

**Vascular Dysfunction,  
Atherosclerosis, and  
Vascular Calcification**

***Insights and Implications  
in Chronic Kidney Disease***



National Kidney  
Foundation®

# INTRODUCTION

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**P**atients with chronic kidney disease (CKD) experience up to 30-fold higher cardiovascular disease (CVD) mortality than the general population<sup>1</sup> with this staggering outcome only incompletely explained by such traditional risk factors as aging, smoking, diabetes, dyslipidemia, or hypertension.<sup>2,3</sup> Research efforts have expanded understanding of the contribution made by vascular pathologies to this burden.

Vascular calcification is a common complication in uremia, due in part to disturbed mineral metabolism and the therapies used to control it,<sup>4</sup> but also due to a complex, active process of osteogenesis in vascular smooth muscle cells (VSMCs).<sup>1,2,5</sup> Furthermore, cardiovascular calcifications in patients with CKD are more prevalent, progressive, extensive and severe compared with the non-CKD population.<sup>6</sup> Computed tomography (CT) and observational studies have provided evidence for calcific complications that encompass development and progression of atherosclerotic plaque calcification associated with events such as myocardial infarction and stroke, as well as arterial stiffness and cardiac valve dysfunction that contribute to ventricular hypertrophy and heart failure, respectively.

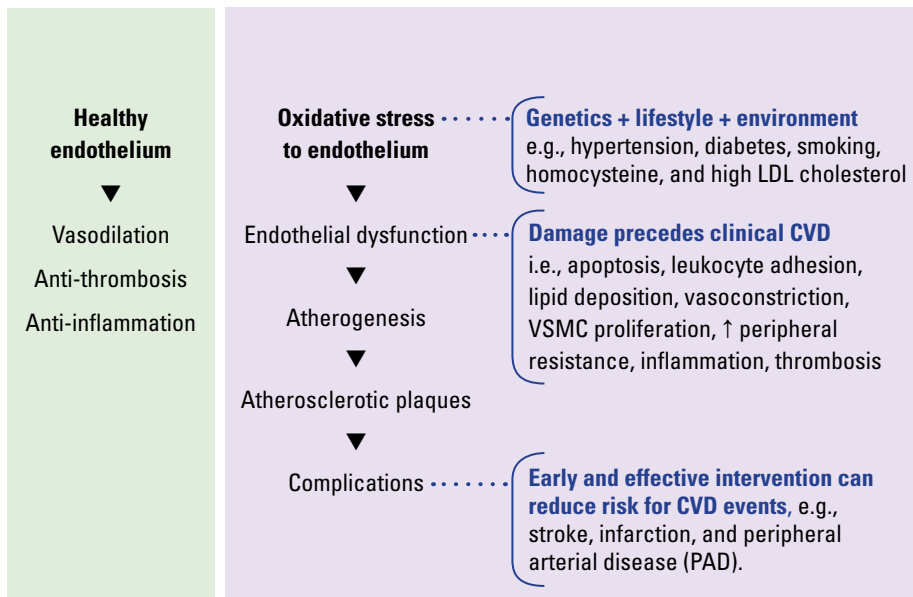
Arterial calcification is an important mechanism through which nephrologists can: (1) appreciate the long-term hemodynamic consequences of hyperphosphatemia in patients with advanced CKD or those receiving dialysis therapies, and (2) appraise current and future therapeutic approaches to reduce risk of serious adverse clinical outcomes.<sup>4</sup>

The purpose of this booklet is to outline vascular dysfunction, atherosclerosis, and vascular calcification, and to highlight elements of the emerging science around vitamin D receptor (VDR) activation as it may pertain to future therapies to mitigate CKD-related calcification.

