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Impaired Vitamin D Metabolism in CKD

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Abstract

Vitamin D metabolism consists of both production and catabolism, which are enzymatically driven and highly regulated. Renal vitamin D metabolism requires filtration and tubular reabsorption of 25-hydroxyvitamin D and is regulated by parathyroid hormone, fibroblast growth factor-23, and 1,25-dihydroxyvitamin D. In CKD, renal production of 1,25-dihydroxyvitamin D from 25-hydroxyvitamin D is reduced. In addition, pharmacokinetic studies and epidemiologic studies of 24,25-dihydroxyvitamin D, the most abundant product of 25-hydroxyvitamin D catabolism by CYP24A1, suggest that vitamin D catabolism is also reduced. New insights into the mechanisms and regulation of vitamin D metabolism may lead to novel approaches to assess and treat impaired vitamin D metabolism in CKD.

Historical Context

In the early 19th century, before vitamin D was discovered, cod liver oil was used to successfully treat Rickets.¹ In 1922, experiments demonstrated that the anti-rachitic substance in cod liver oil was not vitamin A, a known cod liver oil component.² The new substance was named vitamin D because vitamins A, B, and C were already ascribed to other substances.³ In 1931, ergocalciferol (vitamin D₂) was purified and crystallized. Cholecalciferol (vitamin D₃) was subsequently isolated in 1935.¹ It was not until the 1970s that 1,25-dihydroxyvitamin D (1,25(OH)₂D) was found to be the potent hormonal form of vitamin D and that it was primarily made in the kidneys.^{4–8} In the 1980s, it was reported that 1,25(OH)₂D was effective for the treatment of secondary hyperparathyroidism as well as osteitis fibrosa in people treated with hemodialysis.^{9,10} These and other seminal studies established the importance of vitamin D metabolism in bone and mineral homeostasis.

In recent years, substantial excitement and controversy have arisen regarding actions of 1,25(OH)₂D on targets other than bone and mineral homeostasis.^{11–14} This is based on three lines of investigation. First, the vitamin D receptor has been found in most tissues of the body.^{11,15} Second, animal models and *in vitro* experiments have shown many actions of 1,25(OH)₂D in tissues other than bone and the parathyroid glands.¹¹ Third, epidemiologic research has demonstrated associations of low circulating concentrations of vitamin D metabolites (25-hydroxyvitamin D (25(OH)D) and 1,25(OH)₂D) with a number of diseases,

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including auto-immune diseases, cardiovascular disease, and cancer.^{11,16–22} As a result, assessment of vitamin D-related biomarkers and treatment with vitamin D-related interventions have become common in patients with and without chronic kidney disease (CKD).¹⁹

As the clinical relevance of vitamin D has evolved, so has our understanding of vitamin D metabolism. It is now clear that multiple vitamin D metabolites are formed in the body through both activating metabolism (production) and inactivating metabolism (catabolism). This metabolism is highly regulated by an intricate endocrine system and becomes disrupted in the setting of CKD. The goal of this article is to review the current state of knowledge regarding vitamin D metabolism, focusing on new insights in its regulation and in particular on vitamin D catabolism.

Production of 1,25-dihydroxyvitamin D

Vitamin D metabolism consists of both production and catabolism, which are enzymatically driven and regulated. Vitamin D production (Figure 1) begins in the skin, where 7-dehydrocholesterol (7-DHC, a cholesterol precursor) is converted by ultraviolet radiation (UVB at a wavelength of approximately 300nm) to pre-vitamin D₃. Pre-vitamin D₃ is then converted by heat to vitamin D₃ (cholecalciferol), which can enter the circulation, bind to vitamin D binding protein (DBP), and travel to the liver. The vitamin D₃-DBP complex is taken up by the hepatocyte, where the microsomal enzyme CYP2R1 adds a hydroxyl group at the 25 position to generate 25-hydroxyvitamin D₃ (25(OH)D₃). 25(OH)D₃ is the most abundant vitamin D metabolite in circulation, where approximately 90% is bound to DBP.^{23,24} In the kidney, the 25(OH)D₃-DBP complex is filtered through the glomerulus into the proximal tubule and is then taken up by the proximal tubular cell via the cell surface receptors megalin and cubilin.^{25,26} Within the proximal tubular cell, an additional hydroxyl group may be added to 25(OH)D₃ in the 1- α position by the mitochondrial enzyme CYP27B1, generating 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃).^{13,15} 1,25(OH)₂D₃, delivered to target tissues bound to DBP, binds to the vitamin D receptor (VDR) to regulate a wide variety of genes.

