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REVIEW

# Vitamin D deficiency and toxicity in chronic kidney disease: in search of the therapeutic window

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**Abstract** Both vitamin D deficiency and vitamin D toxicity are associated with cardiovascular complications in chronic kidney disease (CKD). Clinical and experiment data indicate that the association of vitamin D levels with cardiovascular disease is best illustrated as a biphasic, or U–shaped, curve. Children and adolescents with CKD need vitamin D due to the demands of a growing skeleton, to prevent renal rickets. However, this therapy carries the risk of severe side effects and chronic toxicity. Observational studies show that vitamin D deficiency and toxicity are frequently present in patients with CKD. In view of the importance of cardiovascular complications for the long-term survival of young patients, these findings demand a judicious use of vitamin D preparations. In clinical practice, the therapeutic window is rather small, presenting a therapeutic challenge to avoid both vitamin D deficiency and toxicity.

**Keywords** Cardiovascular disease · Chronic kidney disease · Vitamin D · Vitamin D receptor

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## Introduction

Contrary to the common belief that nutritional rickets is a disease of the past, recent epidemiological data show that vitamin D deficiency is a worldwide health problem that affects children in particular [1]. It is associated not only with skeletal disorders, but also with atherosclerotic cardiovascular disease and the development of coronary artery calcifications in the general population [2, 3]. These findings have led to the appreciation of vitamin D as a cardioprotective hormone [4]. Importantly, recent studies have shown that vitamin D deficiency is highly prevalent in adult and pediatric patients with chronic kidney disease (CKD) [5, 6].

In contrast, pediatric nephrologists have been using high doses of 1-alpha hydroxylated vitamin D preparations as a mainstay therapy for renal osteodystrophy in CKD for decades. The earliest reports indicated that the use of 1,25-dihydroxyvitamin D [$1,25(OH)_2D_3$] (calcitriol) may have additional beneficial effects on growth [7], hypertension, insulin resistance, and lipid abnormalities [8, 9] in patients with CKD. However, calcitriol therapy in pediatric CKD has also been associated with significant side-effects, of which cardiovascular complications have lately caused major concerns, especially the association with vascular calcifications [10]. In view of the importance of cardiovascular complications for long-term survival of children with CKD, these findings demand a judicious use of such vitamin D preparations.

This review will delineate the role of vitamin D in cardiovascular complications in CKD and discuss accumulating evidence that both ends of the spectrum of activity of the vitamin D system, i.e. deficiency and excess (toxicity),

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are associated with cardiovascular disease (CVD). In clinical practice, both conditions are frequently encountered in young patients with CKD and pose a major therapeutic challenge.

## Part I: vitamin D: physiology and requirements

### Vitamin D: physiology

The major source for vitamin D in humans is exposure to sunlight. In the epidermis, vitamin D3 (cholecalciferol, D3) is formed from the sterol 7-dehydrocholesterol by solar ultraviolet B (UVB, 290–315 nm) radiation in a photolytic reaction that results in the formation of previtamin D and, following thermal isomerization, vitamin D3 (Fig. 1). Vitamin D can also be taken up by the enteral route, but not many foods contain vitamin D. Dietary sources include a few natural supplies, such as fatty fish (D3), fortified foods (mainly in the USA), such as milk, infant formulas, cereals (D3), and supplements (e.g. prescription vitamins: containing D2, the plant sterol-derived ergocalciferol, or D3). Vitamin D3 formed in the skin is transported in the blood by the vitamin D binding protein (DBP). Enterally absorbed vitamin D (D represents either $D_2$ or $D_3$) is transported via chylomicrons and other lipoproteins, which are rapidly cleared by the liver [11]. Hepatic metabolism of either endogenously synthesized or ingested vitamin D is mediated by a microsomal 25-hydroxylase, currently thought to be primarily a cytochrome P450 (CYP2R1 rather than CYP27A1) [12]. This leads to the formation of the major circulating vitamin D metabolite 25-hydroxyvitamin D [25(OH)D, calcidiol] (Fig. 1). Levels of 25(OH)D are rather stable [13], and the half life of labeled 25(OH)D (measured in human volunteers) is about 4 weeks [14]. Since the activity of the hepatic 25-hydroxylase is apparently without strict feedback control [15], 25(OH)D levels directly reflect the external vitamin D supply by dietary intake of vitamin D (precursors) and light exposure. Consequently, 25(OH)D levels are lower in winter than in the summer, differ in populations according to geographic location, and are influenced by many clinical conditions affecting uptake and synthesis. In the normal population, limitations to epidermal vitamin D3 formation are age, skin pigmentation, sunscreen use, and clothing. In the kidney, circulating 25(OH)D bound to DBP undergoes glomerular filtration and reabsorption by the megalin/cubilin receptor complex in proximal renal tubular cells [16] and is then metabolized by 1-alpha-hydroxylase (CYP27B1) to form $1,25(OH)_2D$, which has a short half life of only 10–20 h in plasma [17]. Importantly, the renal synthesis of this vitamin D metabolite with the highest biological potency is strictly controlled by transcriptional feedback regulation by parathyroid hormone (PTH), calcium (Ca), and phosphorus (P) levels as well as by fibroblast growth factor 23 (FGF-23) secreted from bone. Therefore, and due to the short half life in plasma, circulating levels provide limited information about the nutritional vitamin D status and body vitamin D stores. Like 25(OH)D, most of the total circulating $1,25(OH)_2$ D is bound to DBP and albumin, and only a small fraction is unbound in plasma. It is assumed that the non-protein-bound free fraction reflects the biologically active hormone [18]. Circulating levels of $1,25(OH)_2D$ reflect exclusively renal synthesis of this metabolite [although under rare pathological conditions, such as sarcoidosis, a high extrarenal production of $1,25(OH)_2D$ may contribute to levels in the circulation]. Recent epidemiological data have shown that plasma levels of 25(OH)D and $1,25(OH)_2D$ in normal adults may be affected by single nucleotide polymorphisms in the DBP gene [19].

The 1-alpha-hydroxylase CYP27B1 is also present *in many extrarenal tissues* and has an important role for cellular effects of $1,25(OH)_2D$ in extrarenal target cells [20]. $1,25(OH)_2D$ transcriptionally controls the expression of target genes through the nuclear vitamin D receptor (VDR) acting as a ligand-inducible factor [21].

The VDR is present in most, if not all tissues; high-affinity binding of the preferred ligand $1,25(OH)_2D$ elicits genomic effects after heterodimerization of the VDR with a retinoid X receptor (RXR) [22] and binding to DNA target sequences, i.e. vitamin D-responsive elements (VDRE) with consequent transcriptional regulation of two major cellular functions: control of cell cycle regulation (proliferation) and differentiation. Both 25(OH)D and$1,25(OH)_2D$ are degraded by the enzyme 25-hydroxyvitamin D-24-hydroxylase (CYP24A1) into biologically inactive metabolites. CYP24A1 is activated by $1,25(OH)_2D$ and by FGF-23. Through its degradation of vitamin D metabolites, CYP24A1 has an important role as a catabolic regulator of both renal and extrarenal activity of the vitamin D system [23, 24].

In addition to the genomic effects mediated by the VDR, membrane binding of $1,25(OH)_2D$ results in rapid non-genomic effects, such as calcium uptake, protein kinase C activation, and other intracellular pathways [25, 26]. However, our understanding of the clinical relevance of these signal transduction pathways is incomplete at the present time [27].

