Vitamin D Deficiency: Causes & Treatment

Chapter 5

Vitamin D Deficiency in Children with Chronic Kidney Disease

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Abbreviations: 1,25(OH)2D: calcitriol; 7-DHC: 7-dehydrocholesterol; 25(OH)D: 25-hydroxyvitamin D; BMD: bone mineral density; CKD: chronic kidney disease; CKD-MBD- CKD: Mineral and Bone Disorder; *CYP2R1* gene: encodes the 25-hydroxylase enzyme; *CYP24A1* gene: encodes the 24-hydroxylase enzyme; *CYP27B1* gene: encodes the 1α-hydroxylase enzyme; FGF23: fibroblast growth factor 23; GFR: glomerular filtration rate; IOM: Institute of Medicine; KDIGO: Kidney Disease Improving Global Outcomes; NKF/KDOQI: National Kidney Foundation/Kidney Disease Outcomes Quality Initiative; PD: peritoneal dialysis; PTH: parathyroid hormone; RCT: randomized controlled trial; RDA: recommended dietary allowance; SHPT: secondary hyperparathyroidism; UV: ultraviolet; Vitamin D2 (ergocalciferol); Vitamin D3 (cholecalciferol); VDBP: Vitamin D binding protein; VDR -Vitamin D receptor

1. Introduction

Chronic kidney disease (CKD) is associated with disturbances of mineral and bone metabolism, which includes abnormalities of hypocalcemia, hyperphosphatemia, and vitamin D metabolism. The alteration of mineral bone metabolism can lead to secondary hyperparathyroidism, metabolic bone disease, and extra-skeletal calcifications. The primary focus of the current chapter is to review vitamin D deficiency among children with CKD and to discuss the available therapeutic options available in the treatment and prevention of vitamin D deficiency and its related effects.

2. Vitamin D: Normal Metabolism

Vitamin D is a fat-soluble pro-hormone that is produced endogenously in the skin and can be found in certain foods. Humans acquire approximately 80% of their vitamin D from sunlight-induced cutaneous synthesis, while the rest comes from diet and supplement [1]. The two major forms of vitamin D are ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). Vitamin D2 is commonly found in vegetable sources and “fortified” foods whereas vitamin D3 is found in animal-based foods and is synthesized in the skin. Dietary sources of vita-

min D are important during the winter months, when there is insignificant synthesis of vitamin D3 in the skin; the only significant sources of vitamin D (D2 or D3) are animal liver, fatty fish, egg yolks, fish oils, and commercially irradiated mushrooms [2].

Vitamin D2 is manufactured through the ultraviolet irradiation of ergosterol from yeast; whereas, vitamin D3 is from the conversion of 7-dehydrocholesterol (7-DHC) to previtamin D in the epidermal layer of the skin by solar ultraviolet (UV) B radiation (wavelength 290-315 nm) [3]. Previtamin D from the skin is then rapidly converted to vitamin D3 by a temperature-dependent process. Excess previtamin D3 can be destroyed by sunlight; thus, excessive exposure to sunlight does not cause vitamin D3 intoxication [3].

Both vitamin D2 and D3 have similar metabolism; they both are incorporated into chylomicrons after ingestion and absorbed into the lymphatic system. From the circulation, they either enter the liver to undergo further processing or are taken up by the adipose tissue for storage. For vitamin D to become biologically active, it must undergo two steps of activation.

In the first step, after vitamin D is transported by the vitamin D-binding protein (VDBP) into the liver, it undergoes hydroxylation at the carbon 25 position by the 25-hydroxylase enzyme (coded by the *CYP2R1* gene) to become 25-hydroxyvitamin D [25(OH)D]. 25(OH)D3 is the main circulating form of vitamin D and has a relatively long half life of 2-3 weeks [4]. 25(OH)D circulates bound to VDBP (85–90 %) or albumin (10–15 %), with <1 % in its free forms [5]. VDBP is a 58 kDa protein that has a higher binding affinity than albumin for the vitamin D metabolites [5,6]. The binding of vitamin D to VDBP affects their half-life, rate of uptake by target cells, and clearance through hepatic metabolism and biliary excretion.

In the second step, 25(OH)D is converted to $1,25(OH)_2D$ (calcitriol) in the kidney. The VDBP- 25(OH)D complexes are filtered by the glomerulus and are reabsorbed via megalin/cubilin receptor-mediated endocytosis at the level of the proximal tubular brush border [5,6]. Once inside the tubular cell, 25(OH)D is released and converted in the mitochondria by 25-hydroxyvitamin D-1α-hydroxylase enzyme (coded by the *CYP27B1* gene), to form $1,25(OH)_2D$. This final product is the biologically active form of vitamin D responsible for maintaining calcium and phosphorus homeostasis.