Vitamin D can also be taken in from the diet either as vitamin D₂ (ergocalciferol) or vitamin D₃ (cholecalciferol). As with skin-derived vitamin D₃, diet-derived vitamin D₂ and vitamin D₃ are converted to 25-hydroxyvitamin D₂ (25(OH)D₂) or 25-hydroxyvitamin D₃ (25(OH)D₃), respectively, in the liver. Though subtle differences exist and may have clinical relevance, further metabolism of 25(OH)D₂ generally parallels that of 25(OH)D₃.²⁷ Therefore, throughout the remainder of this review, 25(OH)D is used to refer to 25(OH)D₂ and 25(OH)D₃, while 1,25(OH)₂D is used to refer to 1,25(OH)₂D₂ and 1,25(OH)₂D₃.

Catabolism of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D

Catabolism (Figure 2) is an essential and often overlooked component of vitamin D metabolism. Both 25(OH)D and 1,25(OH)₂D undergo catabolism via multiple side chain hydroxylations to become more polar metabolites, which are subsequently excreted in both the urine and the feces.²⁸ With the discovery of multiple hydroxylation products of 25(OH)D and 1,25(OH)₂D, it was originally believed that vitamin D catabolism was carried out by multiple enzymes.²⁹ Subsequent work, however, demonstrated that one enzyme, CYP24A1, is capable of catalyzing all of the hydroxylation steps in the catabolism of both 25(OH)D and 1,25(OH)₂D.^{30–32}

CYP24A1 is a mitochondrial enzyme that is dependent on NADPH, adrenonexin, and adrenonexin reductase for activity.³³ Structurally, it is similar to other cytochrome P450 enzymes with 12 α -helices and 4 β -sheets surrounding a heme group. It also has an aromatic

cluster, which is conserved among mitochondrial P450 proteins.³⁴ CYP24A1 catabolizes 25(OH)D to 24,25,26,27-tetranor-23-hydroxyvitamin D and 1,25(OH)₂D to calcitric acid (or via a second pathway to 23,26-lactones) through multiple hydroxylations.^{30–32} This process is thought to proceed with release of the intermediate products at each step of the hydroxylation pathways, raising the possibility that measurement of intermediate products may reflect CYP24A1 activity.³¹

CYP24A1 has been found in many of the tissues that express the vitamin D receptor and is thought to be present in all of them.³⁵ In the kidney, CYP24A1 is found in the proximal and distal tubule as well as the glomerular parietal epithelial cells and the glomerular mesangial cells.³⁶ The CYP24A1 gene is highly inducible by 1,25(OH)₂D in all tissues in which it is found, thus acting as a local control mechanism to prevent tissue-level 1,25(OH)₂D intoxication.³⁷ The importance of this feedback mechanism was demonstrated when inactivating mutations of CYP24A1 were identified in all eight children in a cohort with hypercalcemia after vitamin D supplementation.³⁸ An inactivating mutation of CYP24A1 was also demonstrated in a case of an adult with hypercalcemia, elevated 1,25(OH)₂D, and undetectable serum 24,25(OH)₂D.³⁹

Another P450 enzyme, CYP3A4, also plays a role in vitamin D catabolism. CYP3A4 is a microsomal cytochrome P450 found in multiple tissues including liver, small intestine, colon, kidney, esophagus, and leukocytes.⁴⁰ CYP3A4 is among the most abundant cytochrome P450 enzymes in the liver, and it metabolizes the majority of drugs that undergo oxidative metabolism.^{41,42} In addition to drug metabolism, CYP3A4 also catabolizes 25(OH)D and 1,25(OH)₂D in a manner similar to CYP24A1, with production of 1,24,25-trihydroxyvitamin D and 24,25-dihydroxyvitamin D. It also catabolizes 25(OH)D via a unique pathway, producing 4β,25-dihydroxyvitamin D.⁴³ Interestingly, the substrate binding cavity of CYP24A1 is structurally more similar to that of CYP3A4 than any other of the known CYP protein structures.³⁴ The quantitative contribution of CYP3A4 to vitamin D catabolism, compared with CYP24A1, is poorly defined.

Normal Regulation of vitamin D metabolism

The generation of vitamin D₃ in the skin is not regulated, and bioavailability of vitamins D₃ and D₂ from the gastrointestinal tract is generally high.⁴⁴ In addition, hepatic 25-hydroxylation of vitamins D₃ and D₂ is thought to be a constitutive, unregulated, process. Teleologically, regulation of these steps is probably not necessary due to the low bioactivity of these vitamin D metabolites. 25(OH)D is the first vitamin D metabolite in the activation pathway that has substantial biologic activity at the level of the vitamin D receptor, but it is at least 100-fold less active than 1,25(OH)₂D.^{45,46}

Our understanding of the regulation of 1,25(OH)₂D production has increased dramatically over the past decade. Production and catabolism of 1,25(OH)₂D by the kidney is tightly regulated through a complex system of hormones that together maintain calcium and phosphorus homeostasis (Figure 3). These hormones include parathyroid hormone (PTH), fibroblast growth factor-23 (FGF-23), and 1,25(OH)₂D itself.

