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# Cardiovascular risk factors in chronic kidney disease: does phosphate qualify?

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**Risk factors for disease states are rigorously defined. This analysis considers the definition of a risk factor as applied to the question of whether the serum phosphorus level is a risk factor for cardiovascular disease. Observational studies strongly suggest that phosphorus is associated with cardiovascular risk, and definitive prospective animal studies are supportive. A plausible mechanism of action has been discovered demonstrating that phosphorus stimulates osteoblastic transition of cells in the neointima of atherosclerotic plaques, which, if prevented, blocks vascular calcification. However, prospective studies demonstrating that modulation of the putative risk factor affects clinical outcomes are lacking, and phosphorus, as yet, does not qualify as a cardiovascular risk factor. This is a clarion call for additional research.**

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Among the 25 million American patients with stage 2–5 chronic kidney disease (CKD), cardiovascular disease causes a disproportionately high mortality risk. Patients with CKD are more likely to die (often of cardiovascular causes) than to progress to dialysis.<sup>1</sup> The risk of death is especially high in late-stage kidney disease; a 30-year-old patient with end-stage renal disease faces an equivalent risk of death to a 90-year-old without CKD.<sup>2</sup> The cardiovascular risk factors generally considered do not explain the heightened cardiovascular risk in CKD.<sup>3</sup> Vascular calcification<sup>4,5</sup> and hyperphosphatemia<sup>6</sup> drive cardiovascular risk in CKD, and they are related.

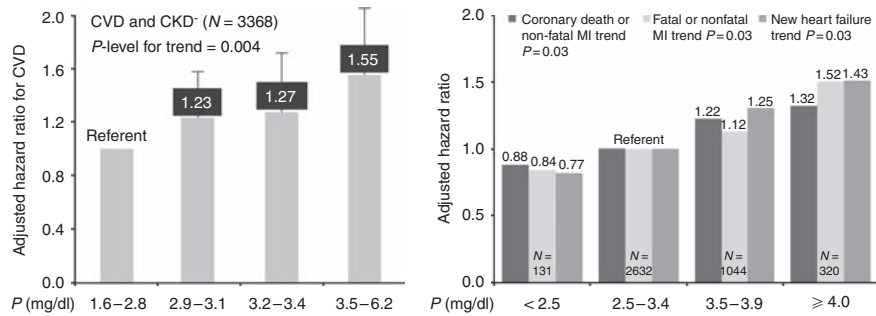
Risk factors are rigorously defined by four types of evidence: (1) observational studies; (2) definitive prospective translational or clinical studies; (3) mechanism of action studies; and (4) outcome studies showing risk reduction when the putative factor is corrected. Serum phosphorus, at the current state of our knowledge, is associated with the first three of these four lines of evidence. One early observational outcome study has also shown risk reduction benefits with correction of phosphate levels in patients with CKD. This review will summarize current evidence for phosphate as a cardiovascular risk factor in the general population and among patients with CKD.

## OBSERVATIONAL STUDIES OF PHOSPHATE AND CARDIOVASCULAR RISK

Serum phosphorus and mortality risk have been analyzed in multiple observational studies. In patients with end-stage renal disease on dialysis (40,538 hemodialysis patients), those with serum phosphorus > 6.0 to 7.0 mg/dl had a relative risk of death 1.25 times that of those with serum phosphorus ≤ 5 mg/dl. The lowest serum phosphate category (< 3 mg/dl) showed slightly increased risk (1.2 versus 4.5 mg/dl).<sup>7</sup> In a retrospective cohort study of 6730 US veterans with CKD not receiving dialysis (those transplanted or without phosphorus measurements were excluded), the adjusted hazard ratio for death rose to 1.90 in patients with serum phosphorus > 5.0 mg/dl (compared with patients with serum phosphorus < 2.5 mg/dl).<sup>8</sup>

In the general population, serum phosphorus has been associated with the risk of cardiovascular disease (Figure 1). The Framingham Offspring Study,<sup>9</sup> deleted of participants with histories of CKD, revealed that increasing serum phosphorus was associated with a continuous increasing