### The vitamin D system: a principal regulatory network

The "classical" physiological function of activated vitamin D $[1,25(OH)_2D]$ is to maintain serum calcium and phosphorus levels within the normal physiological range, thus regulating bone mineralization and a multitude of metabolic functions (Fig. 1). However, this view has been expanded to include a much wider range of physiological actions of vitamin D by the discovery of the presence of the VDR and of 1-alpha-hydroxylase (CYP27B1) in many

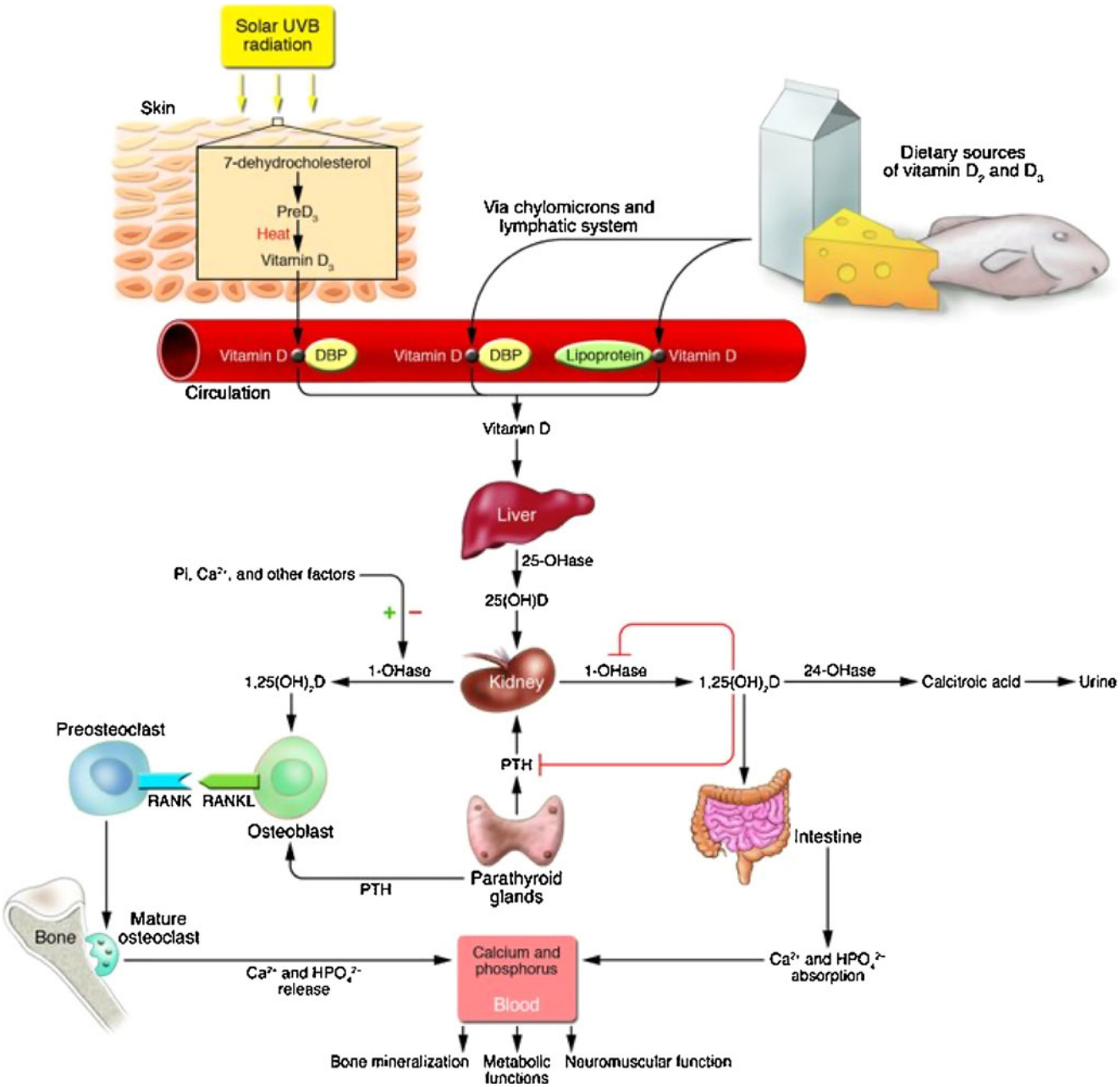


**Fig. 1** Overview of vitamin D metabolism: classical actions. *UVB* Solar ultraviolet B radiation, *DBP* vitamin D binding protein, *25(OH) D* 25-hydroxyvitamin D, *1,25(OH)$_2$D* 1,25-dihydroxyvitamin D, *OHase* hydroxylase, *PTH* parathyroid hormone. Used with permission from [33]

extrarenal tissues, including the skin, prostate, breast, placenta, lymph nodes [28], intestinal cells, dendritic cells, monocytes and macrophages [29], osteoblasts [30] as well as tumor cell lines and parathyroid cells [31]. These cells are capable of the local production of 1,25(OH)$_2$D with autocrine and paracrine functions in extrarenal tissues (Fig. 2). It is currently thought that after uptake of the vitamin D/DBP complex (via megalin and cubilin cell surface receptors), CYP27B1 boosts the local production of 1,25(OH)$_2$D to augment effects of circulating 1,25(OH)$_2$D produced by the kidney [20]. As an example, the human osteoblast could function as an almost autonomous 1,25 (OH)$_2$D-producing cell, independent of renal 1,25(OH)$_2$D production, by expressing megalin and cubilin as well as 25-hydroylase and 1-alpha-hydroxylase activity [24, 30]. In the immune system, macrophage activation leads to upregulated expression of CYP27B1 and thus synthesis of 1,25 (OH)$_2$D (Fig. 2), resulting in increased antibacterial activity by vitamin D-induced synthesis of cathelicidin (CD) [32]. Chronic stimulation as in sarcoidosis may even

