Published in final edited form as:

Am J Kidney Dis. 2011 November ; 58(5): 826–834. doi:10.1053/j.ajkd.2011.07.020.

The Role of Phosphorus in the Development and Progression of Vascular Calcification

Jessica Kendrick, MD^{1,2} and Michel Chonchol, MD¹

¹University of Colorado Denver, Aurora, CO, USA

²Denver Health Medical Center, Denver, CO, USA

Abstract

Vascular calcification is associated with significant cardiovascular morbidity and mortality in patients with chronic kidney disease (CKD). Factors unique to CKD patients, such as hyperphosphatemia, predispose these patients to early and progressive vascular calcification. Hyperphosphatemia appears to be involved in a number of mechanisms that trigger and advance progression of vascular calcification including (1) transition of vascular smooth muscle cells (VSMC) from a contractile to an osteochondrogenic phenotype and mineralization of VSMC matrix through sodium-dependent phosphate cotransporters; (2) induction of apoptosis of VSMC; (3) inhibition of monocyte/macrophage differentiation into osteoclast-like cells; (4) elevation of fibroblast growth factor 23 levels; and (5) decreases in klotho expression. Whether vascular calcification can be prevented or reversed with strategies aimed at maintaining phosphate homeostasis is currently unknown. The current review discusses these mechanisms in-depth, exploring the interplay among vascular calcification promoters, inhibitors and substrate that affect phosphorus handling leading to vascular calcification in individuals with CKD.

Cardiovascular disease (CVD) is the most common cause of mortality in patients with chronic kidney disease (CKD) and may be due in part to excess vascular calcification. In CKD patients, the presence and extent of arterial calcification independently predicts CVD and mortality.¹ The hallmark of vascular calcification is calcium phosphate deposition, which can occur in the blood vessels, myocardium, and cardiac valves.² Calcification occurs at two distinct sites, the intimal and medial layers of the vasculature. Intimal deposition is associated with atherosclerotic plaques, while medial deposition is associated with vascular stiffening and arteriosclerosis.^{3,4} Although both forms of calcification occur in patients with CKD, medial calcification appears to be more common.² Vascular calcification is associated with adverse outcomes including myocardial infarction, stroke, and all-cause and CVD mortality in both the general and CKD population.^{5–8} However, vascular calcification occurs decades earlier in CKD patients than in the general population.⁹ A major area of interest concerns the reasons behind the development and accelerated progression of this abnormal calcification in patients with kidney disease.

© 2011 The National Kidney Foundation, Inc. Published by Elsevier Inc. All rights reserved

Corresponding author's address: Jessica Kendrick, MD Assistant Professor, Division of Renal Diseases and Hypertension, University of Colorado Denver, Denver Health Medical Center, 660 Bannock Street, Mail Code 4000, Denver, CO 80204. Phone: 303-436-7458; Fax: 303-436-3509; Jessica.Kendrick@ucdenver.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Financial Disclosure: The authors declare that they have no relevant financial interests.

Calcification is a highly regulated process. Under normal conditions, cell-mediated processes control inducers and inhibitors responsible for mineralization, and the process of vascular calcification is avoided;² however, an imbalance of these regulators results in the initiation of calcification. The key factors responsible for calcification appear to differ across disease states. In the general population, the development of vascular calcification is mainly associated with age and atherosclerotic risk factors, while in the CKD population, vascular calcification is associated with additional non-traditional factors that may be unique to CKD and thus predispose these patients to early and more accelerated calcification. Traditional and non-traditional risk factors for CVD and vascular calcification in patients with kidney disease are shown in Boxes 1 and 2, respectively.¹⁰ One such factor is serum phosphorus, which has been linked to vascular calcification in several studies and is emerging as a key regulator of calcification in the CKD population.^{9,11,12} This review focuses on the potential mechanisms by which phosphorus may trigger and/or advance progression of accelerated vascular calcification.

The Role of Serum Phosphorus in Vascular Calcification

The apparent participation of serum phosphorus in vascular calcification stems from studies of patients with kidney disease and work detailing genetic syndromes that result in hyperphosphatemia. Mutations in the genes for fibroblast growth factor 23 (FGF-23) and uridine diphosphate (UDP) *N*-acetyl- α -D-galactosamine result in familial tumor calcinosis, which is characterized by elevated serum phosphorus and vascular calcifications.^{13,14} This syndrome of hyperphosphatemia and vascular calcification has also been observed in mice with a targeted deletion of the FGF-23 gene¹⁵ or *klotho*¹⁶, a gene required for FGF-23 function. Hyperphosphatemia is also associated with prevalence and progression of vascular calcification in dialysis patients.⁹ Similar findings were reported by Raggi et al;¹² in a study of 205 adult patients on maintenance dialysis, they noted that the extent of coronary artery calcification was more pronounced in the patients with higher serum phosphorus levels. The association between higher serum phosphorus levels and vascular calcification has also been reported in patients with CKD stage 3–4. In 439 participants from the Multi-Ethnic Study of Atherosclerosis (MESA) with moderate CKD (mean estimated GFR of 51 ml/min/1.73m²) and no clinical CVD, each 1-mg/dL increase in serum phosphorus was associated with a 21% ($p=0.002$), 33% ($p=0.001$), 25% ($p=0.16$) and 62% ($p=0.007$) greater prevalence of coronary artery, thoracic, aortic and mitral valve calcification, respectively, after adjusting for demographics and estimated GFR.¹⁷ Recently, serum phosphorus levels have also been linked to vascular calcification in the general population. In 3015 individuals from the CARDIA (Coronary Artery Risk Development in Young Adults) study¹⁸, participants with serum phosphorus levels greater than 3.9 mg/dL had a 52% greater risk of coronary artery calcification 15 years later versus participants with a serum phosphorus level less than 3.3 mg/dL.

