Phosphate: an old bone molecule but new cardiovascular risk factor

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Phosphate handling in the body is complex and involves hormones produced by the bone, the parathyroid gland and the kidneys. Phosphate is mostly found in hydroxyapatite, however recent evidence suggests that phosphate is also a signalling molecule associated with bone formation. Phosphate balance requires careful regulation of gut and kidney phosphate transporters, SLC34 transporter family, but phosphate signalling in osteoblasts and vascular smooth muscle cells is likely mediated by the SLC20 transporter family (PIT1 and PIT2). If not properly regulated, phosphate imbalance could lead to mineral disorders as well as vascular calcification. In chronic kidney disease-mineral bone disorder, hyperphosphataemia has been consistently associated with extra-osseous calcification and cardiovascular disease. This review focuses on the physiological mechanisms involved in phosphate balance and cell signalling (i.e. osteoblasts and vascular smooth muscle cells) as well as pathological consequences of hyperphosphataemia. Finally, conventional as well as new and experimental therapeutics in the treatment of hyperphosphataemia are explored.

Introduction

Phosphate is the second most abundant mineral found in the human body where most is bound to calcium in the form of hydroxyapatite crystals. Hydroxyapatite, which is actively formed by osteoblasts, is deposited onto the collagen matrix to form bone [1]. It has long been recognized that abnormal phosphate homeostasis leads to the development of bone diseases, including osteoporosis, osteomalacia and adynamic bone [2–5]. However, phosphate dysregulation is becoming increasingly linked to the development of cardiovascular disease [6, 7]. Although there are no randomized controlled studies demonstrating the benefit of lowering serum phosphate, numerous studies have shown that hyperphosphataemia is a major risk factor for all-cause and cardiovascular morbidity and mortality in patients with chronic kidney disease (CKD) [8, 9] and in end stage renal disease [10]. Furthermore, recent studies have also shown that, even within the normal range of phosphate, individuals at the high-normal end are at an increased risk of cardiovascular disease [11, 12].

The objective of this review is to analyze the regulation of circulatory phosphate with a focus on the mechanisms involved in phosphate cellular uptake, bone remodelling, kidney elimination, as well as the process by which phosphate dysregulation leads to cardiovascular disease with a focus on CKD. Finally this paper will review preclinical and clinical studies that investigate the involvement of phosphate in cardiovascular function and the efficacy of pharmacological agents that modify phosphate in cardiovascular disease.

Normal phosphate homeostasis

Eighty-five percent of total body phosphate is found in bone. In adults, bone is a dynamic organ that continually undergoes bone formation as well as bone resorption [1]. The remaining 15% of total body phosphate is found within cells. Cellular phosphate participates in cell signalling via the phosphorylation of proteins and lipids and participates in energy storage in the form of ester bonds of nucleic acids and phospholipids [13–17]. Less than 1% of

total phosphate is found in the blood [3]. However, circulatory phosphate is a mediator between bone, the parathyroid glands, intestines and kidneys, all of which are the major players in the regulation of phosphate.

There is an abundance of phosphate in the average North American diet. Most of the phosphate in a typical Western diet is generally found in proteins (such as meat, milk and eggs) and in healthy individuals, roughly two thirds of the ingested phosphate is absorbed [3, 18]. In contrast, a large fraction of phosphate in plants is in the form of phytate which cannot be broken down and absorbed by the gut [18]. However, phosphate salts are being added as preservatives and additives in a large number of processed foods and fast foods [18, 19]. These phosphate salts are readily absorbed and could increase daily dietary phosphate intake by 1 g.

There are a number of phosphate pools that dietary phosphate can enter before it is either incorporated into the mineral phase of bones or eliminated by the kidneys. These phosphate pools consist of cellular phosphate, the skeletal mineralization front (i.e. osteoid) and blood. When cellular processes require more phosphate, phosphate enters cells predominantly through sodium-phosphate co-transporters. There are two main sodium-phosphate solute carrier families: SLC34 and SLC20, also known as sodium phosphate transporter (NaPi) type II and type III, respectively [20–22]. In the gut, the NaPi type IIb predominantly transports phosphate from the lumen (Figure 1). This carrier is electrogenic and requires three sodium molecules for every phosphate it carries [23, 24]. Studies have shown that these carriers become up-regulated in cases of phosphate deprivation [25]. Elsewhere, including the vasculature, phosphate is mostly transported intra-cellularly by the ubiquitous type III transporters, also known as PiT1 and PiT2 transporters [24]. These carriers have a high affinity for phosphate and also become up-regulated when cellular processes, such as glycolysis or oxidative metabolism, require increased phosphate [24, 26]. In healthy individuals, excess circulatory phosphate, if not used by cellular processes or incorporated into bone, is dealt with by elimination by the kidneys.