# VASCULAR DYSFUNCTION, ATHEROSCLEROSIS AND CALCIFICATION

## THE ENDOTHELIUM REFLECTS VASCULAR HEALTH

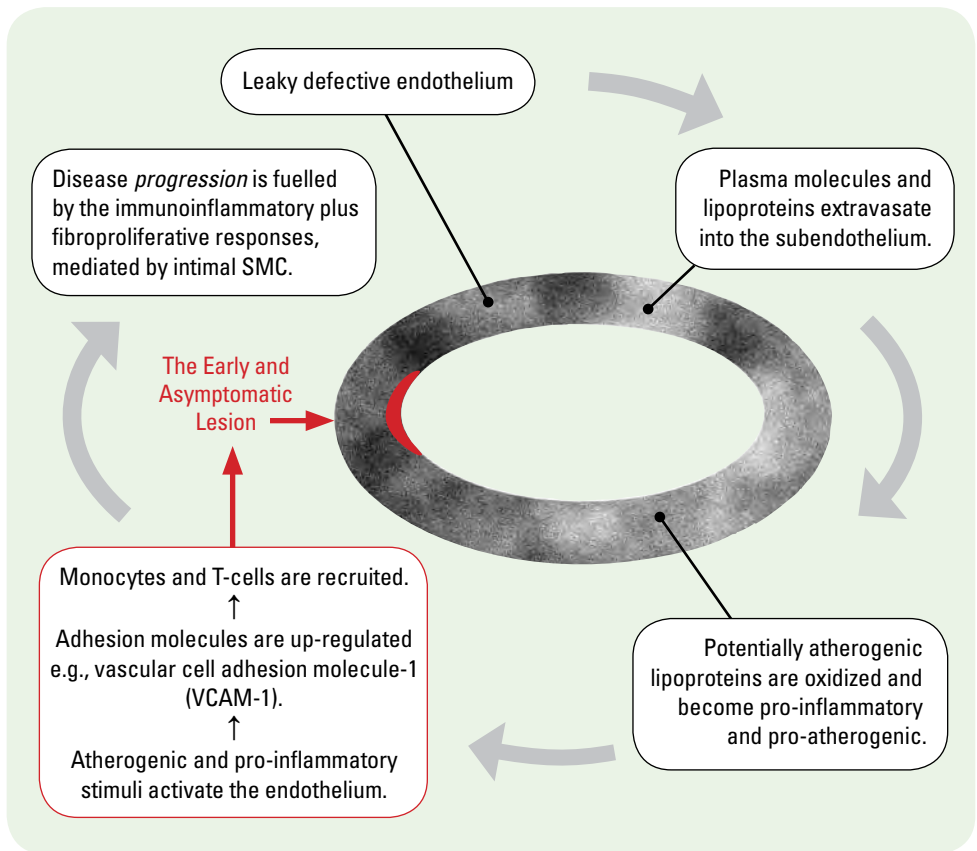
A functional paradigm of the endothelium has long been believed to have at its core the homeostasis of vasoreactivity factors. Such factors are central to understanding endothelial cell integrity and, therefore, endothelial dysfunction, which refers to impairment of endothelium-dependent vasodilation. *Disruption of endothelium-derived relaxing factors may signal an early stage in atherosclerosis in coronary arteries that precedes development of obstructive coronary artery disease (CAD).*<sup>7,8,9</sup>



[Adapted from Kasprzak JD, Kłosińska M, Drożdż J. 2006.<sup>8]</sup>

# ATHEROSCLEROSIS

- A chronic, immunoinflammatory, fibroproliferative disease of large and medium-sized arteries, fuelled by lipids. (Figure 1.)
- Major cell players are endothelial cells, leukocytes, and intimal smooth muscle cells (SMC).
- The cellular and humoral activity may be responsible for destabilizing the plaque and initiating atherothrombotic events.
- Focal calcification within atherosclerotic plaques is common, increases with age, and is due to both active (osteogenic) and passive (cellular necrosis) processes.<sup>2</sup>



**FIGURE 1.** Key Cellular and Molecular Processes in Endothelial Dysfunction

## THE VITAMIN D RECEPTOR: HERE, THERE, EVERYWHERE

The VDR is expressed widely in organ and cellular systems in the body. Aside from its role in mineral homeostasis, vitamin D exerts effects in cardiovascular, epithelial and immune system tissues. Impairment of VDR activation has been implicated in the dysfunction of vascular smooth muscle and endothelium, and in accelerated atherosclerosis, calcification and cardiac hypertrophy.<sup>10,11</sup> The role in cardiac contractility played by the VDR in cardiomyocytes has been identified in animal studies.<sup>12</sup>

Vitamin D deficiency, determined by serum 25-hydroxyvitamin D [25(OH)D] level, is thought to be common and present in up to 50% of the general population, irrespective of CKD status.<sup>6</sup> Cross-sectional and epidemiological evidence evaluating vitamin D status and CVD risk has been gathering. Among the risk factors associated with low vitamin D are hypertension, elevated triglyceride level, microalbuminuria, and diabetes. In the Framingham Offspring Study, incident cardiovascular events in subjects *without a history of CVD* appeared to be higher where vitamin D deficiency was severe (25(OH)D <10 ng/mL).<sup>13</sup> Interestingly, during the 7-year follow up of 36,282 postmenopausal women in the Women's Health Initiative (WHI) study, calcium and vitamin D supplementation neither increased nor decreased risk for stroke, myocardial infarction, heart failure or coronary heart disease (CHD) death. The authors described possible reasons for this finding, including that the vitamin D dose of 400 IU/day was low; that fracture, not CVD, was the event that the trial was designed to evaluate; or that poor adherence reduced the treatment effect.<sup>14</sup>

Although very few studies have examined vitamin D supplementation and cardiovascular mortality, pre-clinical research is ongoing into the mechanisms by which vitamin D may exert protective effects on inflammatory cytokines, glycemic control, the renin-angiotensin-aldosterone system (RAAS), and directly on the vasculature.<sup>13</sup> Emerging science suggests that VDR activators may favorably affect aortic injury in atherosclerosis<sup>15</sup> and progress of calcification,<sup>16</sup> and thus may have a protective role to play in future therapies that reduce CVD morbidity in patients with CKD.<sup>17</sup> (See page 16)

