Roles of phosphate and fibroblast growth factor 23 in cardiovascular disease

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Abstract | Disturbances in phosphate homeostasis are common in patients with chronic kidney disease. As kidney function declines, circulating concentrations of phosphate and the phosphate-regulatory hormone, fibroblast growth factor (FGF)-23, rise progressively. Higher serum levels of phosphate and FGF-23 are associated with an increased risk of adverse outcomes, including all-cause mortality and cardiovascular events. The associations between higher FGF-23 levels and adverse cardiovascular outcomes are generally independent of serum phosphate levels, and might be strongest for congestive heart failure. Higher serum phosphate levels are also modestly associated with an increased risk of cardiovascular events even after accounting for FGF-23 levels. This observation suggests that FGF-23 and phosphate might promote distinct mechanisms of cardiovascular toxicity. Indeed, animal models implicate high serum phosphate as a mechanism of vascular calcification and endothelial dysfunction, whereas high levels of FGF-23 are implicated in left ventricular hypertrophy. These seemingly distinct, but perhaps additive, adverse effects of phosphate on the vasculature and FGF-23 on the heart suggest that future population-level and individual-level interventions will need to simultaneously target these molecules to reduce the risk of associated cardiovascular events.

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Introduction

The discovery of fibroblast growth factor (FGF)-23 has advanced our understanding of the role of phosphate homeostasis in the pathogenesis of cardiovascular disease.[1] FGF-23 is a circulating endocrine hormone that is secreted by osteocytes, primarily in response to increases in dietary phosphate intake and circulating concentrations of $1,25(OH)_2D_3$, the hormonally active form of vitamin D.[2,3] The classic actions of FGF-23 on mineral metabolism are mediated by binding of FGF-23 to heterodimeric complexes consisting of FGF receptors and the specific FGF-23 co-receptor, Klotho.[4] Activation of Klotho–FGF receptor complexes in the kidney augments urinary phosphate excretion by downregulating the activity and expression of the sodium-dependent phosphate transporters NaPi-2a and NaPi-2c in the proximal tubule apical membrane. FGF-23 also inhibits expression of *CYP27B1,* which encodes 25-hydroxyvitamin D_3 1α-hydroxylase, and stimulates expression of *CYP24A1,* which encodes the catabolic $1,25\text{-}(OH)_2D_3$ 24-hydroxylase. As a result, $1,25(OH)_2D_3$ levels are reduced.[5,6] By increasing urinary phosphate excretion and decreasing gastrointestinal phosphate absorption as a result of lower $1,25(OH)_2D_3$ levels, elevated FGF-23 levels maintains serum phosphate levels within the normal range when dietary phosphate intake is high. Conversely, reduced FGF-23 levels maintain normal serum phosphate levels when dietary phosphate intake is low (Figure 1).[7–10]

Levels of FGF-23 rise progressively in patients with chronic kidney disease (CKD) as their glomerular filtration rate (GFR) falls.[11,12] Although the mechanisms are incompletely understood, rising FGF-23 levels help patients with CKD to maintain normal serum phosphate levels until their kidney function becomes critically low. Despite the important physiological effect of FGF-23 to maintain phosphate homeostasis in the context of differences in diet and kidney function, a growing body of evidence suggests that higher FGF-23 levels are a risk factor for cardiovascular disease, and are possibly a modifiable mechanism of disease.[13–17] However, since higher serum phosphate levels are also linked to an increased risk of cardiovascular disease and mortality,[18–22] therapies that target FGF-23 alone and precipitate worsening of hyperphosphataemia could be harmful.[23] To design effective therapeutic strategies, a detailed understanding of the complex interrelationships between phosphate, FGF-23 and cardiovascular disease is required.

In this Review, we discuss relevant data from epidemiological, physiological and experimental studies that evaluate phosphate and FGF-23 as cardiovascular risk factors. We consider to what extent the relationships between FGF-23, phosphate and cardiovascular disease are distinct and, most importantly, whether or not they are modifiable targets to prevent cardiovascular disease.

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Key points

- Higher circulating levels of phosphate and fibroblast growth factor (FGF)-23 are associated with increased risk of cardiovascular disease in populations with or without chronic kidney disease
- Higher phosphate concentrations induce vascular calcification and endothelial dysfunction *in vitro* and in animal models; observational studies in humans have corroborated these findings
- Higher levels of FGF-23 might have direct hypertrophic effects on cardiac myocytes, which could explain their association with left ventricular hypertrophy and congestive heart failure in patients
- Simultaneous control of FGF-23 and serum phosphate levels might be useful strategies to reduce cardiovascular disease in at-risk populations, including those with chronic kidney disease

