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Review Article

Phosphate and fibroblast growth factor 23 in diabetes

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Diabetes is associated with a strongly elevated risk of cardiovascular disease, which is even more pronounced in patients with diabetic nephropathy. Currently available guideline-based efforts to correct traditional risk factors are only partly able to attenuate this risk, underlining the urge to identify novel treatment targets. Emerging data point towards a role for disturbances in phosphate metabolism in diabetes. In this review, we discuss the role of phosphate and the phosphate-regulating hormone fibroblast growth factor 23 (FGF23) in diabetes. We address deregulations of phosphate metabolism in patients with diabetes, including diabetic ketoacidosis. Moreover, we discuss potential adverse consequences of these deregulations, including the role of deregulated phosphate and glucose as drivers of vascular calcification propensity. Finally, we highlight potential treatment options to correct abnormalities in phosphate and FGF23. While further studies are needed to more precisely assess their clinical impact, deregulations in phosphate and FGF23 are promising potential target in diabetes and diabetic nephropathy.

Introduction

Diabetes mellitus is among the most common non-communicable chronic diseases in the world. Patients with diabetes have a three-fold increased risk of developing cardiovascular disease [1,2]; this risk is driven by microvascular (retinopathy, neuropathy, nephropathy) and macrovascular (peripheral vascular disease, stroke, and coronary artery disease) complications. In fact, over the last two decades global mortality rate attributable to vascular complications in diabetes has increased by 37.9% [3].

Many risk factors for cardiovascular disease in diabetes have been identified. These include traditional risk factors such as hypercholesterolemia, smoking, hypertension, (diabetic) nephropathy and aging [4,5], while hyperglycemia in itself is also a risk factor for cardiovascular disease [6,7]. The benefits of interventions targeting these risk factors in diabetes, e.g. lifestyle modifications, using proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors [8] and sodium glucose co-transporter 2 (SGLT2) inhibitors [9] have been shown in clinical trials. At the same time, despite major efforts to correct these traditional risk factors through the implementation of prevention programs, the residual cardiovascular risk in patients with diabetes remains excessive, fuelling the search for additional risk factors and targets for intervention.

Calcification of the vascular wall, specifically the tunica media layer, is a hallmark of advanced diabetes and an independent risk factor for cardiovascular disease [10]. Deregulations in mineral metabolism, and calcium and phosphate in particular, set the stage for accelerated vascular calcification. This paradigm has been extensively studied in patients with chronic kidney disease (CKD), where deregulation of phosphate and calcium metabolism parallel progressive loss of kidney function [11]. In addition, emerging evidence indicates that early in the progression of diabetes without complications, phosphate metabolism is already disturbed [12,13]. Phosphate contributes to several biological systems, for example as component of phospholipid membranes in cell structures, as component of energy metabolism in adenosine

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triphosphate (ATP) formation and as an important urinary pH buffer. Both hypophosphatemia and hyperphosphatemia may occur in diabetes, the latter especially in the context of diabetic nephropathy [14]. In this review, we discuss the role of phosphate and the phosphate-regulating hormone fibroblast growth factor 23 (FGF23) in diabetes, addressing deregulation of phosphate metabolism in patients with diabetes in acute and chronic settings. Finally, we discuss several inexpensive interventions that exist to correct phosphate deregulations. While the clinical potential of such interventions for diabetes patients with preserved kidney function remains unclear in the absence of trial data, this may be a relevant subject to address in future studies.

Phosphate physiology

Phosphate is an essential mineral, implying that dietary sources are essential to meet the body's requirement. Protein-rich food products such as meat, fish, nuts, and dairy products contain organic phosphate. Inorganic phosphate is present in many food additives, and therefore highly processed foods, including fast food, contain high amounts of inorganic phosphate. Inorganic phosphate is efficiently absorbed in the intestine, and therefore processed foods are important sources of bioavailable phosphate. Intestinal phosphate uptake occurs via active transport by sodium–phosphate cotransporters (NaPi–IIb), and is positively regulated by active vitamin D [15]. Additionally, passive phosphate transport takes place through a paracellular pathway, which is diffusion-driven and is mostly determined by dietary phosphate intake.

Phosphate homeostasis is tightly regulated. The vast majority of phosphate in the body, approximately 85%, is stored in bone as calcium phosphate [16]. The remaining 15% is divided between the extraosseous intracellular (14%) and extracellular (1%) compartments. In the extracellular fluid, phosphate can be bound to different minerals, including calcium. The exchange of phosphate with bone is mainly controlled by calcitriol and parathyroid hormone (PTH) [17]. Phosphate is excreted by the kidney tubules in part via NaPi2a and NaPi2c, which are regulated by the hormones PTH and FGF23, the latter through interaction with the co-receptor Klotho [18,19].

Knock-out experiments in mice revealed that FGF23, which is predominantly expressed in osteocytes, also strongly controls 1-α-hydroxylase, an enzyme which converts inactive 25(OH)-vitamin D into active 1,25$(OH)_2$-vitamin D [20,21]. The main effect of 1,25$(OH)_2$-vitamin D is to increase gut absorption of calcium and phosphate. FGF23 deficiency leads to an increase in 1,25 vitamin D, which may promote ectopic calcium depositions [22,23]. The net result of FGF23 inhibiting both renal phosphate reabsorption and 1-α-hydroxylase is a decrease in plasma phosphate level. In turn, 1,25$(OH)_2$-vitamin D and an increase in plasma phosphate stimulate FGF23 production in bone, resulting in a negative feedback loop [24]. Taken together, in the healthy individual, phosphate metabolism is closely regulated by orchestrated actions of several hormones and other factors, including FGF23, PTH, and 1,25$(OH)_2$-vitamin D [25]. In chronic diseases, including diabetes and CKD, these factors may be deregulated, resulting in impairments in phosphate homeostasis that may eventually contribute to adverse outcomes.

Phosphate deregulation in diabetes

Studies addressing phosphate metabolism in stable diabetes outpatients show conflicting results. Although most studies demonstrate normal phosphate levels similar to healthy controls [26], some other studies suggest that phosphate levels are lower in stable diabetes patients with preserved kidney function compared with controls without diabetes [27]. Additionally, several studies have shown that improved glycemic control is associated with increased serum phosphate levels and reduced urinary phosphate excretion in stable diabetes outpatients [27–29]. These observations can potentially be explained by competition of glucose with phosphate as a consequence of an altered electrochemical Na^+ gradient at the tubular epithelium, leading to relatively low phosphate levels in diabetes [30–32]. In this respect, it is interesting to note that recent data from our group and others indicate that treatment with SGLT2 inhibitors leads to higher phosphate levels, independent of changes in estimated glomerular filtration rate (eGFR) or albuminuria [33,34]. This may be explained by the fact that SGLT2 inhibitors prevent cotransport and reabsorption of sodium and glucose, preserving the sodium gradient for the sodium-dependent phosphate transport proteins NaPi-2a and 2c, and thereby stimulating phosphate reabsorption at the proximal tubule [35]. This concept is in-line with animal studies showing that the transporters of phosphate, glucose, and alanine all make use of the same sodium gradient, thereby limiting each other [31].