# CALCIFICATION

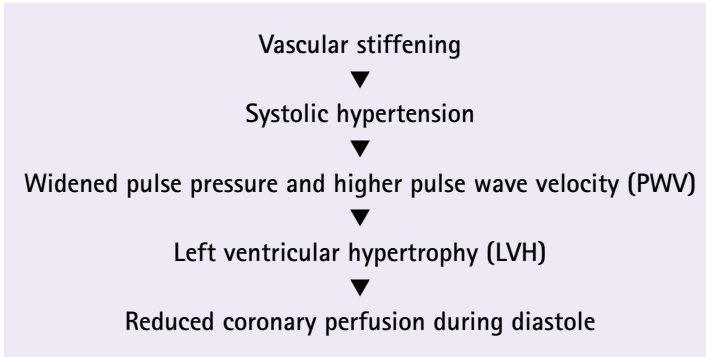
## The Two Major Types of Calcification Affect Different Layers of the Artery<sup>1,2,4,18</sup>

**Atherosclerotic plaque** occurs within the intimal layer. Calcification of the lesions is common, but exhibits a patchy, discontinuous course along the artery. Arterial intimal calcification (AIC) is advanced atherosclerosis, driven by cellular necrosis, inflammation, and lipid deposition.

- Plaques and occlusion develop and the lesions impinge on the lumen:
  - Advanced disease → compromised blood flow → tissue ischemia → necrosis
  - Plaque rupture → thrombus formation → arterial occlusion → acute ischemic events
- AIC has been shown to develop in older individuals and those with clinical history of diabetes, atherosclerotic complications (e.g., vascular nephropathy, calcified common carotid artery [CCA]), longer history of smoking, higher LDL cholesterol levels, and higher C-reactive protein levels.
- End stage renal disease (ESRD)-specific risks for AIC included elevated serum phosphate, lower serum albumin, higher calcium intake, and hemodialysis (HD) duration.<sup>18</sup>
- More recent work has reported that most large, conduit artery (carotid and femoral) calcification is intimal, and related to atherosclerosis risk factors, e.g., older age, elevated C-reactive protein, and carotid intima-media thickness. (Figure 2, Panels A and B) In large arteries, the presence of medial calcification is significantly reduced. (Figure 2, Panel C) Both calcified plaque and the presence of calcium in the intima are atherosclerosis-related calcification, the calcified plaque being a more advanced stage of atherosclerosis.<sup>19</sup>

**Monckeberg's sclerosis** occurs in the medial wall (or tunica media). Calcification increases vascular stiffness and reduces vascular compliance. Arterial medial calcification (AMC) is observed in elastic lamella of the medial layer of conduit arteries.

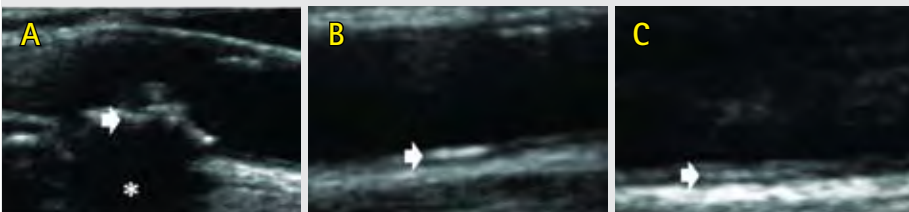
AMC is typically less occlusive of the arterial lumen than AIC, but causes:



London, et al,<sup>18</sup> reported that:

- AMC was more closely associated with HD duration and the *absence of clinical history of CVD at the start of HD therapy*.
- Atherosclerotic plaque could be found in larger arteries, although fewer of the patients with AMC had calcified CCA intimal plaques compared with the patients with AIC.

**FIGURE 2.** Carotid arteries showing (A) highly-calcified plaque, (B) intimal calcification, and (C) medial calcification. [Photo courtesy of B.Coll, MD. Used with permission.]



Vascular calcification exhibits different pathophysiology according to the hemodynamic and structural differences between arteries in different regions of the body.

<b>Conduit Arteries</b> e.g., carotid, coronary, brachial, aorta, iliac	<b>Peripheral Arteries</b> e.g., pedal, digital
<ul style="list-style-type: none"> <li>• Function is to drive the stroke volume delivered by the heart.</li> <li>• Media is poor in VSMC, but rich in elastin.</li> <li>• More prone to atherosclerosis</li> </ul>	<ul style="list-style-type: none"> <li>• Function is to regulate tissue blood flow according to metabolic needs.</li> <li>• Media is dense in VSMC.</li> <li>• More easily calcified e.g., CUA</li> </ul>

**Arteriosclerosis** refers to the reduced arterial compliance due to increased fibrosis, loss of elasticity, and vessel wall calcification affecting the media of large and middle-sized arteries. Age and arterial hypertension are causes. Mechanically, increased arterial stiffness increases systolic pressure because reflected waves are prematurely returned in late systole. Pulse wave velocity and left ventricular (LV) afterload increase, thereby altering coronary perfusion.<sup>20</sup> Changes in aortic PWV independently predict survival in ESRD and the general population.<sup>21</sup>

**Atherosclerosis** refers to intimal lesions, histologically classified as type I to type VI along a continuum of minimal changes to clinically significant lumen stenosis. A type I lesion contains enough atherogenic lipid protein to form scattered macrophage foam cells; type II lesions consist of the foam cells with lipid-laden VSMCs and fatty streaks; type III lesions show extracellular lipid droplets and disruption to the intimal SMCs; type IV is a disruptive atheroma with characteristic lipid core; type V lesions add fibrous connective tissue layers to the lipid core and may calcify (type Vb) or fibrose (type Vc); and type VI lesions demonstrate fissures, hematoma or thrombus. Morbidity and mortality is due largely to types IV and V lesions that disrupt the surface.<sup>22</sup>

