Laboratory Abnormalities in CKD-MBD: Markers, Predictors, or Mediators of Disease?

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Summary: Chronic kidney disease–mineral and bone disorder (CKD-MBD) is characterized by bone abnormalities, vascular calcification, and an array of laboratory abnormalities. The latter classically include disturbances in the parathyroid hormone/vitamin D axis. More recently, fibroblast growth factor 23 (FGF23) and klotho also have been identified as important regulators of mineral metabolism. Klotho deficiency and high circulating FGF23 levels precede secondary hyperparathyroidism in CKD patients. Levels of FGF23 and parathyroid hormone increase along the progression of CKD to maintain mineral homeostasis and to overcome end-organ resistance. It is hard to define when the increase of both hormones becomes maladaptive. CKD-MBD is associated with adverse outcomes including cardiovascular disease and mortality. This review summarizes the complex pathophysiology of CKD-MBD and outlines which laboratory abnormalities represent biomarkers of disease severity, which laboratory abnormalities are predictors of cardiovascular disease, and which laboratory abnormalities should be considered (direct) uremic toxins exerting organ damage. This information may help to streamline current and future therapeutic efforts.

Keywords: Calcium, hyperphosphatemia, vitamin D, uremic toxins, FGF23, klotho, PTH

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alcium and phosphate are essential to many vital physiological processes. Consequently, the maintenance of Ca and P homeostasis is essential to a healthy existence. Several organs contribute to the exquisite regulation of calcium and phosphate homeostasis by facilitating intestinal absorption, bone (de)mineralization, and renal excretion/reabsorption of both ions. Regulation of these processes occurs by a number of hormones. The biologically active forms of vitamin D (1,25-dihydroxyvitamin D3, further referred to as calcitriol), parathyroid hormone (PTH), and calcitomin have been studied extensively in this regard. More recently, fibroblast growth factor 23 (FGF23) and klotho were identified as new players essential to the regulation of calcium and phosphate homeostasis (Fig. 1).

Acknowledging the important role of the kidneys in the homeostasis of calcium and phosphate, it is not surprising that disturbances in mineral metabolism occur early in the course of chronic kidney disease (CKD). Current data postulate that increased FGF23 may be the earliest alteration in mineral metabolism in CKD.2,3 Gradually increasing FGF23 levels cause the early decrease in calcitriol levels.4,5 This frees PTH from feedback inhibition, leading to secondary hyperparathyroidism. All of these changes occur long before changes in serum phosphate and calcium levels become evident. The primary stimulus for enhanced FGF23 secretion in early CKD remains obscure. On the basis of scant human and experimental data, it has been proposed that decreased expression of klotho, which functions as a co-receptor for FGF23, may be one step further upstream from FGF23 excess.6 The pattern of metabolic changes, and especially the observation of a modest reduction in serum phosphate during early CKD, however, argues against the latter hypothesis. Additional work must be performed to determine if phosphate plays any direct role in the early increase in FGF23. Certainly, early experimental work has been able to show that strict reduction of phosphate intake prevents PTH increase in early CKD.7

To emphasize the close association and interaction between laboratory abnormalities, bone disease, and vascular calcification, KDIGO in 2006 coined the term CKD-mineral and bone disorder (CKD-MBD).8 Bone disease starts in patients with CKD stage 2, and becomes almost universal in patients with CKD stage 5.9,10 There is significant heterogeneity of histologic abnormalities and patients may go through phases with

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different, partly treatment-associated abnormalities, or the initial changes may progress with deterioration of kidney function. Strikingly, there has been a marked increase of the prevalence of low bone turnover disease over the past 2 decades. This most probably reflects demographic changes (ageing population with high prevalence of diabetes) and overzealous suppression of PTH through the use of calcium-based phosphate binders and pharmacologic doses of calcitriol. Extra-PTH through the use of calcium-based phosphate binders and pharmacologic doses of calcitriol. Extra-PTH through the use of calcium-based phosphate binders and pharmacologic doses of calcitriol. Extra-PTH through the use of calcium-based phosphate binders and pharmacologic doses of calcitriol. Extra-PTH through the use of calcium-based phosphate binders and pharmacologic doses of calcitriol. Extra-PTH through the use of calcium-based phosphate binders and pharmacologic doses of calcitriol. Extra-PTH through the use of calcium-based phosphate binders and pharmacologic doses of calcitriol. Extra-PTH through the use of calcium-based phosphate binders and pharmacologic doses of calcitriol. Extra-PTH through the use of calcium-based phosphate binders and pharmacologic doses of calcitriol.