Hormonal regulation of phosphate

The major hormonal regulators of phosphate homeostasis are vitamin D, fibroblast growth factor 23 (FGF-23) and parathyroid hormone (PTH) (Figure 1). Although phosphate-sensing receptors have not been discovered and the mechanism by which phosphate imbalance is detected is still unknown, FGF-23 and PTH are released in the circulation to lower positive phosphate balance. In contrast, 1,25 dihydroxyvitamin D₃, or calcitriol, a steroid hormone that is primarily synthesized in the kidneys, has a direct transcriptional effect on the gut where it modifies phosphate transport [25, 27]. Consequently, vitamin D also

Figure 1

Hormonal regulation of elevated phosphate. Dietary phosphate (PO₄) as well as calcitriol stimulate gut uptake of PO₄ via sodium phosphate (NaPi) type II transporters. Calcitriol and blood PO₄ concentrations signal parathyroid hormone (PTH) release from the parathyroid gland and fibroblast growth factor 23 (FGF-23) release from osteoblasts. PTH and FGF-23 signal the proximal convoluted tubules and distal tubules to decrease phosphate uptake via the NaPi type II transporters, hence increasing renal phosphate elimination. FGF-23 production is directly regulated by serum PO₄. In contrast, FGF-23 in a negative feedback loop inhibits the productions of PTH and calcitriol

has two potential phosphataemic effects that are mitigated, in part, by PTH-induced phosphaturia as well as by the activation of FGF-23 expression in osteoblasts (Figure 1).

FGF-23 is a 251 amino acid protein belonging to the FGF family of proteins. The N-terminus contains the FGF domain, but the C-terminus distinguishes it from other FGFs [28, 29]. That is, unlike other FGFs, FGF-23 is produced by osteoblasts and osteocytes and released into the circulation [28, 29] where it has a direct effect on kidney re-absorption of phosphate. Understanding the role of FGF-23 in maintaining phosphate homeostasis was enhanced by the identification of the genes responsible for hypophosphataemic rickets [2, 30]. The genetic disorder X-linked hypophosphataemia (XLH) is caused by a mutation in PHEX which is a phosphate regulating gene located on the X chromosome [31]. This disorder is characterized by elevated FGF-23 concentrations, renal phosphate wasting, growth retardation, osteomalacia and rickets. The murine homologue of XLH is the hyp mouse. Treatment of the hyp mouse with antibodies against FGF-23 corrects serum phosphate concentrations and improves growth [32]. Transgenic mice developed to over-express FGF-23 also develop osteomalacia and rickets [33,

34]. On the other hand, deletion of FGF-23 in mice leads to hyperphosphataemia, secondary hyperparathyroidism and extra-osseous calcification [35].

Klotho is a 130 kDa transmembrane protein predominantly found in the kidneys. FGF-23 functions by binding to FGF receptors in the presence of Klotho on the basolateral membrane of renal tubules [36, 37]. This signalling mechanism leads to extracellular signal-regulated kinases 1 and 2 (ERK1/2) phosphorylation and downstream reduced expression of renal NaPi-IIa/c in the proximal as well as distal tubules of nephrons [36, 38]. FGF-23 also reduces concentrations of 1,25 dihydroxyvitamin D₃ (the active form of vitamin D) by inhibiting 1- α -hydroxylase (CYP27B1) and activating 24-hydroxylase (CYP24A1) which is the enzyme responsible for the breakdown of 1,25 dihydroxyvitamin D₃ [39–42]. The net effect is reduced gut absorption of calcium and phosphate (i.e. due to less vitamin D) [43, 44] and increased phosphate wasting by the kidneys (i.e. due to less NaPi) [36, 38].

Regulation of phosphate by PTH is similar to FGF-23. PTH decreases NaPi-IIa in proximal tubules by binding to the PTH receptor, a G protein-coupled receptor [44–46]. Depending on its concentration, PTH mediates this signal either by cyclic adenosine monophosphate (cAMP)/protein kinase A pathway (in high concentration) or phospholipase C/protein kinase C (in low concentration) pathway [47–49]. In comparison with FGF-23, the effects of PTH may be more acute and short term.

Inorganic phosphate is a signalling molecule in bone remodelling and vascular calcification

There exist many parallels between the process of normal bone formation and the pathological mineralization of vascular smooth muscle cells. Normal bone formation is a process requiring cellular proliferation and differentiation of osteoblasts, osteoid deposition and mineralization [50]. Experimental studies have identified that inorganic phosphate, which is fundamental to hydroxyapatite crystallization, is an integral signalling molecule in this process. That is, pre-osteoblasts *in vitro* cannot differentiate without the presence of elevated extracellular inorganic phosphate [51, 52]. Early studies by Bingham *et al.* demonstrated that increasing media phosphate of rat bone organ cultures not only increased growth and mineralization but also increased collagen content and hydroxyproline synthesis, suggesting that phosphate is needed not only for mineralization, but that it also may regulate certain cellular functions [51]. Microarray studies have identified many transcriptional factors that are regulated by extracellular phosphate early in the osteoblast differentiation process. Among these, Cbfa1/RUNX2, a transcriptional factor of the RUNT family, is up-regulated in response to phosphate, and is responsible for the formation of osteoblasts [53, 54]. Inor-

ganic phosphate is then also essential to the maturation and mineralization of differentiated osteoblasts [55]. Concentrations of alkaline phosphatase (ALP), a membrane bound enzyme with an extracellular catalytic subunit, rise early in the differentiation process of osteoblasts [50]. The main role of ALP is to break down pyrophosphate or beta-glycerophosphate into inorganic phosphate [52, 56]. This initial local rise in inorganic phosphate at the early stages of matrix maturation appears to be essential to differentiation and mineralization. Subsequent studies have confirmed that blocking ALP early in the differentiation process abrogates mineralization, whereas blocking ALP late in the process (i.e. once mineralization has begun) does not [52, 55, 57].