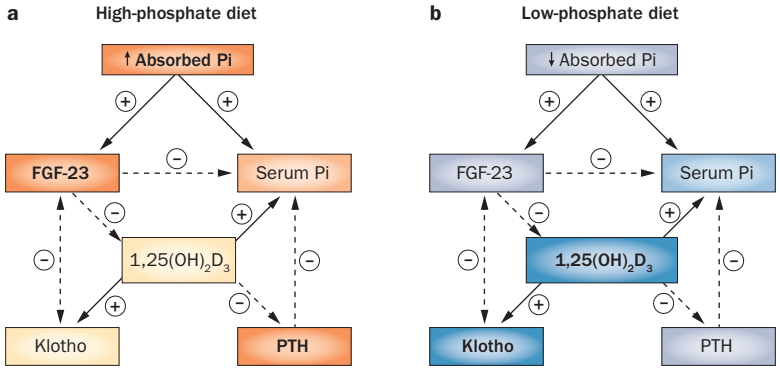

Figure 1 | The physiology of phosphate homeostasis in the context of low-phosphate and high-phosphate diets. **a** | In response to high dietary phosphate loads, levels of FGF-23 rise, which leads to inhibition of 25-hydroxyvitamin-D_3 1α-hydroxylase expression and a reduction in $1,25(OH)_2D_3$ (the hormonally active form of vitamin D) levels. Normally, $1,25(OH)_2D_3$ suppresses PTH release and stimulates intestinal absorption of phosphate; thus, FGF-23-mediated decreases in $1,25(OH)_2D_3$ levels raise PTH levels and reduce phosphate absorption. High PTH and FGF-23 levels stimulate phosphaturia, which minimizes the effect of a high-phosphate diet on serum phosphate levels. Dark coloured boxes denote increased level, light coloured boxes denote decreased level. **b** | Diets that are low in phosphate result in reduced FGF-23 levels and increased $1,25(OH)_2D_3$ levels, which suppress PTH release and augment phosphate absorption in the intestine. Under both dietary conditions, the changes in serum phosphate levels are small, and are generally maintained within the normal range (0.74–1.52 mmol/l). Additional feedback loops involving FGF-23, PTH and Klotho are not depicted. According to the dietary reference intake, adequate intake for phosphorus in healthy nonpregnant adults is 700 mg per day[127] and typical intake in the USA is in the approximate range 800–1,600 mg per day.[128] Dark coloured boxes denote increased level, light coloured boxes denote decreased level. Abbreviations: FGF-23, fibroblast growth factor 23; Pi, inorganic phosphate; PTH, parathyroid hormone.

Epidemiological data

Phosphate levels and cardiovascular disease

Hyperphosphataemia was first identified as a risk factor for increased mortality in patients undergoing haemodialysis.[24] Multiple subsequent studies confirmed these findings, and similar results were reported for patients with less-advanced CKD who were not on dialysis.[25–30] Higher serum phosphate levels have also been consistently linked to higher rates of cardiovascular events or cardiovascular-disease-related mortality in the general population,[18–21] and in individuals with underlying coronary artery disease.[22] In most of the studies outside the dialysis population, serum phosphate levels were predominantly normal, yet higher levels were associated with adverse outcomes independent of kidney function and traditional cardiovascular risk factors,[18–22] and the findings were detectable among subpopulations with unequivocally normal GFR (for example, ≥90 ml/min/1.73 m^2).[18,19] These findings suggest existence of a biological dose–response relationship between cardiovascular disease and serum phosphate levels across the normal and high ranges—analogous to other classic cardiovascular risk factors, such as blood pressure.[18,19,22,29]

The relationships between serum phosphate levels and distinct subtypes of cardiovascular events have not been extensively studied, but higher serum phosphate levels are independently associated with stroke,[20] fatal and nonfatal myocardial infarction[22] and congestive heart failure.[18,22] The finding that higher serum phosphate levels are not linked to one specific subtype of cardiovascular event might suggest an underlying mechanism that involves multiple vascular beds. Alternatively, this lack of specificity raises the possibility that residual confounding factors resulting from incompletely captured differences in patients' health status or kidney function, might explain the findings.

Another gap in the literature is the shortage of studies focused on the relationship between serum phosphate levels and incident cardiovascular disease. Prospective cohort studies that exclusively assess incident cardiovascular events, as opposed to those that are inclusive of participants with prevalent cardiovascular disease, would provide stronger evidence that serum phosphate levels clearly precede the initial manifestations of disease. The Framingham Offspring Study investigators reported that higher serum phosphate levels were associated prospectively with increased risk of the composite end point of incident coronary artery disease, stroke, peripheral vascular disease and congestive heart failure,[19] but this relationship was not confirmed in a case–control study in the prospective Health Professionals Follow-Up Study.[31] Finally, FGF-23 levels were not measured in all of the studies that investigated serum phosphate levels and cardiovascular disease, which renders it difficult to determine the magnitude of risk that is directly attributable to serum phosphate levels versus FGF-23.

FGF-23 levels and cardiovascular disease

Higher serum levels of FGF-23 are consistently associated with a graded increase in all-cause mortality in prospective studies of various populations of patients, either with or without CKD.[15,16,32–34] Increased cardiovascular mortality has been proposed to account for this association, based on the results of studies in elderly adults and kidney transplant recipients, in whom higher serum FGF-23 levels are associated with an increased risk of death from cardiovascular causes.[35,36] Similar to the literature regarding serum phosphate, few studies have examined whether or not higher FGF-23 levels are exclusively associated with cardiovascular causes of death, rather than with other leading causes, such as cancer and infection.