**Calciophylaxis/calcific uremic arteriolopathy (CUA)** refers to a potentially life-threatening calcification entity of ESRD, characterized by subcutaneous small vessel media calcification, panniculitis, tissue ischemia, dermal necrosis and ulcerating, painful wounds. Sepsis and amputation are among the morbidities of this obliterative disease. The muscles of the torso, lumbar area and lower limbs are affected. No single treatment approach is superior; aside from management of secondary hyperparathyroidism (SHPT), adjunctive strategies have been studied for their role in ulcer healing (hyperbaric oxygen) and reduction of the vascular calcium load (sodium thiosulfate).<sup>23</sup>



# TRADITIONAL LOCATIONS WHERE CALCIFICATION HAS BEEN STUDIED

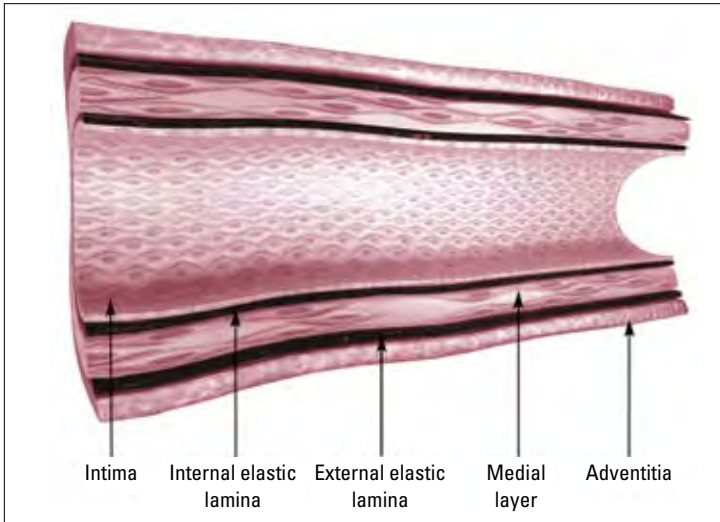


FIGURE 3. The walls of normal blood vessels are composed of distinct layers.

[Reproduced with permission of Remedica Medical Education and Publishing from Ashley EA, Niebauer J, eds. *Cardiology Explained*. 2004]

## 1. **Intimal** calcification and calcification of atheromatous plaques:

- Possibly a healing response to the abnormal deposition of lipids and oxidation products in the subendothelial space.

## 2. **Medial** calcification (Monckeberg's sclerosis) associated with:

- Disturbances of Ca, P and vitamin D metabolism (ESRD)
- LVH from increased left ventricular overload
- Rhythm disturbances

Injury to the **internal elastic lamina** (IEL) may be an under-studied aspect of arterial remodeling in atherosclerotic arteries. The IEL is a membrane of elastin and fibers that separates the intima from the media.<sup>24</sup>

- Membrane *enlargement* may be a compensatory response to expanding plaque size and plaque hemorrhage, and contribute to intimal thickening and luminal narrowing in coronary arteries. IEL *shrinkage* is associated with plaque erosion.
- Smoking, hypercholesterolemia, vessel size and morphological variables within the plaque (such as calcified lipid core size) have been studied in relation to IEL expansion and luminal patency in coronary, carotid and renal arteries.
- The IEL also calcifies in Monckeberg's sclerosis, however the sequence and process of calcific deposits in the media and IEL are among the inconsistencies in the literature regarding this histological finding.<sup>25</sup>

# ASSESSING CALCIFICATION AND ATHEROSCLEROSIS

A noninvasive method of identifying and quantifying calcification in coronary arteries and valves is noncontrast cardiac computed tomography (electron-beam or multislice).<sup>1,26,27</sup> Advantages include reproducibility, safety and convenience. The issues of CT cost or availability aside, one disadvantage is that CT imaging techniques cannot differentiate whether the calcium is in the intima or the media, or identify or quantify *early vascular calcium load* in incident dialysis patients.

## Coronary Artery Calcification Score (CACS)

In the asymptomatic adult population, Agatston calcium scores stratify risk for a cardiovascular event<sup>28</sup> and appear to better predict the risk for future coronary events than age/gender-specific percentile ranking.<sup>29,30</sup>

0-10	Low risk
11-100	Intermediate risk
>100	Signals the progression from intermediate to high risk and thus the need for more aggressive therapy
>400	Highest risk

Although the burden of *calcified atherosclerosis* can be estimated, *non-calcified atherosclerosis* that poses a risk is not captured in the calcification score. Recent research using carotid ultrasound to quantify carotid intima-media thickness (CIMT) suggests that this technique can offer a similarly non-invasive and reproducible way to monitor subclinical atherosclerosis.<sup>31</sup>

