subsequent toxicity. Since vitamin D toxicity, as a result of dietary supplementation, was more commonly reported in the earlier part of this century, and more recently, due to accidental food fortification errors [26], hypervitaminosis D is therefore considered mostly as a problem that is not relevant to the present time. However, the majority of the people might not be aware of the fact that uncontrolled vitamin D consumption, as a supplement, could induce occult vitamin D intoxication; more importantly, such intoxication could produce skeletal changes that one would expect in vitamin D deficiency, making it harder to identify the original cause, unless the serum level of 25(OH)$_2$D is monitored for determining either hypervitaminosis D or hypovitaminosis D [27]. Of relevance, hypercalcuria with gradual loss of bone mineral density is usually the consequence of hypervitaminosis D, while no such urinary calcium wasting is found in hypovitaminosis D.

The uncontrolled consumption of vitamin D supplements by elderly patients (53 to 73 years old) with osteoporosis has shown to induce occult vitamin D intoxication, and the resultant effect being diminished bone mass; discontinuation of use of dietary supplements resulted in the normalization of serum levels of 25(OH)$_2$D leading to the recovery of

bone mineral density (annual increase of 1.9±0.6%) and the normalization of the ratio of urinary calcium to creatinine [27]. This study is informative, as its 3-year follow-up phase showed that the increase in bone mineral density persisted after initial recovery [27]. The observations that hypervitaminosis D is associated with hypercalciuria and bone loss in the affected patients are comparable to the results obtained in some of the experimental studies with excessive vitamin D activities; for instance, genetically altered mice that were ablated for the sodium phosphate co-transporters 2a (NaPi2a) have very high serum levels of $1,25(OH)_2D_3$ and exhibit hypercalciuria and skeletal anomalies [28]. Similarly, both *Fgf-23*- and *klotho*-deficient mice have extremely high serum levels of $1,25(OH)_2D_3$ [6,24,29] and yet develop skeletal changes like osteopenia and osteomalacia (Fig. 2) that are normally seen in a vitamin D-deficient state [24,25,30–32]. Hypervitaminosis D in both *Fgf-23*- and *klotho*-deficient mice resulted in severe hyperphosphatemia with vascular and soft tissue calcifications [24,25,30,31,33–35]. Moreover, hypervitaminosis D in *Fgf-23* null mice and *klotho* mutant mice induces numerous premature aging-like features such as infertility, kyphosis, atherosclerosis, skin atrophy, muscle wasting, T-cell dysregulation, pulmonary emphysema, osteoporosis/osteopenia and altered mineral ion metabolism, and the resultant effect being shortened lifespan [36,37].

Again, both *Fgf-23*- and *klotho*-deficient mice have increased renal expression of the *1α(OH)ase* gene, accompanied by elevated serum levels of active vitamin D, $1,25(OH)_2D_3$; a significant rescue of premature aging-like features has been achieved by either reducing vitamin D activities or genetically ablating vitamin D activities from *Fgf-23*- and *klotho*-ablated mice. Reducing vitamin D activities in *klotho* mutant mice by feeding a vitamin D-deficient diet has resulted in disappearance of ectopic calcifications, gain of fertility and, most importantly, prolonged survival, suggesting that the premature aging-like features in *klotho* mutant mice are downstream events resulting from increased activity of vitamin D [6]. In the same line, when vitamin D activities were genetically ablated from *Fgf-23* null mice by deleting the *1α(OH)ase* gene, most of the premature aging-like features in *Fgf-23* null mice were rescued [6]; for instance, the generalized atrophic changes in skin, intestine and other organs of *Fgf-23* null mice were rescued in *Fgf-23*$^{-/-}$/*1α(OH)ase*$^{-/-}$ double mutants, and the resultant effect being significant increased overall survival of vitamin D-ablated *Fgf-23* null mice. It is therefore reasonable to conclude that hypervitaminosis D, by altering mineral metabolism, could induce features consistent with premature aging. Further studies will determine whether the results of the in vivo mouse studies have any clinical relevance or significance [38]. Since aging is a complex biological process that includes multi-organ, multi-system pathologies [39–45], it will be interesting to know whether dysregulation of FGF-23 or KLOTHO might induce some of the pathological events that are more likely to be present in elderly individuals, such as vascular calcification and skeletal mineral defects. It is worth mentioning that the phenotype of *Fgf-23* null animals mimics patients with familial tumoral calcinosis (FTC), an autosomal recessive disorder characterized by ectopic calcifications and elevated serum levels of phosphate due to inactivating mutations in the *FGF-23* gene [46,47]. It will be of clinical interest to see whether mutations in the human