Interestingly, higher serum phosphorus level is also linked to vascular stiffness in patients with and without CKD. In patients with moderate CKD, a serum phosphorus level greater than 4.0 mg/dL is associated with a 4-fold greater risk of high ankle-brachial index, a hallmark of peripheral arterial stiffness, compared to a phosphorus level less than 3.0 mg/dL.¹⁹ In a study of over 500 participants with normal kidney function from NHANES (the National Health and Nutrition Examination Survey), individuals with serum phosphorus levels of 3.7–5.0 mg/dL had almost a five-fold increased risk of high ankle-brachial index compared to those with phosphorus levels of 3.1–3.4 mg/dL.²⁰ Thus, even serum phosphorus levels within the normal laboratory range are associated with vascular calcification and stiffness, suggesting that phosphate load and/or abnormal regulation of serum phosphorus and not just overt hyperphosphatemia is a key mediator of vascular

calcification. Furthermore, higher serum phosphorus concentrations are associated with death and cardiovascular events in patients with and without kidney disease.^{21–25}

If phosphorus is a major player in vascular calcification, then methods to reduce serum phosphorus levels may prevent or at least slow progression of calcification. In uremic rats fed high-phosphate diets, treatment with sevelamer or calcium carbonate decreased myocardial and hepatic calcification.²⁶ In dialysis patients, two randomized trials have shown that reducing serum phosphorus levels with sevelamer slows the progression of vascular calcification.^{27,28} Similar findings were reported in a trial involving patients with stage 3–4 CKD.²⁹ However, two other recent randomized trials did not find any decrease in vascular calcification with the use of sevelamer or calcium-based binders.^{30,31} Thus, additional large randomized trials using agents that reduce serum phosphorus levels are needed to prove a causal relationship. To date, no randomized trials have compared phosphate binding agents with placebo in order to evaluate whether targeting phosphate metabolism reduces or prevents vascular calcification and/or cardiovascular events.

Mechanisms of Vascular Calcification

The many factors hypothesized to play a role in vascular calcification are summarized in Table 1; those that influence phosphorus handling are discussed in this review. There are multiple mechanisms by which abnormal phosphorus handling may contribute to the initiation and/or progression of vascular calcification (Fig 1).

Phosphate and osteochondrogenic phenotype change of vascular smooth muscle cells

To determine whether phosphorus directly affects vascular calcification, several *in vitro* studies have been done using human vascular smooth muscle cells (VSMC). When VSMC are exposed to high levels of inorganic phosphate (> 2.4 mM), consistent with levels seen with hyperphosphatemia, calcification is induced in the extracellular matrix surrounding the VSMC.³² This calcification has features similar to calcification that occurs in bone, including matrix vesicles and bioapatite.³³ Furthermore, phosphate directly induces phenotypic changes in VSMC, causing them to transform from a contractile phenotype into an osteochondrogenic phenotype. When VSMC are exposed to elevated phosphate concentrations *in vitro*, there is increased gene transcription of messenger RNAs encoding proteins involved in matrix mineralization and bone formation, such as osteocalcin and CBFA1/RUNX2 (core-binding factor subunit 1 α /runt-related transcription factor 2) and simultaneous down-regulation of transcription factors for smooth muscle cells.^{34,35} Notably, these changes also occur in human and animal models of calcification. For example, calcified inferior epigastric arteries from dialysis patients express CBFA1/RUNX2 and osteopontin in both the media and intima layers.³⁶ Giachelli et al³² has hypothesized that this phenotypic change in VSMC may serve to repair or adapt to a mineralizing environment, given the increased expression of several mineral-regulating molecules in the VSMC. The mechanisms responsible for controlling this phosphate-induced phenotypic change are currently unknown. However, it is known that this phosphate-induced phenotypic change in VSMC is dependent on the activity of sodium-dependent phosphate cotransporters (Na/Pi cotransporters). Type III Na/Pi cotransporters, specifically PiT-1, have been characterized in VSMC.² *In vitro* and animal studies demonstrate that phosphate and the PiT-1 cotransporter on VSMC play key roles in controlling vascular calcification. *In vitro* treatment of VSMC with an inhibitor of the Na/Pi cotransporter results in a decrease in phosphate uptake, calcification, and osteochondrogenic phenotype.^{35,37,38} Furthermore, the expression of osteogenic differentiation markers CBFA1/RUNX2 and osteopontin are blocked in VSMC that have been transduced with PiT-1 knockdown cells *in vitro*.³⁹ Interestingly, different disease states and cytokines appear to increase the expression of the Na/Pi cotransporter.² Mizobuchi et al.⁴⁰ showed *in vivo* that PiT-1 mRNA levels are

elevated in calcified aortas of rats with kidney disease and severe secondary hyperparathyroidism, whereas no calcification was found in aortas of control animals. Additionally, PDGF (platelet-derived growth factor) induces PiT-1 expression and calcification in cultured VSMC.⁴¹ This increased expression of the Na/Pi cotransporter in response to disease states may result in an increased susceptibility to calcification even at serum phosphorus levels within the normal laboratory range. It is currently unknown whether Na/Pi cotransporters play a role in vascular calcification in human disease but this data demonstrates that PiT-1 phosphate transport is required for calcification of human VSMC *in vitro*.