## Is CACS of value as a prognostic marker for CVD in dialysis patients?

- Coronary artery calcification is common in advanced CKD and is almost always due to atherosclerosis. There is greater frequency and severity of coronary artery calcification in patients on dialysis, as demonstrated by an up to five-fold higher coronary artery calcium score than in age-matched non-CKD patients.<sup>32</sup> Whilst the above grades for CACS are not different for ESRD versus non-ESRD populations, the incongruous scores between the two groups are worth illustrating. CAC scores in maintenance HD patients are substantially higher and progress more rapidly than in patients without kidney failure but who have suspected and documented coronary artery disease. This was highlighted in a study that found a mean CACS in dialysis patients of 4,290 compared with 406 in the non-dialysis patients.<sup>32</sup>

More recently, Haydar, et al, reported a mean calcium score in ESRD patients of 2370. Within the cohort, there was a much higher mean score (2869) in those with abnormal coronary angiography, and a much lower mean (559) in those with normal angiography, although still of a magnitude that would be indicative of significant CAD in the non-CKD population.<sup>33</sup>

- Observational studies suggest that CACS is an independent predictor of mortality in chronic HD patients after adjusting for age, gender, dialysis vintage and diabetes mellitus, and that a high CACS should prompt early intervention to manage modifiable risk factors such as dyslipidemia and hyperphosphatemia.<sup>34</sup> Shantouf, et al, have more recently shown that total and vessel-specific CAC independently predict mortality in patients receiving maintenance HD.<sup>35</sup>
- Both vascular stiffness and vascular calcification have been found to occur in patients with *earlier stage CKD*.<sup>26,36</sup> Progressive uremia and dialysis vintage have been reported to worsen vascular and valvular calcifications<sup>26,27</sup> whilst age, systolic blood pressure and diabetes seem to increase the fibrosis and loss of elasticity typical of arterial stiffness (arteriosclerosis).<sup>26</sup> (See Table 1.)

Risk Factor	Intimal/Atherosclerotic Calcification	Medial/Monckeberg's Calcification
Dyslipidemia	Yes	No
Advanced age	Yes	Yes
Elevated blood pressure	Yes	Medial lesions worsen BP
Male gender	Yes	No
Smoking	Yes	No
Inflammation	Yes (local)	Yes (systemic mediators)
Diabetes/glucose intolerance	Yes	Yes
Reduced GFR	No	Yes
Hypercalcemia	No	Yes
Hyperphosphatemia	Yes	Yes
PTH abnormalities	No	No
Vitamin D administration	No	Yes
Duration of dialysis	No	Yes

TABLE 1. Vascular Calcification Risk Factors. [Adapted from Goodman WG, et al., 2004.<sup>4</sup>]

- Baseline CAC score has been reported to predict all-cause mortality in *incident* hemodialysis patients.<sup>37</sup> Low or zero CAC score is associated with minimal progression that may be further limited with careful control of mineral metabolism.<sup>38</sup> In fact, non-calcified patients with CKD have a high likelihood of remaining free of cardiovascular calcification over months to years.<sup>6</sup>
- CACS has been shown to be higher in those with hypertension,<sup>32</sup> and to correlate with prevalence of myocardial infarction and angina,<sup>27</sup> and aortic valvular calcification.<sup>32</sup>
- As research progresses on local and systemic regulators of mineralization, biomarkers could help individualize calcification risk assessment. If accelerated calcification could be predicted, treatment for a susceptible patient could be tailored, for example, to calcium-free phosphate binders<sup>1</sup> or selective VDR activators that have differential effects on calcification markers, for example Cbfa1(inducer) and fetuin-A (inhibitor). (See Glossary, page 19)
- Medications used to control calcium, phosphorus and parathyroid hormone (PTH) imbalance in ESRD have been investigated for their impact on CAC score. (See page 14) Recently, the ADVANCE Study did not find significant differences in the primary outcome (percentage change in Agatston coronary calcium score) between treatment groups (cinacalcet plus low-dose vitamin D sterols versus flexible doses of vitamin D sterols without cinacalcet) after 52 weeks of follow-up. Volume coronary score was also analyzed in a post hoc analysis, revealing a significant decrease in the patients assigned to receive cinacalcet.<sup>39</sup> The clinical implications of coronary volume score changes should be taken cautiously until more studies address the relationship between volume score and cardiovascular events.

# DIRECTIONS IN TREATMENT: CAN RISK BE MODIFIED OR REDUCED?

Although the evidence is limited from randomized controlled trials (RCT) in patients with CKD that reducing progression of arterial calcification impacts mortality,<sup>6</sup> the magnitude of CVD risk in these patients, and the prominence of vascular calcification as a component of this risk, underscore a range of implications that are worth considering in the clinical setting.

The CT-based CAC score is the reference standard for detecting cardiovascular calcification in the general and CKD population. However, widely available and less expensive methods, such as lateral abdominal x-ray (for aortic and iliac artery calcifications), PWV measurements (for hemodynamic effects) and echocardiography (for valvular calcification) yield useful assessment information with which to:<sup>4,6</sup>

- **Determine risk** for patients for whom the physician decides that such information impacts therapeutic decision making, for example, regarding phosphate binder therapy in a patient with significant hyperphosphatemia, or in a transplant wait-listed patient.
- **Heighten awareness** among clinicians about the prevalence and risk relationships of calcification and adverse clinical outcomes in patients of all ages with CKD and those with pre-existing coronary artery disease.
- Prompt review of the patient's management plan in order to identify aggravating factors and **implement CVD risk reduction measures**, such as minimizing atherosclerotic risk factors and controlling biochemical parameters of CKD-mineral and bone disorder.
- **Monitor changes over time** so as to evaluate the effectiveness of treatments aimed at modifying disease progression.