Conversely, hypophosphatemia may be the cause of poorer glycemic control [36]. In rats, low serum phosphate levels appeared associated with high resting cytosolic calcium concentrations, leading to decreased ATP production in pancreatic islets, in turn resulting in decreased insulin secretion [37]. Conversely, phosphate supplementation in both healthy persons and glucose-intolerant hypophosphatemic patients contributed to improved insulin sensitivity [38,39]. Taken together, it could be concluded that serum phosphate levels are disturbed in early progression

Table 1 Factors potentially influencing FGF23 in diabetes

Factor	Effect on FGF23
Increase in pro-inflammatory cytokines	Increase
Loss of kidney function	Increase
Administration of leptin	Increase
Increase in advanced glycation end products (AGEs)	Increase
Increase in glycerol-3-phosphate (G3P)	Increase
Release of insulin	Decrease
Adminstration of SGLT2 inhibitors	Decrease

of diabetes and that, conversely, phosphate deregulation adversely influences glucose metabolism. Obviously, more, preferably prospective, studies are needed to investigate the intricate relationship between phosphate and glucose homeostasis in diabetes.

Although these preliminary data should be interpreted with caution, deregulations in mineral metabolism are commonly observed in patients with diabetes [12,13]. Hyperglycemia is associated with increased bone mineral density, decreased markers of bone turnover, and increased risk of fractures [40–44].

FGF23 deregulation in diabetes

Several recent studies have shown elevated FGF23 levels in patients with type 2 diabetes, even with preserved kidney function, compared with the general population [45]. Insulin resistance and deficiency, both hallmarks of diabetes [46], may directly influence FGF23. While the main effect of insulin is to maintain glycemic balance, insulin harbors additional non-glycemic effects that might directly or indirectly influence FGF23 (Table 1).

First, several studies found a link between insulin resistance and elevated FGF23 levels [47,48]. Preclinical studies showed that insulin is a negative regulator of FGF23, by activation of the phosphoinositide 3-kinase (PI3K)/protein kinase B (PKB)/Akt signaling transcription factor forkhead box protein O1 (FOXO1) [49]. In an elegant set of experiments, it was shown that insulin treatment in cultured cells and mice inhibited FGF23 gene expression [49]. Furthermore, elevated FGF23 levels were found in insulin-deficient mice, which can be normalized by treatment with insulin [49]. Therefore, insulin might be able to directly decrease FGF23, independent of inflammation or changes in kidney function. On the other hand, studies in obese adolescents found an inverse association between FGF23 and insulin resistance [50]. It, therefore, cannot be excluded that the relationship of insulin with FGF23 differs between young subjects and elderly patients with type 2 diabetes.

Second, it has over the past years been recognized that several inflammatory cytokines, including tumor necrosis factor α (TNF-α) and interleukin-6 (IL-6), which are produced by adipocytes, hepatocytes and macrophages, play a prominent role in the pathophysiology of insulin resistance [51]. In many patients with diabetes, coexisting obesity fuels a state of chronic low-grade inflammation [52]. Inflammation might be one of the driving factors of elevated FGF23 levels in diabetes, since pro-inflammatory factors are heavily involved FGF23 production and metabolism [53]. Support for this hypothesis arises from studies that identified a very robust Kidney Risk Inflammatory Signature (KRIS), a group of 17 proteins from the TNF receptor superfamily, associated with an increased risk of developing end-stage kidney disease (ESKD) in three independent type 1 and 2 diabetes populations [54].

Third, leptin, a hormone produced by adipocytes, might also influence FGF23 in obese patients. A study has found that leptin administration in ob/ob mouse directly induces FGF23 expression [55]. In line, in a cross-sectional study in approximately 2000 elderly individuals, it was found that serum leptin was associated with FGF23 [56]. Whether weight reduction would trigger leptin-mediated changes in FGF23 is currently unknown.

Another important consequence of prolonged hyperglycemia is the production of advanced glycation end products (AGEs) [57]. A major consequence of hyperglycemia is the overproduction of reactive oxygen species (ROS) in cellular mitochondria as a result of increased intracellular glucose flux [58]. Increased oxidative stress subsequently results in structural DNA damage, and an increased production of AGEs. AGE formation is a process in which glucose-derived products (dicarbonyls) bind with fats and proteins [59]. AGEs are found in atherosclerotic plaques, e.g. glycated LDL, and thus directly stimulate plaque formation. In addition, AGEs play an indirect role in the pathophysiology of cardiovascular disease by various routes of cell signaling. The production of AGEs is therefore closely linked to the pathogenesis of diabetic nephropathy. One of the main features of diabetic nephropathy is protein accumulation in the extracellular matrix (ECM) [60]. It appears that production of AGEs is directly related to accumulation of these proteins in the ECM. In addition, AGEs stimulate formation of pro-inflammatory cytokines, such as transforming

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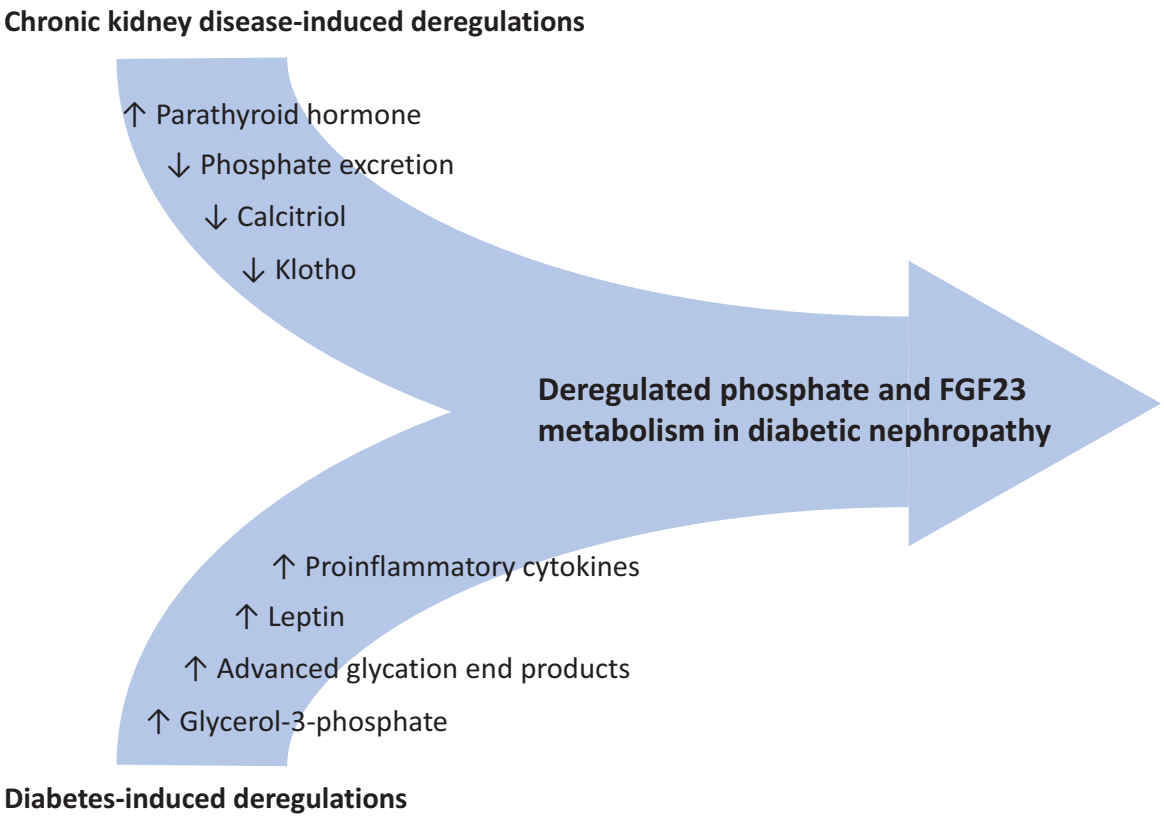