Phosphate-induced apoptosis of VSMC

Studies have found a link between apoptosis of VSMC and vascular calcification; accordingly, it has been suggested that apoptosis is a key regulator of VSMC calcification.^{42–44} Matrix vesicles appear to play a role in apoptosis-induced vascular calcification. Matrix vesicles are generated by budding from chondrocytes and osteoblasts and hold within all of the factors needed for initiation of calcification. Matrix vesicles or at least structures very similar to matrix vesicles have been identified in VSMC calcification.³³ The matrix vesicles derived from VSMC may be remnants of apoptotic cells. For example, in advanced carotid artery atherosclerotic plaques, Kockx et al.⁴³ found that the matrix vesicles were derived from VSMC and they contained the proapoptotic protein BAX (BCL2-associated X protein). Furthermore, studies have suggested that cell death leads to the production of matrix vesicles.^{42,45} Thus, it is these matrix vesicles/apoptotic bodies that initiate calcification because they have the capacity to concentrate and crystallize calcium.⁴⁴ Evidence supports the role of apoptosis in calcification, with studies finding that apoptosis in VSMC occurs before the onset of calcification.⁴⁴ Furthermore, when apoptosis is inhibited in VSMC by a caspase inhibitor there is a significant reduction in calcification.⁴⁴ However, Giachelli et al.³² found that calcification of VSMC does not appear to require apoptosis for the initiation of calcification; nonetheless, apoptosis appears to accelerate the calcification process.

Phosphate has been shown to induce apoptosis in terminally differentiated chondrocytes. The role of phosphate in apoptosis appears to be associated with chondrocyte maturation and extracellular matrix mineralization.⁴⁶ Exposure to elevated phosphorus concentration causes an increase in intracellular phosphate which triggers chondrocyte apoptosis.⁴⁷ It appears that Na/Pi cotransporters play a role in the increase in intracellular phosphate as competitive inhibitors of the Na/Pi transporter block apoptosis in chondrocytes.⁴⁷ How increased phosphorus concentrations results in apoptosis is unclear, but this process may be related to disruptions in normal mitochondrial energy metabolism.⁴⁷ Phosphate has also been shown to induce apoptosis in human aortic smooth muscle cells.⁴⁸ When human aortic smooth muscle cells were exposed to high phosphate (similar to levels seen with hyperphosphatemia), calcification and apoptosis were induced in a dose and time dependent manner.⁴⁸ Interestingly, the use of HMG-CoA (β -hydroxy- β -methylglutaryl-CoA) reductase inhibitors (statins) blocks phosphate-induced calcification in human aortic smooth muscle cells.⁴⁸ The inhibitory effect of statins reflected prevention of apoptosis rather than inhibition of Na/Pi cotransporters. This may be the reason for the decrease in vascular calcification seen with the use of statins in clinical trials.^{49,50} More studies are needed to confirm that phosphate-induced apoptosis plays a role in vascular calcification in humans.

Phosphate and inhibition of osteoclast differentiation

In bone formation there is a delicate balance between mineral deposition by osteoblasts and mineral resorption by osteoclasts. Disruptions in this balance lead to disease states such as osteoporosis. It has been hypothesized that a similar balance between mineral deposition and

resorption exists in the vasculature and disruptions result in calcification of the vessel walls.⁵¹ As discussed earlier, the calcified vascular wall contains VSMC that have an osteoblast-like phenotype. However, the calcified wall also contains monocytes and macrophages that are able to differentiate into cells with an osteoclast-like phenotype.⁵² It is thought that these osteoclast-like cells may be important regulators in arterial homeostasis and extracellular mineralization.⁵² Thus, an imbalance that favors the osteoblast-like phenotype may both initiate and cause progression of calcification. Recent studies have found that uremic toxins favor the osteoblast-like phenotype.⁵¹ Elevated phosphorus concentrations to levels seen in advanced kidney disease significantly decrease the differentiation of monocytes/macrophages into osteoclast-like cells *in vitro* by downregulating RANK (receptor activator of nuclear factor κ B) ligand-induced signaling.⁵³ This reduction in osteoclast activity may be an additional way that elevated phosphorus levels results in vascular calcification. Additional studies are needed to evaluate whether activating these osteoclast-like cells in the vessel wall prevents or reverses vascular calcification.

FGF-23 and vascular calcification

FGF-23 is a hormone secreted by osteocytes. It is the main regulator of serum phosphorus and plays a key role in vitamin D metabolism and secondary hyperparathyroidism.⁵⁴ FGF-23 results in renal phosphate excretion through inhibition of proximal tubule renal phosphate reabsorption and also inhibits 1- α -hydroxylase activity, resulting in decreasing calcitriol and decreased phosphate reabsorption in the intestine.⁵⁴ FGF-23 weakly binds its receptor (FGFR1c) and thus requires a co-factor, klotho, for activity. Given the requirement for klotho, FGF-23 is presumed to be active only in tissues that manufacture klotho (distal tubule of kidney, parathyroid glands, brain). FGF-23 levels increase early in the course of kidney disease to maintain serum phosphorus within the normal range, which is why phosphorus levels do not become noticeably elevated until the glomerular filtration rate falls to < 20 ml/min/1.73 m². In addition, FGF-23 suppresses parathyroid hormone (PTH) secretion, PTH gene expression, and parathyroid cell proliferation, thereby closing the loop in the bone-parathyroid axis.^{55,56} In advanced CKD there is down-regulation of the parathyroid klotho-FGFR1c, and FGF-23 fails to decrease PTH expression and parathyroid cell proliferation.⁵⁶ Interestingly, FGF-23 is stimulated not only by increased phosphate but also by PTH,⁵⁵ and recent data have demonstrated that FGF-23/klotho signaling is not necessary for the phosphaturic and anabolic actions of PTH.⁵⁷ Thus, PTH still exerts its functions even in the presence of extremely high serum FGF-23 levels.

Several recent studies have linked higher FGF-23 levels with death and cardiovascular events in patients with and without kidney disease, although the mechanism for this relationship remains unclear.^{58–62} In moderately uremic mice fed high-phosphate diets, serum FGF-23 levels rather than serum phosphorus levels were associated with extensive arterial-medial calcifications.⁶³ In dialysis patients, FGF-23 has been independently linked to coronary artery, aortic and peripheral vascular calcification.^{64–66} These data suggest that another mechanism by which phosphate affects vascular calcification may be via phosphorus-mediated elevation of FGF-23.