PHOSPHATE

Phosphate Balance

Inorganic phosphorus is essential for multiple biological functions such as intracellular signal transduction, the production and function of cell membranes, and energy exchange. In the healthy state, serum phosphate levels are maintained within the physiological range by the regulation of dietary absorption, bone formation and resorption, and renal excretion, as well as by equilibration with intracellular stores. Given the important role of the kidneys, it is not surprising that phosphate metabolism is disturbed early in CKD. Although it is common to indicate that phosphate retention is typical for CKD, there are no data to suggest that total-body phosphate load is actually increased, at least not in predialysis CKD. It should be emphasized that hyperphosphatemia only occurs once the estimated glomerular filtration rate (GFR) decreases to less than 30 mL/min/1.73 m² (ie, when compensatory mechanisms [mediated to a large extent by high PTH and FGF23] start to fail). Thus, hyperphosphatemia is a late marker of a disturbed phosphate metabolism.

Figure 1. FGF23 and klotho effects on the parathyroid glands and on the kidneys.

Phosphate as a Uremic Toxin

Phosphate and cardiovascular disease

CKD patients suffer from a high cardiovascular mortality rate. It was not until the late 1990s when nephrologists became aware of phosphate as an independent predictor of cardiovascular mortality. Phosphate is a main factor in the development of vascular calcification and therefore it affects coronary pathobiology as well as myocardial performance by inducing arterial stiffness, valvular rigidity, and left ventricular hypertrophy. Epidemiologic observations are corroborated by abundant experimental data. Phosphate, in synergism with calcium, drives accelerated calcification by its distinct effect on vascular smooth muscle cells (VSMCs), in particular through hydroxyapatite nanocrystal formation. Key processes include osteochondrocytic differentiation, apoptosis, and vesicle release, and perturbation of calcification inhibitor levels (Fig. 2). Osteochondrocytic differentiation is characterized by active cellular phosphate uptake, down-regulation of VSMC-specific genes, de novo induction of the osteoblast transcription factor cbfa-1 (runx2), release of precalcified matrix vesicles, and local expression of bone proteins including alkaline phosphatase, osteocalcin, osteopontin, and collagen I, all within the vessel wall.

Figure 2. Phosphate as a uremic toxin. Hyperphosphatemia leads to active uptake of phosphate into human aortic smooth muscle cells (SMCs). The consecutive increases in intracellular phosphate concentrations cause down-regulation of SMC-specific genes and up-regulation of the osteoblast transcription factor cbfa-1 (or runx2). Subsequently, the human aortic SMCs act similar to bone cell–producing precalcified matrix vesicles and a variety of bone-specific proteins (alkaline phosphatase, osteocalcin, osteopontin, collagen I). This specific process of a phenotypic cell change is termed osteochondrogenic transdifferentiation. Cbfa-1, core-binding factor-1; NPC, sodium-phosphate co-transporter PIT-1.
Independent of the deleterious effect of high phosphate on VSMCs, recent studies have shown that high phosphate levels directly affect endothelial function both in vitro and in vivo. For example, di Marco et al\textsuperscript{29} showed annexin II down-regulation and inhibition of endothelial-dependent angiogenesis after phosphate exposure in vitro.