Figure 1. Schematic overview illustrating the hypothesized role of diabetes-induced and CKD-induced deregulations in phosphate and FGF23 metabolism, and how these factors coincide in diabetic nephropathy

Details are provided in the text (sections on phosphate/FGF23 in diabetes and diabetic nephropathy).

growth factor β 1 (TGF-β1), and expression of growth factors, which are both associated with diabetic kidney injury [61]. Conversely, therapies that inhibit the production or formation of AGEs lead directly to an improvement in renal function in populations with diabetic kidney disease, which thus support the role of AGEs in diabetic nephropathy [62]. Throughout the body, including in the myocardium, AGEs bind to their receptor advanced glycation end products (RAGE) and initiate a signaling pathway that leads to inflammation, artherogenesis and vasoconstriction, with detrimental consequences [63,64]. AGEs may act as mediators of diabetes-related complications such as cataract, retinopathy, and cardiovascular events [65]. Interestingly, AGEs have been shown to induce FGF23 gene expression in bone cells [66]. In line, in an experimental model of kidney disease, AGE-lowering treatment caused a decrease in FGF23 [67]. Although the benefits of AGE-lowering therapies are likely beyond FGF23 reduction, this illustrates interesting cross-talk between two pathophysiologically important processes relevant in diabetes.

Finally, SGLT2 inhibitors induce a small but significant reduction in plasma FGF23 levels, independent of changes in eGFR or albuminuria [33,34]. This effect may be secondary to the changes in phosphate in response to these drugs, as outlined before.

The aforementioned processes may chronically influence phosphate and FGF23 in patients with diabetes with or without impaired kidney function. Diabetic ketoacidosis (DKA), an acute deregulation in glucose homeostasis with severe metabolic consequences, also profoundly impacts on phosphate homeostasis, which will be addressed separately in more detail.

Phosphate and FGF-23 in diabetic nephropathy

In addition to diabetes, CKD also has major impact on phosphate and FGF23 homeostasis (Figure 1) [68,69]. Deregulated bone and mineral metabolism is one of the hallmarks of CKD in general, and the combined effects of diabetes and kidney disease further provoke relevant adverse deregulations of mineral metabolism. In parallel with declining kidney function, CKD patients develop secondary (and ultimately tertiary) hyperparathyroidism, remarkably

elevated FGF23 levels, hyperphosphatemia, and deficiency of active vitamin D. As kidney function declines, serum phosphate increases, initially due to decreased phosphate clearance. This stimulates the production of FGF23 levels in osteocytes, through a currently unclear mechanism, resulting in promotion of phosphaturia. In parallel with progressive loss of kidney function, plasma FGF23 levels increase strongly, reaching values that are more than a thousand-fold higher in patients with ESKD, compared with healthy individuals [70]. The strong and early increase in plasma FGF23 is mediated by loss of Klotho expression, an important coreceptor for FGF23 in the kidney proximal tubule, which also reduces phosphaturia during progressive CKD. In fact, an increased plasma FGF23 level is considered one of the first signs of deregulated mineral metabolism, occurring relatively early in the course of CKD [71]. Already from stage 1–2 CKD, elevated FGF23 levels may occur [45,72]. Moreover, increased PTH and FGF23 levels result in deficiency of active vitamin D [73]. Finally, it has also been reported that insulin may act of the kidney proximal tubular cells to induce an anti-phosphaturic effect [74]. This finding was replicated in dogs, where an increase was shown in phosphate reabsorption in proximal tubuli in response to insulin [75].

Progression of kidney function decline eventually results in hyperphosphatemia and excessive levels of PTH and FGF23. Subsequent kidney transplantation, the preferable treatment for most ESKD patients, results in significant reductions in plasma FGF23 and PTH, which—in the context of restored kidney function after transplantation—may initially lead to hypophosphatemia [76]. These observations position kidney function as a strong driver of FGF23 levels, and FGF23 is also considered a biomarker for loss of kidney function [77,78].

In addition to phosphate itself, several other factors may contribute to elevated FGF23 levels in diabetes (and diabetic nephropathy). Another interesting regulator of FGF23 that could be relevant to patients with diabetes was identified in a recent study addressing the question which factor(s) could cause acute kidney injury to induce changes in bone signaling (i.e., induction of FGF23 production). Using a mass spectrometry-based approach, the authors demonstrated that glycerol-3-phosphate (G3P) is a metabolite that was strongly correlated with an increase in FGF23 in response to acute kidney injury [79]. This elegant study further found that during acute kidney injury, the increase in FGF23 by kidney-derived G3P was mediated by G-3-P acyltransferase-mediated synthesis of lysophosphatidic acid (LPA) in bone [79]. This could be particularly relevant in the context of diabetes, since metformin effectively suppresses gluconeogenesis by inhibiting mitochondrial glycerol phosphate dehydrogenase, which is an enzyme involved in G3P production [80]. To our knowledge, no studies have so far explored the potential effects of metformin on FGF23 levels in animals or patients. Therefore, although there might be a link between mitochondrial dysfunction and FGF23 in diabetes, more evidence is needed to establish this hypothesis.