It is unclear if elevated FGF-23 levels directly results in vascular calcification or if FGF-23 inhibits calcification, as patients with inactivating mutations of FGF-23 have an increase rather than a decrease in vascular calcifications. Furthermore, a recent study in dialysis patients evaluating changes in aortic arch calcification scores found that FGF-23 levels were higher in regressors than in non-progressors and progressors.⁶⁷ FGF-23 was also negatively associated with changes in aortic arch calcification scores during the 5 year follow-up period (β value = -0.001 , $p=0.0115$).⁶⁷ Thus, extremely high FGF-23 levels may result in inhibition of calcification in the vessel wall in dialysis patients. The mechanism by which

FGF-23 effects vascular calcification is unknown, but it is possible that FGF-23 has both direct and indirect actions on vascular calcification. It is unknown if Klotho is found on VSMC but VSMC do contain FGF receptors.⁶¹ Even though FGF-23 typically requires Klotho for its activity, it has been hypothesized that the extremely high levels of FGF-23 seen in dialysis patients may induce Klotho-independent functions.⁶⁶ Further large, prospective studies are needed to specify whether FGF-23 may be a marker or a potential mechanism for vascular calcification.

Klotho deficiency and vascular calcification

Klotho is a single-pass transmembrane protein that functions as a co-receptor for FGF-23.⁵⁴ Klotho is also secreted into the blood, urine and cerebrospinal fluid.⁶⁸ CKD appears to be a state of Klotho deficiency, and renal Klotho expression is decreased in human nephrectomy samples from end-stage kidneys.⁶⁹ Furthermore, secreted Klotho is also decreased in the blood and urine in people with CKD.⁶⁸ In humans, the decrease in urine Klotho becomes apparent in early stages of CKD and continues as kidney disease progresses.⁶⁸

Klotho deficiency in mice results in hyperphosphatemia and ectopic calcification.¹⁶ Hu et al.⁶⁸ showed in an experimental model of CKD that transgenic mice overexpressing Klotho have preserved Klotho levels, better kidney function, enhanced phosphaturia and less ectopic calcification than wild-type mice with CKD. Thus, data suggests that Klotho may be an inhibitor of vascular calcification. The anticalcification effect of Klotho is likely due to both direct and indirect actions of Klotho. Indirectly, Klotho protects against calcification through its actions on phosphorus and vitamin D metabolism. One of the key roles of Klotho is to promote renal phosphate excretion through its interaction with FGF-23. This control of serum phosphorus results in reduced calcification. Overexpression of Klotho also decreases kidney disease progression.⁶⁸ Preservation of kidney function results in less disturbances in mineral metabolism and other risk factors for vascular calcification. Recent data by Hu et al.⁶⁸ further suggest that Klotho has a direct effect on the vasculature. In an experimental model of CKD, animals lacking Klotho had increased expression of the Na/Pi cotransporter and the osteogenic transcription factor CBFA1/RUNX2 in VSMC, suggesting that decreased Klotho drives calcification.⁶⁸ Furthermore, when Klotho is added to VSMC *in vitro*, it reduces phosphate uptake by suppressing the activity of the Na/Pi cotransporter and prevents the phenotypic change of VSMC to an osteochondrogenic phenotype. It is unclear how Klotho mediates its effects on VSMC. Further studies are needed to confirm the role of Klotho in vascular calcification.

Conclusion

Vascular calcification is associated with significant morbidity and mortality in patients with CKD. An elevated serum phosphorus level is a risk factor for death, cardiovascular events and vascular calcification in CKD patients. Hyperphosphatemia contributes to several mechanisms that trigger or advance the progression of vascular calcification and is emerging as a key regulator of calcification in patients with kidney disease. The question remains as to whether strategies that reduce serum phosphorus prevent or reverse vascular calcification.

Acknowledgments

Support: This work is supported by National Institute of Diabetes and Digestive and Kidney Disease grants R01 DK081473 and R01 DK078112 and K23DK087859-01A1.