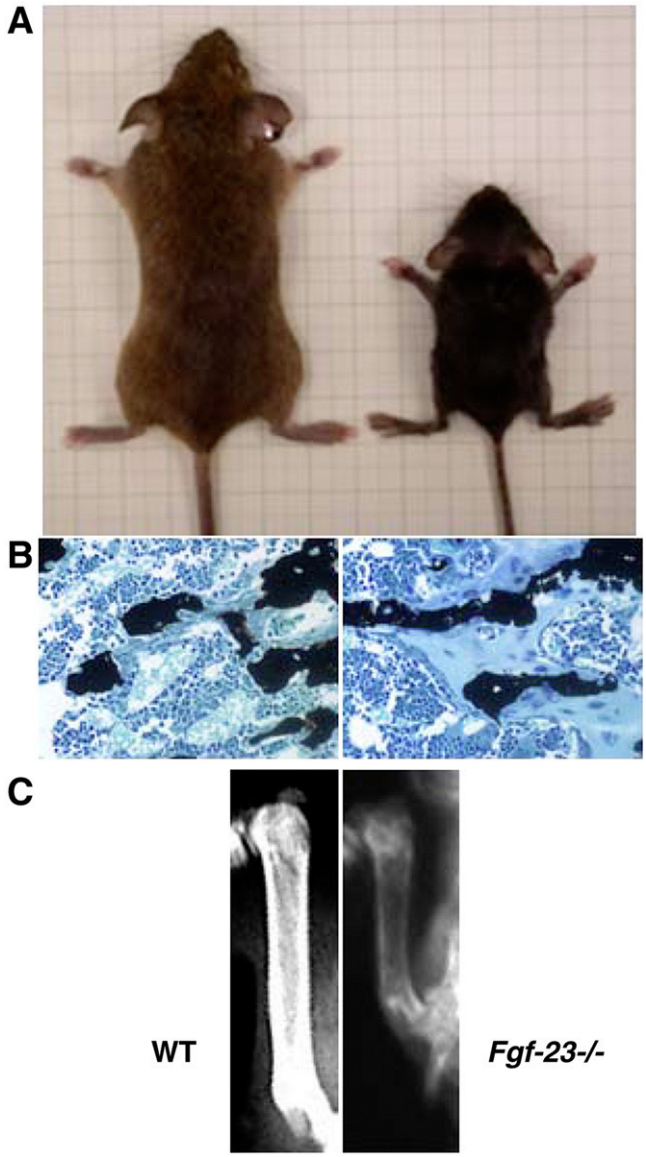

Fig. 2. (A) Gross appearance of an 8-week-old *Fgf-23* null mouse (right) and age-matched control littermate (left). Note the significantly reduced body size of *Fgf-23* null mouse; these mice have shorter life span and show multiple features of premature aging, including infertility, kyphosis, atherosclerosis, skin atrophy, muscle wasting, T-cell dysregulation, pulmonary emphysema, rickets, with increase vitamin D levels and altered mineral ion metabolism [24,30–32]. (B) Histological analysis of trabecular bone shows heterogeneous distribution of mineral contents with unmineralized osteoid (light blue) formation in the femur of a 4-week-old *Fgf-23* null mouse (right). (C) Radiographic analysis shows increased translucency, indicating a significant decrease in bone mineral density in the *Fgf-23* null mouse (right), compared to the age-matched control littermate (left).

KLOTHO gene could produce similar symptoms as tumoral calcinosis; such speculation is based on the fact that both Fgf-23 and klotho appear to exert similar functions, perhaps using similar signaling cascade [30,35]. In contrary to knockout animals, the phenotype of *FGF-23* transgenic animals mimics patients with autosomal dominant hypophosphatemic rickets (ADHR) carrying mutations in the *FGF-23* gene [48]; these mutations prevent proteolytic cleavage of the FGF-23 protein, with net effect being phosphate wasting and skeletal defects in the affected patients, perhaps due to enhanced biologic activities of FGF-23. Equally, polymorphisms in the human *KLOTHO* gene are suggested to correlate with the occurrence of age-related pathologies, including osteoporosis and coronary artery diseases, and predicted to influence overall survival [49]. Of relevance, both FGF-23 and klotho are vitamin D inducible genes.

The use of vitamin D supplements that might need further activation in the kidney is likely to exert additional workload to the potentially failing kidney of elderly individuals. A recent National Health and Nutrition Examination Survey (NHANES) revealed elevated serum creatinine levels in nearly 3% of the US population. This survey also revealed that 11% of people over the age of 65, without obvious renal disease, had 60% less renal function when compared to normal individuals [50]. Considering that the incidence of age-associated renal diseases is generally underestimated, the possible effects of drugs that need to be metabolizing in the kidney are not clearly known. Since vitamin D toxicity is considered to be an unlikely phenomenon, health professions that deal with treatment of hypovitaminosis D are more concerned with the replacement therapy, without considering the possible adverse effect of such treatment on the potentially failing kidneys of elderly individuals. The vitamin D in the supplement is usually present in $25(OH)_2D$ form. One can argue that such workload on the kidney could be avoided by providing active metabolites; however, such treatment has the potential to increase the risk of renal stone formation, as demonstrated by about 17% increased rate of kidney stone formation in osteoporotic patients treated with vitamin D and calcium supplemented [9]. Since hip fractures are considered to be a more serious health problem than kidney stones, one might argue that the overall benefit of the treatment outweighs the eventual risks.