CYP24A1 encodes the key enzyme (24-hydroxylase) involved in the catabolism of both 25(OH)D and $1,25(OH)_2D$ to form $24R,25(OH)_2D3$ and $1a,24,25(OH)_3D3$, respectively. In vitro and animal studies have shown that renal CYP24A1 is suppressed by the parathyroid hormone (PTH) and induced by fibroblast growth factor 23 (FGF23) [5]. The 24-hydroxylation is the first of a five step process in the C-24 oxidation pathway to form the biologically inactive, water soluble calcitroic acid [2,3,5,7]. *CYP24A1* is expressed in most tissues and is regulated by $1,25(OH)_2D$. In normal settings, the inactive metabolites of 25(OH)D circulate at less than 10–15% of the total concentration of 25(OH)D [8].

3. Vitamin D: Physiologic Functions of Vitamin D

Vitamin D hormone functions through a single vitamin D receptor (VDR), a superfamily of nuclear receptors, to initiate or suppress gene transcription of a wide variety of vitamin D dependent genes in vitamin D target cells [2,9]. VDR is located in the target cells of enterocytes, osteoblasts, distal renal tubular cells and parathyroid gland cells [10]. Active vitamin D is tightly regulated by PTH, calcium, phosphorus, and FGF23 levels.

When calcitriol binds to the VDR in the small intestinal cells, a cascade of events leads to the increase of calcium channel expression and entry. Active vitamin D enhances phosphorus absorption in the small intestine as well. Without vitamin D, only 10-15% of dietary calcium and about 60% of phosphorus are absorbed. With active vitamin D, intestinal calcium absorption increases to 30-40% and phosphorus absorption to approximately 80% [3]. In general the dietary calcium is favored to support serum calcium concentrations under normal conditions; however, if this system fails, then calcium mobilization from bones and reabsorption in the kidneys is required to maintain calcium and phosphorus homeostasis.

PTH regulates calcium metabolism by increasing tubular reabsorption of calcium in the kidney, increasing mobilization of calcium from the skeleton by the activation of osteoblasts, and by increasing the renal production of $1,25(OH)_2D$ [8]. PTH also causes phosphaturia, resulting in low to normal serum phosphorus level. The presence of active vitamin D can also suppress the parathyroid gene and proliferation of the parathyroid gland cells [7].

FGF23, a bone derived hormone, down-regulates calcitriol synthesis by decreasing the expression of *CYP27B1* and upregulates calcitriol catabolism via *CYP24A1*. It also increases phosphaturia by internalizing the sodium-phosphate transporter (NaPi2a) channels from the luminal proximal tubular cells via a Klotho-FGF23 signal transduction. FGF23 is stimulated by both phosphate intake and vitamin D treatment.

4. Vitamin D: Extra-Renal Calcitriol Synthesis and Effects

Extra-renal 1α-hydroxylase is also expressed in nonrenal tissues to locally produce $1,25(OH)_2D$, where it is believed to act in an autocrine or paracrine manner to promote additional functions of vitamin D outside the classical endocrine functions in calcium or phosphate homeostasis [2]. The VDR appears also in the placenta, macrophages, skin keratinocytes, promyelocytes, lymphocytes, colon cells, pituitary gland cells, breast cells, prostate and ovarian cells (2,3,7]. Studies shows that 1α-hydroxylase activity in extra-renal cells may contribute to tissue function, cell proliferation, and immunoregulation [6]. It is probably regulated by cytokines and growth factors as part of an inflammatory response [2]. Although it is beyond the scope of this article to discuss every organ system or conditions associated with extra-renal calcitriol synthesis [3], we will discuss the effect of vitamin D in the immune and cardiovas-

cular systems here.

4.1. Immune System

Calcitriol is a potent immunomodulator, whose biological effects are mediated through VDR. VDR is present in most immune cells, including T lymphocytes, neutrophils and antigen presenting cells (APCs) such as macrophages and dendritic cells [11]. $1,25(OH)_2D$ can be synthesized by monocytes and macrophages via the upregulation of extra-renal 1α-hydroxylase [12]. $1,25(OH)_2D3$ primarily affects dendritic cell maturation and macrophage differentiation, and reduces cytokine release [11]. In monocytes, vitamin D acts as a transcription factor for antibacterial peptides such as cathelicidin and beta-defensin 4A [3]. Cathelcidins are a family of antimicrobial peptides found in lysosomes, macrophages and polymorphonuclear leukocytes that serve a role to defend against bacterial infections.

Resting T-cells express almost undetectable levels of VDR, but the receptor levels increase as T-cells proliferate following an antigenic activation [13]. $1,25(OH)_2D$ can inhibit T-cell activation and proliferation by modifying the capacity of APCs to express co-stimulatory molecules, such as CD40, CD80 and CD86; it can inhibit the release of IL-12, a cytokine responsible for stimulating T-helper 1 cell development; and, it can inhibit T-helper 17 cell development and increase the production of T-helper 2 and T regulatory cells [11]. There is also evidence that $1,25(OH)_2D$ may inhibit the differentiation of B cells into plasma cells, thus modulating the production of antibodies [13]. Hence, the suppression of the adaptive immune system could be useful in treating a variety of autoimmune disorders, whereas, the stimulation of the innate immune response could be a useful first line of defense against microbial pathogens [11].