References

1. Blacher J, Guerin AP, Pannier B, Marchais SJ, London GM. Arterial calcifications, arterial stiffness, and cardiovascular risk in end-stage renal disease. *Hypertension*. 2001; 38(4):938–942. [PubMed: 11641313]
2. Giachelli CM. The emerging role of phosphate in vascular calcification. *Kidney Int*. 2009; 75:890–897. [PubMed: 19145240]
3. Hunt JL, Fairman R, Mitchell ME, et al. Bone formation in carotid plaques: a clinicopathological study. *Stroke*. 2002; 33:1214–1219. [PubMed: 11988593]
4. Edmonds ME, Morrison N, Laws JW, Watkins PJ. Medial arterial calcification and diabetic neuropathy. *Br Med J (Clin Res Ed)*. 1982; 284:928–930.
5. Locker TH, Schwartz RS, Cotta CW, Hickman JR. Fluoroscopic coronary artery calcification and associated coronary disease in asymptomatic young men. *J Am Coll Cardiol*. 1992; 19:1167–1172. [PubMed: 1564217]
6. Hollander M, Hak AE, Koudstaal PJ, et al. Comparison between measures of atherosclerosis and risk of stroke: the Rotterdam Study. *Stroke*. 2003; 34:2367–2372. [PubMed: 12958327]
7. Detrano R, Guerci AD, Carr JJ, et al. Coronary calcium as a predictor of coronary events in four racial or ethnic groups. *N Engl J Med*. 2008; 358:1336–1345. [PubMed: 18367736]
8. London GM, Guerin AP, Marchais SJ, Metivier F, Pannier B, Adda H. Arterial media calcification in end-stage renal disease: impact on all-cause and cardiovascular mortality. *Nephrol Dial Transplant*. 2003; 18:1731–1740. [PubMed: 12937218]
9. Goodman WG, Goldin J, Kuizon BD, et al. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. *N Engl J Med*. 2000; 342:1478–1483. [PubMed: 10816185]
10. Sigrist MK, Taal MW, Bungay P, McIntyre CW. Progressive vascular calcification over 2 years is associated with arterial stiffening and increased mortality in patients with stages 4 and 5 chronic kidney disease. *Clin J Am Soc Nephrol*. 2007; 2:1241–1248. [PubMed: 17928470]
11. Block GA. Prevalence and clinical consequences of elevated Ca x P product in hemodialysis patients. *Clin Nephrol*. 2000; 54:318–324. [PubMed: 11076108]
12. Raggi P, Boulay A, Chasan-Taber S, et al. Cardiac calcification in adult hemodialysis patients. A link between end-stage renal disease and cardiovascular disease? *J Am Coll Cardiol*. 2002; 39:695–701. [PubMed: 11849871]
13. Benet-Pages A, Orlik P, Strom TM, Lorenz-Depiereux B. An FGF23 missense mutation cause familial tumoral calcinosis with hyperphosphatemia. *Hum Mol Genet*. 2005; 14:385–390. [PubMed: 15590700]
14. Ichikawa S, Lyles KW, Econs MJ. A novel GALNT3 mutation in a pseudoautosomal dominant form of tumor calcinosis: evidence that the disorder is autosomal recessive. *J Clin Endocrinol Metab*. 2005; 90:2420–2423. [PubMed: 15687324]
15. Stubbs JR, Liu S, Tang W, et al. Role of hyperphosphatemia and 1,25-dihydroxyvitamin D in vascular calcification and mortality in fibroblastic growth factor 23 null mice. *J Am Soc Nephrol*. 2007; 18:2116–2124. [PubMed: 17554146]
16. Kuroo M, Matsamura Y, Aizawa H, et al. Mutation of the mouse klotho gene leads to a syndrome resembling ageing. *Nature*. 1997; 390:45–51. [PubMed: 9363890]
17. Adeney KL, Siscovick DS, Ix JH, et al. Association of serum phosphate with vascular and valvular calcification in moderate CKD. *J Am Soc Nephrol*. 2009; 20:381–387. [PubMed: 19073826]
18. Foley RN, Collins AJ, Herzog CA, Ishani A, Kalra PA. Serum phosphorus levels associate with coronary atherosclerosis in young adults. *J Am Soc Nephrol*. 2009; 20:397–404. [PubMed: 18987306]
19. Ix JH, De Boer IH, Peralta CA, et al. Serum phosphorus concentrations and arterial stiffness among individuals with normal kidney function to moderate kidney disease in MESA. *Clin J Am Soc Nephrol*. 2009; 4:609–615. [PubMed: 19211667]
20. Kendrick J, Ix JH, Targher G, Smits G, Chonchol M. Relation of serum phosphorus levels to ankle brachial pressure index (from the Third National Health and Nutrition Examination Survey). *Am J Cardiol*. 2010; 106(4):564–568. [PubMed: 20691317]

21. Tonelli M, Sacks F, Pfeffer M, Gao Z, Curhan G. Relation between serum phosphate level and cardiovascular event rate in people with coronary disease. *Circulation*. 2005; 112:2627–2633. [PubMed: 16246962]
22. Dhingra R, Sullivan LM, Fox CS, Wang TJ, D'Agostino RB Sr, Gaziano JM, Vasan RS. Relations of serum phosphorus and calcium levels to the incidence of cardiovascular disease in the community. *Arch Intern Med*. 2007; 167:879–885. [PubMed: 17502528]
23. Chonchol M, Dale R, Schrier RW, Estacio R. Serum phosphorus and cardiovascular mortality in type 2 diabetes. *Am J Med*. 2009; 122:380–386. [PubMed: 19332233]
24. Kestenbaum B, Sampson JN, Rudser KD, Patterson DJ, Seliger SL, Young B, Sherrard DJ, Andress DL. Serum phosphate levels and mortality risk among people with chronic kidney disease. *J Am Soc Nephrol*. 2005; 16:520–528. [PubMed: 15615819]
25. Block GA, Hulbert-Shearon TE, Levin NW, Port FK. Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic hemodialysis patients: a national study. *Am J Kidney Dis*. 1998; 31:607–617. [PubMed: 9531176]
26. Cozzolino M, Dusso AS, Liapis H, et al. The effects of sevelamer hydrochloride and calcium carbonate on kidney calcification in uremic rats. *J Am Soc Nephrol*. 2002; 13:2299–2308. [PubMed: 12191974]
27. Chertow GM, Burke SK, Raggi P. Sevelamer attenuates the progression of coronary and aortic calcification in hemodialysis patients. *Kidney Int*. 2002; 62:245–252. [PubMed: 12081584]
28. Block GA, Spiegel DM, Ehrlich J, et al. Effects of sevelamer and calcium on coronary artery calcification in patients new to hemodialysis. *Kidney Int*. 2005; 68:1815–1824. [PubMed: 16164659]
29. Russo D, Miranda I, Ruocco C, et al. The progression of coronary artery calcification in predialysis patients on calcium carbonate or sevelamer. *Kidney Int*. 2007; 72:1255–1261. [PubMed: 17805238]
30. Qunibi W, Moustafa M, Muenz LR, et al. A 1-year randomized trial of calcium acetate versus sevelamer on progression of coronary artery calcification in hemodialysis patients with comparable lipid control: the Calcium Acetate Renagel Evaluation-2 (CARE-2) study. *Am J Kidney Dis*. 2008; 51:952–965. [PubMed: 18423809]
31. Barreto DV, Barreto Fde C, de Carvalho AB, et al. Phosphate binder impact on bone remodeling and coronary calcification-results from the BRiC Study. *Nephron. Clin Pract*. 2008; 110:c273–283. [PubMed: 19001830]
32. Giachelli CM, Speer M, Li X, Rajachar R, Yang H. Regulation of vascular calcification, roles of Phosphate and Osteopontin. *Circ Res*. 2005; 96:717–722. [PubMed: 15831823]
33. Wada T, McKee MD, Stietz S, Giachelli CM. Calcification of vascular smooth muscle cell cultures: Inhibition by osteopontin. *Circ Res*. 1999; 84:1–6. [PubMed: 9915769]
34. Tyson KL, Reynolds JL, McNair R, Zhang Q, Weissberg PL, Shanahan CM. Osteo/chondrocytic transcription factors and their target genes exhibit distinct patterns of expression in human arterial calcification. *Arterioscler Thromb Vasc Biol*. 2003; 23:489–494. [PubMed: 12615658]
35. Jono S, McKee MD, Murry CE, Shioi A, Nishizawa Y, Mori K, Morii H, Giachelli CM. Phosphate regulation of vascular smooth muscle cell calcification. *Circ Res*. 2000; 87:E10–17. [PubMed: 11009570]
36. Moe SM, Duan D, Doehle BP, O'Neill KD, Chen NX. Uremia induces the osteoblast differentiation factor Cbfa1 in human blood vessels. *Kidney Int*. 2003; 63:1003–1011. [PubMed: 12631081]
37. Sugitani H, Wachi H, Murata H, Sato F, Mecham RP, Seyama Y. Characterization of an *in vitro* model of calcification in retinal pigmented epithelial cells. *J Atheroscler Thromb*. 2003; 10:48–56. [PubMed: 12621165]
38. Chen NX, O'Neill KD, Duan D, Moe SM. Phosphorus and uremic serum upregulate osteopontin expression in vascular smooth muscle cells. *Kidney Int*. 2002; 62:1724–1731. [PubMed: 12371973]
39. Li X, Yang HY, Giachelli CM. Role of the sodium-dependent phosphate cotransporter, Pit-1, in vascular smooth muscle cell calcification. *Circ Res*. 2006; 98:905–912. [PubMed: 16527991]