Mineralization then proceeds with an accumulation of calcium and phosphate in the matrix vesicles. Prior to budding off the plasma membrane, osteoblasts supply these vesicles with calcium and phosphate transporters [58, 59]. The accumulation of minerals in these microstructures and their subsequent calcification serve as nucleation sites for the mineralization of the extracellular matrix [60]. Studies using microarrays have now identified numerous genes which are up-regulated or down-regulated in phosphate rich osteoblast cultures, and of particular note, is the up-regulation of the phosphate transporter PiT1 in response to extracellular phosphate [54, 61] among others (e.g. osteopontin, type-I collagen, alkaline phosphatase and osteocalcin [50, 62]).

Pre-osteoblastic cells (MC3T3-E1) treated *in vitro* with elevated phosphate exhibit phosphorylation of ERK1/2, but do not show activation of the other mitogen activated protein kinase (MAPK) signalling proteins (p38 or the c-jun N-terminal kinase (JNK)) [62, 63]. The phosphorylation of ERK is followed by osteopontin production and expression (a bone protein involved in the matrix maturation process) [62]. Inhibitors of a number of other pathways, including PI3-kinase, protein kinase A and protein kinase G, do not inhibit phosphate induced osteopontin expression suggesting a high degree of specificity in the signalling mechanism induced by increased inorganic phosphate [62]. The phosphate signalling pathway is most likely mediated by PiT transporters since PiT RNA interference abrogates ERK signalling and differentiation in phosphate mediated calcification [64, 65]. Bone morphogenic protein 2 (BMP-2), which is a potent osteogenic protein involved in osteoblast differentiation and bone formation, increases bone matrix mineralization via a mechanism that may be different from phosphate induced mineralization in MC3T3-E1 cells. BMP-2 also increases PiT1 expression and phosphate transportation. However it is blocked by a specific inhibitor of JNK pathway [66].

It is now recognized that calcification of vascular smooth muscle cells occurs by a process that is similar to that of normal bone formation. There is substantial evidence that extracellular phosphate stimulates phenotypic transformation of VSMCs via the PiT1 transporter [67].

Similar to bone formation, the Cbfa1/RUNX2 transcriptional factor is one of the first genes to become up-regulated by vascular smooth muscle cells (VSMC) in response to elevated phosphate [68, 69]. *In vitro* experiments with VSMCs have consistently demonstrated that increasing concentrations of intracellular phosphate directly stimulate gene transcription of proteins involved in osteoblast function/bone formation [e.g. Cbfa-1, bone morphogenic proteins (BMP) 2 and 4, osteocalcin, and osteopontin (OPN)] and down-regulates the expression of VSMC contractile proteins (α -actin, SM22, and myosin heavy chain) [70]. Similar to bone, VSMCs treated with elevated phosphate also demonstrate phosphorylation of ERK1/2. Inhibition of ERK phosphorylation by the MAPK inhibitor U0126 prevents osteogenic differentiation and promotes VSMC lineage markers [71]. BMP-2 also has a similar effect in the vasculature as in bone. BMP-2 treated VSMC cultures show increased PiT1 mRNA and protein expression, as well as osteogenic transcription factor Cbfa1/RUNX2 expression [72]. Although phosphate is a key signalling molecule in the development of vascular calcification (VC), it is important to note that these *in vitro* models do not account for the potential additive effects of a dynamic circulation. Furthermore, VC is a multifaceted process and evidence suggests that in addition to phosphate, the uremic environment, apoptotic bodies and extracellular calcium also contribute to this process [10, 73–75].

Animal models with targeted disruption of genes as well as *in vitro* studies have been central in identifying a number of locally acting and circulating inhibitors of VC that are active even in the basal state. These inhibitory factors include ones which are circulating [fetuin-A [76], inorganic pyrophosphates (PPI) [77], BMP-7 [78]] as well as locally acting ones, matrix Gla protein (MGP) [79], OPN [80] and osteoprotegerin (OPG) [81].