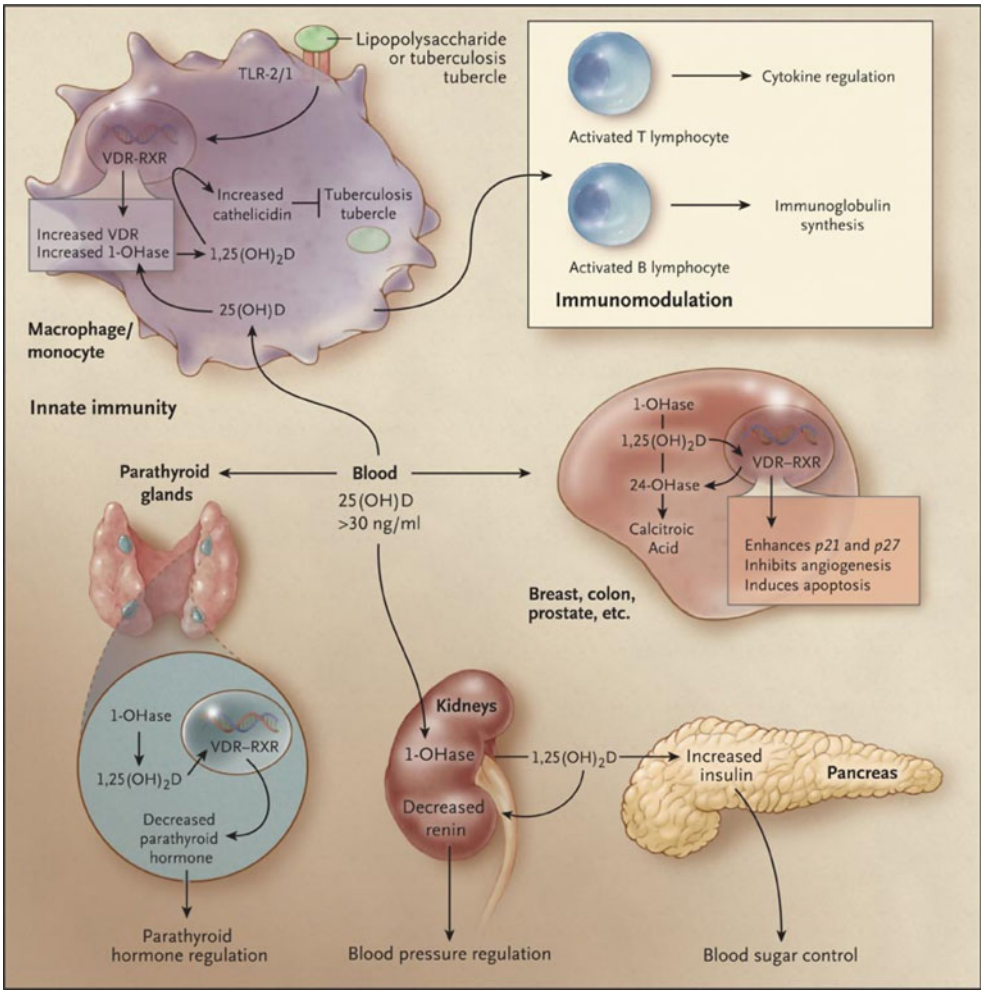


**Fig. 2** Non-classical actions of vitamin D. *VDR-RXR* Nuclear vitamin D receptor-retinoid X receptor. Used with permission from [2]

result in elevation of the 1,25 $(OH)_2D$ serum levels and hypercalcemia [33]. Thus, multiple "non-classical" actions of vitamin D include a multitude of autocrine effects on gene expression in the cardiovascular and immune system, skin, muscle, pancreas, brain, adipocytes, and other tissues. It has indeed been estimated that 3% of the human genome is regulated by the vitamin D endocrine system [34]. Vitamin D homeostasis seems to be critical for many tissues to maintain normal cellular proliferation and differentiation—which could explain why vitamin D deficiency is associated with a large variety of diseases, including cancer, neuromuscular, cardiovascular, renal, and metabolic disorders.

Importantly, the non-classical actions of 1,25$(OH)_2D$ mediated by CYP27B1 are dependent upon circulating 25 (OH)D, the substrate for the extrarenal 1-alpha-hydroxylase in target tissues [24].

Thus, the vitamin D system involves several receptors and ligands and may represent a principal regulatory network controlling cell proliferation and differentiation. An undisturbed function of this network has special relevance to the cardiovascular system.

## Vitamin D requirements

Normal infants require approximately 400–1000 IU of vitamin D daily, and preterm infants need higher daily doses. Revised guidelines of the American Academy of Pediatrics now recommend that all infants and children, including adolescents, have a minimum daily intake of 400 IU of vitamin D beginning soon after birth [35]. However, children and adults without adequate sun exposure may require approximately 800–1000 IU per day [2]. Vitamin D-deficient infants need pharmacological doses of vitamin D because of depleted body stores; the currently recommended regimen is to give a total of 5–15 mg (200,000–600,000 IU) of vitamin D2 or vitamin D3 orally with adequate dietary calcium, either as a single-day therapy or as daily doses of 2,000–4,000 IU/day (50–100 μg/day) for 3–6 months [33]. Similarly, treatment of deficiency states in adults requires about 50,000 IU per week [2]. This would correspond to a dose of approximately 4,000 IU/$m^2$/day. It should be mentioned that there is ongoing discussion as to whether increased vitamin D intake should be recommended to

reduce the high prevalence of vitamin D deficiency and insufficiency in the general population [2].

## Part II: vitamin D deficiency and toxicity

### Definitions

The serum level of 25(OH)D is used for assessing vitamin D status. Vitamin D deficiency can be reliably diagnosed if serum levels of 25(OH)D are <20 ng/ml [2] (normal laboratory levels may differ depending on local conditions). Some authors have used the term severe deficiency for levels <10 ng/ml [4]. "Relative" vitamin D deficiency (= vitamin D insufficiency) is considered at serum levels of 10–30 ng/ml, and substitution with vitamin D preparations should aim for serum levels of at least 30 ng/ml [4] or preferably [20] 40–80 ng/ml (vitamin D sufficiency); highly elevated levels >80 ng/ml (>200 nmol/l) [20], typically >150 nmol/l [36], indicate vitamin D toxicity. Data obtained in the healthy population of adults [37] and adolescents [38] show that secondary hyperparathyroidism (SHPT) occurs at a threshold of 25(OH)D levels of <32 ng/ml. Therefore, although some controversy exists regarding these definitions, there is emerging consensus that the threshold between insufficiency and sufficiency of vitamin D is around 30–40 ng/ml (or 75/100 nmol/l) [20]. More controversy exists regarding an optimal level of 25(OH)D assuring vitamin D homeostasis. While some recommend maintaining a 25-hydroxyvitamin D level of at least 20 ng/ml—and preferably 30–50 ng/ml—in both healthy children and adults, as well as children and adults suffering from CKD [36], others have argued for higher levels of 40–80 ng/ml [20].

### Vitamin D deficiency

Vitamin D deficiency/insufficiency is now regarded as an important risk factor for a variety of common diseases in the general population, including osteoporosis, cancer, autoimmune diseases, and CVD [2]. As defined by low serum levels of 25(OH)D, vitamin D deficiency and insufficiency is endemic both in the European and the U.S. population [36], including children [1, 39].

In fact, vitamin D deficiency may be especially pronounced in infants and young children with the highest demand for calcium and phosphorus due to a rapidly growing skeleton. If untreated, the full blown clinical picture of rickets develops, characterized by bone deformities, growth failure, bone pain, muscular weakness, and failure to thrive in infants, as well as extraskeletal manifestations, including hypocalcemia, tetany and seizures, laryngospasm, cardiomyopathy, and death [33]. Rickets, by no means a disease of the past, with frank bone abnormalities may be observed in healthy children with 25(OH)D levels <15 ng/ml [33]. Paradoxically, rickets is a major health problem in the sunniest areas of the world due to avoidance of sun exposure and deficient dietary supply with vitamin D [33].

### Vitamin D toxicity

Acute vitamin D intoxication with D2 or D3 supplements is a rare event. It is characterized by hypercalcemia, hypercalciuria, and nephrocalcinosis and is usually iatrogenic or due to accidental overdose [40]. However, high-dose maintenance therapy with active vitamin D preparations (calcitriol, alpha calcidiol) in patients with CKD is fraught with the risk of overdose (chronic toxicity) in both adults and children [41–43]. Symptoms include hypercalcemia, hyperphosphatemia, oversuppression of PTH, adynamic bone disease, and ectopic calcifications. Chronic toxicity seems to be especially frequent in dialysis patients, and the risk is further amplified by the use of calcium-containing phosphate binders [44, 45].

## Part III: vitamin D and the cardiovascular system

### Cardiovascular effects of vitamin D deficiency

Large-scale epidemiological studies have provided compelling evidence that vitamin D deficiency is associated with atherosclerosis and CVD adverse events in the general population [44–50].