40. Mizobuchi M, Finch JL, Martin DR, Slatopolsky E. Differential effects of vitamin D receptor activators on vascular calcification in uremic rats. *Kidney Int.* 2007; 72:709–715. [PubMed: 17597697]
41. Kakita A, Suzuki A, Nishiwaki K, et al. Stimulation of Na-dependent phosphate transport by platelet-derived growth factor in rat aortic smooth muscle cells. *Atherosclerosis.* 2004; 174:17–24. [PubMed: 15135246]
42. Kim KM. Apoptosis and calcification. *Scanning Microscopy.* 1995; 9:1137–1178. [PubMed: 8819895]
43. Kockx MM, DeMeyer GRY, Muhring J, Jacob W, Bult H, Herman AG. Apoptosis and related proteins in different stages of human atherosclerotic plaques. *Circulation.* 1998; 97:2307–2315. [PubMed: 9639374]
44. Proudfoot D, Skepper J, Hegyi L, Bennett M, Shanahan C, Weissberg P. Apoptosis regulates human vascular calcification in vitro. Evidence for initiation of vascular calcification by apoptotic bodies. *Circ Res.* 2000; 87:1055–1062. [PubMed: 11090552]
45. Anderson HC. Molecular biology of matrix vesicles. *Clin Orthop.* 1995; 314:266–280. [PubMed: 7634645]
46. Mansfield K, Rajpurohit R, Shapiro IM. Extracellular phosphate ions cause apoptosis of terminally differentiated epiphyseal chondrocytes. *J Cell Physiol.* 1999; 179:276–286. [PubMed: 10228946]
47. Mansfield K, Pucci B, Adams CS, Shapiro IM. Induction of apoptosis in skeletal tissues: phosphate-mediated chick chondrocyte apoptosis is calcium dependent. *Calcif Tissue Int.* 2003; 73:161–172. [PubMed: 14565598]
48. Son BK, Kozaki K, Iijima K, et al. Statins protect human aortic smooth muscle cells from inorganic phosphate-induced calcification by restoring gas6-axl survival pathway. *Circ Res.* 2006; 98:1024–1031. [PubMed: 16556867]
49. Shavelle, Dm; Takasu, J.; Budoff, MJ.; Mao, S.; Zhao, XQ.; O'Brien, KD. HMG CoA reductase inhibitor (statin) and aortic valve calcium. *Lancet.* 2002; 359:1125–1126. [PubMed: 11943265]
50. Callister TQ, Raggi P, Cooil B, Lippolis NJ, Russo DJ. Effect of HMG-CoA reeducates inhibitors on coronary artery disease as assessed by electron-beam computed tomography. *N Engl J Med.* 1998; 339:1972–1978. [PubMed: 9869668]
51. Massy ZA, Mentaverri R, Mozar A, Brazier M, Kamel S. The pathophysiology of vascular calcification: are osteoclast-like cells the missing link? *Diabetes Metab.* 2008; 34:S16–S20. [PubMed: 18358422]
52. Doherty TM, Uzui H, Fitzpatrick LA, et al. Rationale for the role of osteoclast-like cells in arterial calcification. *FASEB J.* 2002; 16:577–582. [PubMed: 11919160]
53. Mozar A, Haren N, Chasseraud M, et al. High extracellular inorganic phosphate concentration inhibits RANK-RANKL signaling in osteoclast-like cells. *J Cell Physiol.* 2008; 215:47–54. [PubMed: 17894387]
54. Stubbs J, Liu S, Quarles LD. Role of fibroblast growth factor 23 in phosphate homeostasis and pathogenesis of disordered mineral metabolism in chronic kidney disease. *Semin Dial.* 2007; 20(4):302–308. [PubMed: 17635819]
55. Kawata T, Imanishi Y, Kobayashi K, et al. Parathyroid hormone regulates fibroblast growth factor-23 in a mouse model of primary hyperparathyroidism. *J Am Soc Nephrol.* 2007; 18:2683–2688. [PubMed: 17855636]
56. Ben-Dov IZ, Galitzer H, Lavi-Moshayoff V, et al. The Parathyroid is a target organ for FGF-23 in rats. *J Clin Invest.* 2007; 117:4003–4008. [PubMed: 17992255]
57. Yuan Q, Sato T, Densmore M, et al. FGF23/Klotho signaling is not essential for the phosphaturic and anabolic functions of PTH. *Diabetes Metab.* 2008; 34(Suppl 1):S16–S20. [PubMed: 18358422]
58. Gutierrez OM, Mannstadt M, Isakova T, et al. Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. *N Engl J Med.* 2008; 359(6):584–592. [PubMed: 18687639]
59. Mirza MA, Larsson A, Lind L, Larsson TE. Circulation fibroblast growth factor-23 is associated with vascular dysfunction in the community. *Atherosclerosis.* 2009; 205:385–90. [PubMed: 19181315]