PTH, generally secreted in response to low serum calcium, has long been known to increase circulating 1,25(OH)₂D. In the kidney, PTH induces transcription of the CYP27B1 gene by stimulating protein kinase A and protein kinase C signaling pathways.^{47,48} PTH also down-regulates CYP24A1 mRNA transcription in the kidney by destabilizing CYP24A1 mRNA.^{49,50} The regulation of extra-renal 1,25(OH)₂D production and catabolism by PTH is not fully elucidated, but it appears that PTH has less effect on regulation of 1,25(OH)₂D production in non-renal tissues that have been examined thus far. For example, CYP27B1 expression in monocytes, respiratory epithelial cells, and epidermal keratinocytes is not

regulated by PTH.^{15,51} Interestingly, PTH increases CYP24A1 mRNA and protein levels in transformed rat osteoblastic cells, suggesting that effects of PTH on vitamin D catabolism in bone are opposite to those in the kidney.^{50,52,53}

FGF-23 is a recently characterized hormone produced in the bone by osteoblasts and osteocytes.^{54–58} It is a potent inducer of phosphaturia, which is thought to be its primary physiologic role. It also decreases CYP27B1 transcription in the kidney through the mitogen activated protein kinase (MAPK) signaling pathway and has been shown to decrease CYP27B1 renal enzyme activity.⁵⁹ Furthermore, FGF-23 is a potent inducer of CYP24A1 transcription in the kidney.^{54,60} The effects of FGF-23 on vitamin D metabolism in extra-renal tissues are not well characterized. FGF-23 has been shown to increase CYP27B1 mRNA in cultured bovine parathyroid gland cells, which is opposite the effect of FGF-23 on CYP27B1 in the kidney.⁶¹

In the kidney, choroid plexus, and parathyroid gland, FGF-23 is thought to work only in conjunction with the membrane-bound co-factor α -klotho. Low expression of klotho has also been found in several other tissues, but its role there is uncertain. In the kidney, FGF-23 acts by binding to an FGF receptor (type 1, 3, or 4) that is coupled with α -klotho.^{62,63} FGF-23 also has effects on the myocardium independent of klotho.⁶⁴ Furthermore, klotho is found in a soluble circulating form.⁶⁵ Whether klotho has effects on regulation of vitamin D metabolism independent of FGF-23 is not known. Klotho knockout mice have increased expression of renal CYP27B1 and impaired expression of renal CYP24A1, but this is thought to be secondary to the inability of FGF-23 to act in the absence of klotho.⁶⁶ Klotho does, however, have actions independent of FGF-23, as demonstrated by its ability to decrease phosphate reabsorption in the proximal tubule.^{65,67}

In the kidney, 1,25(OH)₂D is a potent regulator of its own production and catabolism through inhibition of CYP27B1 transcription and induction of CYP24A1 transcription, respectfully.⁶⁸ Like PTH, 1,25(OH)₂D does not appear to regulate CYP27B1 in extra-renal tissues. However, 1,25(OH)₂D does potently induce CYP24A1 transcription in extra-renal tissues.¹⁵ In this way, 1,25(OH)₂D tightly regulates its own intra and extra-cellular concentration to prevent 1,25(OH)₂D intoxication.³⁷

One important question that demands attention is whether vitamin D metabolites that are bound to vitamin D binding protein (DBP), bound to albumin, or unbound (free) are most clinically relevant. Experiments in mice have shown that DBP knockout mutants do not display altered calcium homeostasis or differences in target tissue concentration of 1,25(OH)₂D even though they have very low total serum 25(OH)D and 1,25(OH)D concentrations.^{69,70} However, mice without megalin, which is necessary for renal tubular uptake of DBP, develop vitamin D deficiency and bone disease.²⁵ These results seem to be conflicting, with one set of experiments demonstrating that DBP is unnecessary for calcium homeostasis and the other experiments demonstrating the necessity of DBP. Recent human data offer additional insight. These studies demonstrate that the combination of free 25(OH)D and albumin-bound 25(OH)D correlate most precisely with other biomarkers of vitamin D metabolism in some populations, suggesting that this fraction of total 25(OH)D is particularly important.^{71,72} Specifically, in dialysis patients, estimated free and albumin-bound 25(OH)D correlated most closely with serum corrected calcium and PTH concentrations. In young healthy adults, this fraction of 25(OH)D was more strongly associated with bone mineral density than was total 25(OH)D or free 25(OH)D alone. One potential explanation for these findings is that DBP binds 25(OH)D too tightly for it to be biologically active, while albumin binds 25(OH)D much more weakly, allowing for greater biologic availability. Furthermore, megalin mediates proximal tubular cell uptake of albumin, which could explain the findings in megalin knockout mice.⁷³ Further research is

required to determine whether measurements or estimation of specific 25(OH)D fractions is useful for clinical care.