In a cross-sectional survey involving 4,839 participants in the USA (NHANES), peripheral arterial disease (defined as an ankle-brachial index <0.9) was inversely associated with 25(OH)D levels across all quartiles of measurements; this graded association remained significant after adjustment for age, gender, race, and other variables, including CKD [46]. In another sample of 8,351 adults taken from the same database, vitamin D deficiency, defined as a 25(OH)D level <30 ng/ml, was highly prevalent in adults with coronary heart disease (79%), heart failure (83%), and stroke (74%) [47]. In the prospective Framingham offspring study, vitamin D deficiency was associated with incident CVD (first event) in a sample of 1,739 adults [48]. In another prospective cohort study of 3,258 consecutive patients scheduled for coronary angiography at a single tertiary center, both low 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D levels were independently associated with all-cause and cardiovascular mortality during a median follow-up period of 7.7 years [49]. These observations together with the results of several other cross-sectional studies [50, 51] and a large nested case-control study [52] suggest an important link between vitamin D status and

CVD risk in the general population. Furthermore, this association seems strengthened by a graded relationship between degrees of vitamin D deficiency, i.e. the 25(OH)D levels and CVD in these studies. Consequently, the multivariable-adjusted hazard ratio was 1.53 for levels of 10 to <15 ng/ml and 1.80 for levels <10 ng/ml in the Framingham study [46].

Population-based epidemiological studies have revealed that several major risk factors involved in CVD are inversely associated with vitamin D levels, such as systolic blood pressure [53, 54], insulin resistance [54–56], inflammatory markers and disease activity [57], incidence of diabetes [58], body mass index and obesity [58, 59], and the metabolic syndrome [60].

There are many potential mechanisms that could explain a central role for vitamin D deficiency in the pathogenesis of CVD. First, animal experiments have shown that 1,25 $(OH)_2D$ is a regulator of the renin–angiotensin system. In wild-type mice, inhibition of 1,25$(OH)_2D$ synthesis leads to an increase in renin expression, whereas 1,25$(OH)_2D$ injection leads to renin suppression [61]. Moreover, renin expression and plasma angiotensin II production are highly increased in vitamin D receptor–null (VDR-null) mice, leading to hypertension, cardiac hypertrophy, and increased water intake [61]. Secondly, the vitamin D system seems to be involved in the regulation of coagulation; increased thrombus formation and tissue factor activity were observed in VDR knockout (VDR-KO) mice [62]. In human aortic smooth muscle cells, activation of the VDR by calcitriol and paricalcitol led to downregulation of thrombogenic protein expression [63]. Thirdly, vitamin D deficiency was shown to be associated with inflammation markers, such as high levels of circulating matrix metalloproteinase-9 (MMP-9) and C-reactive protein (CRP), which was partially reversible by vitamin D administration [64]. Fourthly, calcitriol is a regulatory hormone in adipocyte lipid and energy metabolism [65–67] and affects adipocyte cytokine production and, thereby, inflammatory responses [68]. Finally, it has recently been shown that low 25(OH)D levels are associated with an increased risk of developing coronary artery calcifications, both in subjects with or without CKD [3]

## Cardiovascular effects of vitamin D metabolites in vivo and in vitro

Microarray studies in human coronary artery smooth muscle cells have shown that activation of the VDR is followed by the differential regulation of >150 genes, with important differences between vitamin D analogs [69]. Although this may result in a complicated interplay of contrasting effects in vivo, one consistent theme is the regulation of cell proliferation (inhibited) and differentiation (induced). However, the net effect of VDR activation may not only depend on the type of activator but also on dose. While vascular homeostasis is observed under physiological conditions (and within a narrow therapeutic window), both vitamin D deficiency and vitamin D overdose result in vascular damage.

Experimental vitamin D deficiency leads to distinct cardiac pathology. Increased cardiac contractility, hypertrophy, and fibrosis were observed in VDR-KO mice. Longitudinal studies demonstrated the development of cardiomyocyte hypertrophy and heart failure, with a >40% increase in the heart weight/body weight ratio, which was not explained by blood pressure changes [70]. In vitro, 1,25 $(OH)_2D$ has both genomic and rapid non-genomic effects on cultured rat cardiomyocytes, resulting in significant changes in the structure and function of these cells [71]. Furthermore, extracellular matrix remodeling by MMPs seems to be regulated by the vitamin D system: VDR-KO mice showed an upregulation of matrix-degrading MMP together with a downregulation of tissue inhibitors [72]. Taken together, these data indicate a principal role of vitamin D homeostasis in cardiomyocyte physiology, possibly by maintaining differentiation and preventing cardiomyocyte hypertrophy [70].

The beneficial effects of vitamin D in the arterial system seem to be conferred by several mechanisms. First, downregulation of the expression of cytokine-induced adhesion molecules by 1,25$(OH)_2D$ in cultured endothelial cells [73] and mononuclear cells [73] may contribute to protection from inflammation. Secondly, the vitamin D system seems to be a regulator of the response to endothelial injury. It could be shown that endothelial stress leads to a stimulation of proliferation and migration of cultured vascular smooth muscle cells (SMCs) mediated by release of DBP, which in turn could be inhibited by vitamin D metabolites [74]. Thirdly, protection from beta-cell dysfunction [75] and maintenance of insulin sensitivity [55] could indirectly contribute to cardiovascular homeostasis. Remarkably, adherence to prescribed dietary vitamin D supplementation during infancy was associated with a reduced risk of type 1 diabetes in a population-based study with >30 years of follow-up [76].

Finally, the suppression of PTH synthesis must be considered to be beneficial in terms of its role as an independent risk factor for CVD in the general population [77, 78]. PTH has multiple deleterious effects on cardiac and vascular functions in patients with CKD [79–81].

At the other end of the spectrum of vitamin D effects, the administration of toxic vitamin D doses in rats leads to aortic calcification with an upregulation of MMP [82]. The combination of hypervitaminosis D and nicotine administration leads to the destruction and calcification of medial elastic fibers in the aorta and left ventricular hypertrophy in

rats [83]. High-dose 1,25(OH)$_2$D treatment thus mediates elastin degradation and calcification in healthy rats, and this might occur together with decreased elastin production, since tropoelastin, the principal precursor of elastin, is downregulated in a dose-dependent fashion by 1,25(OH)$_2$D in vitro [84, 85]. High doses of vitamin D metabolites have also been employed in animals with experimental uremia, and these deserve special consideration (see below). In addition, studies in mutant mice overexpressing 1-alpha hydroxylase (the FGF-/-and Klotho-/-mouse) have clearly shown that the hypervitaminosis D state mediates vascular and soft tissue calcification, mineral metabolism disturbances, and an ageing phenotype [86, 87].

## Part IV: vitamin D in CKD

### Vitamin D and the incidence, progression, and mortality of CKD

It has been shown that the non-Hispanic African American population in the USA has a higher incidence of end-stage renal disease (ESRD) even after adjustment for multiple risk factors [88]. This could be due to lower levels of 25 (OH)D (<15 ng/ml), as shown by a study in >13,000 individuals followed for over 9 years that revealed a significantly increased risk for incident ESRD, even after multivariable adjustment, including ethnicity [89]. Similarly, a prospective single-center study found an independent significant association of 25(OH)D levels with disease progression and death in patients with CKD stage 2–5 [90]. Moreover, all-cause mortality studied in >3,000 adults with CKD stage 1–4 was significantly increased in participants with 25(OH)D levels <15 ng/ml, and to a lesser extent, in those with levels of 15–30 ng/ml—after adjustment for cardiovascular and other risk factors, indicating a graded relationship between serum 25(OH)D levels and death in patients with CKD [91]. Theoretically, vitamin D deficiency could favor the progression of CKD by promoting hypertension, proteinuria, inflammation, or other mechanisms [89]. Thus, recent studies show that vitamin D deficiency is not only a consequence, but also a cause of CKD and that it is associated with disease progression and all-cause mortality.