Phosphate transporters

The SLC20 NaPis (PiT1 and PiT2) were initially identified as retrovirus receptors without any knowledge of their transport activity. Experimental evidence eventually showed that these receptors were electrogenic sodium phosphate transporters [21, 22]. Unlike the transporters of the SLC34 family, which are mainly found in the gut and kidneys, PiT transporters are found ubiquitously and are present in bone cells (osteoblast and osteoclasts) and chondrogenic cells, VSMCs, parathyroid cells and hepatic cells as well as gut tissue and kidney tissue [66, 69, 82–86]. PiT transporters are essential for osteoblastic differentiation. That is, early in the differentiation and mineralization process and following the activation of ERK1/2 by phosphate or JNK by BMP-2, PiT1 mRNA levels begin to rise [66, 69, 83, 87]. There is now a growing body of evidence to suggest that PiT1 and PiT2 are not solely phosphate transporters and might be

involved in other cellular functions such as differentiation and proliferation. Yoshiko *et al.* demonstrated that the transport of phosphate to the cytosol by PiT transporters was essential to mineralization. However, they also demonstrated that a cellular paracrine/autocrine stanniocalcin 1 (STC) was produced in response to inorganic phosphate and increased PiT1 and ALP expression via mechanisms yet to be discovered [88]. PiT transporters may also directly regulate cellular processes in response to phosphate without actual transport activity. For instance, PiT2 mutants which have been engineered without transport activity undergo cell surface conformation changes in response to inorganic phosphate [89]. As well, transport deficient PiT1 mutants have been shown to be critical in cell proliferation in response to extracellular inorganic phosphate [65]. Since PiT1 knockout mice do not survive gestation, the recent generation of a conditional knockout of PiT1 transporter (from Giachelli *et al.*'s group) will hopefully provide further knowledge of their activity and function [90, 91].

Taken together, sodium phosphate PiT transporters are essential to phosphate mediated osteoblast differentiation [88]. However, they are also recognized to play a role in pathological calcification. When VSMCs are exposed to high phosphate, they also demonstrate increased PiT1 mRNA levels, although increased transport activity has yet to be studied [87, 92]. A recent study by Villa-Bellosta in VSMC cultures suggests the acute elevation in PiT1 mRNA expression plays a role in the increased endoplasmic reticulum PiT1 expression, a cell process which may mediate the phosphate-induced elevation of pro-calcifying proteins and a reduction in inhibitory proteins [93].

Phosphate impacts on osteoclast differentiation and activity

Increased osteoclast activity also causes an imbalance in bone remodelling. This imbalance is responsible for diseases such as osteoporosis, periodontal disease and rheumatoid arthritis [94]. *In vitro* studies demonstrate that high or low phosphate concentrations inhibit the activity of osteoclasts [95, 96]. DMP1 null mice, which are hypophosphataemic due to elevated FGF-23 concentrations, have osteoblast abnormalities as well as low osteoclast numbers despite having an elevated PTH [32]. PTH, which is elevated 5 to 10-fold in these mice, normally would activate osteoclasts to increase bone resorption [96], but in the DMP1 null mice, chronic hypophosphataemia appears to decrease osteoclast activation [32]. Correction of hypophosphataemia via treatment with FGF-23 antibodies also corrects the low osteoclast number [32].

The direct impact of abnormal osteoclast function on the pathogenesis of VC is not clear. However proteins involved in osteoclast activation and function have also

been associated with soft tissue calcification. The receptor activator of nuclear factor κ B (RANK), the RANK ligand (RANKL) and osteoprotegerin (OPG) have been associated with VC in animal models, as well as in post-menopausal women and in patients with chronic kidney disease [81, 97–99]. In bone, RANKL and OPG have been shown to be expressed in osteoblasts and function as regulators of osteoclast activation. The receptor RANK is expressed in osteoclasts. The major role of RANKL in bone is the stimulation of osteoclast activity and inhibition of osteoclast apoptosis [100]. In contrast, the major role of OPG is to bind to RANKL and inhibit its function [101]. OPG-deficient mice develop severe osteoporosis as well as vascular medial calcification [81]. A recent study by Osako *et al.* (2010) showed that RANKL induced calcification in VSMCs and the mechanism could be partly due to the fact that RANKL also reduced the soft tissue calcification inhibitor matrix Gla protein (MGP) [97]. At present, it is not clear whether phosphate and RANKL share a common pathway in VSMC calcification. However as demonstrated by the DMP1 null mice phosphate dysregulation impacts osteoclast activity, and a disruption in osteoclast function could potentiate a RANKL induced pathway in VC.

Chronic kidney disease and abnormal phosphate regulation

The definition of the chronic kidney disease-mineral bone disorder (CKD-MBD) is 'a systemic disorder of mineral and bone metabolism due to CKD manifested by either one or a combination of biochemical abnormalities (phosphate, calcium, parathyroid hormone secretion or vitamin D metabolism), abnormalities in bone turnover, mineralization, volume, linear growth or strength and vascular or other soft tissue calcification [102]. Bone remodelling, or changes in bone structure and function during adulthood, is severely disrupted in patients with CKD. Extraskelatal calcification is frequently observed in patients with advanced CKD and may be exacerbated, in part, by some of the therapies used to correct mineral and bone changes. Hyperphosphataemia typically occurs by stage 4 CKD (estimated glomerular filtration rate, eGFR, $<30 \text{ ml min}^{-1}$) yet marked changes in counter-regulatory hormones involved in phosphate homeostasis begin by stage 2 CKD (eGFR $60\text{--}90 \text{ ml min}^{-1}$) [103]. The pathogenesis of hyperphosphataemia in CKD is due to decreased renal clearance which creates a positive balance that increases the concentration of phosphate in the exchangeable pools, including the circulation. Consequently, the positive phosphate balance leads to elevated hormones involved in phosphate regulation such as fibroblast growth factor 23 (FGF-23) and PTH [103]. Due to loss of renal function, the capacity of vitamin D₃ production is also decreased, leading to decreased calcium absorption, hypocalcaemia and stimulation of PTH secretion. Chronically elevated concentrations of PTH