4.2. Cardiovascular System

VDR is also found on endothelial cells, smooth muscle cells and myocytes [9]. One of the earliest stages of the atherosclerosis process is the impairment of endothelial function [14]. Endothelial dysfunction involves a complex mechanism that includes overproduction of reactive oxidative species, inflammatory cytokines and pro-atherogenic lipoproteins, along with an imbalance between molecules for vasodilation (e.g., nitric oxide (NO), produced by endothelial cells) and vasoconstriction (e.g., endothelin-1) [15,16]. In addition, calcitriol directly regulates endothelial NO synthase [17] and suppresses the expression of renal renin production [18]. Flow mediated dilation, release of NO in response to sheer stress, is the gold standard in measuring endothelial function [16].

While vitamin D may improve endothelial function in some studies, a large meta-analysis of 16 adult studies showed no significant improvement of endothelial function with vitamin D supplementation, regardless of the type of vitamin D, method of administration and baseline

25(OH)D levels [15]. Another meta-analysis summarized of 46 prospective trials suggested no effects of 25(OH)D supplementation on systolic or diastolic blood pressure reduction [19]. However, this does not completely exclude a role for vitamin D in modulation of the renin-angiotensin-aldosterone system but suggests that the effect may be small and possibly sub-clinical.

5. Definition of Vitamin D Deficiency

The serum concentration of 25(OH)D is the best marker for vitamin D status because:

a) there is no negative feedback to limit the conversion of pre-vitamin D metabolite from cutaneous synthesis or diet to 25(OH)D

b) has no significant storage in the liver

c) has a half life in vivo of approximately 2-3 weeks

d) and serum/plasma 25(OH)D is stable and resistant to repeated freeze-thaw cycles [20].

The serum 1,25 (OH)D level does not accurately reflect the vitamin D status because:

a) its conversion depends on the availability of its substrate 25(OH)D;

b) its conversion is tightly regulated by circulating PTH, FGF23, calcium and phosphate; and

c) the half-life in vivo is approximately 4-6 hours [20].

The recommendations for the current vitamin D guidelines comes from prior studies that showed a direct relationship between PTH and vitamin D levels. For example, it was shown that PTH levels began to plateau at their nadir when 25(OH)D levels were between 30 and 40 ng/mL (70-100 nmol/L) [21]. Furthermore, healthy adults that were given 50,000 IU of vitamin D once a week for 8 weeks for a 25(OH)D level of between 11 and 25 ng/mL, had an increase in their 25(OH)D levels by more than 100% at the end of 8 weeks, and that the mean decrease on PTH levels declined by 55% in subjects who had 25(OH)D between 11 and 15ng/mL and decreased by 35% for those with 25(OH)D levels of between 16 and 19 ng/mL [22]. Those subjects who had 25(OH)D > 20 ng/mL had no significant change in their PTH level. Thus, it was suggested that vitamin D deficiency should be defined as 25(OH)D <20 ng/mL. It is also observed that intestinal calcium transport increased by 45 to 65% in women when 25(OH)D levels increased from 20 to 32ng/ml (50-80 nmol/L) [23]. It also appears that vitamin D intoxication does not occur until blood levels are 150–200 ng/mL [8]. Vitamin D intoxication is defined as a 25(OH)D level >150 ng/mL that is associated with hypercalcemia, hypercalciuria and, often, hyperphosphatemia [3].

Based on published guidelines [24-29] (Table 1), the critical lower limit of serum vitamin D concentration is not well defined. The Pediatric Endocrine Society [25] and Endocrine Society Clinical Guideline [27] base their recommendations on the effects of vitamin D on the prevention of nutritional rickets, PTH suppression and optimal gut calcium absorption. Other experts recommend that serum 25(OH)D should be greater than 30 ng/ml [8, 29, 30] as concentrations below this are associated with hyperparathyroidism, lower bone mineral density (BMD) and hip fractures [20]. Institute of Medicine [27] recommends their definition based solely on the effects of vitamin D on bone health outcomes.