The results of several studies revealed associations between higher FGF-23 and an increased risk of cardiovascular events, which was independent of kidney

function[13–16,37–39] and serum levels of other mineral metabolites.[13,15,16,39] In the Heart and Soul Study, higher FGF-23 was associated with increased risk of a composite of myocardial infarction, stroke, transient ischaemic attack and hospitalization for congestive heart failure in participants with a history of stable coronary artery disease.[15] In that study, higher FGF-23 was also associated with increased risk of stroke or transient ischaemic attack and congestive heart failure as distinct end points.[15] In the Homocysteine in End-stage and Advanced Kidney Disease study, which included patients with advanced CKD and end-stage renal disease (ESRD), higher FGF-23 was associated with a composite of atherosclerotic events, including myocardial infarction, amputation and stroke, but heart failure events were not evaluated.[16]

In other studies, atherosclerotic and congestive heart failure events were considered as distinct end points on the basis of differences in their pathophysiology.[13–15] In these studies, higher FGF-23 was more robustly associated with congestive heart failure than with atherosclerotic events.[13–15] In a study of 3,860 participants with CKD from the Chronic Renal Insufficiency Cohort (CRIC) study, our own research group reported that participants with FGF-23 levels in the highest quartile had an approximate threefold higher risk of adjudicated congestive heart failure hospitalization than did those with levels in the lowest quartile, after adjustment for kidney function and traditional cardiovascular risk factors.[13] This association was stronger than that described between FGF-23 levels and atherosclerotic events.[13] Although classification of congestive heart failure can be difficult in the setting of advanced CKD, which is also characterized by volume retention, the results of the CRIC study were similar in subsets of patients with early stage CKD (for example, estimated GFR ≥45 ml/min/1.73 m^2) and low-grade proteinuria, and even when we exclusively considered cardiovascular events that met the more-stringent adjudication criteria for classifying congestive heart failure as definite. By contrast, elevated FGF-23 levels were not significantly associated with definite atherosclerotic events in the CRIC study.

Other reports suggest that FGF-23 is expressed by cardiac myocytes under conditions of stress and in atherosclerotic plaques,[40,41] and that FGF-23 levels are elevated in patients with systolic heart failure.[42–44] These findings raise the possibility of reverse causality, meaning that heart failure could be a cause rather than a consequence of the elevation in FGF-23 levels. To address this possibility, our group carried out a time-lagged analysis of the CRIC study data, which demonstrated that higher baseline FGF-23 levels were associated with incident heart failure events that occurred ≥1 year after FGF-23 levels were measured. This finding indicates that the elevation in FGF-23 levels probably preceded even the early, subclinical phase of cardiac dysfunction that can occur before incident heart failure becomes clinically evident.[13] Higher FGF-23 levels were also strongly associated with incident heart failure in an elderly population in the Cardiovascular Health Study (≥65 years of age),[14] and, similar to the findings in the CRIC study, higher FGF-23 was not associated with incident atherosclerotic events in the Health Professionals Follow-Up Study.[13,31]

In contrast to early outcome studies that focused exclusively on serum phosphate levels because FGF-23 had not yet been discovered, subsequent outcome studies have simultaneously investigated both FGF-23 and serum phosphate levels as potential risk factors. In most studies of mortality and cardiovascular events, the magnitude of the effects of FGF-23 was larger than that of serum phosphate and was independent of serum phosphate.[13,15,16,32,34,35] However, in some studies, serum phosphate was also an independent, albeit modest, predictor of adverse outcomes even after adjusting for FGF-23 levels. For example, in the CRIC study, each 0.16 mmol/l increment in serum phosphate concentration was associated with a 9% increase in the risk of atherosclerotic events and a 10% increase in the risk of congestive heart failure events, independent of FGF-23.[13] Serum phosphate levels were also modestly associated with the cardiovascular composite end point in the Homocysteine in End-stage and Advanced Kidney Disease study, after adjustment for FGF-23.[16] These data suggest the possibility of independent effects of higher levels of phosphate and FGF-23 on cardiovascular disease, perhaps mediated by distinct pathophysiological mechanisms.

Interpreting the data

The strong associations between serum phosphate, FGF-23 and cardiovascular disease raise the provocative question of whether or not these represent causal relationships that justify embarking on interventional studies. However, confirming causality is a major challenge in epidemiological research. Principles initially proposed by Sir Bradford Hill[45] provide a useful framework for considering the likelihood that a collective body of epidemiological evidence supports causality, although these factors are not absolute criteria *per se* and neither are they all necessary nor sufficient to invoke causality.[46]

Acknowledging these caveats, several characteristics of the epidemiological data suggest that serum phosphate and FGF-23 levels might be causally implicated in cardiovascular events (Figure 2). In particular, researchers have described consistent findings across populations (consistency) and dose–response relationships (biological gradient); biological plausibility has been established in experimental animal data (plausibility); and these biochemical factors are aligned with existing knowledge of risk factors for CKD, such as poor diet and poverty (coherence). The magnitude of the effect linking higher FGF-23 levels with cardiovascular disease is greater than that of serum phosphate, which provides reassurance that residual confounding factors are less likely (strength). However, the substantial diurnal variation in serum phosphate levels can result in an underestimation of its effect.[47] Further data are needed to confirm that serum phosphate and FGF-23 levels are specifically associated with cardiovascular events rather than other adverse events, which would strengthen the claim that they are not merely markers of overall poor health status (specificity). The results of several prospective studies demonstrate that