cause excessive bone resorption and this may, in fact, contribute to hyperphosphataemia as the reservoir function of the skeleton is lost. The vasculature and the soft tissues become a 'new' reservoir for unexcreted phosphate. Progressive deposition of phosphate in the vasculature is associated with VC [75, 104]. Consequently, the functional outcome of VC is elevated pulse wave velocity and pulse pressure, both of which contribute to left ventricular remodelling and mortality in patients with CKD [105–107]. We have shown in an experimental model of renal failure that CKD rats with VC have elevated pulse wave velocity, pulse pressure, systolic blood pressure and lowered diastolic blood pressure. These circulatory changes as well as elevated FGF-23 were associated with the severity of left ventricular hypertrophy in this model [108].

FGF-23 is being increasingly recognized as a key player in the maintenance of normal serum phosphate concentrations in early stages of kidney disease. Whereas phosphate concentrations in the blood may not be increased in the earlier stages of CKD, in contrast, FGF-23 concentrations have been found to be markedly elevated and can range from 100- to 1000-fold higher than in healthy subjects [103]. It is currently believed that the early impact of FGF-23 on phosphaturia is homeostatic and beneficial. However, later in disease it may have direct organ toxicity. For instance, recent studies have linked FGF-23 with left ventricular hypertrophy (LVH) and cardiovascular disease [109, 110].

Studies of phosphate dysregulation in humans with chronic kidney disease

Database studies of dialysis patients consistently show an increased rate of mortality with increasing concentrations of serum phosphate [10, 105, 111–113]. A number of cross-sectional studies performed in patients with CKD have also shown an association between higher concentrations of phosphate and severity of VC as well as progression of CKD [8, 9, 114–116]. Similarly, concentrations of FGF-23 have been proven to be a strong and independent risk factor for all-cause mortality in CKD patients [117].

It has also been demonstrated that there is a graded, independent relation between higher concentrations of serum phosphate and risk of cardiovascular events and mortality in a large cohort of non-CKD subjects, most of whom had serum phosphate concentrations within the normal range [6]. Foley *et al.* have also recently demonstrated that being in the highest quintile of 'normal' serum phosphate concentrations was associated with a greater prevalence of LVH in community-dwelling adults [6]. There is also evidence linking FGF-23 and adverse patient outcomes in a non-CKD cohort as well [118]. Within the general population, FGF-23 concentrations, even within the normal range, are associated with left ventricular mass

Table 1

Clinical outcomes of sevelamer vs. calcium based binders

	Patients	Comparison	Primary end point	Result
Suki <i>et al</i> [123] (Dialysis Clinical Outcomes Revisited, DCOR)	Prevalent HD patients	Sevelamer hydrochloride vs calcium carbonate or calcium acetate	All-cause mortality and cause-specific mortality	No difference between the two treatment arms
Chertow <i>et al</i> [129] (Treat to Goal)	Prevalent HD patients	Sevelamer hydrochloride vs calcium carbonate or calcium acetate	Change in CAC score over 12 months	Change in CAC score significantly higher in the calcium arm
Qunibi <i>et al</i> [128] (the Calcium Acetate Renagel Evaluation-2, CARE-2)	Prevalent HD patients	Sevelamer hydrochloride + atorvastatin vs calcium carbonate + atorvastatin	Change in CAC score over 12 months	Change in CAC score did not differ significantly between the treatment arms
Block <i>et al</i> [127] (Renagel in New Dialysis, RIND)	Incident HD patients	Sevelamer hydrochloride vs calcium carbonate or calcium acetate	Change in CAC score over 6, 12 and 18 months	Change in CAC score significantly higher in the calcium arm
Barreto <i>et al</i> [126] (Bone Remodelling and Coronary Calcification, BRIC)	Prevalent HD patients	Sevelamer hydrochloride vs calcium carbonate	Change in CAC score over 12 months	Change in CAC score did not differ significantly between the treatment arms
Asmus <i>et al</i> [120]	Prevalent HD patients	Sevelamer hydrochloride vs calcium carbonate	Change in CAC score over 24 months	Change in CAC score significantly higher in the calcium arm

CAC, coronary artery calcification; HD, haemodialysis.

and increased risk for the presence of LVH in elderly subjects [118]. These findings suggest that the extra skeletal manifestations of elevated phosphate, such as VC and stiffening, may begin at substantially lower concentrations of phosphate dysregulation than previously appreciated and the implications of phosphate dysregulation are therefore not limited to individuals with advanced kidney failure.