Impaired 1,25(OH)₂D production in CKD

In CKD, 1,25(OH)₂D production is reduced due to alterations in CYP abundance, CYP activity, and delivery of substrate to CYP enzymes (Table 1).^{74–76} These mechanisms of decreased 1,25(OH)₂D production are well-described though their relative contributions remain debated.

With the recent delineation of actions of FGF-23 and the finding of markedly elevated circulating FGF-23 in CKD, there has been interest in FGF-23-mediated down-regulation of CYP27B1 as a cause of decreased 1,25(OH)₂D production in CKD. Multiple studies have demonstrated that FGF-23 decreases CYP27B1 mRNA expression in the renal tubules.^{54,59} A rat anti-glomerular basement membrane antibody model of CKD with elevated circulating concentrations of FGF-23 and PTH demonstrated decreased renal CYP27B1 expression, increased renal CYP24A1 expression, and decreased circulating 1,25(OH)₂D concentration. Administration of FGF-23 antibodies increased renal CYP27B1 mRNA, decreased renal CYP24A1 mRNA, and restored circulating 1,25(OH)₂D to normal.⁷⁷ These effects were not mediated by PTH, because circulating PTH concentration fell with FGF-23 antibody administration. Together, these studies consistently demonstrate that FGF-23 acts to reduce renal CYP27B1 expression. Studies have also shown that PTH increases CYP27B1 expression in renal tubular cells.^{47,48} Thus, two of the most markedly elevated hormones in CKD regulate CYP27B1 in a reciprocal manner. Other metabolic abnormalities commonly present in CKD, including diabetes, acidosis, and hyperuricemia, may also decrease CYP27B1 expression.^{78–81} Increased serum phosphorus is associated with lower circulating 1,25(OH)₂D concentration, though it is not clear whether this represents a direct effect, is mediated by FGF-23, or is confounded by other factors.

Given the competing effects of FGF-23 and PTH on CYP27B1 transcription, several studies have addressed the net effects of CKD on CYP27B1 expression and activity, with mixed results. One study of 3/4 nephrectomy rats showed significantly elevated CYP27B1 mRNA expression.⁸² Another study of adenine-induced CKD in rats showed increased CYP27B1 mRNA and protein expression compared to normal.⁸³ A study of human kidney biopsies demonstrated no difference in CYP27B1 expression with decreased creatinine clearance despite decreased circulating 1,25(OH)₂D concentrations. This study did find, however, that increased renal inflammation was associated with elevated CYP27B1 mRNA expression in the same biopsies.⁸⁴ Therefore, while FGF-23 clearly down regulates CYP27B1 transcription, it is not clear whether the decreased circulating 1,25(OH)₂D concentration associated with CKD is due primarily to decreased CYP27B1 transcription induced by excess FGF-23 or related metabolic abnormalities.

Instead of, or in addition to, FGF-23-mediated reduction of CYP27B1 transcription, decreased 1,25(OH)₂D production in CKD may be due to impaired delivery of 25(OH)D to CYP27B1 in the renal proximal tubule and/or decreased CYP27B1 activity. In CKD, circulating 25(OH)D concentration is often low due to obesity, low sun exposure, decreased cutaneous synthesis caused by dark skin or azotemia, and limited dietary vitamin D intake.^{85–87} Moreover, with declining GFR, less 25(OH)D is filtered, and therefore less 25(OH)D is available for reabsorption into the proximal tubule. In addition, absorption of filtered 25(OH)D may be impaired in CKD. Expression of megalin, which is essential for 25(OH)D uptake, is diminished in CKD,⁸² and with albuminuria, filtered 25(OH)D may be lost in the urine instead of undergoing proximal tubular reabsorption.^{88,89} Once 25(OH)D substrate is absorbed into proximal tubular cells, its metabolism to 1,25(OH)₂D may still be

impaired by reduced CYP27B1 activity. Diabetes (a common cause of CKD) and metabolic acidosis⁷⁸ (a common result of CKD) have been associated with low circulating 1,25(OH)₂D independent of circulating 25(OH)D concentration and estimated GFR.^{78,79} Potential explanations for these observations include diminished enzyme activity or a general reduction in proximal tubule metabolic capacity.