Thus, both diabetes and CKD seem to trigger deregulations in phosphate and FGF23 metabolism, culminating in a high risk of such deregulations in diabetic nephropathy (Figure 1). Hyperphosphatemia is more prevalent among persons with diabetes compared with those without, and this contributed to progression of kidney disease [81–83]. Furthermore, elevated levels of FGF23 were also a significant independent predictor of progression of CKD in patients with diabetic nephropathy [69,84].

Phosphate and FGF23 in DKA

In addition to the aforementioned changes in phosphate metabolism in the chronic setting, patients with DKA may develop acute deregulations in phosphate and (potentially) FGF23. DKA is an acute complication of diabetes, mostly observed in type 1 diabetes. DKA is characterized by the presence of hyperglycemia and acidosis, which results from insulin deficiency. During DKA, gluconeogenesis is enhanced by the convergence of insulin deficiency, secretion of counter-regulatory hormones such as cortisol and glucagon and increased supply of precursors such as amino acids (by proteolysis). Insulin deficiency during DKA often is the result of infection, therapy non-adherence or, in approximately 20% of all cases, unrecognized (new onset) diabetes. The mainstay of treatment of DKA is administration of insulin and fluid- and electrolyte resuscitation. Despite these treatments, the mortality rate of DKA is still 2–14%.

A schematic overview illustrating the course of plasma phosphate in DKA, and potential factors driving changes in plasma phosphate, are provided in Figure 2. Large shifts in phosphate between the intra- and extracellular compartments appear during DKA [85]. Initially, in the absence of insulin and the presence of hyperglycemia, phosphate (as well as many other electrolytes) shifts to the extracellular compartment due to hypertonicity [86]. In addition, the cellular uptake of phosphate from the extracellular compartment is reduced. While alkalosis stimulates glycolysis by the enzyme 6-phosphofructo-1-kinase and the intracellular uptake of phosphate, acidosis (as occurs in DKA) has the opposite effect [87,88]. In the setting of osmotic diuresis and hyperphosphatemia, a large amount of phosphate is filtered by the glomerulus into the pre-urine, of which little is reabsorbed. This reduced reabsorption can be explained by acidosis, that has a direct effect on the brush borders of the proximal tubule [89], where transepithelial phosphate transport is partly regulated by pH levels. At higher pH levels, phosphate uptake via NaPi co-transport is stimulated

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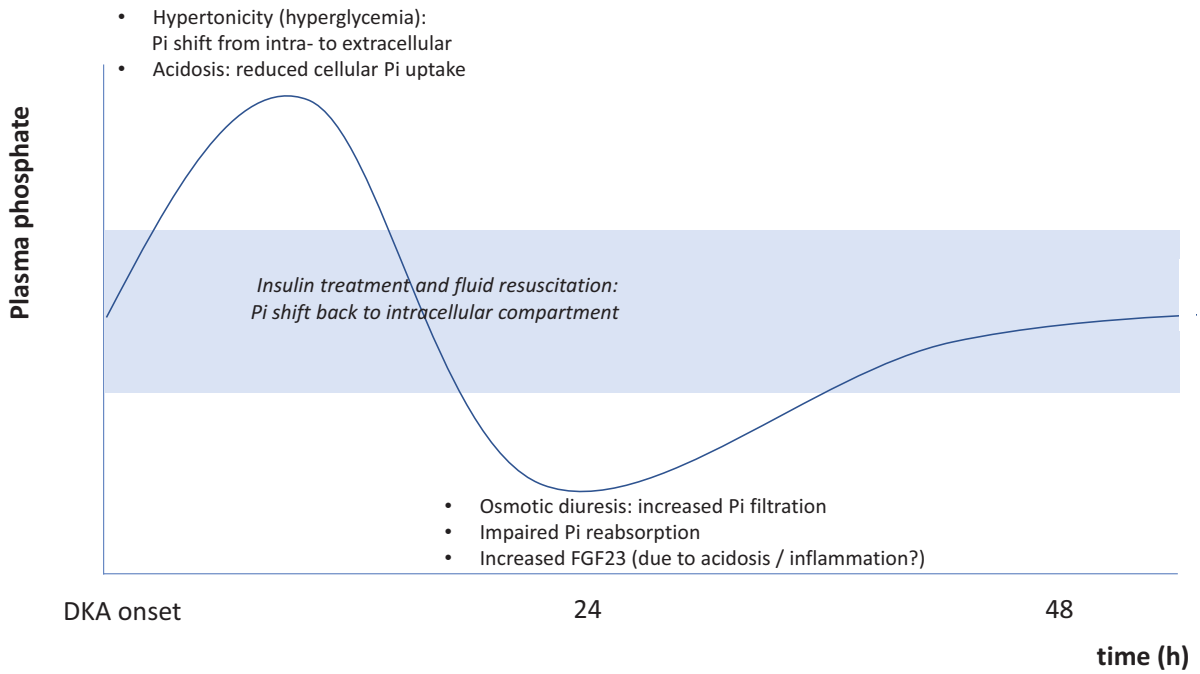

Figure 2. Schematic overview illustrating the hypothesized course of plasma inorganic phosphate in DKA, and potential factors driving changes in plasma phosphate during DKA

The x-axis represents time since DKA onset, the y-axis depicts the hypothesized course of plasma phosphate.

by improving the affinity of the channels for sodium, as documented in rabbits [90]. This massive phosphaturia seems to serve a purpose, since phosphate is used as an important urine buffer during metabolic acidosis. Phosphaturia can be further explained by an inhibition of type IIa Na-Pi co-transport due to the high degree of glucose reabsorption. The role of FGF23 during DKA is currently unknown. However, since hypophosphatemia is common during recovery from DKA, FGF23 may play a role in this. It appears that FGF23 levels are increased in a pro-inflammatory state [53,91]. DKA is also accompanied by increased levels of proinflammatory cytokines, potentially contributing to elevated FGF23 levels in DKA. Based on circumstantial evidence, we can only speculate that FGF23 also contributes to hypophosphatemia in the context of DKA [92].

At initial presentation (before treatment is started), individuals with DKA show normophosphatemia or even hyperphosphatemia, with an estimated prevalence of 31 and 66%, respectively [93]. Hypophosphatemia is very rare at initial presentation (3%). An explanation for this initial low prevalence of hypophosphatemia could be that the shift of phosphate from the intra- to extracellular compartment annihilates and often even exceeds the net urinary loss of phosphate. During fluid and insulin administration as part of the treatment of DKA, phosphate shifts back to the intracellular compartment thereby promoting hypophosphatemia. It seems that the nadir of serum phosphate appears when acidosis has almost been corrected, on average ∼22 h after start of therapy [93].