60. Hsu HJ, Wu MS. Fibroblast growth factor 23: a possible cause of left ventricular hypertrophy in hemodialysis patients. *Am J Med Sci.* 2009; 337:116–122. [PubMed: 19214027]
61. Gutierrez O, Januzzi J, Isakova T, et al. Fibroblast growth factor-23 and left ventricular hypertrophy in chronic kidney disease. *Circulation.* 2009; 119:2545–2552. [PubMed: 19414634]
62. Kendrick J, Cheung A, Kaufman J, Greene T, Roberts W, Smits G, Chonchol M, The HOST Investigators. FGF-23 associates with death, cardiovascular events, and initiation of chronic dialysis. *J Bone Miner Res.* 2011; 26(9):2026–2035. [PubMed: 21590742]
63. El-Abbadi MM, Pai AS, Leaf EM, et al. Phosphate feeding induces arterial medial calcification in uremic mice: role of serum phosphorus, fibroblast growth factor-23, and osteopontin. *Kidney Int.* 2009; 75:1297–1307. [PubMed: 19322138]
64. Nasrallah MM, El-Shehaby AR, Salem MM, Osman NA, El Sheikh E, Sharaf El Din UA. Fibroblast growth factor-23 (FGF-23) is independently correlated to aortic calcification hemodialysis patients. *Nephrol Dial Transplant.* 2010; 25:2679–2685. [PubMed: 20176609]
65. Jean G, Bresson E, Terrat JC, et al. Peripheral vascular calcification in long-hemodialysis patients: associated factors and survival consequences. *Nephrol Dial Transplant.* 2009; 24:948–955. [PubMed: 18852190]
66. Balci M, Kirkpantur A, Gulbay M, Gurbuz OA. Plasma fibroblast growth factor-23 levels are independently associated with carotid artery atherosclerosis in maintenance hemodialysis patients. *Hemodial Int.* 2010; 14:425–32. [PubMed: 20955275]
67. Tamei N, Ogawa T, Ishida H, Yoshitaka A, Nitta K. Serum fibroblast growth factor-23 levels and progression of aortic arch calcification in non-diabetic patients on chronic hemodialysis. *J Atheroscler Thromb.* 2011; 18:217–223. [PubMed: 21139318]
68. Hu MC, Shi M, Zhang J, et al. Klotho deficiency causes vascular calcification in chronic kidney disease. *J Am Soc Nephrol.* 2011; 22:124–136. [PubMed: 21115613]
69. Koh N, Fujimori T, Nishiguchi S, et al. Severely reduced production of klotho in human chronic renal failure kidney. *Biochem Biophys Res Commun.* 2001; 280:1015–1020. [PubMed: 11162628]
70. Rennerberg RJ, Schurgers LJ, Kroon AA, Stehouwer CD. Arterial calcifications. *J Cell Mol Med.* 2010; 14:2203–2210. [PubMed: 20716128]
71. Hruska KA, Mathew S, Lund RJ, Memon I, Saab G. The pathogenesis of vascular calcification in the chronic kidney disease mineral bone disorder: the links between bone and the vasculature. *Semin Nephrol.* 2009; 29:156–165. [PubMed: 19371806]
72. Covic A, Kanbay M, Voroneanu L, Turgut F, Serban DN, Serban IL, Goldsmith DJ. Vascular calcification in chronic kidney disease. *Clin Sci (Lond).* 2010; 119:111–121. [PubMed: 20443781]

Figure 1. Mechanisms by which phosphate may contribute to the initiation and/or progression of vascular calcification. Abbreviations: VSMC, vascular smooth muscle cells; FGF-23, fibroblast growth factor 23.

Box 1**Risk Factors for Cardiovascular Disease in Kidney Disease****Traditional Risk Factors**

- Age
- Male sex
- Diabetes mellitus
- Hypertension
- Smoking
- Dyslipidemia
- Family history
- Obesity

Nontraditional Risk Factors

- Kidney function decline
- Albuminuria
- Anemia
- Inflammation and oxidative stress
- Disorders of mineral metabolism
 - Hyperphosphatemia
 - Changes in vitamin D metabolism
 - Secondary hyperparathyroidism
 - Elevated FGF-23 levels
- Activation of the sympathetic nervous system

Abbreviation: FGF-23=fibroblast growth factor-23

Box 2**Risk Factors for Vascular Calcification in Kidney Disease****Traditional Risk Factors**

- Age
- Diabetes mellitus
- Hypertension
- Dyslipidemia
- Smoking

Nontraditional Risk Factors

- Kidney function decline
- Dialysis vintage
- Disorders of mineral metabolism
 - Hyperphosphatemia
 - Hyperparathyroidism
 - Changes in vitamin D metabolism
 - Elevated FGF-23 levels
- Inflammation and oxidative stress
- Osteogenesis factors (CBFA1/RUNX2)