Impaired vitamin D catabolism in CKD

Kidney disease also disrupts vitamin D catabolism. Within the kidney, CYP24A1, like CYP27B1, is subject to competing hormonal regulation by FGF-23 and PTH. The net effects of elevated concentrations of each of these hormones on vitamin D catabolism in CKD are debated. One prevailing hypothesis posits that elevated circulating FGF-23 concentration prevails over other signals, including PTH, to induce CYP24A1 transcription, which in turn leads to accelerated 25(OH)D and 1,25(OH)₂D catabolism.^{50,90,91} This hypothesis is based primarily on mRNA studies in rat models of CKD, which show increased CYP24A1 mRNA in diseased compared to healthy renal tissue.^{77,83} This mechanism has been used to explain the low circulating 25(OH)D concentrations commonly found in people with CKD. However, a study of human biopsy tissue showed no difference in CYP24A1 transcription with lower eGFR.⁸⁴ Thus, neither renal CYP24A1 expression nor net renal vitamin D catabolism is completely understood in CKD.

In CKD, vitamin D catabolism may also be impaired in non-renal tissues. The relative contribution of extra-renal CYP24A1 to overall vitamin D catabolism has not been determined. It is possible that non-renal tissues contribute substantially to vitamin D catabolism, and that this contribution is decreased in CKD due to systemic 1,25(OH)₂D deficiency.

Several studies have assessed the net effects of CKD on vitamin D catabolism. Horst et al measured 24,25(OH)₂D in anephric and control pigs. He found that the anephric pigs were able to generate substantial circulating concentrations of 24,25(OH)₂D after administration of very large doses of cholecalciferol, but that the appearance was delayed and concentrations were lower than in normal controls.⁹² Gray found that the half-life of 25(OH)D was twice as long in anephric versus normal humans (42 versus 23 days, respectively).²⁸ Studies in CKD not requiring dialysis are conflicting. While Dusso observed no significant difference in the metabolic clearance rate of 1,25(OH)₂D in dogs,⁷⁴ Hsu found a 22% lower metabolic clearance rate of 1,25(OH)₂D in humans with CKD compared to normal control subjects.⁹³ These results suggest that extra-renal tissues are able to catabolize circulating 25(OH)D and 1,25(OH)₂D, but that renal CYP24A1 is the primary site of vitamin D catabolism, which may be reduced in CKD.

24,25-dihydroxyvitamin D as a biomarker of vitamin D catabolism

Circulating 24,25(OH)₂D concentration has offered new insight into vitamin D catabolism in CKD. 24,25(OH)₂D is the predominant initial product of 25(OH)D catabolism by CYP24A1. Most 24,25(OH)₂D generated by CYP24A1 likely quickly undergoes further metabolism by CYP24A1 to more polar metabolites. However, substantial quantities of 24,25(OH)₂D appear in blood. 24,25(OH)₂D generally circulates in concentrations of 1–10 ng/mL, higher than any vitamin D metabolite other than 25(OH)D and 100-fold higher than 1,25(OH)₂D. In addition, 24,25(OH)₂D has a circulating half-life of approximately 7 days,⁹⁴ long enough that single measurements offer reasonably precise estimates of short-term blood concentrations. While the exact relationship of circulating 24,25(OH)₂D concentration to CYP24A1 activity and 25(OH)D half-life has not been determined using gold standard methods, it is likely that circulating 24,25(OH)₂D concentration, relative to circulating 25(OH)D concentration, provides a reasonable estimate of net 25(OH)D catabolism.

With this in mind, a number of studies have measured circulating 24,25(OH)₂D concentration in order to investigate vitamin D catabolism in CKD. Several small studies in dialysis patients have shown very low to undetectable concentrations of circulating 24,25(OH)₂D.^{28,92,95–98} Another study examined circulating 24,25(OH)₂D in 76 non-dialysis CKD patients with a range of eGFR.⁹⁹ This study found a direct correlation between estimated creatinine clearance and 24,25(OH)₂D concentration ($r = 0.25$, $p < 0.05$). It also showed relationships of 24,25(OH)₂D with 25(OH)D and serum albumin in multiple regression analysis. A limitation of these studies was measurement of 24,25(OH)₂D by competitive protein binding assays. Given that differences in structures of circulating vitamin D metabolites are subtle, cross-reactivity is an important concern with antibody-based assays. For example, a recent study¹⁰⁰ demonstrated that an antibody-based assay of plasma 1,25(OH)₂D correlated poorly with plasma 1,25(OH)₂D measured by mass spectrometry, suggesting that the antibody-based assay had substantial cross-reactivity with other circulating vitamin D metabolites. Therefore, we developed a novel high-throughput mass spectrometry assay for circulating 24,25(OH)₂D. This assay measures 24,25(OH)₂D concentration across its physiologic range (lower limit of detection 0.5ng/ml) with excellent reliability (inter-assay imprecision 8.58% at 1.5ng/ml).¹⁰¹

We measured serum 24,25(OH)₂D concentration among 278 participants in the Seattle Kidney Study and found that lower eGFR was associated with lower circulating 24,25(OH)₂D and 1,25(OH)₂D concentrations but not with 25(OH)D concentration.¹⁰² Black race, diabetes, lower serum bicarbonate, lower 25(OH)D, and higher urinary albumin to creatinine ratio were also independently associated with lower 24,25(OH)₂D concentration. These results suggest that CKD is a state of both decreased 1,25(OH)₂D production and decreased 1,25(OH)₂D catabolism.