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**Figure 1 | Observational studies from the general population, which suggest that phosphorus is a cardiovascular risk factor.** The left panel assembles data from Dhingra *et al.*<sup>9</sup> Cardiovascular disease (CVD) was scored as fatal/non-fatal MI, angina, cerebrovascular events, peripheral vascular disease, or heart failure. Hazard ratios are adjusted for age, sex, body mass index, diabetes, blood pressure, treatment of hypertension, smoking, alcohol consumption, total cholesterol/high-density cholesterol ratio, hemoglobin, serum albumin, estimated glomerular filtration rate, proteinuria, and high-sensitivity C-reactive protein. The right panel is adapted with permission from Tonelli *et al.*<sup>10</sup> Hazard ratio values are adjusted for baseline age, sex, race, smoking status, diabetes, waist-to-hip circumference, fasting glucose, glomerular filtration rate, hemoglobin, serum albumin, aspirin use, and left ventricular ejection fraction. *N* = 4127. CKD, chronic kidney disease; MI, myocardial infarction.

risk of cardiovascular disease (heart attack, stroke, angina, peripheral vascular disease, or heart failure). In a *post hoc* analysis of the Cholesterol and Recurrent Events study<sup>10</sup> (Figure 1), serum phosphate showed a graded independent relationship to risk of death and new cardiovascular events in patients who had suffered heart attacks previously and had normal kidney function. In both the Cholesterol and Recurrent Events study and the US Third Health and Nutrition Examination Survey populations, elevation of serum alkaline phosphatase and serum phosphate together conferred greater risk than either parameter alone.<sup>11</sup>

How might serum phosphorus affect atherosclerosis? In CKD, we associate hyperphosphatemia causally with vascular calcification, which stiffens arteries and leads to cardiovascular events. In young and middle-aged adults without CKD, serum phosphorus levels were associated with vascular stiffness and coronary artery calcium levels in the Multi-Ethnic Study of Atherosclerosis. In middle-aged participants with mild-to-moderate CKD,<sup>12</sup> the ankle-brachial index increased in parallel with serum phosphorus levels within the normal range. In the Coronary Artery Risk Development in Young Adults study, which was a 15-year prospective observational study,<sup>13</sup> 10% of the participants with 15-year data experienced significant coronary calcification during follow-up, related to the serum phosphorus level at the beginning of the follow-up period.

**DEFINITIVE PROSPECTIVE TRANSLATIONAL OR CLINICAL STUDIES: HOW DOES PHOSPHORUS FUNCTION AS A RISK FACTOR FOR CARDIOVASCULAR MORTALITY?**

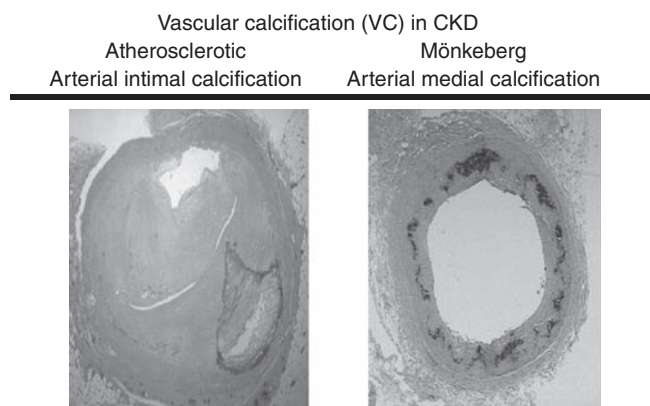
The complexity of human disease causing cardiovascular risk requires a translational model to dissect the role of phosphorus in vascular calcification and coronary artery disease development in CKD. There are two types of translational studies that address the issue of phosphorus as a cardiovascular risk. In the first, genetic engineering was used to produce deficiency of the fibroblast growth factor-23 skeletal hormone, which is responsible for regulating renal phosphate

excretion. Mice with fibroblast growth factor-23 deficiency develop hyperphosphatemia, excess calcitriol and vascular calcification, and have shortened life spans. Feeding these mice low-phosphate diets corrects hyperphosphatemia, eliminates vascular calcification, and lengthens their life span.

The second type of study that demonstrates the function of inorganic phosphate as a cardiovascular risk factor comprises studies in animal models of atherosclerosis in which hyperphosphatemia is induced by CKD. We have used the low-density lipoprotein receptor-deficient mouse (*ldlr*<sup>-/-</sup>) fed a high-fat diet with ablative CKD. *LDL*<sup>-/-</sup> mice fed high-fat diets develop hypercholesterolemia, metabolic syndrome, and vascular calcification. CKD added to this model induces hyperphosphatemia (beginning at stage 3 CKD) and increases vascular, especially aortic, calcification.<sup>14</sup> Administration of phosphate binders and bone morphogenetic protein (BMP)-7 in this model prevented hyperphosphatemia by reducing phosphate absorption and sending serum phosphate into the skeleton.<sup>15,16</sup> In mice with established vascular calcification (atherogenic diet begun at 10 weeks, CKD induced by 14 weeks, and phosphate binder or BMP-7 administered from 22 to 28 weeks), control of phosphate for weeks 22–28 with sevelamer carbonate or BMP-7 diminished vascular calcification and prevented cardiac hypertrophy.<sup>15,16</sup> The study with BMP-7 originally addressed concerns that BMP-7 might stimulate vascular calcification, but in fact showed BMP-7 to be therapeutic, decreasing aortic calcification below control levels and stimulating bone formation. The stimulation of bone formation was the mechanism of BMP-7-induced correction of hyperphosphatemia, and a component of the action against vascular calcification.