The incidence, determinants, and consequences of hypophosphatemia in DKA are underreported. Recently we performed a retrospective study [94] and demonstrated that, among persons with type 1 diabetes and DKA, 66% initially presented with hyperphosphatemia, while during therapy 74% of patients developed hypophosphatemia, of whom 23% developed a severe hypophosphatemia (serum phosphate < 0.32 mmol/l). The strongest predictor for hypophosphatemia in DKA was the degree of acidosis. Although the precise mechanisms driving these observations have not yet been studied, a partial explanation could be the effect of metabolic acidosis on FGF23 release, which has been documented mainly in CKD populations [95,96]. In CKD, metabolic acidosis results from a reduced capacity for renal clearance of acid. Studies in mice show that FGF23 is stimulated in response to metabolic acidosis, suggesting that the effect of acidosis on hypophosphatemia in DKA may be mediated at least in part by FGF23.

International guidelines imply repeated plasma phosphate measurements during hospital admission for DKA [97]. This recommendation is based on studies suggesting that hypophosphatemia in DKA contributes to lower 2,3-diphosphoglycerate levels [98]. Furthermore, this recommendation is based on the assumption that severe hypophosphatemia (<0.32 mmol/l) may lead to clinical symptoms including muscle weakness, altered mental status,

Table 2 Overview of studies investigating phosphate (A) and FGF23 (B) in relation to clinical outcomes in diabetes

Author	*n*	Follow-up (years)	Age (years)	eGFR (ml/min/1.73 m^2)	Phosphate (mg/dl)	Outcome: hazard ratio (95% CI)[1]
(A)						
Silva et al.	107	2.8 ± 0.7	57.2 ± 7.1	52.89 ± 20.15	3.99 ± 0.85	CV mortality 1.08 (1.02–3.41)
Silva et al.	119	76 months	62.6 ± 12.1	44.9 ± 25.2	4.32 ± 1.19	CV mortality: 1.44 (1.16–3.52)
Choncol et al.	950	4.8 ± 1.3	57.9 ± 8.4	67.0 (18.5)	3.6 ± 0.52	CV mortality: 5.00 (1.70–14.72)

Author	*n*	Follow-up (years)	Age (years)	eGFR (ml/min/1.73 m^2)	FGF23 (RU/ml)	Outcome: hazard ratio (95% CI)[1]
(B)						
Silva et al.	107	2.8 ± 0.7	57.2 ± 7.1	52.89 ± 20.15	135.0 ± 135.2	CV mortality: 2.05 (1.01–8.25)
Titan et al.	55	2.6 ± 0.8	58.4 ± 10.0	53.0 ± 20.6	92.0 ± 42.9	Composite endpoint[2]: 1.09 (1.01–1.16)
Tuñón et al.	173	2.15 ± 0.99	62.8	73.75 ± 20.84	72.2 (56.7–104.9)	Composite endpoint[3]: 1.27 (1.13–1.43)
Yeung et al.	310	5.8 (3.3–6.5)	61.5 ± 8.7	88.5 ± 14.8	84.2 (67.0–117.6)	All-cause mortality: 2.55 (1.58–4.10) MACE: 1.68 (1.08–2.61)
Frimodt et al.	200	6.1 (5.9–6.6)	59.9 ± 9	91.1 ± 18.3	71 (52–108)	All-cause mortality: 1.57 (1.11–2.18)
Chan et al.	513	6.6 (5.8–7.5)	55.0 (49.0–62.0)	91.3 (76.4–111.3)	112.4 (79.0–165.8)	All-cause mortality: 1.74 (1.44–2.09)

[1]Adjusted for potential confounders.
[2]Composite endpoint of all-cause mortality, doubling of serum creatinine, or requirement for dialysis.
[3]Composite endpoint of acute ischemic events (acute coronary syndrome, stroke, or transient ischemic attack), heart failure, or death.
Abbreviations: CI, confidence interval; CV, cardiovascular; MACE, major adverse cardiac event.

epilepsy, tremors, and heart failure. It should be noted, however, that studies investigating clinical consequences of hypophosphatemia in DKA did neither find reduced tissue oxygenation, nor increased morbidity or mortality [99–101]. Furthermore, literature with respect to (adverse) outcomes of hypophosphatemia in DKA is anecdotal [102,103].

Management of hypophosphatemia in DKA

Current guidelines recommended phosphate supplementation therapy in DKA at levels below 0.32 mmol/l. However, it appears that phosphate supplementation does not influence any clinical outcome, including the duration of the DKA, insulin requirement for correction of the DKA, enzyme levels associated with muscle breakdown, glucose reduction, morbidity, and mortality [101]. At the same time, phosphate supplementation may provoke the risk of hypocalcemia, through calcium binding [99,104], as well as risk of hyperphosphatemia during the later stage of DKA (Figure 2). In fact, it appears that hyperphosphatemia developed in 24% of DKA patients who received phosphate supplementation [94]. Well-designed prospective studies and trials addressing the clinical impact of phosphate deregulations in DKA and recommendations for supplementation are needed to substantiate current guideline recommendations.

Consequences of phosphate deregulations in diabetes

As outlined in detail above, many factors may contribute to deregulations in phosphate and its regulating hormone FGF23 in diabetes. The remainder of the present paper will address the adverse consequences of deregulated phosphate and FGF23, and strategies to target these deregulations. Strong associations between high FGF23 levels and cardiovascular and all-cause mortality have been reported in many studies in populations ranging from healthy individuals to ESKD patients [69,70,105,106]. Notably, several studies have shown that FGF23 and phosphate are associated with major adverse cardiac events and premature mortality in patients with type 2 diabetes with preserved or only mildly impaired kidney function (Table 2). This supports the concept that diabetes in itself could induce an increase in FGF23 [26]. Although some data implicate FGF23 also in vascular calcification [107], other studies do

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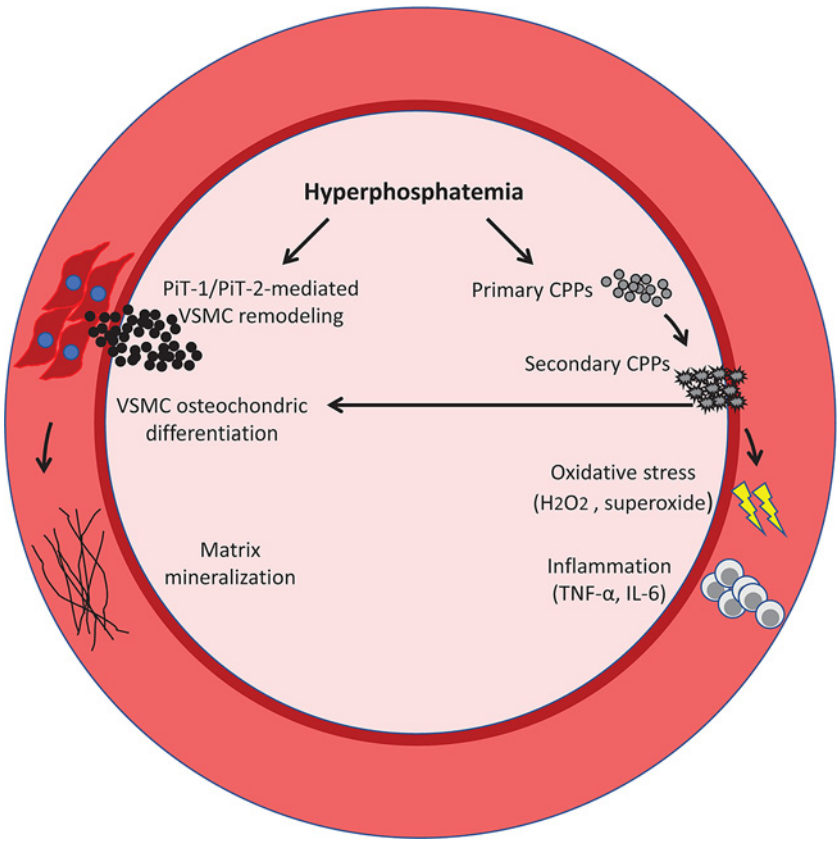