Finally, high phosphate may contribute to the accelerated aging observed in CKD patients; phosphate appears to be a key element in defining the Klotho-/- phenotype, and reducing serum phosphate levels in Klotho-/- mice ameliorated premature aging-like features and prolonged survival.\textsuperscript{30}

**Phosphate and renal disease progression**

Several studies have reported an association between a disturbed phosphate metabolism and accelerated loss of renal function.\textsuperscript{31–37} The negative effect of phosphate on cardiovascular status may condition an accelerated progression of renal disease. In addition, according to the precipitation-calcification hypothesis, this accelerated loss may be explained (at least partly) by deposition of calcium phosphate crystals in the renal parenchyma, resulting in nephrocalcinosis,\textsuperscript{38} which in its turn leads to inflammatory response and renal damage, inciting a vicious circle.\textsuperscript{39} Dietary phosphorus restriction or phosphate binders reduce the occurrence of nephrocalcinosis and attenuate the progressive loss of renal function in animal studies.\textsuperscript{31,38,40–44}

**Phosphate as a Therapeutic Target**

High phosphate levels may be blamed for being a key contributor to cardiovascular mortality in CKD. This defines phosphate as a very relevant uremic toxin and therefore as a main target for therapy (Fig. 3). Current guidelines suggest maintaining serum phosphate levels in the normal range in CKD stages 3 to 5 and decreasing serum phosphate levels toward the normal range in dialysis patients. Dietary restriction of phosphate has long been the cornerstone of therapy for disordered mineral metabolism. Recent data point to phosphate additives as an important and theoretically avoidable source of dietary phosphorous.\textsuperscript{45–47} Dietary measures, overall, are not sufficient to control hyperphosphatemia. Therefore, adjunctive therapy with oral phosphate binders is used in most patients with CKD not yet on dialysis, and most studies yielded disappointing results, both with regard to biochemical and intermediate cardiovascular end points.\textsuperscript{50,51} In aggregate, these data question the appropriateness of phosphate binder therapy in patients who have moderate to advanced CKD and normal or near-normal serum phosphorus concentrations.

**CALCIUM**

**Calcium Balance**

Given the crucial role of calcium in many metabolic processes, serum calcium concentrations are tightly regulated, mainly by the actions of PTH and calcitriol. Serum calcium concentrations are maintained in the normal range until very late in CKD when they decrease slightly. The key issue when considering calcium as a potential uremic toxin is that serum calcium levels poorly reflect calcium balance in CKD.\textsuperscript{52} Recently, rigorous calcium balance studies were performed by two research groups in CKD patients not yet on dialysis.\textsuperscript{16,53} Although participants of both studies were in neutral or slightly negative balance when consuming the normal calcium diet, they went into markedly positive calcium balance when treated with calcium supplements for up to 3 weeks. Additional long-term experiments are required to
investigate whether prolonged exposure yields similar results and to elucidate the fate of the retained calcium.\textsuperscript{17,54}

**Calcium as Uremic Toxin**

Although the subject of ongoing controversy, several lines of evidence have suggested that calcium loading is an important culprit of cardiovascular disease in CKD. The poor relationship between serum calcium levels and calcium balance\textsuperscript{52} probably explains the observations from epidemiologic trials that even high calcium levels are usually only weak predictors of adverse outcomes, but that calcium exposure may be a cardiovascular risk factor.\textsuperscript{19,55,56}

One of the key studies pointing to calcium toxicity in uremia was published in 2010.\textsuperscript{57} The investigators used arterial samples obtained during abdominal surgery in children with normal renal function, with impaired renal function, and on dialysis, respectively. These intact arteries, free of any signs of atherosclerosis, were first exposed ex vivo to hyperphosphatemia to induce osteochondrocytic differentiation and calcification of VSMC. Although samples from healthy children were resistant to such phenotypic changes, there was some moderate calcification induction already in CKD arteries, and a severe calcification induction in vessels from children on dialysis. When the ex vivo calcium concentrations were increased, osteochondrocytic differentiation and calcification became synergistically accelerated—an experimental proof of the concept that calcium may not just be a passive compound in the process of intravascular hydroxyapatite formation.

A recent clinical pilot study was supportive for the concept of calcium toxicity in the context of cardiovascular calcification. Block et al\textsuperscript{50} performed a placebo-controlled trial on the effects of three different phosphate binders (sevelamer carbonate, lanthanum carbonate, and calcium acetate) on serum phosphate levels and parameters of phosphate metabolism in patients with CKD stages 3 to 4. Binders were all capable of modestly decreasing serum phosphate levels, and urinary phosphate excretion was reduced by 22%. Unexpectedly, there was an increase in coronary calcification scores with binder therapy over 9 months, and no progression in the placebo group. Looking at the 3 binders separately, this progressive calcification was observed exclusively in the group treated with the calcium-containing binder.