**MECHANISM OF ACTION STUDIES**

A scientific consensus more advanced than mere plausibility has developed with regard to the mechanism by which high serum phosphate leads to vascular calcification and cardiovascular risk. Pathologically, there are two types of

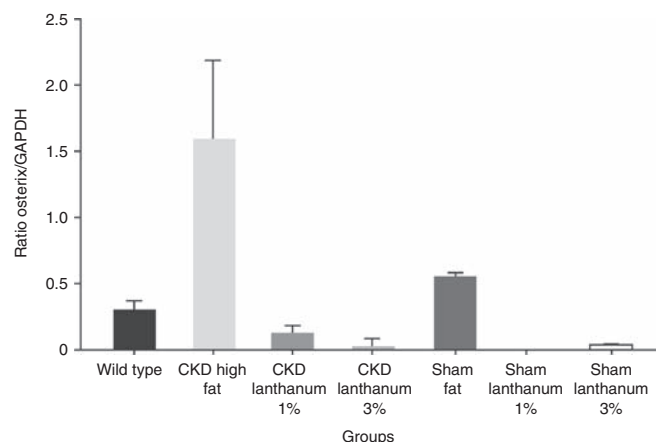


© In CKD both forms of VC are observed in the large arteries, calciphylaxis is mainly Mönkeberg, coronary artery calcification and cardiac valve calcification are atherosclerotic

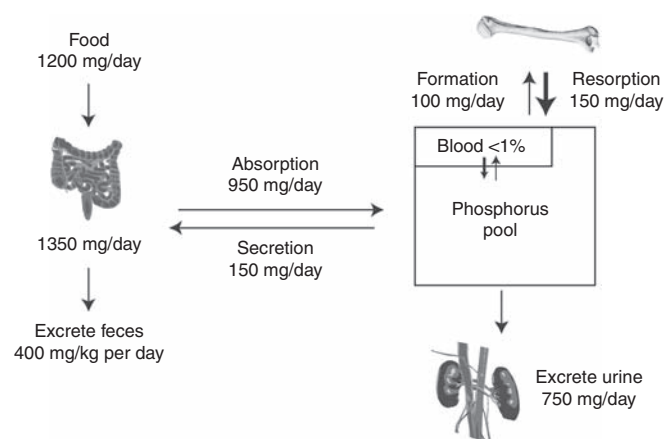
**Figure 2 | The two major types of large artery calcification stimulated by chronic kidney disease (CKD) are atherosclerotic neointimal calcification and medial calcification.** Reproduced with permission from Dr Gerard London.

large artery calcification stimulated by CKD, calcification of the smooth muscle tunica media and atherosclerotic calcification of smooth muscle cells in plaque neointima (Figure 2). Atherosclerotic calcification is more important in cardiovascular mortality risk, and tunica media calcification is more important in vascular stiffness.<sup>17</sup> In atherosclerotic calcification, neointimal cells express an osteoblastic phenotype, either by migration and differentiation of pericytes or by dedifferentiation, migration, and redifferentiation of smooth muscle cells. Initial renal injury induces dedifferentiation of vascular smooth muscle cells, which makes them migratory and vulnerable to osteoblastic induction by BMP-2. Additional studies have shown phosphorus to be a molecule capable of stimulating signal transduction.<sup>18,19</sup> We have shown inorganic phosphate to stimulate expression of osterix both *in vivo* in our *ldlr*<sup>-/-</sup> high-fat fed CKD mice and *in vitro* in human aortic smooth muscle cells derived from atherosclerotic donors with early CKD<sup>20</sup> (Figure 3). Osterix is an osteoblast transcription factor required for cellular stimulation of matrix mineralization.<sup>21</sup> Primary mouse or human smooth muscle cells *in vitro* transitioned from normal to high-phosphate culture medium (1 to 2 mmol/l) will mineralize their extracellular matrix in 2–3 weeks.<sup>20</sup> Blocking of osterix expression in the presence of high phosphorus prevents mineralization.