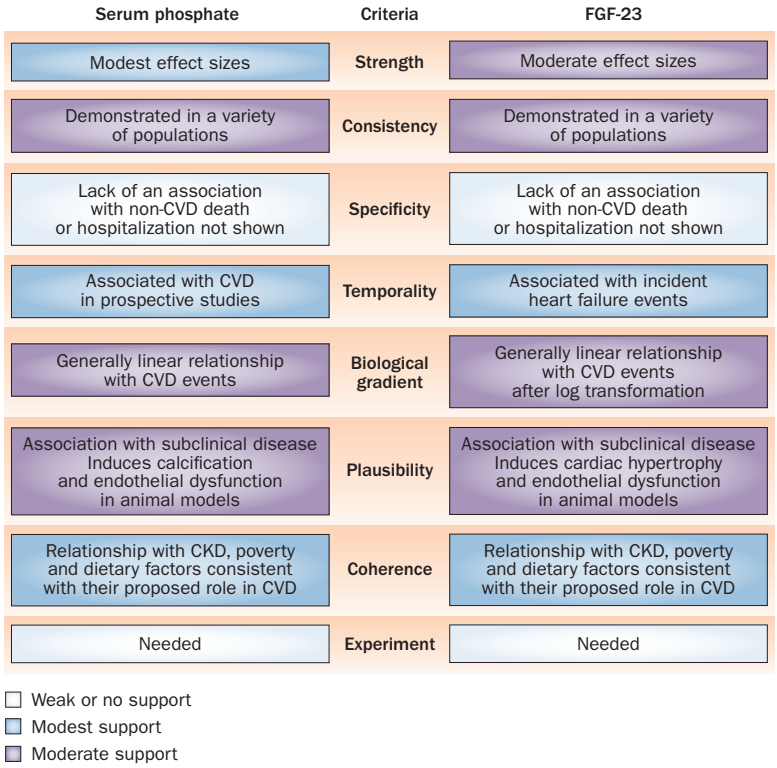

Figure 2 | Qualitative interpretation of the evidence supporting causal relationships between circulating levels of phosphate and FGF-23 with CVD. Eight major characteristics of causality as initially outlined by Sir Bradford Hill (centre column)[45] are considered, in turn, for serum phosphate levels (left column) and FGF-23 (right column). Abbreviations: CKD, chronic kidney disease; CVD, cardiovascular disease; FGF-23, fibroblast growth factor 23.

both FGF-23 and serum phosphate levels are associated with future cardiovascular events, but given the expected latency of these factors in the pathogenesis of cardiovascular disease and the possibility that they might instead be markers of already established disease,[41] more analyses of incident events are needed (temporality). In aggregate, however, compelling, albeit preliminary, evidence indicate that higher serum phosphate and FGF-23 levels might be modifiable risk factors for cardiovascular disease, particularly in CKD. Data from randomized controlled clinical trials—or the evaluation of 'natural experiments', such as changes in food policy or other factors that affect either FGF-23 levels or dietary intake of phosphate at the population level—would strengthen this body of evidence (experiment).

Other noncausal constructs that could also explain the epidemiological data must be noted. For example, the adverse effects of higher FGF-23 or serum phosphate levels could be epiphenomena attributable to other factors, such as Klotho deficiency or decreased kidney function. Klotho deficiency is a consequence of CKD, and might also promote cardiovascular disease.[5,48–50] However, the lack of validated assays for measuring circulating Klotho levels is a barrier to investigation of this hypothesis in humans,[51] and limits our understanding of the extent to which circulating Klotho levels correlate with tissue expression of the membrane-bound form.[49] Preliminary data on associations between soluble Klotho levels and clinical outcomes are limited and conflicting;[52,53] thus, indirect evidence has been used to invoke a possible role for Klotho deficiency in cardiovascular disease. For example, many studies have demonstrated stronger relationships between higher serum FGF-23 levels and adverse outcomes in patients with CKD than in those without CKD.[14,35,54–56] The stronger associations in CKD might relate to Klotho deficiency, which could either accentuate Klotho-independent toxic effects of FGF-23,[17] or eliminate Klotho-dependent protective effects.[50] In addition, the association between higher serum FGF-23 levels and cardiovascular events was especially strong in patients with low fractional excretion of phosphate, which could indicate tubular cell resistance to the actions of FGF-23 due to underlying Klotho deficiency.[57]

As circulating levels of FGF-23 and phosphate rise with the progression of CKD,[12] another alternative interpretation of the epidemiological data is that higher FGF-23 and serum phosphate levels indicate greater impairment in kidney function that is not entirely captured by the measurement or estimation of GFR. Indeed, FGF-23 levels increase extremely early in the course of progressive CKD and in mouse models of acute kidney injury, suggesting an exquisite sensitivity of FGF-23 levels to even subtle changes in kidney function.[58,59] Ultimately, definitive evidence of causal links between FGF-23, phosphate and cardiovascular disease in humans can only be derived from a randomized clinical trial demonstrating that lowering FGF-23 and serum phosphate levels reduces the risk of adverse cardiovascular outcomes. In the interim, however, animal data can provide additional insight.

Potential mechanisms

Vascular calcification

Animal studies

A major mechanism linking abnormal phosphate homeostasis to cardiovascular disease might be the promotion of vascular calcification. Incubation of human vascular smooth muscle cells (VSMCs) in media with increasing concentrations of phosphate results in transition from a smooth muscle to a mineralizing phenotype.[60,61] In explanted human blood vessels, high phosphate conditions stimulate apoptosis of VSMCs and the release of calcifying vesicles.[62] Similarly, vascular calcification can be induced in rodent models by high-phosphate diets[63] and other experimental manipulations that result in hyperphosphataemia, including genetic deletion of Klotho or FGF-23,[6,48] and blocking the actions of FGF-23 using inhibitory antibodies.[23] A central role for excess phosphate in vascular calcification is supported by experiments in which deletion of NaPi-2a normalizes serum phosphate levels, reverses calcification and restores normal lifespan in Klotho-deficient (*Klotho*$^{-/-}$) mice, that otherwise exhibit severe hyperphosphataemia.[64]