In addition, 11 randomized clinical studies on the comparison of calcium-free versus calcium-containing phosphate binders in hyperphosphatemic patients recently were reviewed together by means of a meta-analysis (n = 4,622 patients in different CKD stages).\textsuperscript{58} This systematic review showed a 22% survival benefit with calcium-free binders, associated with inhibition of cardiovascular calcification progression (Fig. 4). The remaining open question now is whether calcium-free binders are the superior choice in hyperphosphatemia treatment, or whether calcium-containing binders are primarily harmful. Data from the recent Current Management of Secondary Hyperparathyroidism: a Multicentre Observational Study (COSMOS) trial seem to favor the first explanation, showing beneficial outcome associations of dialysis patients on calcium-containing binders versus those patients without phosphate binder treatment.\textsuperscript{59}

Figure 4. Calcium as a uremic toxin. Data from a recent systematic review showed a significant 22% survival disadvantage if hyperphosphatemic CKD patients were treated with calcium-containing versus calcium-free phosphate binders. Accelerated cardiovascular calcification is suspected as the major pathophysiologic mechanism behind this observation. Modified with permission.\textsuperscript{58}
still may qualify as a uremic toxin, but in this context high calcium loads or a markedly positive calcium balance may be the essential harmful culprits rather than the immediate serum calcium concentrations. However, one should always take into account the limitations with regard to the proper determination of calcium load and balance.

Calcium as a Therapeutic Target

Defining the optimal calcium intake in CKD is challenging given ongoing uncertainties concerning calcium balance in the different stages of disease, as mentioned earlier. Current guidelines suggest limiting elemental calcium intake in the presence of hypercalcemia, arterial calcification, and/or adynamic bone disease, and if serum PTH levels are persistently low.15

FGF23

FGF23 is a bone-derived hormone that plays an important function in regulating mineral metabolism.4 The physiological actions of FGF23 are to promote phosphaturia, decrease production of calcitriol, and suppress secretion of PTH. These effects are mediated through activation of the FGF receptor (FGFR) and its co-receptor klotho. FGF23 levels increase early in the course of CKD to reach levels in CKD stage 5D exceeding normal values by a factor of up to 1,000. In predialysis CKD, high FGF23 values may represent a compensatory mechanism to overcome FGF23 resistance and to maintain normophosphatemia.

FGF23 Resistance

It increasingly is recognized that CKD is a state of FGF23 resistance because both factors have been shown to be involved.63 Recent experimental evidence also points to the protein-bound uremic toxins indoxyl sulfate (IS) and p-cresyl sulfate as potential culprits. Administration of IS to hypertensive rats reduced renal expression of klotho, promoted cell senescence, and increased renal fibrosis. Cell signaling pathways involve induction of reactive oxygen and nuclear factor-κB activation, and subsequent interference with RNA transcription.64

Recently, Sun et al65 showed that IS and p-cresyl sulfate cause epigenetic modification and silencing of the klotho gene, resulting from increased DNA methyltransferase expression and DNA hypermethylation. The toxicity of p-cresyl sulfate is discussed in more detail in the article by Sirich et al of this issue.66 Another possible mechanism of FGF23 resistance is impaired FGF23 signaling caused by accumulation of the C-terminal tail of FGF23, which competes with full-length ligand for binding to the klotho/FGFR complex,67 although this scenario may not be the case in patients undergoing dialysis.68,69 Parathyroid resistance to FGF23 may explain why high PTH levels may co-exist with high FGF23 levels in patients with advanced CKD.62