## Phosphate Binder Therapy and Calcification

Phosphate binder choice may be important in modifying progression of vascular calcification because of the potential to lower the patient's exogenous calcium load; however, the superiority of one compound over another in terms of reducing mortality is less clear. Several studies have investigated the comparative effect of calcium salts and sevelamer-hydrochloride (HCl), the non-calcium-containing binder and bile acid sequestrant, on progressive coronary artery and aortic calcification, as determined by sequential electron beam CT.

Although comparable in terms of lowering hyperphosphatemia, calcium-containing binders were reported in both incident<sup>37,40</sup> and prevalent<sup>41</sup> hemodialysis patients to result in more hypercalcemia and more rapid progression of coronary calcification compared with sevelamer-HCl. However, in another study comparing calcium acetate with sevelamer-HCl, patients experienced similar progression of CAC, even with the addition of atorvastatin to the regimen to lower LDL cholesterol.<sup>42</sup> The Dialysis Clinical Outcomes Revisited trial (DCOR) reported a trend toward lower mortality in hemodialysis patients older than 65 years of age who were treated with sevelamer versus calcium-containing binders; however, there was no survival benefit demonstrated in the overall study population.<sup>43</sup> In patients with non-dialysis CKD, one study has shown CAC score progression to be lowest in the patients treated with sevelamer-HCl, compared to patients treated with a low phosphate diet alone or a low phosphate diet plus calcium carbonate.<sup>44</sup>

Taken together, these trials have shown that, in addition to their hypophosphatemic effects, phosphate binder choice may achieve attenuation of CAC progression and lowering of LDL cholesterol; however, superiority of agents for reducing cardiovascular mortality has not been proved.

## Anti-atherosclerotic Strategies

Looking at cholesterol crystallization as it may pertain to the calcification of atherosclerotic plaques, and the general role of lipid deposition as a component of atherosclerosis, the question arises about the effect of lipid-lowering treatment on progressive calcification and cardiovascular events. This has been studied using hydroxy-methyl glutaryl-CoA reductase inhibitors (statins) in both hemodialysis and non-CKD populations. These agents do not appear to reverse progression of arterial calcification, despite favorably affecting the patient's atherogenic profile and cardiovascular events.<sup>6</sup>

Arad, et al, studied treatment with atorvastatin and vitamins C and E in a double-blind, placebo-controlled RCT in asymptomatic adults aged 50 to 70 years with coronary calcium scores higher than the 80th percentile for age and gender. Although reductions in total and LDL cholesterol and triglycerides were achieved, a significant reduction in atherosclerotic CVD events was not seen and no effect on calcium score was achieved.<sup>45</sup> In diabetic hemodialysis patients, the 4D trial failed to show benefit on CVD outcomes with atorvastatin treatment.<sup>46</sup> More recently, the AURORA study group reported effective LDL lowering in hemodialysis patients aged over 50 years treated with rosuvastatin; however, this had no effect on composite primary end point of death from cardiovascular causes, nonfatal myocardial infarction, or nonfatal stroke. Hyperphosphatemia was highlighted as a strong risk factor for these end points.<sup>47</sup>

The benefit of statin therapies may lie in younger dialysis patients, those healthy enough for kidney transplantation, or with fewer years of dialysis duration at start of therapy, or with the non-dialysis CKD population, in whom a recent Cochrane review found significantly reduced all-cause and cardiovascular mortality in those receiving statin therapy.<sup>48</sup>

Recently, results were reported for the Study of Heart and Renal Protection (SHARP),<sup>49</sup> a large-scale, international, randomized trial in patients on dialysis and with non-dialysis CKD to assess the effects of lowering LDL cholesterol on time to first major vascular event, and on rate of CKD progression. The investigators compared ezetimibe 10mg daily and simvastatin 20mg daily with placebo and found that the intervention afforded a risk reduction benefit for major atherosclerotic events in both groups of patients without serious side effects.<sup>50</sup>

## EMERGING SCIENCE ABOUT VITAMIN D RECEPTOR ACTIVATION

Vitamin D receptor activators, such as 19-nor-1 $\alpha$ ,25(OH) $_2$ D $_2$  (paricalcitol) or 1 $\alpha$ -(OH)D $_2$  (doxercalciferol) and 22-oxa-1 $\alpha$ -(OH) $_2$ D $_3$  (maxacalcitol), effectively suppress parathyroid hormone and are routinely used to control development and progression of CKD-related secondary hyperparathyroidism, their approved indication. The term “selective” indicates that VDR activation is lower in the gastrointestinal tract and bones than in other organs, accounting for the lower calcemic and phosphatemic effect seen with these agents, compared with calcitriol.<sup>51</sup> The growing understanding about selectivity among physiological actions of vitamin D agents has even prompted a novel term, *D-mimetic*, for VDR activators such as maxacalcitol and paricalcitol that exhibit less calcemia<sup>52</sup> because they differ from calcitriol in terms of biological and gene activation profiles, and therefore modulate VDR functions differently.<sup>53</sup> The World Health Organization has reclassified the selective agents as “other anti-parathyroid agents”, reflecting the gathering data about their diverse physiological actions.<sup>54</sup> The observation of differential tissue effects could be explained by active VDR ligands differing from one another and differing between tissues not directly involved in calcium homeostasis. Mechanisms of tissue-specific target gene activation and inhibition could enable variation in the transport, storage or effect of vitamin D agents to the VDR.<sup>55</sup>