Table 1: Definitions for Vitamin D Levels (ng/ml)*

Vitamin D status	AAP [24]	Pediatric Endocrine Society [25]	National Osteoporosis Society [26]	Institute of Medicine [27]	Endocrine Society [28]	K/DOQI 2009 [29]
Vitamin D Toxicity	-	>150	>150	-	>150	-
Vitamin D Risk of Toxicity/ Excess	-	>100	-	> 50	> 100	-
Vitamin D Sufficiency/ Adequacy	≥20	≥20	≥ 20	≥20	≥ 30	≥ 30
Vitamin D Insufficiency	16-20	15-20	10-19	12-20	21-29	16-30
Vitamin D Deficiency	<15	≤15	<10	<12	<20	<5-15
Severe Vitamin D Deficiency	-	≤ 5	-	<5	-	<5

*to convert from ng/ml to nmol/L multiply by 2.496 AAP - American Academy of Pediatrics

6. Impact of CKD on Vitamin D Metabolism

6.1. Definition of CKD

CKD is defined as any abnormality of the kidney structure, such as having markers of kidney damage and/or decreased glomerular filtration rate (GFR) < 60ml/min/1.73m^2, present for > 3 months, with implications for health [31]. The markers of kidney damage include albuminuria (ACR >30mg/g; AER > 30mg/24 hours), abnormal renal histology, renal imaging, urinary sediment, electrolyte and/or tubular disorders, and history of kidney transplantation. Furthermore, CKD is further divided into stages based on the glomerular filtration rate (see Table 2).

Table 2: GFR categories in CKD [31]

CKD category	GFR (ml/min/1.73m^2)	Terms
Stage 1	>90	Normal or high
Stage 2	60-89	Mildly decreased
Stage 3a	45-59	Mildly to moderately decreased
Stage 3b	30-44	Moderately to severely decreased
Stage 4	15-29	Severely decreased
Stage 5	<15	Kidney failure

6.2. Prevalence of Vitamin D Deficiency in CKD

It is estimated that more than one billion people have vitamin D insufficiency or deficiency worldwide [3]. It is recognized that 30–50% of both the European and US population are vitamin D insufficient or deficient [8]. According to the NHANES 2001-2004 report, about 9% of the USA healthy pediatric population aged 1 to 21 years is vitamin D deficient (vitamin D level < 15ng/ml) and 61% are vitamin D insufficient (vitamin D level 16-29 ng/ml) [32]. The NHANES 2001-2006 report states that 18% of children aged 1 to 11 years old are vitamin D deficient. Younger age (< 6 years old), girls and non-his panic blacks had the highest prevalence of vitamin D deficiency [33]. In healthy infants and toddlers (8-24 months of age), the prevalence of vitamin D deficiency (vitamin D level < 20ng/ml) is 12.1% [34].

The prevalence of pediatric CKD ranges from 15 to 74.7 cases per million children [35]. About half of the children with CKD have congenital abnormalities of the kidney and urinary tract (CAKUT), such as renal hypodysplasia/aplasia, obstructive uropathy and reflux nephropathy [36, 37]. The prevalence of vitamin D insufficiency and deficiency in children with CKD ranges between 16% and 82% [37].

In a cohort of European children with CKD stages 3-5, 94.2% had vitamin D levels below 30 ng/ml, with 68.2% having vitamin D deficiency (< 16 ng/ml) [37]. In a cohort of 78 children with CKD stages 2-4 in India, vitamin D deficiency was present in 92%: 34% had insufficiency, 50% had mild deficiency and 6.7% had severe deficiency [35]. In a cohort of 167 children, vitamin D deficiency (<20 ng/ml) was present in 12.5% of healthy controls compared to 32% with CKD [38]. Among the different subgroups of children with CKD, the severity of vitamin D deficiency increases with advanced stages [38].

6.3. Risk Factors for Vitamin D Deficiency in CKD

● Limited sun exposure - Children with CKD are at a greater risk for vitamin D deficiency because they are less active and have less sunlight exposure that would otherwise promote cutaneous generation of endogenous vitamin D3 [39]. It is observed that as skin pigmentation increases from type III to types V and VI, the exposure time necessary to maximize previtamin D3 formation increases from 30 minutes to 1 hour and 3 hours, respectively, likely due to melanin competition with 7-DHC [40]. However, regardless of the skin type, previtamin D3 reaches a maximum and plateaus at about 15% of the original 7-DHC and further UV-B radiation increases only in lumisterol-3 (biologically inactive photoisomer of vitamin D3) [40]. As the latitude changes, with a decrease in UV radiation from the equator to the North, the exposure time required to maximize previtamin D3 formation also increases [40].

● Chronic kidney disease - When the GFR falls < 50ml/min/1.73m2, the kidney cannot convert 25(OH)D to 1,25$(OH)_2$D. The proposed reasons are the following ([39]:

a) reduced renal mass is accompanied by reduced availability of 1a-hydroxylase

b) raised phosphate and FGF23 downregulate 1**α**-hydroxylase

c) 1**α**-hydroxylase is suppressed in an acidic and uremic milieu

d) reduced renal megalin expression

e) reduced availability of the substrate 25(OH)D

f) and secondary hyperparathyroidism depletes body stores of vitamin D by promoting *CYP24A1*