FGF23 as Uremic Toxin

Recent epidemiologic studies have shown strong associations between increased FGF23 levels and poor renal37,70 and cardiovascular outcomes both in CKD patients and in the general population.71–74 Of note, adjustment for important covariates including phosphate, calcitriol, and vitamin D therapy did not affect the result substantially. Several explanations can be raised for these findings. First, FGF23 may be a kidney function-dependent surrogate for another risk factor. High on the list concerning this hypothesis is klotho deficiency because both factors have been shown to be related. It is well established that high FGF23 levels correlate with klotho deficiency.6 Klotho deficiency may potentiate the development of arterial calcification and thereby confer an increased risk of cardiovascular morbidity and mortality.75 This line of reasoning is supported by a recent study in older community-dwelling adults, linking low soluble klotho levels to all-cause mortality, even after adjustment for age, classic cardiovascular risk factors, and traditional markers of calcium-phosphate metabolism.76

The latter findings, however, could not be reproduced in a cohort of 312 CKD patients.77 Second, FGF23 may directly exert toxic effects. It has been shown in a series of elegant clinical and experimental studies that left ventricular hypertrophy (LVH) may be triggered by FGF23 in a klotho-independent manner and can be reduced by nonspecific FGF-receptor blockade.78,79 Intriguingly, Seiler et al80 showed the same LVH association in a cardiovascular cohort with no or only slightly impaired renal function. It is unlikely that in these individuals LVH is mediated by activation of nonspecific FGF receptors. This again fuels the thesis that FGF23 is a surrogate of another risk factor.

FGF23 as a Therapeutic Target

At present, it remains unclear whether FGF23 is a marker, predictor, or mediator of disease. However, directly antagonizing FGF23 might not be the right therapeutic approach, at least not in CKD patients not yet on dialysis because antagonizing FGF23 in these patients means antagonizing the phosphaturic defenses
of the body. This may confer important health risks as was shown recently in a study by Shalhoub et al. In the latter study, the use of specific FGF23 antibodies in an animal model of CKD led to increased mortality, despite some favorable effects on the majority of classic parameters of CKD-MBD and particularly on the development of secondary hyperparathyroidism. Hyperphosphatemia was assumed to be the mediator of mortality leading to accelerated calcification in animals after FGF23 antagonism. Treatment strategies that indirectly target FGF23 such as dietary phosphate restriction and phosphate binder therapy might be a better alternative. However, it should be emphasized that the efficacy, potency, and even safety of these treatment strategies remain to be shown.

PTH

PTH is a single-chain polypeptide of 84 amino acids that is secreted by parathyroid cells at a constant, low rate. PTH secretion is stimulated primarily in response to reductions in serum calcium concentration. The entire PTH molecule (1-84) is also called whole or biointact PTH; this is to differentiate it from other smaller-molecular-weight carboxy-terminal fragments (C-PTH), which also are normally present in plasma. The hormonal fragments arise from metabolism of 1-84 PTH by peripheral organs as well as from secretion of C-PTH fragments from the parathyroid glands. Cleavage of PTH (1-84) by liver cells generates C-PTH fragments that subsequently are removed from the circulation by the kidneys. Amino-terminal fragments (N-PTH) also are produced by Kupffer cells during cleavage of I-PTH but rapidly are degraded. Parathyroid cells secrete both PTH (1-84) and C-PTH. Although hypercalcemia reduces the secretion of both PTH (1-84) and C-PTH, the reduction of PTH (1-84) secretion is more marked than that of C-PTH. Cleavage of PTH (1-84) by liver cells generates C-PTH fragments that subsequently are removed from the circulation by the kidneys. Thus, impairment of renal function causes accumulation of C-PTH. The primary physiological actions of PTH are to increase calcium levels and to induce phosphaturia. PTH exerts its effects by binding the parathyroid hormone/parathyroid hormone–related peptide receptor (PTHR1). Major target organs of PTH include the bone and the kidney.