### Cardiovascular effects of vitamin D preparations in experimental uremia

Both experimental and clinical studies with vitamin D treatment cannot be compared without consideration of the VDR activator and the dose range. This has been clearly shown in an animal model of CKD with aortic calcifications [low-density lipoprotein (LDL) receptor knockout mice with surgically induced CKD fed a high fat diet]: both calcitriol and paricalcitol provided protection from aortic calcification development at low doses (sufficient to suppress PTH), but strongly enhanced the degree of aortic calcification at higher doses; importantly, protective and toxic doses were different for both vitamin D preparations [92]. It follows from the above that treatment with VDR activators should be closely monitored, with the aim of avoiding toxicity and maintaining normal target organ function.

Cardiovascular effects of vitamin D preparations are of special interest in the clinical setting of CKD. Most animal data on vitamin D effects in uremia have been obtained in subtotally nephrectomized (SNX) rats. Using a low, non-hypercalcemic dose of calcitriol [6 ng/kg subcutaneously (s.c.)] in 3-month old SNX rats, researchers observed a reduction of proteinuria and amelioration of cardiac remodeling (collagen type I and III deposition, MMP-1 expression) after 12 weeks, independent of an elevated blood pressure [93]. The same group reported that treatment of SNX rats with a (non-hypercalcemic) dose of 30 ng/kg s.c. for 12 weeks produced (at a similar degree of hypertension) increased intima thickness and incipient calcification of the aorta and increased expression of osteoblastic phenotype markers in the aortic wall [94]. We have shown that significant cardiovascular morbidity [with hypertension, left ventricular hypertrophy, extensive arterial calcifications, aortic aneurysm formation (accompanied by malnutrition and impaired body growth)] and increased mortality were present in SNX rats treated for 6 weeks with a high (albeit non-hypercalcemic) oral dose of 250 ng/kg calcitriol [95]. Taken together, these studies indicate that in SNX rats, lower doses of calcitriol have beneficial cardiovascular effects, whereas high doses are toxic. Indeed, at a dose of 20 ng/mg given thrice weekly intraperitoneally, calcitriol prevented aortic calcification in LDL receptor knockout mice with CKD, and the VDR activator paricalcitol was protective at a dose of 100 ng/kg, but toxic at 400 ng/kg [92]. If given for a short period, i.e. for only 2–8 days, at a dose of 1000 ng/kg s.c. in rats with intact renal function, calcitriol produced aortic media calcifications; interestingly, these changes were largely reversible over several weeks [96].

Aortic calcification is a cardinal feature of vitamin D toxicity in experimental uremia. At protective dosages, VDR activators can reduce osteoblastic gene expression in the aorta, but higher dosages stimulate aortic calcification [92]. Calcium is mainly deposited in the form of microcrystalline apatite, as it physiologically occurs in bone; however, magnesium-containing whitlockite could be identified in addition to apatite by physicochemical methods [97], probably explained by increased gastrointestinal magnesium absorption induced by calcitriol.

Altogether, CVD in experimental animals with CKD is characterized by arteriosclerosis, vascular calcification, cardiac interstitial fibrosis, and a change in the phenotype of arterial smooth muscle cells toward osteoblasts, which is significantly aggravated by treatment with (high dose) calcitriol. The cardiovascular effects of VDR activators are most likely due to (1) complex regulatory changes in cellular functions mediated by the VDR in the heart and the arterial tree and (2) independent effects of calcium, phosphorus, and PTH levels—although all of the latter are influenced by the vitamin D system.

Treatment with therapeutic doses of VDR activators other than calcitriol, such as paricalcitol or doxercalciferol, has differential effects on aortic calcification, calcium and phosphorus levels, and aortic osteoblast markers in SNX rats [92, 98–100]. While these effects are clearly in contrast to those seen with high-dose calcitriol treatment, the doses employed may not be equivalent. In parallel to the vascular effects, VDR activators were found to have contrasting properties on bone resorption, which were similarly dose-dependent [101]. While it is apparent from these animal studies that VDR activators in general have a dose-dependent deficiency and toxicity, the therapeutic window for beneficial effects is variable for different preparations.

### Clinical studies of cardiovascular effects of vitamin D therapy in adult patients with CKD

Patients with CKD are known to have a high risk for CVD. This seems to be related to the presence of ectopic calcifications and a spectrum of disorders of mineral metabolism and bone [81].The recently introduced term chronic kidney disease-related mineral and bone disorders (CKD-MBD) summarizes these findings [102].

Opinions currently vary as to which stage of CKD vitamin D therapy should be initiated in adult patients with CKD. The Kidney Disease Outcomes Quality Initiative (K/DOQI) guidelines (2003) recommend the measurement of 25(OH)D levels in adult patients with stage 3 and 4 CKD and elevated PTH concentrations [103], and the newer Kidney Disease Improving Global Outcomes (KDIGO) clinical practice guidelines recommend measuring 25(OH)D levels in patients with stages 3–5D [104]. Vitamin D (calcitriol) therapy is targeted to reach PTH levels in the desired range and continued under close observation of calcium and phosphorus levels. Importantly, both adult and pediatric K/DOQI guidelines recommend the initiation of vitamin D therapy only after elevated serum calcium and phosphorus concentrations have been corrected [105]. However, it should also be mentioned that in clinical practice, recommended target levels are often difficult to achieve [106].

Recent retrospective studies in adult dialysis patients have suggested a survival benefit for patients receiving vitamin D therapy. In this population, characterized by a high mortality rate, a significant survival advantage was found over a 2-year period in patients using injectable vitamin D preparations [107]. The use of intravenous (i.v.) paricalcitol (vs. calcitriol) was associated with a significant survival benefit in a large historical cohort study of adult patients on dialysis [108]. Similarly, the intake of oral alfacalcidol was associated with a reduced risk for cardiovascular death in ESRD patients [109], and the use of oral calcitriol was associated with better survival in non-dialyzed CKD patients [110]. In another historical cohort of chronic hemodialysis patients from six Latin American countries, the use of oral active vitamin D preparations was associated with a survival benefit in those patients receiving mean daily doses of less than 1 μg, with the highest reduction associated with the lowest dose [111].

However, such conclusions have been questioned by findings of the Dialysis Outcomes and Practice Patterns Study (DOPPS), which also pointed out the methodological problems of pooling data from various dialysis centers [112]. Likewise, a recent meta-analysis concluded that the beneficial effects of vitamin D therapy in the CKD population were unproven [113]. Prospective, randomized, carefully controlled clinical trials are clearly warranted to clarify this issue.

## Part V: vitamin D therapy in children with CKD

### Rationale

Children with CKD constitute a high-risk group for calcitriol deficiency. Several factors contribute to this condition. First, there is a decreased renal synthesis of $1,25(OH)_2D$ with diminished glomerular filtration rate (GFR), and serum levels of $1,25(OH)_2D$ decrease dramatically with progression of CKD [114]. Recent studies have shown that serum levels of the phosphaturic factor FGF-23 increase early in CKD and are associated with calcitriol deficiency through the downregulation of calcitriol synthesis by CYP27B1 and upregulation of the catabolizing enzyme, CYP24A1 [115]. Thus, calcitriol deficiency precedes the development of hyperphosphatemia and seems centrally involved in the pathogenesis of SHPT. Secondly, patients may lose 25(OH)D bound to DBP with the urine in proteinuric states or with peritoneal dialysate [116], and this may result in significant comorbidity even though clinical symptoms may be absent. Bone loss is indeed present in patients with persisting nephrotic syndrome, especially if high steroid doses are administered [117]. Also, osteomalacia is a characteristic finding in young adult nephrotic patients with normal renal function and correlates with the degree of proteinuria and low levels of 25(OH)D [118].