Treatment of hyperphosphataemia in the prevention of bone mineral disorders

Despite the recognition that phosphate is an emerging cardiovascular risk factor, there are no dietary recommendations for phosphate intake for individuals with normal kidney function who are at high cardiovascular risk. For patients with CKD, the KDIGO clinical practice guidelines (Kidney Disease: Improving Global Outcomes) recommend targeting 'normal' phosphate concentrations. However, a randomized controlled trial has yet to examine whether treating hyperphosphataemia to specific target goals improves clinical outcomes in patients with CKD. Although patients with CKD attempt to maintain a normal serum phosphate via restriction of dietary phosphate, normal concentrations are often difficult to achieve. The conventional treatment for phosphate imbalance is oral phosphate binders which include calcium-based phosphate binders (calcium carbonate or calcium acetate) and non-calcium based phosphate binders (sevelamer, lanthanum). These agents aim to bind dietary phosphate within the gut and prevent its absorption. Vitamin D, bisphosphonates and calcium sensing antagonists are agents that also impact on phosphate homeostasis. Modification of the

conventional thrice weekly haemodialysis treatment regimen to a quotidian dialysis schedule is a further option in select patients.

Phosphate binders

After dietary restriction, calcium-based phosphate binders (either calcium carbonate or calcium acetate) are the usual first line of treatment for CKD patients with elevated phosphate concentrations. They have relatively similar phosphate binding potency; however hypercalcaemia is a recognized risk. More recently, the excess calcium that these patients are exposed to, in association with the loss of the skeletal reservoir, has been linked to an increased risk of VC [119–121]. As such, calcium-free phosphate binding agents have been developed and are now widely available. Sevelamer hydrochloride is an anion-exchange resin used by close to 20% of haemodialysis patients world-wide [122]. Major problems related to sevelamer include cost, pill burden to achieve adequate phosphate lowering, gastrointestinal side effects and metabolic acidosis. A new formulation called sevelamer carbonate has been developed to prevent acidosis but most comparative studies have been performed using the hydrochloride formulation. Lanthanum carbonate is the most recent non-calcium based phosphate binder to be approved for use in patients with CKD. This metal-based drug is associated with less hypercalcaemia but gastrointestinal side effects are common.

One randomized trial in prevalent dialysis patients, The Dialysis Clinical Outcomes Revisited Study (DCOR), has been adequately powered to detect a difference in mortality between patients taking a calcium-based phosphate

binder and sevelamer [123] (Table 1). The primary end points of all-cause mortality and cause-specific mortality were not different between the two groups. A secondary analysis suggested a survival benefit in favour of sevelamer but only in those individuals who were older than 65 years of age. In contrast, the longitudinal follow-up of the Renagel in New Dialysis (RIND) study participants, who were incident dialysis patients, demonstrated that being randomized to sevelamer was associated with a reduced risk of mortality compared with calcium [124]. Similar findings were reported by Di Iorio in patients with stage 3–4 CKD. In this randomized controlled trial of 212 patients, sevelamer provided a benefit in all-cause mortality over 36 months when compared with calcium [125]. Five randomized clinical trials (Table 1) have evaluated the effectiveness of sevelamer compared with calcium-based binders in preventing the progression of coronary artery calcification in patients with CKD and the results are inconsistent [120, 126–129]. Three of these clinical studies, Treat to Goal, RIND and Asmus *et al.*, demonstrated a beneficial effect of sevelamer on the progression of coronary artery calcification whilst the Calcium Acetate Renagel Evaluation (CARE) 2 study and the Bone Remodelling and Coronary Calcification (BRiC) studies did not (Table 1). However, the severity of VC in the patients included in the trials varied. Some patients already had significant calcification at the start of the phosphate binder treatment, while others did not. This heterogeneity was found to be significant in the progression of calcification. Those patients with calcification already present on the baseline scan were more likely to benefit from sevelamer. If calcification was not present at the moment of phosphate treatment initiation, there was no difference between sevelamer and calcium based binders [120, 127, 130]. It is a limitation of these studies that the degree of bone abnormalities present in these patients was not accounted for as the risk of VC with calcium based phosphate treatment is enhanced when low bone turnover conditions are present. London *et al.* reported this association in a study of patients with and without bone biopsy proven adynamic bone disorder treated with calcium carbonate [131]. They showed an increased vascular stiffness in patients treated with calcium carbonate with this pre-existing bone disease, suggesting that the calcium burden associated with calcium carbonate exacerbated VC when low bone turnover was present. As such, the KDIGO guidelines give a weak recommendation to avoid calcium-based phosphate binders in patients with low bone turnover, VC or hypercalcaemia [132].

Animal models provide a controlled environment for comparing new drugs with conventional drugs. The clinical trials of phosphate binder therapies may be confounded by the inherent heterogeneity in significant co-morbidity factors such as diabetes, dyslipidaemia, CKD duration and smoking. In animal models of CKD, sevelamer and calcium carbonate are equally effective at reducing hyperphosphataemia and secondary hyperparathyroidism [133, 134].