Figure 3. Hyperphosphatemia drives key processes that promote vascular calcification in diabetes

On one hand, high phosphate levels induce VSMC remodeling, mediated by PiT-1 and PiT-2 phosphate transporters. This triggers VSMC osteochondric differentiation as well as matrix mineralization, leading to vascular wall stiffening. On the other hand, high phosphate levels promote the conversion of primary into secondary calcicprotein particles (CPPs), which in turn promote oxidative stress and inflammation.

not support a direct link [108,109]. Alternatively, it could very well be that FGF23 contributes to adverse outcomes through mechanisms other than vascular calcification. Preclinical studies point towards off-target FGF23 effects on cardiomyocytes through FGF receptor 4 (FGFR4), inducing left ventricular hypertrophy [110,111]. Epidemiological studies also point towards a role for FGF23 in inflammation [53,112]. Specifically, FGF23 may facilitate inflammatory cell influx through regulation of chemokine signaling and integrin activation in CKD [113]. Whether FGF23 also contributes to the pro-inflammatory state in diabetes is unclear, but it seems that FGF23 may play a role in the host defense against bacteria, which likely goes beyond the CKD setting [114]. Finally, FGF23 has been linked with endothelial dysfunction [115] and renin–angiotensin system activation [116] as potential players driving the relationship between FGF23 and adverse outcomes [117]. Thus, although FGF23 may contribute to adverse outcomes through pathways other than vascular calcification, higher phosphate levels have been much more consistently linked with vascular calcification.

Since hyperphosphatemia is highly prevalent in patients with advanced CKD, its pathological consequences are best characterized in this population. Yet, similar pathophysiological processes may play a role in the development of vascular calcification in diabetes, even when kidney function is still in the normal range. Some of the key processes known to date are presented in Figure 3, and are discussed below. The metabolic derangements in progressive CKD set the stage for accelerated vascular calcification, particularly of the lamina media [14,118]. This type of calcification does not only entail passive calcification but also active remodeling by type III sodium-dependent phosphate transporters, PiT-1 and PiT-2, driving Pi-induced osteochondrogenic differentiation and matrix mineralization in vascular smooth muscle cells (VSMCs) [119,120]. In turn, switch to an osteogenic phenotype induces arterial stiffness, reduced vascular compliance, and systolic hypertension [121]. Along with structural changes of VSMCs in the

medial vessel layer, deposits of crystallized calcium and phosphate in the intimal layer also contribute to cardiovascular disease [11]. Hyperphosphatemia, particularly when accompanied by hypercalcemia, is strongly associated with vascular calcification and stiffness, as well as with a higher risk of cardiovascular disease and premature mortality, both in CKD and non-CKD populations [122–124], while low serum phosphate levels are generally considered cardioprotective [125]. However, recent findings in a large-scale cohort suggest that low serum phosphate levels are also associated with an increased risk of cardiovascular disease and premature mortality [126]. This might support the existence of a U-shaped relation between serum phosphate and cardiovascular disease.

Under physiological conditions, calcium and phosphate only precipitate in the form of hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) in bones and teeth. Although calcium and phosphate are excessively present in other tissues and in blood, it appears that the threshold to calcification is much higher in these tissues compared with bone and teeth. This supports the hypothesis that biomineralization is a closely regulated process that is context-specific and is influenced by other calcification inhibiting and stimulating factors. Fetuin A has been identified as a major endogenous inhibitor of calcification [127]. Indeed, in the presence of fetuin A, calcium and phosphate do not precipitate in serum as hydroxyapatite, but rather form soluble colloidal particles, called calciprotein particles (CPPs). Importantly, fetuin A is unable to convert already formed hydroxyapatite back into CPPs. In a time-dependent maturation process, these primary CPPs (CPP1s) can develop into secondary CPPs (CPP2s), characterized by an altered shape and increased diameter. In contrast with the apparently limited significance of CPP1s [128], CPP2s promote oxidative stress (H_2O_2) and inflammation. Furthermore, CPP2s also contribute to the transformation of VSMCs to an osteogenic phenotype, as described above. In addition, CPP2s induce generation of TNF-α, further catalyzing the transformation process towards more CPP2s [129].

A major breakthrough in the quantification of CPP conversion was achieved by the group of Pasch with the development of the serum T50 test [130]. The T50 test is able to quantify the transformation time from CPP1 to CPP2 in serum, thus reflecting serum calcification propensity. Across many studies, plasma phosphate was consistently the strongest determinant of serum T50, even after multivariable adjustment [131,132]. Recently we performed a study in 932 patients with type 2 diabetes and discovered that glycosylated hemoglobin (HbA1c), reflecting long-term glycemic control, was inversely associated with serum T50 levels upon multivariate analysis [133]. Low serum T50 levels have also been associated with markers of inflammatory stress (such as TNF-α), as well as with markers of bone resorption [131]. In various populations with both preserved and impaired renal function, serum T50 has been shown to predict all-cause mortality.