The role of FGF-23 in vascular calcification has not been studied as extensively as that of phosphate. Studies *in vitro* indicate that FGF-23 does not potentiate calcification in human VSMCs or mouse aortic rings under normal or high phosphate conditions, nor does it potentiate intracellular uptake of phosphate even in VSMCs

incubated with soluble Klotho.[60] Another group reported that although FGF-23 does not promote calcification of rat aortic rings under normal phosphate conditions, in high-phosphate conditions, FGF-23 may potentiate calcification of rat aortic rings or VSMCs that express membrane-bound Klotho.[65] Conversely, other investigators report that FGF-23 can actually mitigate calcification in the vasculature when Klotho is present, possibly by inhibiting cellular phosphate uptake.[50] A central role of phosphate rather than FGF-23 in vascular calcification is also supported by mouse models of FGF-23 overexpression,[66–68] which do not exhibit vascular calcification, and mouse models of Klotho and FGF-23 deletion, which exhibit both severe hyperphosphataemia and extensive calcification.[6,48]

Human studies

Data from epidemiological studies support the concept that high serum phosphate levels are a risk factor for vascular calcification. In a subgroup of 641 patients with CKD who took part in the Multiethnic Study of Atherosclerosis, higher serum phosphate concentrations were independently associated with an increased number of calcified cardiovascular sites, including the coronary arteries, thoracic aorta and mitral and aortic valves.[69] Other studies have confirmed the association between higher phosphate and valvular calcification or sclerosis,[70] and linked these anatomical changes with functional measures of calcification, such as increased peripheral arterial stiffness as indicated by an ankle brachial pressure index score >1.30.[71,72]

Although the results of several small observational studies showed links between higher FGF-23 levels and vascular calcification,[73–78] most did not fully adjust for serum phosphate, GFR or length of time on dialysis in patients with ESRD, and other groups have reported conflicting results.[79–81] In one of the largest vascular calcification studies to date, our research group reported that higher serum phosphate levels, but not higher FGF-23 levels, were associated with an increased prevalence and severity of coronary artery calcification, independent of kidney function and other traditional cardiovascular risk factors, in approximately 1,500 CRIC participants with CKD stages 2–4.[60] To our knowledge, our study was the first to demonstrate a statistically significant association between serum phosphate levels and vascular calcification that is independent of FGF-23 levels, which is important given that previous landmark studies of the effect of serum phosphate on vascular calcification did not measure FGF-23 levels. Our findings are consistent with the *in vitro* data and strongly favour elevated serum levels of phosphate, but not FGF-23, as a major risk factor for vascular calcification in CKD.

Endothelial dysfunction and atherosclerosis

Animal studies

Hyperphosphataemia might also have adverse effects on endothelial cells. Incubating cultured endothelial cells in high phosphate concentrations induces apoptosis,[82–84] stimulates production of reactive oxygen species,[82,85] impairs secretion of nitric oxide (NO) in response to acetylcholine[84] and inhibits angiogenic behaviour.[83] Analogous to the effects of high phosphate conditions on VSMCs,[86] many of these effects can be blocked by inhibiting cellular uptake of phosphate,[82,84] suggesting that high intracellular phosphate is a key mediator that directly induces endothelial cell dysfunction. High phosphate conditions also induce vasoconstriction and impair relaxation in intact aortic rings, suggesting that the results observed *in vitro* translate to intact vessels *ex vivo*.[56] In contrast to the proapoptotic effects of phosphate, FGF-23 inhibits apoptosis and stimulates proliferation of endothelial cells and VSMCs *in vitro*,[50,87] suggesting that FGF-23 might have counter-regulatory effects against the actions of phosphate.

Human studies

In observational studies, higher serum phosphate levels are associated with an increased extent of coronary[79,88] and carotid[89] atherosclerosis. Although most studies in this area were cross-sectional, one prospective cohort study of 3,015 healthy young adults showed that baseline serum phosphate levels >1.26 mmol/l (normal range 0.74–1.52 mmol/l) were associated with an increased risk of atherosclerosis, ascertained by CT 15 years later.[88] Higher FGF-23 levels were associated with greater severity of atherosclerosis as detected using angiography in some,[55,90] but not all studies.[79] To date, these data are too sparse to determine whether or not an elevation in FGF-23 levels clearly precedes the development of atherosclerosis, or is simply a marker of established disease.[41]

In a small observational study of patients with CKD stages 3–4, but without cardiovascular disease or diabetes mellitus, higher levels of FGF-23 were independently associated with decreased flow-mediated dilatation, which is a clinical measure of endothelial function.[91] Lower FGF-23 levels have also been associated with increased endothelium-dependent and endothelium-independent vasodilatation in individuals aged ≥70 years, most of whom had normal kidney function.[92] Similarly, the dramatic fall in FGF-23 levels and amelioration of other alterations in mineral metabolism that occur after renal transplantation correlate with improved endothelial function.[93]