PTH levels are usually in the normal range in a majority of patients with CKD stage 2, but it rises in a significant percentage of patients with progressive kidney failure [41]. In CKD stages 2-3, hyperphosphatemia stimulates FGF23 synthesis to decrease phosphate retention [42,43]. FGF23 also suppresses renal 1**α**-hydroxylase enzyme, resulting in a reduction of active vitamin D level [42] and induces 24-hydroxylase expression, which is responsible for the degradation of $1,25(OH)_2D$ [44]. As CKD progresses, maximal FGF23 effects on renal phosphate excretion are reached and phosphate retention ensues. Moreover, the rising PTH levels with advanced CKD causes release of calcium and phosphorus from the bone [45]. Early increases in FGF23 may be the first sign of altered osteocyte function in pediatric CKD patients [42]. In late CKD, hypocalcemia, hyperphosphatemia, and low 1,25 (OH2)D levels combine to stimulate PTH secretion and development of secondary hyperparathyroidism (SHPT).

● Nutrition and Uremia - Nutritional factors also contribute to suboptimal vitamin D status in CKD. Many patients with CKD have decreased food intake of natural sources of vitamin D due to poor appetite and dietary restrictions. Uremia may also be associated with impaired GI absorption of vitamin D [44]. It is also observed that rats with uremic toxins have decreased 25(OH)D synthesis secondary to PTH-mediated reduction in liver CYP450 isoforms (namely, CYP2C11, 2J3, 3A2, 27A1), which impedes the C-25 hydroxylation of vitamin D3 [46]. In this study, rats with CKD and parathyroidectomy had improvement in their CYP450 expression and calcidiol levels when compared to those with CKD and no parathyroidectomy [46].

● Reduced megalin- It is observed that 25(OH)D tubular reabsorption is impaired due to decreased renal megalin in rats [47]. As a compensatory mechanism, downregulation of *CYP24A1* may represent an appropriate response to calcitriol deficiency as a result of GFR loss [5,48], as seen *in vivo* rat studies [46,47]. These findings may explain why some CKD patients have poor efficacy of vitamin D3 therapy on serum calcidiol levels.

● Proteinuria - Proteinuria may be a contributing factor due to increased urinary excretion of VDBP [6, 39]. In a recent multicenter study of 61 children with idiopathic nephrotic syn-

drome, the prevalence of vitamin D deficiency was 100% at diagnosis and 53% at 2–4 months of follow-up after vitamin D supplementation [49]. Furthermore, among 182 children and adolescents with CKD and ESRD, glomerular disease, particularly FSGS, was an independent risk factor for lower 25(OH)D concentrations, adjusted for age, race, season, CKD severity, and hypoalbuminemia [38, 50]. Among non-dialysis participants, FSGS was also associated with significantly lower $1,25(OH)_2D$ concentrations, adjusted for the concentration of 25(OH) D, intact PTH, and FGF23 [5].

● Dialysis - Serum vitamin D level is lower in peritoneal dialysis (PD) and hemodialysis patients compared with CKD and renal transplant patients [51]. Patients on PD have greater loss of VDBP and its bound vitamin D metabolites through the peritoneal membrane compared to hemodialysis and non-dialysis patients [52]. VDBP losses in the urine and dialysate closely mirrors a linear relationship to albumin losses in these fluids [53]. However, total 25(OH)D was not associated with serum VDBP, suggesting that impaired vitamin D metabolism likely involves mechanisms extending beyond urinary losses of binding proteins. This hypothesis is supported by recent evidence that antiproteinuric therapy (i.e, angiotensin converting enzyme inhibitor) in CKD patients reduced urinary VDBP loss, but did not impact serum VDBP or vitamin D concentrations [54]. Also, chronic hemodialysis patients exhibit defective photoproduction of cholecalciferol, despite normal epidermal content of 7-DHC substrate [55].

7. Consequences of Vitamin D Deficiency in CKD

As the vitamin D level decreases in the body, a decrease in intestinal calcium absorption lowers the ionized calcium transiently. Hypocalcemia is recognized by calcium sensors in the parathyroid glands to increase the production and secretion of PTH, which increases calcium reabsorption in renal tubules and increases 1α-hydroxylase activity, which increases calcitriol synthesis [25]. However, with sustained vitamin D deficiency, the prolonged stimulation of the parathyroid glands leads to SPTH.

The activation of osteoblasts by PTH and vitamin D stimulates the transformation of preosteoclasts into mature osteoclasts [10]. Osteoclasts dissolve the mineralized collagen matrix in the bone, which can result in rickets (failure of mineralization of growing bone and cartilage) among children and osteomalacia in in adults. The peak incidence of rickets in normal children is between 3 and 18 months of age [24]. The symptoms of rickets can range from none to varying degrees of irritability, delay in gross motor development, and bone pain. Signs include widening of the wrists and ankles, genu varum or valgum, rachitic rosary, delayed closure of fontanelles, craniotabes and frontal bossing; tooth eruption may be delayed; and may be associated with poor growth [25]. One third of healthy infants and toddlers with a serum 25(OH)D level of 20 ng/ml were noted to have some evidence of bone demineralization by standard radiograph, while only one child had signs of rickets on physical exam [56]. Thus,

subclinical vitamin D deficiency could make detection in a routine clinical practice difficult. In addition, mild rickets has been found in North American infants with serum 25(OH)D levels close to 20 ng/ml [57].