Hypothesis: an evolving concept of impaired vitamin D metabolism in CKD

Recent insights into vitamin D regulation and catabolism suggest that CKD is a state of stagnant vitamin D metabolism characterized by reduced vitamin D catabolism and turnover in addition to reduced 1,25(OH)₂D production. In this paradigm, competing effects of PTH and FGF-23 on the expression of CYP27B1 and CYP24A1 either balance each other or are superseded by a general decrease in vitamin D metabolic function in the kidney. Causes of decreased vitamin D metabolic function could include impaired uptake of 25(OH)D, diminished metabolic capacity of proximal tubular cells perhaps related to oxidative stress and redox imbalance, or a simple reduction in the number of functioning nephrons. If this hypothesis is correct, such diminished enzymatic function parallels that seen in other metabolic and signaling pathways in kidney disease including decreased drug metabolism in the gastrointestinal tract, cytochrome dysfunction in oxidative phosphorylation, dysregulated lipid metabolism, and altered growth hormone signaling.^{103–106} Alternatively, impaired vitamin D catabolism may be a direct consequence of reduced 1,25(OH)₂D production due to reduced 1,25(OH)₂D-mediated stimulation of CYP24A1. Ideally, the precise nature of impaired vitamin D metabolism and relationships of circulating hormones, other biomarkers, and treatments to vitamin D production and catabolism would be established using pharmacokinetic studies across a broad range of kidney function and race/ethnicity.

Implications for diagnostic testing

Current clinical management of bone and mineral metabolism in CKD focuses on evaluation and treatment of 25(OH)D and PTH. Recent advances make it clear that these biomarkers only partially capture vitamin D metabolism and its derangements in CKD.

Circulating 25(OH)D concentration is generally regarded as a biomarker of total vitamin D intake from cutaneous synthesis and dietary consumption, a biomarker of total body

25(OH)D stores, or both.^{11,19,107,108} While these interpretations are founded on high-quality studies and appear to be largely correct, they are also likely oversimplified to some extent. In particular, vitamin D catabolism may also influence steady-state blood 25(OH)D concentrations, particularly in CKD. Most studies of CKD populations report a high prevalence of 25(OH)D deficiency.^{99,109–113} Precise prevalence estimates vary based on geography, demographics, stage of kidney disease, and medication/supplement use. 25(OH)D deficiency is also common in the general population, and data conflict regarding whether 25(OH)D concentrations are lower in CKD than among people with normal kidney function. For example, Ravani et al found a strong positive correlation between eGFR and 25(OH)D in 168 Nephrology clinic patients with CKD.¹¹⁴ In contrast, in the United States population, 25(OH)D concentration was lower in people with eGFR less than 30 ml/min/1.73m² (mean 24.7 ng/ml) but not in those with eGFR 30–59 ml/min/1.73m² (mean 30.4 ng/ml) or eGFR 60–89 ml/min/1.73m² (mean 31.0 ng/ml), compared with eGFR ≥ 90 ml/min/1.73m² (mean 29.4 ng/ml).¹¹⁵ In the Study for Early Evaluation of Kidney Disease (SEEK) and the Seattle Kidney Study, 25(OH)D did not correlate with estimated GFR, while concentrations of 1,25(OH)₂D, PTH, and FGF23 demonstrated strong correlations with eGFR.^{78,101} It is possible that conflicting associations of estimated GFR with 25(OH)D concentration relate to differences in vitamin D catabolism. In some populations, decreased 25(OH)D production due to limited ultraviolet light exposure, impaired cutaneous vitamin D synthesis,⁸⁷ or dietary restriction of vitamin D-containing foods may be balanced by decreased 25(OH)D catabolism. In other populations, an imbalance of these processes may lead to changes in steady state circulating 25(OH)D concentration and/or steady state 25(OH)D body stores. Interestingly, a common genetic polymorphism in CYP24A1 has been reported to influence steady-state 25(OH)D concentration in the general population.¹¹⁶

PTH is directly suppressed by 1,25(OH)₂D.^{117–119} As a result, PTH is frequently used in practice as a read-out of 1,25(OH)₂D activity at the level of the parathyroid gland. While using a functional measure to ascertain 1,25(OH)₂D status has important strengths, this approach also has limitations. For example, PTH secretion is also controlled by other factors, including circulating calcium concentration and parathyroid gland hypertrophy. Moreover, to the extent that PTH reflects 1,25(OH)₂D activity, it does so only within one organ.