Moreover, the risk of cardiovascular calcification is higher with vitamin D treatment, which is particularly important for elderly patients suffering from clinical symptoms due to existing vascular narrowing. Long-term use of active vitamin D metabolites in patients with end-stage renal diseases facilitates formation of abnormal calcification to increase the risk of cardiovascular death of these renal failure patients. Abnormal soft tissue calcification is associated with almost half of the cardiovascular deaths among patients undergoing dialysis. Furthermore, continuous use of high calcium dialysate and

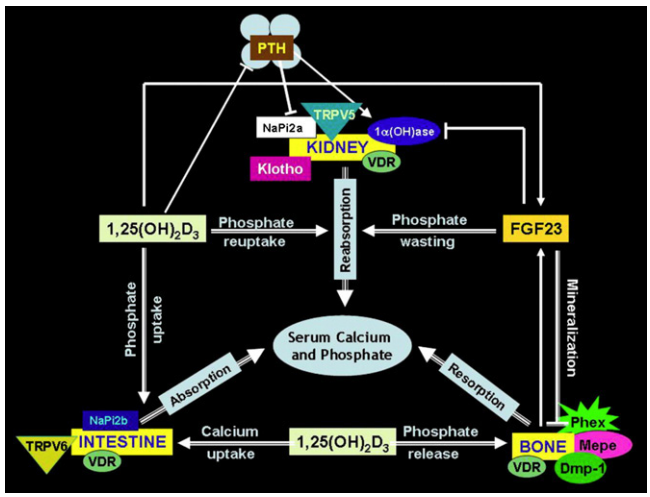

Fig. 3. Simplified schematic diagram of possible molecular interactions of FGF-23 and vitamin D in regulating calcium and phosphate homeostasis. Disruption of any of these components can lead to deterioration of physiological functions and thereby increase the risk of bone diseases, such as osteoporosis, osteomalacia or osteopenia, some of which are more prevalent in advanced age.

prolonged administration of vitamin D are the main underlying cause of tumoral calcinosis in patients with chronic renal failure [51]. Milliner et al. [52] found that the probability of calcinosis was higher in patients receiving vitamin D therapy, and withdrawal of vitamin D therapy from the patients with tumoral calcinosis could markedly regress the lesion [53], emphasizing that reducing vitamin D could ameliorate ectopic calcification. The vitamin D-induced calcification in the disease state might not be comparable to regular aging, but there might be similarities in the underlying mechanisms, as molecules involved in mineral ion metabolisms in normal and disease state are limited and selective (Fig. 3).

The accidental overdose of prescription drugs, or abuse of the medications that produce acute symptoms, is usually dealt with in clinical practice for drug toxicity; however, the fact that is not well appreciated is that excessive and uncontrolled consumption of nutrients (as a supplement format) could also seldom produce chronic toxicity, such as hypervitaminosis D. It has been shown that vitamin D toxicity can be caused by excess oral intake through over-the counter supplements [54]. Given that more than one third of the US population regularly use some form of dietary supplements, the unanticipated prevalence of apparent hypervitaminosis from consumption of dietary supplements may be more widespread than actually apparent.

4. Concluding remarks

Mild vitamin D deficiency is much more prevalent than its overdose. Surveys have found that elderly people often consume less than the required amount of vitamin D through their diet; vitamin D supplementation is therefore a reason-

able approach to minimize the risk for osteoporosis and metabolic bone disease. However, any such recommendations should consider the food habits and dietary supplement use by the individual patient, as most of the patients regularly take some form of dietary supplements, which have wide diversity, in terms of compositions and exact contents; a random uncontrolled use of vitamin D without specific objectives is an unrealistic approach and may induce unexpected complications. Moreover, our ability to perform molecular screening and genetic profiling of individual patients will, in the future, help us to design specific medication according to the specific need of a particular patient, rather than a generalized recommendation. Finally, in this article, we wanted to highlight two important aspects of vitamin D in aging: (1) the effects of hypovitaminosis D and (2) relatively less appreciated effects of hypervitaminosis D in aging [30,55]. Further controlled studies will determine how much vitamin D, in what clinical situation, is too much during aging to exert unwarranted effects.

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