7.1. Bone Health

The consensus guidelines for the management of bone health in children with CKD are largely opinion based, and there is lack of agreement in particular on the target range for PTH [58]. The term used to describe the mineral, skeletal and vascular disease associated with progressive kidney failure is “CKD Mineral and Bone Disorder” (CKD-MBD).

CKD-MBD is manifested by either one or a combination of the following [59]:

1) abnormalities of calcium, phosphorus, PTH, or vitamin D metabolism,

2) abnormalities in bone histology, linear growth, or strength, and

3) vascular or other soft tissue calcification.

“Renal osteodystrophy” is the specific term used to describe the bone pathology that occurs as a complication of CKD and is therefore one aspect of the CKD-MBD. While the definitive evaluation of renal osteodystrophy requires a bone biopsy, this procedure is not routinely performed in the clinical setting. However, bone histomorphometry continues to be the gold standard for the assessment of three essential aspects of bone histology: turnover, mineralization, and volume [42].

With 90% of peak bone mass accrued by 18 years of age and cortical bone comprising 80% of skeletal mass, the growing skeleton is vulnerable to chronic disease, and an abnormal bone density impacts fracture risk [58]. Bone mineralization defects develop even in CKD stage 2, which may precede increases in PTH [41] and have been associated with increased fracture rates observed in CKD [60]. In Chronic Kidney Disease in Children (CKiD) study, the incident fracture burden in pediatric CKD was 12.5%. This fracture incidence was 2-3 times higher than sex-specific rates from the general population, with higher rates in adolescent males compared to females [60].

Defective skeletal mineralization, defined as an increase in osteoid volume/bone volume in combination with a prolongation in osteoid maturation time, is present in 29% and 79% of stage 2 CKD and 4/5 CKD, respectively [41]. Defective mineralization was associated with lower serum calcium levels and increased PTH concentrations. Bone turnover was normal in all patients with stage 2 CKD and increased in only 18% of patients with stage 3 and stage 4/5 CKD, despite the lack of therapy with active vitamin D in the majority of patients [41]. They also noted that the ability of any biochemical parameters (PTH, calcium, phosphorus, vitamin

D, FGF23) to predict bone turnover was very poor [41]. With the use of DEXA (dual energy X-ray absorptiometry) or peripheral quantitative computed tomography (pQCT) to measure BMD in childhood CKD, it is noted that higher PTH levels were associated with lower cortical BMD, lower cortical area, and greater trabecular BMD [58]. This would be anticipated given the high turnover state of hyperparathyroidism. In the only randomized control trial (RCT) of native vitamin D therapy in 40 children with CKD stage 2-3, it was shown that children on ergocalciferol who achieved 25(OH)D levels >30 ng/mL had a delayed development of secondary hyperparathyroidism compared to the placebo group [61].

However, there are few studies in children or adults with CKD that examine the effects of 25(OH)D concentrations on bone, and the optimal target level of 25(OH)D is unclear and may need to be higher than that in the general population. Moreover, there is little evidence to define PTH target levels in children with CKD Stages 2–5D and international guideline committees have suggested different recommendations, with PTH targets ranging from normal in CKD stages 2–4 to 2- to 9-fold above the upper limit of normal in children on dialysis [29, 62] (Table 3).

Table 3: Optimal PTH

CKD category	GFR (ml/min/1.73m2)	Desired PTH level [29]	KDIGO 2017 [62]
Stage 1	>90	35-70 pg/ml	Maintain PTH at approximately 2 to 9 times upper normal limit for assay.
Stage 2	60-89		
Stage 3a	45-59		
Stage 3b	30-44		
Stage 4	15-29	71-110 pg/mL	
Stage 5/D	<15	200-300 pg/mL	

8. Therapeutic Use of Vitamin D in CKD

Food fortification with synthetic vitamin D2 was pioneered and patented in the United States in the 1930 by Harry Steenbock at the University of Wisconsin. With increased fortification of certain foods with vitamin D, rickets was virtually eradicated in North America [2]. Currently, only 1 of 3 children is taking a vitamin D–containing supplement [33]. And although vitamin D supplementation raises 25(OH)D levels, 1 in 10 children taking vitamin D–containing supplements at current doses (100 –400 IU) had a 25(OH)D level of 20 ng/mL and over half of children had a level of 30 ng/mL [33].