However, while circulating levels of DBP are invariably low in nephrotic syndrome, it is as yet unclear whether the free fraction of $1,25(OH)_2D$ is affected by proteinuria [119–122]. Finally, pediatric patients with CKD are often hospitalized and engage in less physical activities, which may contribute to low sunshine exposure and vitamin D supply.

Early studies reported that treatment with calcitriol improves mineral metabolism and linear growth and prevents skeletal deformities in uremic children [7, 123], thus providing the rationale for treatment with 1-alpha hydroxylated vitamin D preparations in nearly all children with CKD [124]. These are used to combat both $1,25(OH)_2D$ deficiency and SHPT, both of which develop early in CKD. Metabolic disturbances resulting from a diminished renal function—decreased renal production of calcitriol, hypocalcemia, and hyperphosphatemia—all stimulate the parathyroid gland to increase the synthesis and secretion of PTH. High PTH levels seem to confer damaging effects to a variety of tissues and, most importantly, are associated with the development of CKD-MBD and vascular calcifications. The synthesis of PTH by the parathyroid gland is suppressed by vitamin D metabolites [125, 126], and current therapy is targeted at the suppression of SHPT.

CKD is also characterized by a high incidence of hypertension and insulin resistance, both of which have been linked to vitamin D deficiency [8]. In fact, i.v. $1,25(OH)_2D$ therapy corrected glucose intolerance, insulin resistance, hypoinsulinemia, and hypertriglyceridemia in the absence of PTH suppression in children on hemodialysis [9].

Importantly, children very frequently have vitamin D deficiency and insufficiency at all stages of CKD [6, 127–129]. The percentage of children with 25(OH)D levels <30 ng/ml in these recent studies ranged from roughly 20 to 80%, indicating that a large percentage of patients, if not the majority, was vitamin D deficient. This deficiency could be due to the reasons listed above, but it also suggests lack of attention to adequate vitamin D supplementation by physicians. It should be emphasized that the inadequate supply with external vitamin D sources in these patients adds to the progressive deficiency of renal synthesis of $1,25(OH)_2D$, resulting in a severe imbalance of the vitamin D system with regard to classical and non-classical actions [130]. Thus, patients may be vitamin D deficient despite therapy with active vitamin D preparations [2].

In view of the importance of a continuous supply of 25(OH)D as substrate for the extrarenal 1-alpha-hydroxylase in target tissues, i.e. autocrine constitutive regulatory effects in the cardiovascular and immune system and other organs, it seems important to keep 25(OH)D levels within a high normal range through all stages of CKD in this high-risk group.

As already mentioned, the FGF-23 axis is strongly stimulated in CKD, resulting in calcitriol deficiency starting at early stages of CKD [115]; this could indicate a principally higher demand for vitamin D in CKD. In addition, there is some evidence for vitamin resistance in renal failure, including diminished expression of the VDR in tissues and impaired VDR-regulated gene transcription [131].

## Clinical practice guidelines for vitamin D therapy

The current American [105] and European (largely opinion-based) guidelines for children [132] take careful consideration of vitamin D therapy. Importantly, target ranges of PTH vary according to stage of CKD, and normal limits for calcium, phosphorus, and alkaline phosphatase are age-dependent. Similar to guidelines for adult patients, measurement of 25(OH)D is recommended for CKD stage 2–4 patients if elevated PTH levels are observed, and supplementation with D2 or D3 is recommended if levels are <30 ng/ml (Table 1). Therapy with oral $1,25(OH)_2D$ (calcitriol) should be initiated in patients with stage 2–4 CKD with 25(OH)D levels >30 ng/ml and elevated PTH levels (Table 2) and routinely in dialysis patients adjusted to PTH levels (Table 3) [105]. However, U.S. and European guidelines disagree on daily versus intermittent use of calcitriol. According to the European guidelines, intermittent high-dose vitamin D therapy should be avoided in pediatric patients, since it may adversely affect bone

**Table 1** Recommended supplementation for vitamin D deficiency/insufficiency in patients with CKD stages 3–4

| Definition | 25(OH)D serum level (ng/ml) | Dose of D2 (ergocalciferol) |
|---|---|---|
| Severe vitamin D deficiency | <5 | 8,000 IU/day orally for 4 weeks or 50,000 IU per week × 4, followed by 4,000 IU/day or 50,000 IU 2× per month × 2 |
| Mild vitamin D deficiency | 5–15 | 4,000 IU/day ×12 weeks or 50,000 IU every other week ×12 |
| Vitamin D insufficiency | 16–30 | 2,000 IU/day or 50,000 IU every 4 weeks |

CKD, Chronic kidney disease; 25(OH)D, 25-hydroxyvitamin D

Supplementation recommended for 3 months, followed by control of serum 25-OH D level

Adapted from the Kidney Disease Outcomes Quality Initiative (K/DOQI) used with permission from [105]

**Table 2** Serum levels of PTH required for initiation of oral vitamin D sterol therapy, and recommended initial doses in patients with CKD stages 2–4

| Stage of CKD | Serum PTH level (pg/ml) or (ng/l) | Dose of oral calcitriol |
|---|---|---|
| 2 | >70 | <10 kg: 0.05 μg every other day; 10–20 kg: 0.1–0.15 μg/day; >20 kg: 0.25 μg/day |
| 3 | >70 | Same |
| 4 | >110 | Same |

PTH, Parathyroid hormone

Calcium and phosphorus levels should be within normal, age-appropriate limits

Adapted from K/DOQI; used with permission from [105]

turnover and growth [132]. The American guidelines state that intermittent administration (i.v. or oral) is more effective than daily oral calcitriol in lowering serum PTH levels. These discrepancies could reflect different practices, since i.v. calcitriol is hardly used in European dialysis centers.

## Current clinical practice of vitamin D therapy

Treatment with comparatively high doses of active vitamin D preparations is standard therapy in children with advanced CKD. At the present time, either alpha-calcidiol or calcitriol are given on a daily basis to keep PTH levels in a defined target range to control SHPT, with dose changes according to K/DOQI guidelines adapted for children. Most centers prefer treatment with calcitriol, since no data on efficacy and safety have been published for alpha-calcidiol.

Current therapy with active vitamin D preparations (calcitriol and other VDR activators) is targeted solely at PTH levels, i.e. adjusted to keep PTH within a desired range. However, the frequent side-effects of calcitriol therapy, especially with respect to CVD, demand a judicious use of this powerful hormone. As described below, data from non-invasive imaging studies indicate that toxicity is frequent if aggressive suppression of PTH is attempted with calcitriol. In this regard, the combination of HPT and vitamin D toxicity may be the most detrimental situation both for cardiac and bone health.