PTH Resistance

The response to PTH has been shown to be decreased and variable in uremic conditions. Suppressed expression of PTHR1 has been shown by several investigators both in uremic kidney tissue and bone, but data with regard to bone have not been unequivocal. A circulating factor is considered responsible for the decreased expression of the PTH1R gene in uremia. In this regard, in vitro and animal studies point to the protein-bound uremic retention molecule IS as the potential culprit. IS and the other protein-bound uremic toxins are discussed in more detail in the article by Sirich et al of this issue. Also, PTH fragments may contribute to resistance to PTH, either through mechanisms involving receptor internalization and/or through mechanisms not involving the PTHR1. Large amino-terminal truncated C-PTH fragments may constitute up to 50% or more of the total PTH immunoreactivity in CKD. Administration of a four-fold molar excess of PTH 7-84 fragments approximately halved the phosphaturic effect of biointact PTH. In experimental animals, the administration of PTH (7-84) prevented the increase in serum calcium otherwise induced by PTH (1-84). The decrease in serum calcium levels induced by administration of PTH (7-84) may be owing to a decrease in osteoclast precursors, which express C-PTH receptors. Thus, PTH (7-84) can inhibit bone resorption, also in part by reducing the rate of formation of new osteoclasts. Furthermore, CKD is a state of inflammation and oxidative stress, and PTH modified by oxidative stress has no end organ activity. Finally, bone morphogenetic protein-7 produced in the kidney is a strong osteoblast differentiation factor, and low serum bone morphogenetic protein-7 levels have been reported in renal failure. It is speculated that low bone morphogenetic protein-7 levels may lead to a reduced number of osteoblasts and decreased PTH response. The clinical relevance of all these resistance factors remains to be shown.

PTH as Uremic Toxin

PTH has been implicated in the pathogenesis of several complications of CKD, including peripheral neuropathy, carbohydrate intolerance, hypertension, and dyslipidemia. PTH produces an increase in intracellular calcium in many cell types with the exception of VSMCs. The increase in cytosolic calcium levels seems to be caused by both an increased influx and a decreased efflux from the cell. High PTH levels inhibit mitochondrial respiration, reduce phosphorylation, and uncouple oxidative phosphorylation in isolated heart mitochondria, which causes adverse effects on the myocardium. An inhibitory effect of PTH on energy production and utilization also was observed in skeletal muscle. In hemodialysis patients, high PTH levels have been associated with abnormalities of left ventricular myocardial function and cardiac hypertrophy cardiac fibrosis. In addition, both high and low PTH levels may foster vascular calcification. Last but not least, PTH is the master regulator of bone remodeling. Bone disease
is almost universal in CKD. Bone turnover, mineralization, and volume may be abnormal. Inappropriate PTH levels may cause high bone turnover disease, low bone turnover disease, and mixed bone disease. Osteitis fibrosa is observed in patients with high PTH levels; in that case, the bone turnover is abnormally high with evidence of marrow fibrosis. A portion of the calcified bone loses its lamellar structure and appears as woven bone. In patients with high PTH, it also is characteristic to observe an increased resorption of cortical bone. Osteomalacia and adynamic bone disease represent low bone turnover. In osteomalacia, the bone surface is covered with uncalcified osteoid, and cell activity (osteoblasts and osteoclasts) is decreased. Bone formation rate is reduced. Adynamic bone disease also is characterized by low bone turnover, but in this case osteoid is almost absent. Mixed bone disorder possesses characteristics of both high and low turnover.\textsuperscript{113} PTH level overall correlates poorly with bone turnover at least partly owing to skeletal resistance to the action of PTH being highly variable. Observational studies mostly showed U-shaped associations between PTH serum levels and mortality, with moderate relationships at the high end of PTH concentration, and strong effects at the low end.\textsuperscript{55}

**PTH as a Therapeutic Target**

The optimal PTH level in the individual patient remains a black box. Current guidelines suggest maintaining PTH levels in the broad range of approximately 2 to 9 times the upper normal limit.\textsuperscript{15} Calcimimetics have been proven to be potent drugs to control PTH levels. Two high-quality, prospective, placebo-controlled treatment studies with intermediate and hard end points in dialysis patients with secondary hyperparathyroidism were completed recently with this drug. In the ADVANCE (A randomizeD VAscular calcificatioN study to evaluate the effects of CinacalcEt) trial (n = 360 hemodialysis patients), the effect of the calcimimetic cinacalcet on cardiovascular calcification progression was tested, with coronary artery calcification progression analyzed by Agatston score as the defined primary end point.\textsuperscript{114} This end point was not reached, but coronary artery calcification progression analyzed by volume score and aortic valve calcification progression, both predefined as secondary end points, were inhibited significantly by cinacalcet. The larger EVOLVE (Evaluation Of Cinacalcet HCl Therapy to Lower CardioVascular Events) trial (n = 3,883 hemodialysis patients) used the same intervention, but a different primary composite end point of time until death, myocardial infarction, hospitalization for unstable angina, heart failure, or a peripheral vascular event.\textsuperscript{115} This primary end point was not reached in the intention-to-treat analysis, however, predefined adjustments for age or time on study drug (lag censoring at 6 months) showed a treatment benefit for cinacalcet. This study suffered from a disproportionally high drop-out rate of patients from the placebo arm, who subsequently were switched to cinacalcet. Clinical outcome studies related to uremia are discussed more extensively in by Liabef et al of this issue.\textsuperscript{116}