Initially, the serum T50 test has been applied in cohorts of patients with poor renal function, including pre-dialysis patients and patients undergoing hemodialysis, as well as patients after kidney transplantation. In a cohort of pre-dialysis patients (average eGFR: 33 ml/min/1.73 m^2), serum T50 was strongly associated with all-cause mortality. After adjustment for several potential confounders, including serum phosphate and kidney function, mortality rate was more than twice as high in the worst serum T50 tertile, compared with the best serum T50 tertile. Similarly, T50 was associated with all-cause mortality in 2785 hemodialysis patients participating in the EVOLVE trial (hazard ratio per 1 SD lower serum T50, 1.15) [134]. Interestingly, it has been suggested that during hemodialysis sessions serum T50 can improve [135], potentially driven by a change in phosphate. This is in contrast with FGF23 which is not influenced by hemodialysis [136]. These observations again underline the prominent relationship between serum phosphate and calcification propensity. Finally, serum T50 was also related with all-cause mortality in two cohort studies in kidney transplant recipients [137,138]. Interestingly, a worse (lower) T50 was also associated with an increased risk of graft failure. Of note, it turns out that serum T50 is not only a predictor of premature mortality in CKD, but also in individuals with preserved kidney function. A recent study from our group among 6231 participants demonstrated that serum T50 is independently associated with cardiovascular and all-cause mortality in the general population. When we specifically focused on high-risk subgroups in this cohort, we can show that among patients with diabetes the association between serum T50 and mortality was relatively strong (hazard ratio: 1.43), also in comparison with various other groups identified by kidney dysfunction, high BMI, or high phosphate levels.

A reduced serum T50 has also been associated with an increased risk of hypertension, independent of kidney function [139]. This is well in-line with the concept that vascular calcification, through vascular stiffening, contributes to the pathophysiology of hypertension. Whether hypertension connects the observed associations between reduced T50 and premature loss of kidney function, for example in kidney transplant recipients as summarized above, remains unknown. The link between serum T50 and pro-inflammatory cytokines including TNF-α, which in turn also regulates the expression of fetuin A, might also be involved.

Besides the impact of phosphate deregulations on the cardiovascular system, which culminates in adverse outcomes, it is also important to realize the impact of these deregulations on bone. Although bone mineral density is generally high in patients with type 2 diabetes [40,44], it still seems that bone strength is lower and a higher incidence

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of bone fractures is observed in patients with type 2 diabetes [140]. Diabetes duration is a significant factor contributing to the risk of fractures [141], and in addition one study showed a higher risk of fractures in case of suboptimal glycemic control [142]. Several bone turnover markers such as osteocalcin and C-terminal cross-linked telopeptide (CTX), are disturbed in patients with type 2 diabetes [41,42,44], and there may be a dose–effect relationship between glucose levels and bone turnovers markers [42]. Furthermore, an animal study found that in mouse model of type 1 diabetes, several bone turnover markers including dentin matrix acidic phosphoprotein 1 (DMP-1) [143] were decreased. This could also be relevant to phosphate metabolism, since DMP-1 is an important inhibitor of FGF23. Loss of DMP-1 function results in excessive production of FGF23, in turn leading to phosphate wasting, osteomalacia, and rickets [144]. Although it is obvious that relatively little is known about the role of phosphate in bone abnormalities in diabetes, this emerging evidence highlights the need for additional studies in this field.

Treatments targeting hyperphosphatemia and increased FGF23

Given the detrimental associations between deregulation phosphate and FGF23 on one hand, and an increased cardiovascular morbidity and mortality risk on the other hand, it seems important to explore potential interventions to target these deregulations. As outlined above, however, the exact impact of diabetes on phosphate homeostasis remains unclear, since phosphate levels may be normal or low in uncomplicated diabetes, whereas hyperphosphatemia may develop in diabetic nephropathy. In a study in stable diabetes outpatients with preserved kidney function, it was found that optimizing phosphate levels through dietary advice with nutritional phosphate has no long-term added value [145]. In this study, it was found that after 4 weeks of adding calcium diphosphate to diet, phosphate levels improved, but at 1 year follow-up this effect had disappeared. In addition, the threshold for phosphate excretion was inversely correlated with hyperglycemia. This suggests that glycemic control may be more relevant to restore phosphate levels in diabetes than dietary phosphate supplementation.

On the other hand, more consistent data point towards elevated FGF23 as a—potentially modifiable—risk factor for adverse outcomes in diabetes. Timely initiation of treatment to reduce FGF23 levels may be relevant, given these associations. Many potential FGF23-lowering strategies have been studied, mostly in the context of CKD. These strategies predominantly focused on factors stimulating FGF23 production, including phosphate and calcium [146,147].

First, a non-pharmacological strategy to lower FGF23 levels is to restrict dietary phosphate intake. It has been shown that variation in phosphate intake can influence FGF23 in the general population. Dietary phosphate restriction resulted in lowering of plasma phosphate and FGF23 levels and, conversely, higher phosphate intake resulted in an increase in plasma FGF23 levels [148–150]. In the CKD population, results have been less consistent, as some studies showed no effects of phosphate restriction, whereas other studies showed a decrease in FGF23 and some studies showed a decrease in FGF23 only by restricting phosphate intake for a period longer than 2 weeks, if it was combined with use of phosphate binders [146,151,152]. These observations may at least in part be explained by counter-regulatory changes in expression of phosphate transporters in the intestinal tract. In addition, the use of different FGF23 assays may partly explain the discrepant results [146]. Additionally, it may be vital to assess the bioavailability of phosphate in food products when aiming for dietary phosphate restriction. Inorganic phosphate is mostly found in processed foods, and it is readily absorbed from the gut. In contrast, organic phosphate found in vegetables and nuts, has a remarkably lower bioavailability due to lower intestinal absorption [153]. It was shown that a vegetarian diet, compared with a meat diet, while both diets contained the same amount of phosphate, can induce a decrease in FGF23 whereas the meat diet induced an increase in FGF23 levels [154].

Phosphate binders reduce intestinal phosphate absorption and may effectively lower plasma phosphate. Various trials investigated the effect of phosphate binders on plasma phosphate and FGF23 levels in CKD patients. Mainly noncalcium-based polymers, such as sevelamer z and lanthanum can significantly decrease FGF23 levels [155–157]. One study in diabetic kidney disease showed that sevelamer can reduce HbA1c, FGF23, and AGEs [158]. On the contrary, calcium-based phosphate binders in several studies did not change FGF23 or even increased FGF23 [155,159]. This is most likely due to the calcium component which could influence FGF23, offsetting the effects of calcium-based phosphate binders. Importantly, the recently published IMPROVE-CKD trial in 138 patients with stage 3b/4 CKD (91% with normal phosphate levels) could not demonstrate an effect of 96 weeks of lanthanum carbonate on arterial stiffness or aorta calcification, compared with placebo. Although this population cannot be compared with normophosphatemic diabetes patients, the findings from the present study suggest that using phosphate binders in normophosphatemic individuals is not useful to reduce vascular calcification.

Sucroferric oxyhydroxide and ferric citrate are iron-based phosphate binders that have both demonstrated phosphate- and FGF23-lowering effects [160–162]. The main advantage of iron-based binders is their ability to (in

theory) simultaneously reduce phosphate uptake and to supplement iron. This is relevant since iron deficiency is common in both CKD and after kidney transplantation [163,164]; moreover, iron deficiency might also increase FGF23 production [165]. At this time, no studies have been conducted involving a head-to-head comparison with traditional phosphate binders regarding their FGF23-lowering effect.