## Side-effects of calcitriol treatment

VDR activators suppress the synthesis of PTH but also activate intestinal calcium and phosphorus uptake, thereby increasing the risk for hypercalcemia, hyperphosphatemia, and an elevated Ca × P product in serum, especially when given together with calcium-containing phosphate binders, which is standard therapy in most centers [133]. Thus, the current standard therapy with calcitriol carries the risk of hypercalcemia and hyperphosphatemia. Both of these disorders of mineral metabolism (with a multifactorial pathogenesis) have been shown to be independently associated with mortality in adult hemodialysis patients [134], and these data have led to a different perception of these metabolic disturbances, which were formerly often neglected as mere laboratory abnormalities of uremia. One could argue, however, that an association with a high incidence of CVD could also be explained by many other factors and may not be applicable to children due to the well-known comorbidities in adult dialysis patients. Nevertheless, studies in young patients with CKD have revealed that vitamin D treatment significantly contributes to the development of subclinical CVD. By examining surrogate endpoints for CVD with non-invasive methods, studies in the pediatric population and in young adults with childhood-onset CKD have found conclusive evidence for an increased risk for CVD in the pediatric population. The clinical phenotype shows incipient vascular changes in the carotid artery and the femoral artery, signs of increased vascular stiffening and, frequently, arterial calcifications [10].

There is accumulating evidence that therapy with active vitamin D preparations is a major factor in the pathogenesis of these cardiac and vascular changes [135]. The use of calcitriol has been found to be significantly correlated with the intima-media thickness of the common carotid artery (cIMT) [136], stiffness of the carotid artery in children on dialysis [137], and coronary artery calcifications [138] in children with renal replacement therapy. In a study examining cardiac calcifications, cIMT and pulse wave

**Table 3** Initial calcitriol dosing recommendations for children and adolescents on maintenance dialysis (stage 5 CKD)

| Serum PTH level (pg/ml) | Calcitriol dose per HD session | Daily calcitriol dose for PD patients |
|---|---|---|
| 300–500 | 0.0075 μg/kg (Maximum dose 0.25 μg) | Same |
| >500–1000 | 0.015 μg/kg (Maximum dose 0.5 μg) | Same |
| >1000 | 0.025 μg/kg (Maximum dose 1 μg) | Same |

HD, Hemodialysis three times weekly; PD, peritoneal dialysis, three times weekly

Calcium and phosphorus levels should be within normal, age-appropriate limits; the calcium-phosphorus (in mmol/l) product should be <55 in adolescents and <65 in infants and children

Adapted from K/DOQI; used with permission from [105]

velocity (PWV) in children on dialysis, vitamin D (alpha calcidiol) dosage was correlated with all vascular measurements [139]. Patients with both low and high $1,25(OH)_2D$ levels had significantly greater carotid IMT and calcification scores than those with normal levels [140]. Thus, $1,25(OH)_2D$ levels in these patients were associated with surrogate endpoints for CVD in a U-shaped distribution (Fig. 3).

The use of calcitriol is a major contributor to the development of adynamic bone disease. This form of renal osteodystrophy is common and estimated to occur in up to 50% of adult dialysis patients [141]. Typically, serum PTH levels are low, and oversuppression of PTH by calcitriol treatment is believed to be the main mechanism involved in the pathogenesis of this disorder. It may be associated with an increased risk for bone fractures in adults [141] and diminished linear growth in prepubertal children treated with large intermittent doses of calcitriol [142]. Data from animal experiments indicate that adynamic bone disease may be a direct consequence of CKD, and SHPT may be a compensatory mechanism to maintain bone turnover. Thus, calcitriol/low phosphate treatment in mice with surgically induced CKD led to PTH suppression and adynamic bone disease, which was reversible by the administration of bone morphogenetic protein 7 (BMP-7) in this model.

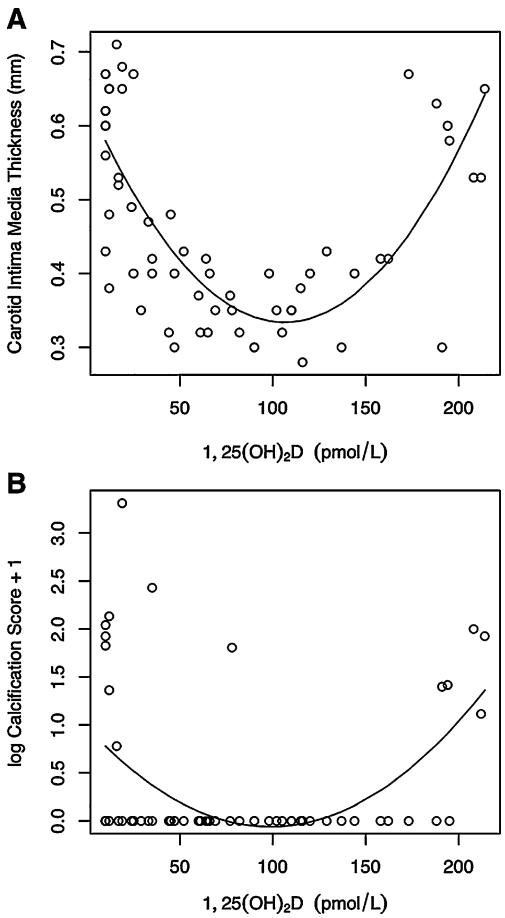


**Fig. 3** Relationship of $1,25\text{-}(OH)_2D_3$ serum levels and vascular changes. Used with permission from [140]

Furthermore, adynamic bone disease is associated with the development of vascular calcifications and aortic stiffness. There is an inverse correlation between bone activity and vascular disease, as estimated by aortic calcifications and arterial stiffness, in adult hemodialysis patients [143]. Osteoporosis is associated with arterial stiffness and atherosclerotic plaque, independent of age [144, 145]. Taken together, these findings suggest a close relation between bone turnover and arterial remodeling, which is as yet largely unexplained.

Finally, treatment with vitamin D preparations may increase the risk for ectopic calcifications at multiple sites. In blood vessels, ectopic calcifications can occur as calcifications of large- and medium-sized arteries, such as myocardial calcifications, or of small-sized vessels in the form of calcific uremic arteriolopathy (calciphylaxis) [146, 147]. Soft tissue calcifications were found in 72 of 120 patients (60%) in a pediatric autopsy study; calcifications were strongly associated with vitamin D therapy and most frequently involved blood vessels, lung, kidney, myocardium, coronary arteries, central nervous system, and gastric mucosa [148]. Clinical studies in adults have shown that the risk for ectopic calcifications is highest with uncontrolled hyperparathyroidism and high bone turnover, on the one hand [44], and low PTH levels/low bone turnover disease, on the other hand [45, 149].

## Other VDR activators

Studies in adult patients suggest that treatment with other VDR activators, such as paricalcitol, is associated with less hypercalcemia and cardiovascular complications. To date, only two clinical studies with paricalcitol have been performed in children. The efficacy of intravenous paricalcitol compared to placebo was studied in children on hemodialysis by measuring the decrease in PTH levels. This randomized double blind, placebo-controlled prospective study [150] included 29 subjects aged 5–19 years. It was performed as a multicenter study at 11 sites in the USA during 2002–2003. Fifteen patients were treated with paricalcitol and 14 with placebo. Paricalcitol decreased PTH levels without a significant effect on serum calcium, phosphorus, and the Ca × P product, and it was tolerated well. In a retrospective analysis of single-center data comparing intravenous calcitriol ($n$=18) with intravenous paricalcitol ($n$=20), it was concluded that paricalcitol was well tolerated and associated with fewer episodes of an elevated Ca × P

product during the treatment period of about 26 weeks [151]. Therefore, published experience in the pediatric age group is currently limited to the intravenous treatment of fewer than 40 patients.

Doxercalciferol has not been studied in children. This vitamin D prohormone (1alpha-OH-vitamin D2) suppresses PTH synthesis at lower doses than paricalcitol (to achieve equivalent suppression) [152], but unlike paricalcitol, it seems to be associated with frequent hypercalcemia and hyperphosphatemia [153, 154]. These findings could indicate a wider therapeutic window for paricalcitol than for calcitriol and doxercalciferol, respectively.