Both the Kidney Disease Outcomes Quality Initiative (NKF/KDOQI) 2009 [29] and Kidney Disease Improving Global Outcomes (KDIGO) 2017 [62] experts recommend checking the serum 25(OH)D concentration to assess vitamin D status of children with and without CKD stages 2-5D at least once a year. It is also recommended to supplement with vitamin D2

or D3 when the serum 25(OH)D level falls < 30ng/ml in CKD and dialysis patients. When vitamin D level is replete, then maintenance dose of vitamin D should be continued and levels monitored at least yearly. Vitamin D deficiency and insufficiency in patients with CKD stage 1–5 should be corrected using treatment strategies recommended for the general population [29, 62].

With respect to the recommended dietary allowance (RDA) of vitamin D in the general population, the Institute of Medicine (IOM) from the US and Canada recommend that without adequate sun exposure, children and adults require 800 to 1000 IU of vitamin D per day [3]. These recommendations cover the needs of >97.5% of the population and assume minimal or no sun exposure; thus, providing further safety for individuals with lower endogenous synthesis of vitamin D [27]. More importantly, the dietary reference intake is developed for "normal healthy persons" and not intended for individuals with specific disease states.

8.1. Vitamin D2 or D3?

Despite their structural differences, both vitamin D2 and D3 are are believed to be equipotent and exhibit identical sets of biological responses around the body through the same VDR mediated regulation of genes as 1α,25$(OH)_2$D3 [2]. A meta-analysis including seven heterogeneous studies indicated that regardless of the dosage, frequency or administration (oral or intramuscular), vitamin D3 was more effective at raising serum 25(OH)D concentrations compared to vitamin D2 [63]. Therapy with cholecalciferol, when compared with ergocalciferol, is more effective at raising serum 25(OH)D in non-dialysis-dependent CKD patients using the same dosage (50,000 IU weekly) [64]. It has also been reported that vitamin D3 increases the total 25(OH)D concentration more than vitamin D2 and that vitamin D2 supplementation was associated with a decrease in 25(OH)D3 [65]. One explanation for this difference could be a faster and more selective catabolism of vitamin D2 by nonspecific cytochrome P450s in the liver and intestine that may limit vitamin D2 action preferentially in target cells where it is expressed [2]. Three randomized trials in healthy children and those with nutritional rickets have examined the effects of vitamin D2 and D3 supplementation [20]. Although the patient cohorts, dosage of vitamin D, frequency of administration and duration of treatment varied widely, there was no difference in 25(OH)D levels between vitamin D2 and D3 supplementation. It must also be noted that the available pharmaceutical vitamin D3 dosages greatly differ from one country to another. Moreover, ergocalciferol (vitamin D2) is the form available by prescription in the United States.

8.2. Treatment With Native Vitamin D Supplementation In Children With CKD

Currently, there is no clear consensus between guideline committees on the type of native vitamin D supplement, its dosage, frequency of administration or duration of treatment in healthy children or children with CKD. Currently, pediatric nephrologists recommend nu-

tritional vitamin D in about 73% of cases with CKD 2-5D, and that about 35% of cases had supplemental vitamin D even with levels > 30ng/ml [66].

All guidelines [24-29] (Table 4) recommend a loading regimen or intensive replacement period for a variable duration of 4–12 weeks followed by a maintenance regimen. Unlike the dosage recommendations for vitamin D treatment in healthy children that are based on age, NKF/KDOQI 2009 recommends escalating doses for intensive replacement depending on the baseline 25(OH)D level. In children with CKD, one RCT showed that normal 25(OH)D levels were more difficult to achieve and maintain in CKD stages 3–4 compared with stage 2 [60], suggesting that higher doses of ergocalciferol may be required in children with CKD stages 3-4. It is not recommended to administer vitamin D analogs to treat vitamin D deficiency. Until further studies in children with CKD and on dialysis are available, there is suggestion of using a treatment schedule guided by age and vitamin D level for native vitamin D supplementation in children with CKD Stages 2–5D [20].