Preliminary evidence suggests that manipulation of dietary phosphate intake to decrease circulating levels of phosphate or FGF-23 may affect cardiovascular risk factors. In a study of 11 healthy adult men, a high-phosphate meal substantially lowered flow-mediated dilatation within 2 h, compared with a low-phosphate meal. In patients with CKD, lowering of serum phosphate and FGF-23 levels, accomplished by 8 weeks of treatment with the phosphate-binding agent, sevelamer, improved flow-mediated dilatation.[94] By contrast, treatment with calcium acetate, another phosphate-binding agent, lowered serum phosphate levels only modestly, did not lower FGF-23 levels and did not improve flow-mediated dilatation. The benefits associated with sevelamer treatment might be due to its more-effective lowering of FGF-23 or phosphate levels, other pleiotropic effects or a combination thereof.[94] More work is needed

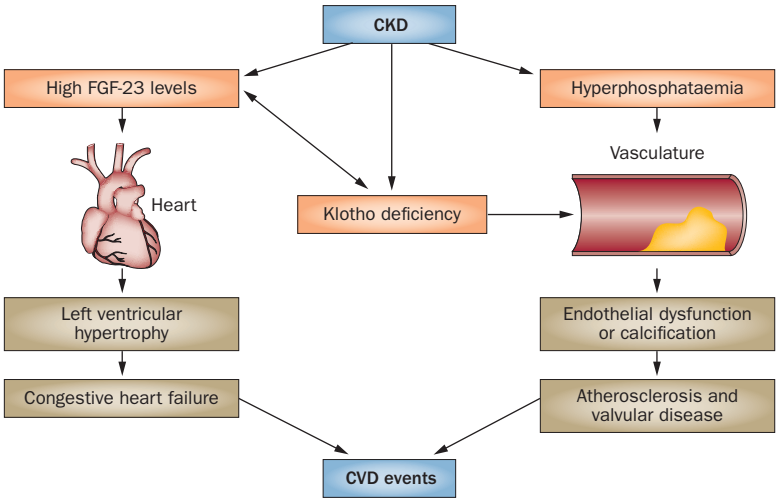

Figure 3 | Proposed mechanisms through which higher circulating levels of phosphate and FGF-23 might contribute separately and additively to clinical CVD events in patients with CKD. Experimental evidence from cell culture and animal models suggests that FGF-23 directly targets the heart to promote left ventricular hypertrophy and phosphate directly induces calcification and dysfunction of the vasculature.[17,61] Together, these insults increase the risk of clinical CVD events. Abbreviations: CKD, chronic kidney disease; CVD, cardiovascular disease; FGF-23, fibroblast growth factor 23.

to pinpoint the mechanisms underlying how phosphate homeostasis alters endothelial function, and the relative contributions of its many components, including dietary phosphate intake, serum phosphate levels, FGF-23, $1,25(OH)_2D_3$, parathyroid hormone, Klotho and perhaps other currently undiscovered factors.

Ventricular hypertrophy

Animal studies

Activation of canonical FGF receptors by FGF-2, the prototypical FGF, is a fundamental molecular mechanism of left ventricular hypertrophy. This finding stimulated the hypothesis that other FGFs, such as FGF-23, have roles.[17] After reporting results in cross-sectional human studies,[95] our group demonstrated that ascending concentrations of FGF-23 stimulated hypertrophic growth of cultured neonatal rat ventricular myocytes, and activated the transcription of genes involved in pathological cardiac hypertrophy.[17] Consistent with these findings, another group demonstrated that ascending concentrations of FGF-23 increased intracellular calcium levels and the contractility of adult mouse ventricular muscle strips, and activated the expression of various genes linked to cardiac hypertrophy.[96] In both studies, the hypertrophic effects of FGF-23 were mediated by Klotho-independent signalling through FGF receptors.

Intramyocardial and intravenous injection of FGF-23 induced left ventricular hypertrophy in wild-type mice, and *Klotho*$^{-/-}$ mice with markedly elevated FGF-23 levels developed severe left ventricular hypertrophy. Furthermore, global blockade of FGF receptors using a small-molecule inhibitor prevented left ventricular hypertrophy in uraemic rats, without lowering blood pressure.[17] These data suggest direct hypertrophic effects of FGF-23; however, it is important to acknowledge that the inhibitor used does not specifically block FGF-23 signalling, and so the hypertrophic effects of other FGFs could have been blocked.

Experiments designed to test the effects of parathyroid hormone on cardiac function and serum phosphate levels provide additional, albeit indirect, support for a role of FGF-23 in cardiac hypertrophy.[97] Parathyroidectomized, uraemic rats were treated with either a physiological or supraphysiological replacement dose of parathyroid hormone, in conjunction with a high-phosphate or low-phosphate diet. Interpretation of the results is clouded by the array of metabolic derangements in these animals; however, myocyte diameters were largest in the animals that exhibited the highest FGF-23 levels.[97] Feeding animals high-phosphate diets that are known to raise both phosphate and FGF-23 levels, promotes increased left ventricular mass and myocardial interstitial fibrosis.[98,99] Although treatment with phosphate binders can mitigate these changes,[100] in the absence of measurement of FGF-23 levels, these studies were unable to address the relative contributions of phosphate and FGF-23 or those of other factors.

In contrast to these findings, uraemic rats treated with FGF-23-neutralizing antibodies did not show a decrease in heart mass or decreased expression of cardiac hypertrophy markers, although these results should be interpreted cautiously because the overall rate of left ventricular hypertrophy in the control group was far less than expected.[23] Additional studies are needed to investigate the effects of FGF-23 on cardiac myocytes, and to determine whether or not FGF-23 also affects myocardial fibroblasts, given the prominence of fibrosis in uraemic cardiomyopathy.[99] If toxic effects of FGF-23 are confirmed, further experimental studies will be needed to define the exact FGF receptors and signalling pathways involved so that therapies can be designed to target the adverse effects of FGF-23 without interfering with its desired physiological effects to maintain normal serum phosphate levels.