Reducing serum phosphorus (for example, with phosphate binders) reverses osteoblastic differentiation of vascular cells and reverses vascular calcification.<sup>20</sup> Osteoblastic transition and calcification of smooth muscle cells (akin to bone formation) in the atherosclerotic plaque is an active and reversible process. Osteoclasts and large multinucleated macrophages are present in plaques and can actually reabsorb calcification when serum phosphorus is decreased.



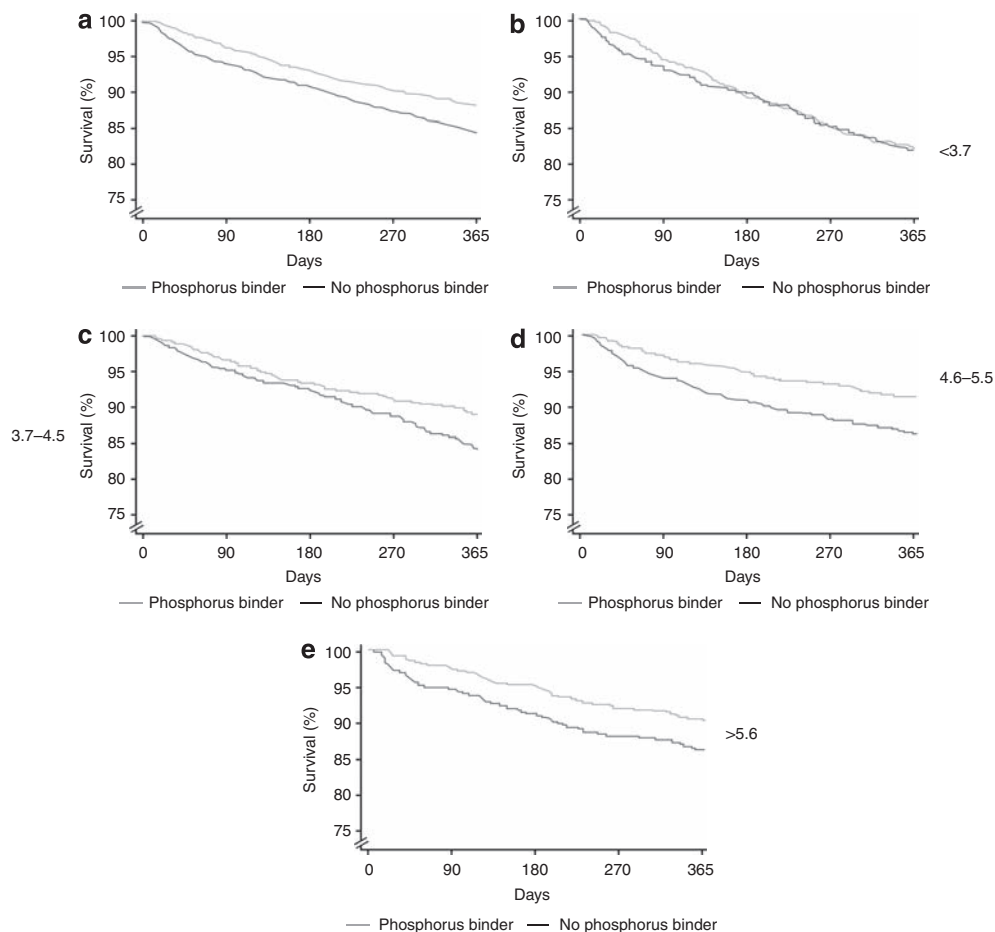
**Figure 3 | Hyperphosphatemia stimulates expression of osterix in the aortas of low-density lipoprotein receptor-deficient mouse (*ldlr*<sup>-/-</sup>) with chronic kidney disease (CKD) fed high-fat diets.** LaCO<sub>3</sub>, a non-calcium-containing phosphate binder, reverses osterix expression. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.