Additional biomarkers are needed to better recognize, study, and treat impaired vitamin D metabolism. This may require multiple biomarkers, each measuring different aspects of the vitamin D metabolic system. A biomarker of tissue-level 1,25(OH)₂D activity is particularly important but has not yet been identified. Tissue-level activity is the net result of VDR expression, 1,25(OH)₂D availability, and 1,25(OH)₂D catabolism. All three of these are likely to vary depending on the tissue examined. The CYP24A1 gene, which is expressed in all tissues that express VDR, is currently thought to be the most transcriptionally responsive gene to 1,25(OH)₂D. To the extent that it may represent 1,25(OH)₂D-dependent induction of CYP24A1 in target tissues, circulating 24,25(OH)₂D concentration may be a candidate biomarker for estimation of tissue-level 1,25(OH)₂D activity, but this has not yet been studied.

Circulating FGF-23 is also a promising biomarker for the assessment of vitamin D metabolism, and of mineral metabolism more broadly. Circulating FGF-23 concentrations increase dramatically in CKD, and higher FGF-23 concentrations are associated with increased risks of cardiovascular disease and death.^{120–123} In addition, FGF-23 has direct effects on the myocardium leading to hypertrophy, and therefore may be causally related to adverse outcomes.⁶⁴ Currently, it remains unclear whether FGF-23 identifies individuals who respond to vitamin D-related therapies, or whether treatments that specifically target

FGF-23 improve patient outcomes in CKD. Studies addressing these questions may identify new approaches to patient evaluation and treatment in the short term.

Implications for treatment

Mounting evidence suggests that disordered vitamin D metabolism has negative health consequences in CKD, but appropriate treatment strategies are not yet clear.¹²⁴ Specifically, current evidence for proper use of 1,25(OH)₂D and 1,25(OH)₂D analogs, nutritional vitamin D (cholecalciferol and ergocalciferol), or other novel therapies is limited.¹²⁵

Active vitamin D analogs and 1,25(OH)₂D have long been used to treat secondary hyperparathyroidism in patients treated with hemodialysis. More recently, animal experimental studies have demonstrated potentially beneficial effects on processes not directly related to bone and mineral homeostasis.^{11,14,19,51} In addition, observational studies suggest that treatment of CKD and ESRD patients with 1,25(OH)₂D or active analogs reduces the risk of mortality.^{126–131} Randomized controlled trials to date, however, have not confirmed beneficial effects of 1,25(OH)₂D or active analogs on targets beyond bone and mineral homeostasis. The VITAL study did show reduction in albuminuria and systolic blood pressure with 1–2 µg of paricalcitol daily among people with type 2 diabetes and pre-dialysis CKD, but follow-up was limited to 24 weeks.¹³² The PRIMO trial randomized 227 pre-dialysis CKD patients to oral paricalcitol or placebo and found no difference in left ventricular mass index during 48 weeks of follow-up.¹³³ A Cochrane review of randomized clinical trials of 1,25(OH)₂D and active analogs, which did not include data from VITAL or PRIMO, concluded that these interventions lowered PTH but had no significant effects on clinical outcomes in CKD.¹¹⁷

It is possible that compensatory regulatory responses to treatment with 1,25(OH)₂D or active analogs reduces the benefit of this therapy. For example, circulating FGF-23 increases in response to 1,25(OH)₂D administration.¹³⁴ Faul et al reported that FGF-23 concentration and left ventricular hypertrophy (LVH) were independently associated in a cohort of CKD patients, and that administration of FGF-23 causes hypertrophy in isolated cardiac myocytes and LVH in *klotho*-deficient mice.⁶⁴ In addition, vitamin D analogs induce CYP24A1, which could then lower circulating 25(OH)D and 1,25(OH)₂D concentrations and mitigate some beneficial effects of treatment.

A large body of evidence now suggests that non-renal 1,25(OH)₂D production is important to maintain beneficial autocrine and paracrine pathways.^{13,14} Therefore, circulating 25(OH)D may be an important therapeutic target. The Institute of Medicine determined that a circulating 25(OH)D concentration of 20 ng/mL was generally sufficient to maintain bone health. A recent cohort study among older adults with predominantly normal estimated GFR reported that risk of hip fracture, myocardial infarction, cancer, and death similarly increased below a threshold 25(OH)D concentration near 20 ng/mL.¹³⁵ However, higher concentrations of 25(OH)D may be beneficial in CKD. In CKD, but not populations with normal estimated GFR, circulating 1,25(OH)₂D and 25(OH)D concentrations directly correlate, even above 25(OH)D concentrations of 20 ng/mL, suggesting substrate dependence of CYP27B1 function above this threshold. Impaired entry of 25(OH)D into cells has been suggested by decreased megalin expression in animal models of CKD,⁸² and this may explain in part different thresholds by estimated GFR.