**VITAMIN D**

When discussing vitamin D in the context of CKD-MBD, it is of outmost importance to distinguish between inactive (vitamin D2 and D3, calcidiol) and active (calcitriol and its synthetic analogues) compounds. Calcitriol is an important modulator of calcium and phosphate absorption in intestine, calcium re-absorption in kidney, and calcium mobilization in bone. In addition to maintaining calcium/phosphate homeostasis, calcitriol regulates cellular proliferation and differentiation of certain cells, modulates the immune response, and affects the cardiovascular system (Fig. 5). Calcitriol exerts its functions mainly by binding to a nuclear vitamin D receptor (VDR), a member of the steroid hormone receptor superfamily, leading to transcriptional regulation of target genes. The VDR is expressed in many tissues, but most prominently in the intestine, kidney, parathyroid gland, and bone.\textsuperscript{117} CKD goes along with low circulating calcitriol levels. This is not surprising because the kidney produces the bulk of this hormone. Mechanisms governing renal calcitriol metabolism in CKD, however, are complex and incompletely understood.\textsuperscript{118,119} Vitamin D metabolism is facilitated by hydroxylation, and recent evidence indicates that calcitriol synthesis by CYP27B1 and catabolism by CYP24A1, as well as tubular reabsorption of the precursor calcidiol, all may be disturbed in CKD.\textsuperscript{120,121} Extrarenal vitamin D metabolism has
gained much interest in recent years. The existence of CYP27B1 has been documented in many tissues and cells including the vasculature, placenta, and monocytes. Data on the impact of CKD on extrarenal vitamin D metabolism are limited and mainly relate to monocytes. Available evidence points to impaired uptake of calcidiol and a blunted immune signal-induced CYP27B1 expression, the latter probably mediated by high FGF23 levels.\textsuperscript{122–124}

Calcitriol Resistance

CKD also is characterized by a state of calcitriol resistance.\textsuperscript{125} Vitamin D hyporesponsiveness may be explained by decreased expression of the VDR or by a decreased binding affinity of VDR with vitamin D–responsive elements.\textsuperscript{126,127} Responsible culprits remain to be identified, but most probably also involve uremic toxins.\textsuperscript{125} Importantly, vitamin D hyporesponsiveness may occur early in the course of CKD and explain why low intestinal calcium absorption and high PTH levels precede low calcitriol levels.\textsuperscript{128,129}

Vitamin D Deficiency as a Uremic Toxin

Calcidiol and calcitriol deficiency are frequent in CKD, and both have been associated with poor outcomes.\textsuperscript{130–133} The underlying mechanisms are complex and ill-defined.

Vitamin D as a Therapeutic Target

Cholecalciferol or ergocalciferol supplementation, in doses to correct calcidiol deficiency, seem to be harmless and without any side effects, however, there are no randomized controlled data yet about its benefits. There is, however, great ambiguity with regard to active vitamin D (analogues). On the one hand, a significant body of observational trials quite consistently suggests that active vitamin D treatment is associated with improved outcomes, mostly independent of other parameters of CKD-MBD and possibly related to some pleiotropic effect of the hormone.\textsuperscript{20,134} On the other hand, active vitamin D analogues increase intestinal calcium absorption and may—in pharmacologic doses—oversuppress bone turnover, thus creating a risk scenario for cardiovascular calcification. No prospective trials are currently available that systematically evaluate the role of active vitamin D analogues on hard outcomes. One randomized controlled study addressed the effect of paricalcitol on the surrogate end point LVH in CKD stages 3 and 4 patients, but showed no benefit versus the placebo arm.\textsuperscript{135} Another recent randomized controlled study addressed the effects of paricalcitol on albuminuria in diabetic CKD stages 3 and 4 patients and found significant reductions with a daily dose of 2 mcg of the compound.\textsuperscript{136}