**Figure 4 | The phosphate balance diagram in chronic kidney disease (CKD) showing positive balance and a skeletal contribution to hyperphosphatemia, which blocks skeletal reservoir function.** Reproduced with permission from Mathew *et al.*<sup>20</sup>

#### EFFECTS OF CKD ON PHOSPHATE HOMEOSTASIS

The phosphate balance diagram is shown in Figure 4. In health, bone formation and resorption balance each other, and there is capacity in the skeletal mineralization fronts to absorb a transient positive phosphate balance, which would be converted into bone. This permits the skeleton to function physiologically as a phosphate and calcium reservoir. In CKD-related mineral and bone disorder, renal osteodystrophy causes excess bone resorption over bone formation in both high- and low-turnover forms of osteodystrophy. This blocks the normal reservoir function of the skeleton. In CKD, increasing fractional excretion of phosphorus (via adaptation of intact nephrons) maintains phosphorus balance in the early stages, but eventually reduction in the tubular reabsorption of phosphate cannot keep pace and phosphate balance



**Figure 5 | Effects of serum phosphate and phosphate binder use on survival in the Accelerated Mortality in Renal Replacement study.**<sup>23</sup> Survival of treated and untreated patients in the overall propensity score-matched cohort (a) and according to quartiles of baseline serum phosphate (b–e). Reproduced by permission from Isakova *et al.*<sup>23</sup>

becomes positive in stage 3 CKD before actual hyperphosphatemia occurs. The increased bone resorption contributes to the exchangeable phosphate pool, and to hyperphosphatemia. When extra phosphorus is present from a positive balance, in this situation it exits from the exchangeable pool into heterotopic deposition sites. Therefore, the skeleton contributes to heterotopic calcification especially in the vasculature in CKD.

The pathophysiology of phosphorus balance in CKD is dependent on a recently discovered skeletal hormone, fibroblast growth factor-23, and its relevant co-receptor, Klotho, which are required for renal phosphate excretion. Their deficiency causes hyperphosphatemia and heterotopic mineralization,<sup>22</sup> which can be rescued by a low-phosphate diet. In this translational model, vascular calcification appears to be the mechanism by which high serum phosphorus increases cardiovascular and mortality risk. Low-phosphate diets improved survival along with preventing vascular calcification.

#### OUTCOME STUDIES OF PHOSPHORUS CORRECTION

Human studies demonstrating that reducing serum phosphorus concentrations decreases cardiovascular risk have not

been conducted. However, some studies have been conducted that are supportive of the conclusion that phosphorus is a cardiovascular risk factor. The Accelerated Mortality on Renal Replacement study<sup>23</sup> (Figure 5) was a prospectively observed cohort study of 10,044 incident hemodialysis patients, comparing those who did with those who did not receive any phosphate-binder treatment during the first 90 days of dialysis. Phosphate binder use was associated with significantly reduced mortality risk on multivariate analysis (on an intent-to-treat or as-treated basis), as well as in a propensity score-matched comparison. Benefits were independent of baseline serum phosphate levels, and thus may reflect lowered levels of the phosphaturic factor fibroblast growth factor-23, which is induced up to 100-fold in untreated hyperphosphatemic dialysis patients.

The Renigel in New Dialysis study,<sup>24</sup> an 18-month randomized, controlled trial of sevelamer HCl versus calcium-containing phosphate binder in 109 new hemodialysis patients, showed that among patients with baseline coronary artery calcification, calcium binder use was associated with more rapid and severe progression of calcification than sevelamer HCl use. A pre-specified secondary end-point analysis of the Renigel in New Dialysis study<sup>25</sup> revealed that,



in 127 new hemodialysis patients randomized to sevelamer versus calcium-containing phosphate binders and followed up for 44 months, baseline coronary artery calcification score was a predictor of all-cause mortality. Sevelamer was associated with a significant survival benefit versus calcium-containing binders in this study.

## CONCLUSIONS

From the discussion above, the following conclusions are drawn. First, observational studies suggest that serum phosphate is a cardiovascular risk factor in patients with CKD and in the general population. Second, translational studies demonstrate that hyperphosphatemia stimulates atherosclerotic vascular calcification by inducing osteoblastic gene expression in the aorta, and that correction of hyperphosphatemia decreases vascular calcification. Third, studies *in vitro* demonstrate that medium inorganic phosphate is an active signaling molecule, the receptor may be Pit1, and phosphate directly stimulates osteoblastic transcription factors. Fourth, human studies are consistent in showing the effects of phosphate and its correction on vascular calcification and cardiovascular mortality. However, these studies are not formal outcome studies and are insufficient evidence without the translational data. Fifth, the strong epidemiologic and mechanistic evidence suggesting that serum phosphate is a cardiovascular risk factor needs to be confirmed by human prospective and outcome studies to satisfy the rigorous definition of phosphorus as a cardiovascular risk factor. Finally, in the meantime, before formal outcome studies are completed, interventions to maintain serum phosphate normal in patients with CKD before end-stage renal disease are clearly warranted.

## DISCLOSURE

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