Vitamin D₂ (cholecalciferol) and vitamin D₃ (ergocalciferol) supplements are commonly used by people both with and without CKD and less commonly in dialysis patients. A meta-analysis reviewing the literature through November 2006 found 18 randomized controlled trials of cholecalciferol or ergocalciferol conducted predominantly among people with normal kidney function. Overall, there was decreased mortality among people taking

vitamin D supplements, with a summary relative risk of death of 0.93 (95% confidence interval, 0.87 – 0.99).¹³⁶ One post-hoc analysis of cholecalciferol supplementation and calcium in people with mild to moderate CKD showed improved bone mineral density at the distal radius compared to placebo.¹³⁷ Other non-randomized studies of nutritional vitamin D supplementation in hemodialysis patients have shown various improvements in intermediate outcomes including increased circulating 1,25(OH)₂D and albumin concentrations as well as decreased calcium, PTH, inflammatory cytokines, left ventricular mass index, and erythropoietin stimulating agent dose.^{138–140} Well-controlled clinical trials are needed to better test the effects of supplementation with vitamins D₂ and D₃ in CKD. The Vitamin D and Omega-3 Trial (VITAL), currently underway, is a large randomized 5 year trial of cancer and cardiovascular disease prevention with cholecalciferol and omega-3 fatty acids in healthy adults, selected without regards to kidney function, and it may provide further guidance on the use of vitamin D supplementation in the general population.¹⁴¹

Inhibition of 1,25(OH)₂D catabolism has been suggested as a novel approach to vitamin D therapy in CKD.⁵⁰ A CYP24A1 inhibitor has been developed, in part to mitigate the ability of cancer cells to escape the anti-proliferative effects of 1,25(OH)₂D by inducing CYP24A1.^{142,143} This inhibitor would theoretically raise tissue and circulating 25(OH)D and 1,25(OH)₂D concentrations, which could be beneficial in CKD. However, use of such an inhibitor must be approached with caution because it may exacerbate a CKD-related impairment of vitamin D catabolism and impair the ability of target tissues to protect against 1,25(OH)₂D intoxication.

Future directions

While our understanding of vitamin D metabolism continues to evolve, many important aspects of diagnosis and treatment remain undefined. Physiologic studies are needed to better define vitamin D metabolism in greater detail across kidney function and other important clinical characteristics, such as race and ethnicity. Improved biomarkers are needed to diagnose perturbations in populations and individuals and to generate and test novel therapeutic interventions. Such biomarkers can also be utilized to better understand the effects of existing interventions on the intricate vitamin D metabolic system. Ultimately, this combined knowledge should be used to select promising populations, interventions, and outcomes to test whether intervening on impaired vitamin D metabolism improves clinical outcomes relevant to CKD.

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Figure 1.
Production of 1,25(OH)₂D. UV-B, ultraviolet B radiation.

Figure 2.

Catabolism of 1,25(OH)₂D and 25(OH)D. 25(OH)D₃, 25-hydroxyvitamin D₃; 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; 23,25(OH)₂D₃, 23,25-dihydroxyvitamin D₃; 24,25(OH)₂D₃, 24,25-dihydroxyvitamin D₃; 1,23,25(OH)₃D₃, 1,23,25-trihydroxyvitamin D₃; 1,24,25(OH)₃D₃, 1,24,25-trihydroxyvitamin D₃; 24,25,26,27-tetranor-23(OH)D, 24,25,26,27-tetranor-23-hydroxyvitamin D.

Figure 3.

Feedback regulation of vitamin D metabolism. 24,25(OH)₂D₃, 24,25-dihydroxyvitamin D₃; 25(OH)D₃, 25-hydroxyvitamin D₃; 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; PTH, parathyroid hormone; FGF-23, fibroblast growth factor-23.

Table 1

Effects of metabolic and hormonal alterations in CKD on vitamin D metabolism in the kidney.

Altered CYP Abundance	
Transcription	
•	↓CYP27B1/↑CYP24A1
–	1,25(OH) ₂ D
–	FGF-23
–	Phosphorus
•	↑CYP27B1/↓CYP24A1
–	PTH
Translation	
•	?

↓CYP activity	
•	Diabetes
•	Acidosis
•	↓ Metabolic capacity
–	Oxidative stress
–	Mitochondrial dysfunction
–	↓ NADPH

↓Delivery to proximal tubular cell	
•	25(OH)D deficiency
–	Obesity
–	Race
–	Diet
–	Sun exposure
•	↓GFR
•	Albuminuria
•	↓Megalin

* 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; FGF-23, fibroblast growth factor-23; PTH, parathyroid hormone; NADPH, nicotinamide adenine dinucleotide phosphate; 25(OH)D₃, 25-hydroxyvitamin D₃; GFR, glomerular filtration rate.