Evidence about the relationship between selective VDR activators and survival advantage, both in HD patients and non-dialysis CKD, is emerging from a range of epidemiological studies. A consistent finding is that treatment with a VDR activator affords a survival benefit, compared with receiving no such treatment.<sup>53</sup> While acknowledging that there are questions not yet answered by well-designed RCTs, and that practitioners may hesitate to extrapolate some observational associations to clinical practice, researchers have cautioned against dismissing the clues from data gained in large observational studies about the effects on hemodialysis patients of VDR activation.<sup>52,56</sup>

Although the conduct of further trials to establish superiority of VDR activators in regards to clinical end points is awaited, pre-clinical research offers insights into their physiological effects. Experimental work by Li, et al, established that VDR knockout mice have increased surrogate markers of CVD, such as elevated blood pressure, elevated activation of the RAAS, and cardiac hypertrophy, and suggested that VDR-mediated mechanisms point to a possible therapeutic role for vitamin D analogues in blood pressure homeostasis.<sup>57</sup>



The comparative effect of calcitriol, doxercalciferol and paricalcitol on aortic calcium content has been studied in animal models, and suggests that VDR activators have different effects on calcification by mechanisms other than their effect on the calcium-phosphorus product. (Figures 4A and 4B) By virtue of their different chemical structure, activators exhibit differential cell and tissue selectivity, and interaction with the VDR, e.g., doxercalciferol may have more consistent bioavailability; paricalcitol has shown lower calcium and phosphorus absorption, lower vascular calcification and less aortic calcium deposition.<sup>53</sup>

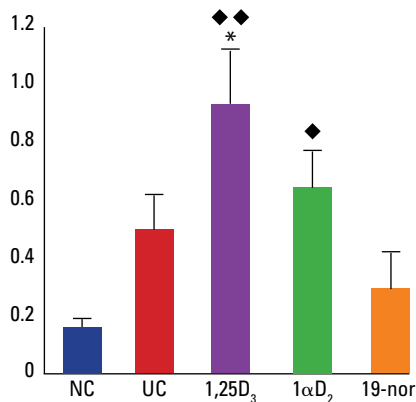
### Recent Experimental Studies of VDR Activators

- Vitamin D agents may have pro- and anti-atherosclerotic properties. For example, in laboratory models, calcitriol appears to influence the gene expression of vascular endothelial growth factor (VEGF), one of the early steps of atherosclerosis development. In an animal model of atherosclerosis, atherosclerotic plaque in the aorta of ApoE-deficient mice was prevented by paricalcitol, by enalapril, and by paricalcitol plus enalapril treatments.<sup>15</sup>
- In a study comparing the effect of three VDR activators on the process of vascular calcification, calcitriol and doxercalciferol but not paricalcitol appeared to increase gene expression of bone-related markers in the aorta, even after titrating the drug doses so as to compare their effect on aortic tissue at similar Ca x P products.<sup>2,16</sup>
- In a comparative study of in vivo effects of paricalcitol and doxercalciferol on cardiac calcification paricalcitol-treated rats showed markedly less aortic calcium at six weeks, compared with those given lower or higher doses of

FIGURE 4A. Effects of 0.04  $\mu\text{g}/\text{kg}$  of calcitriol ( $1,25\text{D}_3$ ), 0.10  $\mu\text{g}/\text{kg}$  of doxercalciferol ( $1\alpha\text{D}_2$ ), or 0.16  $\mu\text{g}/\text{kg}$  of paricalcitol (19-nor) on Runx2 mRNA expression levels in aorta from uremic rats.

Runx2 mRNA levels were analyzed by real-time RT-PCR technique. Each drug was given intraperitoneally three times a week for 1 month. Values are mean  $\pm$  s.e.m. ( $n=6$ ).  $P<0.01$  by analysis of variance. \* $P<0.05$  versus UC; ♦♦ $P<0.01$  and ♦ $P<0.05$  versus 19-nor by *post hoc*, Scheffe test.