## Finding the therapeutic window

At first glance, it appears to be difficult to reconcile the observed survival benefit of vitamin D treatment in adult dialysis patients with the detrimental effects of calcitriol usage documented in children, adolescents, and young adults. Moreover, since vascular calcification may be the "killer" of patients with CKD [155], the various calcification-promoting effects of vitamin D would suggest that vitamin D treatment is essentially dangerous.

A second look at clinical and experiment data, however, reveals that the relationship of vitamin D with CVD is best illustrated as a biphasic, or U–shaped, curve. As shown in this review, both vitamin D deficiency and toxicity are clearly associated with cardiovascular pathology in a dose-dependent fashion. This relationship indicates important constitutive regulatory functions of vitamin D at the bottom of the curve, i.e. in homeostasis. A biphasic relationship has been shown for several of the major risk factors involved in CVD, such as ageing [156], calcification [3, 157], and atherosclerosis in animal models [86] as well as bone mineralization [158–160]. The biphasic principle is further supported by the observed U-shaped relationship (Fig. 3) between calcitriol levels and vascular changes in dialyzed children [140]. Thus, there is some evidence for both the deficiency [6] and toxicity of vitamin D [139] in pediatric CKD patients, both of which may significantly contribute to the observed high risk for cardiovascular complications.

These data also suggest that the therapeutic window is rather small, presenting a therapeutic challenge to avoid both vitamin D toxicity and deficiency (Fig. 4). Treatment with VDR activators, targeted at PTH levels, affects mineral metabolism, bone, and the arterial wall, but few clinical tools are currently available for monitoring the safety and efficacy of such a powerful therapy. Thus, although hypercalcemia is an indicator of vitamin D toxicity, it should be noted that in animals with CKD, vascular calcification can be induced not only by high [95], but also low doses of calcitriol [94, 161] in the absence of hypercalcemia. Similarly, although the PTH level plays a central role in the therapeutic strategy, bone biopsy studies in children have shown that PTH levels are not a good indicator of bone morphology [43, 162, 163]. Therefore, alternative monitoring strategies are needed.

Studies in adult CKD patients indicate that newer VDR activators are not associated with same toxicity as calcitriol [164]. More studies are needed to explore the individual therapeutic window for each of these drugs in the pediatric population with CKD.

In addition, earlier detection of vitamin D deficiency [by regular measurements of 25(OH)D] and supplementation with D2 or D3 should be routinely considered through all stages of CKD [20]. The non-classical actions of vitamin D are of special relevance to patients with CKD. Not only do these patients have a progressive loss of renal 1-alpha-hydroxylase activity with decreasing GFR, but they also very frequently develop a severe substrate deficiency, i.e. low levels of 25(OH)D. To provide sufficient vitamin D for

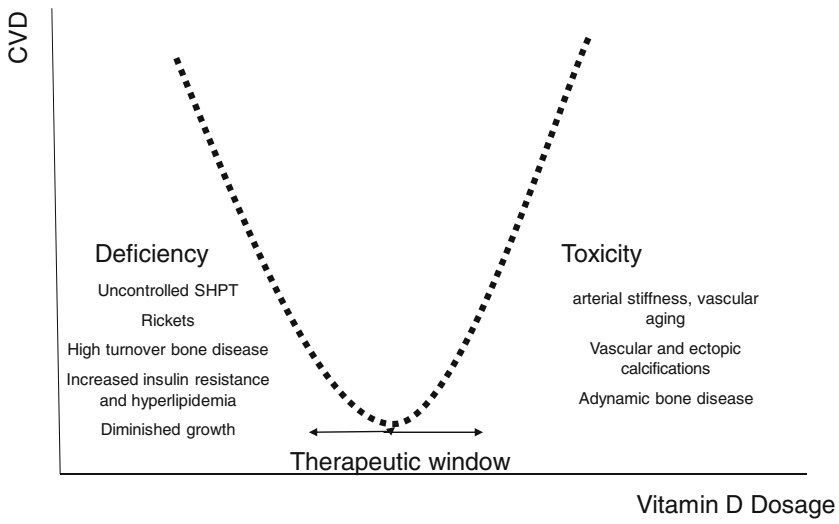


**Fig. 4** Vitamin D and cardiovascular disease in children with CKD. *SHPT* Secondary hyperparathyroidism

these patients, management should include supplementation with 25(OH)D to assure extrarenal synthesis of active vitamin D3 [20]. A long ongoing discussion among pediatric nephrologists has been whether supplementation with D2 or D3 should be included routinely in the management of CKD patients [165]. Clinical experience seems to confirm a salutary effect of adding D3 to the regimen in patients who have been unsuccessfully treated solely with calcitriol (own unpublished observations) or paricalcitol [166]. Apart from this anecdotal experience, however, the critical role of vitamin D for non-classical target tissues seems to be a principal indication for maintaining sufficient 25(OH)D levels in this high-risk population. However, prospective controlled studies are needed to define sufficient levels of 25(OH)D for undisturbed target organ functions in the various stages of CKD. At the present time, it seems prudent to maintain serum levels 25(OH)D and $1,25(OH)_2D$ in the upper normal range. Of note, nutritional vitamin D insufficiency states should be treated with rather high doses of D2 or D3, but pediatric experience is currently limited [128, 167].

In addition, in view of the importance of DBP levels in delivering 25(OH)D to the kidney and peripheral tissues, vitamin D dosages should be reevaluated for patients on peritoneal dialysis [168] and with persistent nephrotic syndrome [169]. Higher vitamin D doses may be required during periods of increased growth velocity [170] and, possibly, growth hormone therapy [171]; on the other hand, oversuppression of PTH by high-dose intermittent calcitriol therapy is associated with diminished linear growth [142]. Careful use of calcium-containing phosphate binders and/or therapy with the newer phosphate binders, such as sevelamer, is an important adjunct to these considerations [172].

Finally, the present guidelines for vitamin D therapy may be inadequate for pediatric patients with CKD. The optimal threshold for 25(OH)D levels and age-group specific tailoring of VDR activator therapy have yet to be developed. Thus, the therapeutic window for treatment with VDR activators needs to be better defined for the pediatric age group by observational studies, including serial measurements of vitamin D levels (25(OH)D and $1,25(OH)_2D$) and other markers. It is obvious that children need D2 or D3 supplements at early stages of CKD and, in addition, substitutional therapy with active vitamin D at later stages to address the physiological needs of a growing organism. However, aggressive treatment of high PTH levels with active vitamin D preparations will result in vascular and bone toxicity. The difficulties in treating SHPT and controlling hyperphosphatemia in patients with CKD are only too well known. There is a current debate on whether SHPT in adult patients should be primarily treated with calcimimetics, while targeting the treatment with vitamin D derivatives aimed at improving cardiovascular and bone health (and for correction of hypocalcemia) [173]. Clearly, carefully controlled studies with calcimimetic drugs are needed in children before such a change in paradigm can be adopted for the pediatric population, and it should be kept in mind that high physiological requirements of vitamin D are present in this age group.

Prospective studies have the greatest potential to identify factors that are associated with the progression of cardiac and vascular changes observed in young patients. Ongoing prospective cohort studies in young patients, such as the CKiD Study [174] and the 4-C-Study [175], therefore aim to explore the prevalence, degree, and progression of CKD and cardiovascular comorbidity in relation to these risk factors, which include vitamin D insufficiency and toxicity.

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