Human studies

In clinical studies, higher serum levels of both phosphate and FGF-23 are associated with increased left ventricular mass and an increased prevalence of left ventricular hypertrophy independent of kidney function in a wide range of settings.[17,18,54,95,101–106] In one prospective study, higher FGF-23 levels at baseline were associated with the development of incident left ventricular hypertrophy during follow-up monitoring in patients with CKD.[17] Although associations between higher levels of FGF-23 and increased left ventricular mass were independent of serum phosphate levels, most human studies of serum phosphate as a risk factor for left ventricular hypertrophy have not incorporated measurement of FGF-23. Occult elevation of FGF-23 levels might, therefore, account for the association of higher serum phosphate with left ventricular hypertrophy and increased ventricular mass. However, elevated serum phosphate levels might also contribute to left ventricular hypertrophy indirectly by increasing left ventricular pressure overload owing to

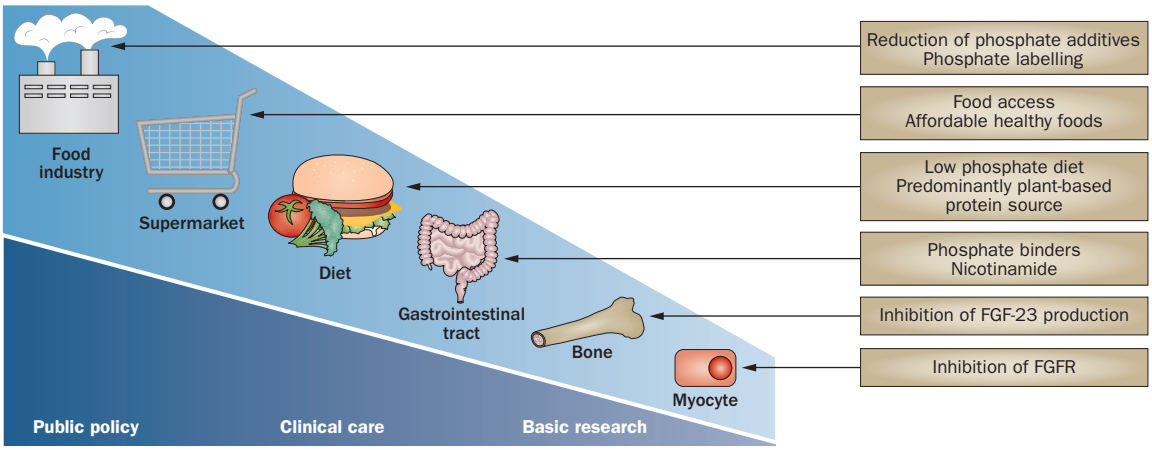

Figure 4 | Potential interventions to target phosphate homeostasis at the population, patient and molecular levels. Approaches to reduce phosphate and FGF-23 levels could engage health policy (regulation of the food industry, subsidies for healthy food), public health (public education, informative food labelling) and clinical medicine (patient education, pharmaceutical development). Abbreviations: FGF-23, fibroblast growth factor 23; FGFR, fibroblast growth factor receptor.

reduced vascular compliance and valvular heart disease as a result of vascular calcification.[69,70,100]

High dietary intake of phosphate raises serum levels of both phosphate and FGF-23, but currently only limited data from human studies relate phosphate intake to cardiovascular disease. Data from an observational study showed that a high phosphate intake was associated with increased left ventricular mass in a population of healthy adults.[107] These intriguing results should, however, be interpreted with caution, given the strong correlations between phosphate intake and total energy intake, as well as intakes of other nutrients, such as sodium, that could affect cardiac mass.

Therapeutic strategies

The existing body of evidence suggests that higher serum phosphate levels are strongly associated with atherosclerosis and endothelial dysfunction, and higher FGF-23 levels are strongly associated with left ventricular hypertrophy and congestive heart failure (Figure 3). These data emphasize the need to focus on treatment strategies that can simultaneously control both factors, instead of targeting one at the expense of the other—as demonstrated in the case of treatment with anti-FGF-23 antibodies, which can precipitate severe hyperphosphataemia.[23]

Dietary approaches

Restricting dietary phosphate intake or preventing its absorption may be a good strategy to target elevated levels of both serum phosphate and FGF-23. In short-term studies, restricting dietary phosphate intake decreased serum levels of both phosphate[7,8] and FGF-23,[7–9] whereas dietary phosphate loading raised FGF-23 levels[7–10] and, occasionally, also raised serum phosphate levels.[10] The majority of studies that investigated the effects of augmented phosphate intake have used supplementation with fairly high doses of inorganic phosphate, which is almost completely absorbed in the gastrointestinal tract.[7–9] Supplementation with modest quantities of phosphate, and differences in phosphate intake resulting from variation in naturally occurring dietary phosphate sources that are not as readily absorbed, have been studied less extensively.