With either treatment calcification is reduced compared with control animals, but in some studies sevelamer is more effective [135]. The effect of phosphate binders has also been shown to impact on bone osteodystrophy in animal models [136–138]. The phenotype of the bone mineral disorder in the adenine induced CKD model includes increased fibrosis, increased bone volume (trabecular), increased osteoid, increased porosity ratio, increased osteoblast and osteoclast number and increased resorption [136–138]. Sevelamer treatment in these rats reduced the bone osteodystrophy significantly, but did not correct the bone abnormalities completely [137, 138]. Transgenic CKD mice (low density lipoprotein receptor knockout), on the other hand, developed bone pathology more similar to adynamic bone disorder in the CKD population [136, 139]. These mice exhibit reduced trabecular bone volume, reduced osteoblast surface, reduced osteoid volume and low bone formation rate. Treatment with sevelamer in this model reversed osteoblast surface to normal levels and significantly increased osteoid volume and bone formation [136, 139]. These benefits of sevelamer in the CKD bone mineral disorder appear to be a direct result of the normalization of hyperphosphataemia without an excess calcium load.

Lanthanum carbonate is a calcium-free phosphate binder that has been shown, in animal models, to be more potent in its ability to reduce phosphate compared with sevelamer and calcium-based binders [140]. Furthermore, long term treatment of CKD animals with lanthanum has shown promising results in the correction of bone osteodystrophy and the reduction of VC [141]. Large clinical trials in humans comparing lanthanum with sevelamer and calcium-based binders are lacking. However, in the few clinical studies available, lanthanum carbonate has shown promising outcomes in the improvement of low bone turnover and renal osteodystrophy compared with calcium-based phosphate binders [142, 143]. No studies have assessed the effect of lanthanum on VC nor have any studies followed patients long term. Therefore, the long term safety of lanthanum is unknown.

Vitamin D

The ideal degree of PTH suppression in the treatment of secondary hyperparathyroidism in CKD with vitamin D analogues is unclear. Suppression of PTH to 'normal' concentrations in CKD might lead to adynamic bone disorder and because of this, the KDIGO guidelines recommend maintaining PTH in the range of approximately 2–9 times the upper reference limit for the assay [132]. However, suppressing intact PTH concentrations with calcitriol (1,25 OH vitamin D₃) treatment has been associated with increased VC presumably due, in part, to elevated gut absorption of calcium and phosphate (a direct effect of calcitriol) [144–146]. However, the vitamin D receptor is present in VSMCs

and osteoblasts, and activation of the receptor might lead to bone and vascular remodelling. Studies utilizing *in vivo* CKD models consistently demonstrate that the administration of 1,25 OH vitamin D₃ causes increased VC. However, the overwhelming majority of these studies utilized a dose of vitamin D that markedly exceeded the equivalent therapeutic dose in humans indicating that, when given in excess, vitamin D causes calcification. Observational studies utilizing a surrogate outcome suggest that taking a vitamin D analogue is a risk factor for coronary artery calcification and/or aortic valve calcification in dialysis patients [147, 148]. The effect of vitamin D concentrations on VC may follow a bimodal pattern. In a study of 61 children on dialysis, calcification scores showed a U-shaped distribution across 1,25 OH vitamin D₃ concentrations [149] suggesting that both 'too little' and 'too much' are problematic. Although the use of vitamin D has been linked to excess calcification in observational studies and pre-clinical studies, observational studies involving large numbers of incident dialysis patients consistently report a survival advantage among patients prescribed the active form of vitamin D [150, 151]. Despite the consistency and strength of the epidemiological observations that favour a form of active vitamin D therapy, to date, no randomized, placebo-controlled trial of vitamin D therapy has been performed in this population that addresses hard clinical end points.

Calcimimetics

Calcimimetics bind to the calcium sensing receptor of the parathyroid gland where they mimic the effects of excess calcium, and thus inhibits PTH release [152]. The clinically available agent, cinacalcet, has been shown to reduce PTH concentrations to a similar range as calcitriol [153]. However, in contrast to calcitriol, cinacalcet has the added benefit of also improving mineral imbalance (decrease in phosphate, decrease in calcium), an effect which might be beneficial in the treatment or prevention of soft tissue calcification as well as bone mineral disorders [153] although the mechanism for reduced phosphate and calcium concentrations is unclear. In uraemic rats, cinacalcet has been shown to reverse osteitis fibrosis in animals with high bone turnover [154]. In experiments in which rats exhibit low bone turnover, cinacalcet treatment resulted in increased bone density and trabecular bone volume [155]. Furthermore, animal studies have also shown treatment with cinacalcet prevents VC [156, 157].