Table 4: Vitamin D Supplementation Varies with Society Recommendations

Vitamin D Supplementation in Healthy Children				**Therapy for Vitamin D Deficiency in Healthy Children**
Society	Age (years)	RDA or Maintenance (IU/day)	Maintenance Upper Limit (IU/day)	Treatment
AAP [24]	Birth-18 yo	400	N/A	N/A
Pediatric Endocrine Society [25]	< 1 mo 1-12 mo > 12mo	400 400 400	N/A	1000 IU/day x 2-3 months 1000-5000 IU/day x 2-3 mo 5000 IU/day x 2-3 mo Stoss therapy: 50,000 IU of vitamin D2 weekly for 8 weeks (teenagers and adults)
National Osteoporesis Society [26]	1-6 mo 6mo-12yo 12-18 yo	400-600	N/A	3000 IU daily x 8-12 weeks 6000 IU x 8-12 weeks 10,000 IU x 8-12 weeks
Institute of Medicine [27]	0-6 mo 6-12 mo 1-3 4-8 9-18	400 400 600 600 600	1000 1500 2500 3000 4000	N/A
Endocrine Society [28]	< 1 1-18 > 18	400-1000 600-1000 1500-2000	2000 4000 4000	2000 IU/day (or 50,000 IU/wk) for 6 weeks 2000 IU/day (or 50,000 IU/wk) for 6-8 weeks 6000 IU/day (or 50,000 IU/wk) for 8 weeks
Vitamin D Supplementation in children with CKD				**Therapy for Vitamin D Deficiency in Healthy Children**
NKF/KDOQI [29]	1-18	200-1000	N/A	Vitamin D < 5 ng/ml: Initial Dose: 8000 IU/day (or 50,000 IU/week) x 4 weeks Maintenance Dose: 4000 IU/day (or 50,000 IU twice monthly) x 2 months Vitamin D 5-15 ng/ml: Initial Dose: 4000 IU/day (or 50,000 IU every other week) x 3 months Vitamin D 16-30 ng/ml: Initial Dose: 2000 IU/day (or 50,000 IU every 4 weeks) x 3 months

RDA – Recommended Dietary Allowances; covering requirement of > 97.5% of the population N/A - not available

8.3. Treatment With Active Vitamin D Analogs in Children With CKD

Vitamin D analogs are chemically synthesized, preactivated versions of active vitamin D2 that mimic the actions of the endogenously synthesized active form in any or all of its calcemic or noncalcemic actions [2]. Vitamin D analogs have been shown to up-regulate VDR and *CYP24A1* while down-regulating renal *CYP27B1* expression at the mRNA and protein levels, thereby, turning off the synthesis of endogenous 1α,25$(OH)_2$D3 [2].

Vitamin D analogs are recommended in patients with CKD stages 3-5 in whom serum PTH levels are progressively rising and remain persistently above the upper normal limit for assay despite the correction of modifiable factors. Even though serum concentrations of PTH and alkaline phosphatase are poor markers of bone status, at present, they are the only tools available in clinical practice to guide active vitamin D therapy. Bone biopsies are highly invasive and rarely performed in clinical practice.

Vitamin D analogs [67] that are available for use in paediatric CKD patients include 1-alfacalcidol, calcitriol, paricalcitol and doxercalciferol (Table 5). There are no head-to head trials of all the vitamin D analogs and only limited data comes from a single RCT comparing calcitriol and doxercalciferol in peritoneal dialysis patients [68]. Moreover, there are no RCTs in children with CKD Stages 2–3 primarily assessing the effect of vitamin D analogs versus placebo or ergocalciferol/cholecalciferol on secondary hyperparathyroidism. In children on haemodialysis, two RCTs showed a significant reduction in PTH with thrice-weekly intravenous calcitriol [69] and thrice-weekly intravenous paricalcitol [70] versus placebo control. A significantly increased risk of hypercalcaemia was reported with intravenous calcitriol [69], but not with paricalcitol [70]. A recent meta-analysis concludes that the overall quality of the evidence available is poor and there are no data to indicate any superiority of paricalcitol over other vitamin D analogs in lowering PTH or reducing the burden of mineral loading [71]. Thus, there are no data supporting the clinical superiority of any vitamin D analogues available in the U.S. compared with calcitriol or placebo.

Vitamin D analogs may also be started prior to repletion of 25(OH)D stores provided the child is normocalcaemic. Vitamin D analogs should be started in the lowest dose to achieve target PTH concentrations and maintain normocalcaemia. Calcitriol is not preferred for stoss therapy (high doses of Vitamin D; “stoss therapy”, from the German word *stossen* meaning “to push”) because it is expensive, has a short half-life and does not build up vitamin D stores [25]. Subsequent titration of vitamin D therapy may be performed based on trends in serum calcium, phosphate and PTH levels.

Table 5: Available Formulations of Vitamin D [24,25,67]

Dosage Form	Strength	Trade Names
Vitamin D2 (ergocalciferol)		
Oral Soultion	8000 IU/ml	Calcidiol, Calciferol, Drisdol
Capsule	50,000 IU	Drisdol
Tablet	400 IU	various
Vitamin D3 (cholecalciferol)		Baby drops
Oral drops	400-, 1000-, 2000 IU/drop	D-Vi-Sol, Just D
Oral solution	400 IU/ml	Dialyvite, Decara
Capsule	400-, 1000-, 2000-, 5000-, 25,000- IU	Thera-D, others
Tablet	400-, 1000-, 2000-, 5000-IU	
1,25(OH)2D (calcitriol)		
Oral solution	1 mcg/ml	Rocaltrol
Capsule	0.25-,0.5-mcg	Rocaltrol
Intravenous	1 mcg/ml	Calcijex, Zemplar

1.0 mcg of vitamin D = 40 IU, 1.0 mg of vitamin D = 40,000 IU

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