Preclinical studies, however, may provide some helpful advice. Mizobuchi et al\textsuperscript{137} studied 5/6-nephrectomized rats and showed that calcitriol and...
doxercalciferol, but not paricalcitol, induced media calcification, despite similar effects on PTH levels. The suggested difference was that the former two compounds induced osteocondrocytic differentiation in the vessel wall, but paricalcitol did not. Lopez et al.\textsuperscript{138} used the same model, but compared calcitriol, paricalcitol, a calcimimetic (AMG-641), and combinations of these compounds. Treatment with the calcimimetic did not induce any cardiovascular calcification, whereas calcitriol and paricalcitol induced severe and moderate, respectively, media calcification. Those calcifications were ameliorated by co-treatment with the calcimimetic. A key article in this context was published by Mathew et al.\textsuperscript{139} who treated uremic low-density lipoprotein–receptor knockout mice with different doses of calcitriol or paricalcitol (Fig. 6). This model represents a combined phenotype of atherosclerosis, CKD, and metabolic syndrome. Moderate doses of both vitamin D analogues blunted plaque calcification, whereas high doses caused significant calcification progression.

In summary, the effects of exogenous calcitriol and its synthetic active analogues are dose-dependent, with adverse effects on the high (pharmacologic) end, and possible benefits on the low (physiological) end of the dose spectrum. The modality of a hormone replacement therapy thus may be a feasible description of the optimal use of these compounds as steroid hormones. However, as with many of the other CKD-MBD–related parameters and therapeutics, there is no randomized controlled proof for these assumptions.

CONCLUSIONS

Laboratory parameters of CKD-MBD are markers of disease severity and predictors of outcome. Circulating levels of PTH, FGF23, and calcitriol are tightly regulated by several feedback mechanisms to maintain bone and mineral homeostasis both in health and disease. All these hormones exert their biological effects by binding their cognate receptors in target organs. One should acknowledge that target organs in CKD may be resistant to the action of these hormones as a consequence of impaired binding to the receptor, decreased expression of the receptor, or postreceptor disturbances. Thus, increasing hormone levels are to some extent required in CKD to overcome end-organ resistance to their action. Hitherto, it has been very difficult, if not impossible, at least on the individual level, to define when the increase turns maladaptive and the hormone becomes a uremic toxin. At present, assessment of the end-organ response (eg, by bone histomorphometry) may be the only means to judge the appropriateness of the PTH exposure in the individual patient accurately. Hyperphosphatemia occurs late in the course of CKD, when compensatory mechanisms start to fail. Despite overwhelming experimental and clinical observational evidence linking high phosphate levels to poor outcomes, randomized controlled intervention trials with hard end points have not been conducted to date. Several aspects of interventions targeting high phosphate levels remain vague. Although several lines of evidence indicate that calcium loading may be harmful, uncertainties also remain with regard to calcium balance in CKD, especially during prolonged loading and with regard to the fate of retained calcium. Current evidence, however, dictates that calcium, either as a phosphate binder or as a supplement, should be prescribed with caution, especially if adynamic bone disease or vascular calcification is present. During the past 2 decades it has become clear that laboratory parameters of CKD-MBD eventually may turn into direct uremic toxins, mediating renal, cardiovascular, and/or bone disease. Additional research is required urgently to prove the benefit of therapeutic approaches targeting these laboratory parameters.

REFERENCES


CKD-MBD abnormalities

78. Seiler S, Cremers B, Rebling NM, Hornof F, Jeken J, Kersting S, et al. The phosphatonin fibroblast growth factor 23 links...
Calcium-phosphate metabolism with left-ventricular dysfunction and atrial fibrillation. Eur Heart J. 2011;32:2688-96.