A major source of naturally occurring organic phosphate is dietary protein. In a crossover study of 19 healthy participants, the percentage of calories from protein was altered in successive feeding periods, without resulting in a change in FGF-23 levels.[108] In other studies, severe restriction of protein intake decreased FGF-23 levels.[109] However, the source of dietary phosphate also determines the extent of its absorption. Phosphate absorption from plant proteins may be lower than from animal proteins, because phosphate in plant proteins is bound within a complex ring structure known as phytate, which is poorly digested in the human gastrointestinal tract. Indeed, a crossover study of nine patients with CKD showed that both phosphate and FGF-23 levels decreased significantly while the patients were consuming diets containing predominantly plant protein, compared with periods when they were consuming animal-protein-based diets despite an identical phosphate content.[110] The wider applicability of this finding is supported by similar results in a large observational cohort study of patients with CKD, in whom a higher proportion of protein intake from plant-based sources was independently associated with lower serum FGF-23 levels.[111] In another large observational study of male health-care professionals, serum FGF-23 levels were modestly increased among those consuming larger amounts of phosphate than others, as assessed by a food intake questionnaire.[112] Although the magnitudes of associations were small in these observational studies, the true associations might have been underestimated because of imprecision in dietary reporting and failure of nutritional databases to comprehensively account for phosphate intake from food additives and for differences in phosphate absorption attributable to different protein sources.[113,114]

Therapeutic approaches to reduce dietary phosphate intake, and thereby lower serum phosphate and FGF-23 levels simultaneously, could target the absolute amount of dietary phosphate intake, the amount of phosphate

additives in the diet, or the proportions of animal versus plant sources of dietary protein (Figure 4). Reducing dietary phosphate intake could be a useful clinical strategy in individual patients, but could also be used to target populations more broadly through public health approaches. For example, regulation could limit the amount of phosphate added to processed foods or require reporting of the phosphate content on food labels. Other public-health initiatives could include the promotion of plant-rich diets, and enhancing access and affordability of healthy foods, including nonprocessed foods, fruits and vegetables, in communities at high risk of CKD. These types of population-based preventative approaches have the advantage of placing minimal risks and burdens on individual patients, but their efficacy and feasibility need to be proven.

Pharmacological approaches

Alternative approaches to reducing serum phosphate levels could be blocking dietary phosphate absorption pharmacologically, or blocking the actions of FGF-23 directly (Figure 4). To date, the results of some,[115–119] but not all,[120–122] short-term studies of patients with CKD support the efficacy of treatment with phosphate binders, with or without dietary phosphate restriction, to reduce both FGF-23 levels and 24 h urinary phosphate excretion, a biomarker of absorbed phosphate. However, the major challenges for these pharmacological strategies will be to determine whether their effects are sufficiently sustainable and acceptable to patients for long-term use,[116,119] and whether they can mitigate cardiovascular disease. Nonetheless, these approaches represent potential strategies to treat individual patients with moderate to severe CKD—who are at risk of cardiovascular complications due to high serum phosphate and FGF-23 levels.

Several other pharmacological approaches are under clinical investigation. Nicotinamide inhibits intestinal NaPi-2b, thereby limiting phosphate absorption in the gastrointestinal tract and reducing serum phosphate levels.[123,124] The effect of nicotinamide on FGF-23 levels is unknown, and no studies of nicotinamide therapy in combination with a phosphate binder have been conducted. This approach could boost their individual effects, as might be hypothesized from the synergistic effects of NaPi-2b deletion and sevelamer treatment in a mouse model of CKD.[125]

Biological agents that directly target FGF-23 are also in development.[23,126] Preclinical studies of inhibitory anti-FGF-23 antibodies in uraemic rats caused severe hyperphosphataemia, hypercalcaemia related to hypervitaminosis D, severe calcification and premature death.[23] These data caution against direct inhibition of FGF-23 without also targeting the distinct adverse effects of high serum phosphate. Anti-FGF-23 antibodies and FGF receptor blockers could still be useful, either in conjunction with serum phosphate-lowering therapy or in patients with ESRD, in whom renal phosphate excretion does not usually contribute substantially to serum phosphate levels. Furthermore, if distinct FGF receptors mediate the desired renal actions of FGF-23, namely, regulating serum phosphate, versus its off-target effects, it might be possible to design molecules that selectively block the unwanted effects of FGF-23.

Conclusions

A strong body of evidence links elevated serum phosphate levels to vascular injury, including vascular and valvular calcification and atherosclerosis. By contrast, initial evidence in the much younger field of FGF-23 research points to direct effects of FGF-23 on the heart that promote myocardial hypertrophy and clinical heart failure. The adverse cardiovascular effects of high levels of phosphate and FGF-23 are probably distinct, which is not only intriguing scientifically, but could also have profound implications for therapeutically targeting the phosphate axis. Ultimately, targeting serum phosphate and FGF-23 levels may be possible through public health policies, patient education and pharmaceutical agents. Although the remaining challenges are substantial, the compelling evidence that serum phosphate and FGF-23 levels are modifiable risk factors for cardiovascular disease, in patients with or without CKD, should continue to drive this field forward.

Review criteria

Articles were identified by searching the PubMed database using a combination of keywords and MeSH terms, including “cardiovascular disease”, “cardiovascular diseases”, “abnormalities, cardiovascular”, “abnormality, cardiovascular”, “congestive heart failure”, “heart failure, congestive”, “cardiovascular events”, ”fibroblast growth factor 23”, “disorder, phosphorus metabolism”, “metabolism disorders, phosphorus”, “phosphorus” and “phosphate”. Additional relevant articles were identified from the reference lists of key articles. All reviewed papers were available in English.

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J.J.S. and M.W. researched the data for the article, provided substantial contributions to discussions of its content, wrote the article and undertook review and/or editing of the manuscript before submission.