Another drug that influences gastrointestinal absorption of phosphate is tenapanor, a non-absorbable oral inhibitor of sodium-hydrogen exchanger 3 (NHE3). A study in dialysis patients showed that tenapanor can induce a decrease in serum phosphate as well as FGF23 levels [166]. Although 12% of patients dropped out of this study due to diarrhea, the dropout rate was lower in a second study, which was recently published. Subsequently, the AMPLIFY trial showed that in hemodialysis patients with hyperphosphatemia, combining tenapanor with phosphate binders could improve phosphate control, compared with placebo, with diarrhea being a reason to discontinue in only 3.4% of patients [167]. Further studies need to be conducted to assess the tolerability in patients and to assess clinically relevant effects, not only in dialysis patients but also in other populations. Calcimimetics, such as cinacalcet and etelcalcetide are treatments used for secondary hyperparathyroidism. Their mode of action is that they inhibit PTH release by the parathyroid gland in response to calcium, by interacting with the calcium-sensing receptor. In animal studies calcimimetics reduced PTH, FGF23 and 1,25$(OH)_2$-vitamin D, as well as plasma calcium, while increasing plasma phosphate [168]. In patients with CKD, calcimimetic treatment had similar effects and resulted in remarkable lowering of FGF23 [169–173]. The EVOLVE trial, a large clinical trial in 3883 hemodialysis patients, could not demonstrate that cinacalcet induced a significant improvement in the risk of mortality or cardiovascular events [174]. Yet, it was subsequently reported that individuals with a $\geq$30% reduction in FGF23 in response to cinacalcet (reached by 68% of patients randomized to cinacalcet) had a lower risk of reaching the primary endpoint (mortality or cardiovascular event) [175]. Although this supports FGF23 reduction as a relevant strategy in CKD patients, it should be noted that these trials have generally been conducted in patients with CKD-associated bone and mineral disorders. No data exist, to our knowledge, on the potential effects of calcimimetics on bone and mineral parameters in patients with diabetes and preserved kidney function.

Vitamin D supplementation can result in lowering of PTH levels, while at the same time it would result in an increase in both phosphate and FGF23 levels, mainly in vitamin D-deficient patients [176,177]. This is likely caused by 1,25$(OH)_2$-vitamin D interacting with the promotor region of the FGF23 gene, stimulating FGF23 production. At the same time, replenishing vitamin D in vitamin D-deficient patient may induce hyperphosphatemia through enhanced intestinal phosphate absorption. The resulting increase in phosphate may further boost FGF23 levels [177]. It has, however, been shown in a CKD rat model that calcitriol reduces expression of FGFR4 (one of the receptors for FGF23), and is subsequently able to reduce left ventricular hypertrophy. This suggests that the cardioprotective effects of calcitriol may be mediated by inhibition of the FGF23/FGFR4 pathway in the heart [178].

In the era of monoclonal antibody therapy, it may seem desirable to target high FGF23 levels with anti-FGF23 antibodies. In fact, the anti-FGF23 monoclonal burosumab has recently been approved for use in adults and children with X-linked hypophosphatemia (XLH), a rare disease characterized by an inappropriate overproduction of FGF23, leading to renal phosphate wasting and hypophosphatemia [179]. Burosumab did not only result in lowering of FGF23 levels, it also resulted in the restoration of phosphate balance and improvement of bone abnormalities in children [180]. The question remains whether anti-FGF23 therapy would be suitable in other settings of increased FGF23. A warning signal was obtained from an animal study using another anti-FGF23 monoclonal in a rat CKD model: the net effect of anti-FGF23 treatment was reduced urinary phosphate excretion, hyperphosphatemia, increased aortic calcification and a high risk of mortality [181]. Along the same lines, FGFR4 antagonists are being developed for treatment of XLH. This novel strategy uses a pan-specific inhibitor to block the FGF23 receptor and downstream signaling effects. A study in mice showed that using this FGFR4 inhibitor could indeed yield these effects; however, at the same time adverse effects were seen including hyperphosphatemia and a higher risk of calcification [182]. Thus, direct targeting of FGF23 or FGFR4 signaling may not be appropriate in CKD, and it may be preferable to target the triggers of high FGF23 (such as phosphate) instead.

If indeed factors directly related to diabetes could increase FGF23, better glycemic control might stabilize or decrease FGF23 levels in patients with type 2 DM. This might be achieved by early initiation of basal insulin on top of glucose-lowering drugs [49], increased physical activity [183], adherence to a healthier diet [184], and continuous glucose monitoring [185]. Future studies should elucidate the effects of improving glycemic control on FGF23 levels in patients with type 2 diabetes.

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Conclusions and further recommendations

Deregulations in phosphate metabolism are relatively common in patients with diabetes. Since early in the progression of diabetes plasma phosphate levels may be normal or even low, these deregulations may be difficult to discern. Phosphate deregulations are more clear in the context of DKA, although patients may progress from initial hyperphosphatemia via hypophosphatemia with a nadir at ∼22 h after DKA onset, to normalization of plasma phosphate. At the same time, recent studies suggest that even in stable diabetes with preserved kidney function, a slightly elevated level of FGF23 can be detected, which has also been associated with an increased risk of adverse outcomes. In diabetic nephropathy, particularly when kidney function is more severely impaired, patients tend to develop hyperphosphatemia, which is accompanied by extremely elevated FGF23 levels. In CKD patients, is has been well documented that both hyperphosphatemia, predominantly through vascular calcification, and high FGF23, among others by promoting left ventricular hypertrophy, strongly drive the excessive cardiovascular risk in these patients.

Although future studies are needed to further address the role of bone and mineral deregulations in patients with type 2 diabetes, the current review provides an overview from what is already known from studies in the field of nephrology. Yet, even in CKD patients more data are needed to assess the clinical impact of phosphate control, and this need extends to patients with diabetes. Accordingly, although the preliminary data that are available seem of interest, future studies will have to establish the role of FGF23 as a cardiovascular biomarker and potential treatment target in diabetes.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

AGE, advanced glycation end product; ATP, adenosine triphosphate; BMI, body mass index; CKD, chronic kidney disease; CPP, calciprotein particle; CPP1, primary CPP; CPP2, secondary CPP; DKA, diabetic ketoacidosis; DMP-1, dentin matrix acidic phosphoprotein 1; ECM, extracellular matrix; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; FGF23, fibroblast growth factor 23; FGFR4, FGF receptor 4; G3P, glycerol-3-phosphate; HbA1c, glycosylated hemoglobin; LDL, low density lipoprotein; PTH, parathyroid hormone; SGLT2, sodium glucose co-transporter 2; TNF-α, tumor necrosis factor α; VSMC, vascular smooth muscle cell.

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