One randomized clinical trial has evaluated the impact of cinacalcet on the progression of coronary artery calcification in dialysis patients. Although the primary end point of coronary artery calcification was not significant, there was a reduction in the progression of valvular calcification in subjects taking cinacalcet (ADVANCE study) [158]. Another study (EVOLVE) evaluating the impact of cinacalcet

on the hard clinical endpoints of mortality and cardiovascular events in patients with CKD was recently published [159]. However, the major results were not significant (7% reductions in relative hazard for composite primary end points, 6% reduction in relative hazard of all-cause mortality). However the low study power limited the interpretation of the results.

Bisphosphonates and pyrophosphate

Bisphosphonates have a similar structure to pyrophosphate, but they are more stable and are not broken down as easily. They have been used worldwide in the treatment of osteoporosis because they inhibit osteoclast differentiation, activity and adhesion and promote osteoclast apoptosis [160, 161]. However, care has to be taken when treating patients with bisphosphonates, as the wrong dose or accumulation of the drug can also inhibit bone mineralization in a similar fashion to pyrophosphate. Pyrophosphate is quickly hydrolyzed by alkaline phosphatase, unlike bisphosphonates which could have a skeletal half-life of more than 10 years [162]. Bisphosphonates have been successful in the treatment and prevention of VC. However, caution is required due to their catabolic actions on bone.

Experimental uraemic models have successfully shown inhibition of VC with treatment of etidronate and ibandronate (two commercially available bisphosphonates). However the doses that inhibited VC also inhibited bone formation [163, 164]. In a clinical study, etidronate treatment in haemodialysis patients was shown to have inhibitory effects on coronary and aortic calcification. However as this was a small study these results require validation in larger well-designed studies [165, 166].

Experimental treatments

Recently, pyrophosphate has been successfully used in experimental models to inhibit soft tissue calcification and might be a better candidate for treatment of VC than bisphosphonates [167]. The drawback to this treatment is that pyrophosphate is not bioavailable orally, and the authors of the experimental model had to deliver the dose by the subcutaneous or intraperitoneal route. Regardless, the inhibition of VC was promising and the dose used did not impact on bone remodelling. The most likely explanation is the local difference in concentration of pyrophosphate in bone vs. arteries. The abundance of non-tissue specific alkaline phosphatase expressed in bone as compared with the arteries would considerably decrease the pyrophosphate concentration in the skeletal microenvironment.

The use of vitamin K represents a promising avenue for the treatment of VC. The active form of matrix Gla protein is

an important inhibitor of VC. Vitamin K is a cofactor for γ -carboxylase, the enzyme responsible for the carboxylation of Glu residues and, therefore, the functional activity of MGP. Recent clinical evidence has shown that CKD patients have a very high prevalence of vitamin K deficiency as well as decreased circulatory active MGP protein [168, 169]. This reduction in vitamin K and MGP function may therefore contribute, in part, to the higher degree of VC observed in these patients [170]. Vitamin K₂ has been able to rescue, in part, the VC phenotype observed in animals treated with high doses of the vitamin K antagonist, warfarin [171]. Currently, two clinical trials in end-stage kidney disease patients testing the beneficial effect of vitamin K₁ in the treatment of VC have been initiated in Europe and Canada.

Bone morphogenic protein 7 (BMP-7) is a bone protein which regulates the transdifferentiation of VSMCs. Unlike BMP-2, which is a promoter of calcification, BMP-7 may play a role in the prevention of vascular differentiation to an osteoblastic phenotype. BMP-7 is also found in circulation and has been suggested to have a hormonal role [172]. In animal models of CKD, treatment with BMP-7 prevented VC and reversed renal osteodystrophy and decreased hyperphosphataemia [173]. Although the mechanism of BMP-7 treatment is unclear, it appears to restore bone anabolic balance by promoting increased phosphate uptake into bone, rather than soft tissue.

Recently, magnesium has been suggested to inhibit VSMC differentiation and calcification. Although magnesium is also an effective phosphate binder, its inhibitory mechanism probably extends beyond just a physiochemical inhibition of hydroxyapatite formation. Magnesium enters cells by the magnesium transporter, transient receptor potential melastatin cation channel (TRPM7), and impacts downstream signalling by reducing osteogenic proteins (osteocalcin, BMP-2) and increasing MGP [174]. These studies were carried out *in vitro* and the reproducibility of this mechanism still has yet to be tested *in vivo*.

Conclusion

In conclusion, circulatory phosphate is closely regulated by the hormones of the bone-kidney axis. The local phosphate environment is tightly regulated. For instance, ALP mediated phosphate increase in the osteoblast microenvironment signals osteoblast differentiation, bone formation and mineralization. Without a closely regulated ecosystem, phosphate imbalance could greatly increase the risk of cardiovascular and bone disease. Hormonal dysregulation, such as occurs in the presence of CKD, substantially increases morbidity and mortality. If the local phosphate balance is tipped to one side, e.g. hyperphosphataemia, experimental data suggest that pathological osseous differentiation occurs through a mechanism similar to bone formation (i.e. PiT1 mediated). Furthermore, epidemiological studies have shown that high phosphate concentra-

tions have consistently been linked to adverse outcomes particularly in the CKD population. Regardless, we still need randomized controlled trials to inform clinical practice with respect to phosphate management.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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