Abbreviations: FGF-23=fibroblast growth factor-23; CBFA1/RUNX2= core-binding factor subunit 1 α /runt-related transcription factor 2

Table 1

Potential Factors in Vascular Calcification

Regulator of Vascular Calcification	Structure and Function	Changes in CKD	Role in Vascular Calcification
<u>Inhibitors</u>			
MGP (Matrix Gla protein) ⁶⁹⁻⁷¹	Vitamin K-dependent protein produced by VSMC and chondrocytes; accrues at sites of calcification; stimulated by an increase in calcium levels	Decreased expression as GFR declines	Inhibits calcification by: 1) regulating transcription factors that inhibit VSMC differentiation into osteoblast-like phenotype; 2) binding to calcium crystals in the vascular matrix, inhibiting calcium crystal formation
BMP-7 (bone morphogenetic protein) ^{69,70}	Expressed in the collecting tubule of the kidney; plays a key role in the development and repair of bone and other tissues	Reduced expression	Inhibits calcification by: 1) promoting the VSMC phenotype; 2) reducing serum phosphorus by restoring normal bone turnover
OPG (osteoprotegerin; encoded by <i>TNFRSF11B</i>) ⁶⁹⁻⁷¹	Soluble circulating ligand of RANK; prevents the binding of RANKL to RANK; expression is high in VSMCs and vascular endothelial cells	Likely increased, indicating a compensatory increase in response to factors promoting vascular calcification	Inhibits calcification by inhibiting osteoclast differentiation and activation
Fetuin A ⁶⁹⁻⁷¹	Serum glycoprotein produced in the liver; an extracellular calcium-regulatory protein; concentrations fall during inflammatory states	Decreased expression	Inhibits calcification by: 1) binding hydroxyapatite structures to prevent precipitation; 2) antagonizes the action of TGF- β 1 and blocks osteogenesis
OPN (osteopontin; encoded by <i>SPP1</i>) ⁶⁹	Acidic, extracellular phosphoprotein; normally present in mineralized tissues; absent in normal arteries but abundant in calcified arteries	Increased expression	Regulates calcification in injured arteries by: 1) inhibiting hydroxyapatite crystal growth; 2) promoting osteoclast function
<u>Promoters</u>			
BMP-2 (bone morphogenetic protein 2) ⁶⁹	Member of the BMP family; plays a key role in the development and repair of bone and other tissues; upregulated by oxidative stress, inflammation and hyperglycemia; decreased by MGP	Increased expression	Induces calcification by decreasing smooth muscle cell markers thereby promoting VSMC differentiation into osteoblast-like cells
RANKL (receptor activator of nuclear factor- κ B) ⁶⁹	Transmembrane protein expressed by T cells and osteoblasts; binds to RANK and regulates cell function, differentiation, and survival; upregulated by vitamin D, PTH, calcium, and inflammatory cytokines; downregulated by TGF- β in osteoblasts	Likely increased expression	Induces calcification by promoting the differentiation and activation of osteoclasts
CBFA1/RUNX2 (core binding factor α 1, runt-related transcription factor 2) ⁷²	Essential transcription factor for osteoblastic differentiation and osteogenesis; upregulated in response to uremic toxins	Increased expression	Induces calcification by promoting differentiation of VSMC into osteoblast-like phenotype
<u>Substrates</u>			
Phosphorus ^{34,35,48,53}	Controlled by PTH, FGF-23, and 1,25(OH) $_2$ D; PTH and FGF-23 promote urinary phosphorus excretion; 1,25(OH) $_2$ D increases the intestinal absorption of phosphorus	Increased	Induces calcification by: 1) promoting osteochondrogenic differentiation of VSMC; 2) inducing apoptosis of VSMC; 3) inhibiting osteoclast differentiation of VSMC; 4) possibly by increasing FGF-23
Vitamin D ^{70,71}	Increases the intestinal absorption of calcium and phosphate and regulates osteoblast and osteoclast differentiation; upregulated by PTH, low calcium, and low phosphate; downregulated by FGF-23 and decreased renal mass	Decreased	Data suggests that vitamin D deficiency promotes vascular calcification; the mechanism is unknown and requires further study; excess vitamin D promotes calcification by: 1) promoting differentiation of VSMC into

Regulator of Vascular Calcification	Structure and Function	Changes in CKD	Role in Vascular Calcification
			osteoblast-like phenotype; 2) stimulating alkaline phosphatase activity; 3) causing hypercalcemia
FGF-23 (fibroblast growth factor 23) ^{70,71}	Produced by osteocytes in response to elevated serum phosphate and 1,25(OH) ₂ D; regulates phosphorus and vitamin D metabolism	Increased	Deficiencies of FGF-23 result in extensive vascular calcifications through hyperphosphatemia and excess 1,25(OH) ₂ D production; data suggests that high FGF-23 levels may result in inhibition or promotion of vascular calcification but the mechanisms are unclear
Klotho ⁶⁷	Transmembrane protein that functions as a coreceptor for FGF-23	Decreased	Inhibits calcification through: 1) promoting phosphaturia; 2) inhibiting the differentiation of VSMC into osteoclast-like phenotype; 3) preservation of kidney function

Abbreviations: VSMC=vascular smooth muscle cell; GFR=glomerular filtration rate; NFκB= nuclear factor-κB; RANK=receptor activator of NFκB; RANKL=receptor activator of NFκB ligand; TGF-β1 = transforming growth factor β1; TGF-β1 = transforming growth factor β, BMP=bone morphogenetic protein; PTH=parathyroid hormone; 1,25(OH)₂D=1,25-dihydroxyvitamin D; FGF-23= fibroblast growth factor-23