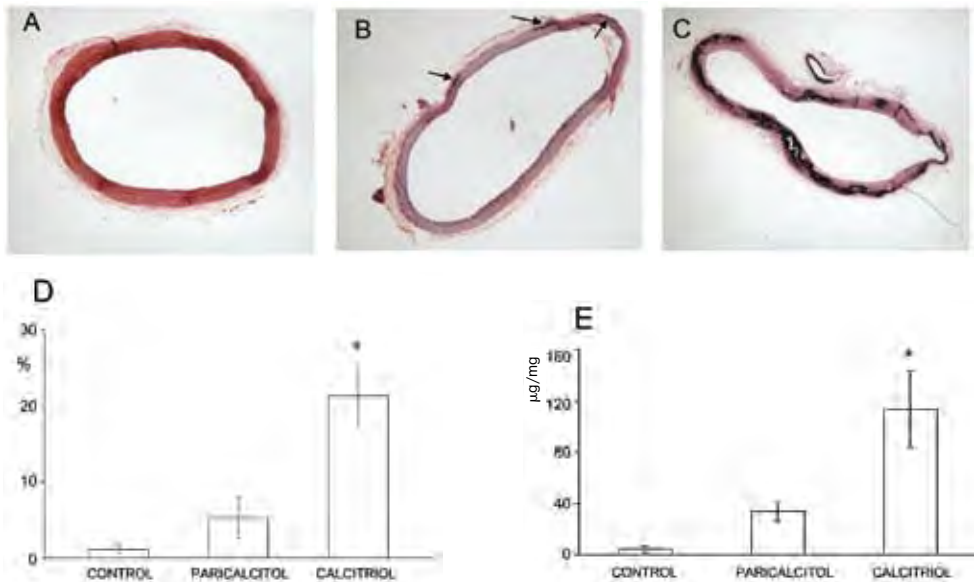
[Adapted from Mizobuchi M, Finch JL, Martin DL, Slatopolsky E. 2007.<sup>16</sup>]



doxercalciferol. In the same study, dose-dependent differential effects on pulse wave velocity were also demonstrated, suggesting that VDR activators differ also in their effect on vascular compliance. Both agents lowered parathyroid hormone levels.<sup>58</sup>

A recent small, randomized controlled clinical study to compare paricalcitol and alfacalcidol is evaluating the suppression of SHPT in patients on maintenance hemodialysis.<sup>59</sup> The authors had a substudy objective to compare the changes in PWV and Augmentation index (Aix) when treated with paricalcitol compared to alfacalcidol. They report an interesting difference after 16 weeks in PWV that, although not of statistical significance, builds on earlier preclinical research<sup>58</sup> and may indicate a difference in the effect on arterial stiffness between these two agents. However, further studies are needed to confirm this.<sup>60</sup>

- The *in vivo* effects of paricalcitol and calcitriol on total calcium content and calcified areas of the abdominal aorta have been investigated in rat models by Cardus, et al.<sup>61</sup> In the same study, the increased gene expression of RANKL in VSMC was 2.5 times higher for calcitriol than for paricalcitol.



**FIGURE 4B.** Effect of the *in vivo* treatment of five-sixths nephrectomized rats with calcitriol (1 µg/kg, three times a week for 8 wk) or paricalcitol (3 µg/kg, three times a week for 8 wk) on aortic calcification.

Representative photographs of von Kossa staining of (A) control animals, (B) animals treated with paricalcitol, or (C) animals treated with calcitriol. (D) Quantification of calcified areas in the aorta. Data are percentage of the media presenting calcification. (E) Quantification of calcium in the aorta. Data are micrograms of calcium per milligram of protein. Data are mean ± SE. \*p < 0.01 vs. control. [Used with permission.]

# GLOSSARY OF SELECTED SYSTEMIC AND LOCAL MINERALIZATION REGULATORS

## CALCIFICATION INDUCERS<sup>1,2</sup>

Alkaline phosphatase (ALP)	Bone-specific ALP acts locally to degrade inorganic pyrophosphate, a potent mineralization inhibitor.
Bone morphogenetic protein-2 (BMP2)	BMPs are cytokines with diverse functions, including osteogenesis, in multiple tissues and in circulation; BMP2 is increased in CKD.
Osteoblast transcription factor Core binding factor $\alpha$ -1 (Cbf $\alpha$ 1/Runx2)	Cbf $\alpha$ 1/Runx2 promotes the change of vascular smooth muscle cells to an osteoblastic phenotype from their mesenchymal precursors in vivo and in vitro. High phosphate concentration upregulates Cbf $\alpha$ 1. <sup>18</sup>
Receptor activator of nuclear factor- $\kappa$ B ligand (RANKL)	Principal regulator of osteoclasts; increases in CKD; levels may predict vascular risk.

## CALCIFICATION INHIBITORS<sup>1,2</sup>

Bone morphogenetic protein 7 (BMP7)	Cytokine expressed in kidney tissue; reduced levels in CKD; reduces serum phosphate levels and calcification in animal models.
Fetuin-A (alpha 2-Heremans-Schmid glycoprotein AHSG)	Serum protein produced in the liver; acts as a negative acute phase reactant and inhibitor of VSMC apoptosis; exerts local and systemic effects; levels are lower in hemodialysis patients, possibly due to inflammation.
Fibroblast growth factor-23 (FGF-23)	Undetermined role, but animal studies suggest deficiency favors hyperphosphatemia, hypercalcemia and medial calcification.
Inorganic pyrophosphate (PPi)	Circulating inhibitor of hydroxyapatite crystal formation.
Matrix Gla protein (MGP)	A low molecular weight protein found in bone, cartilage, kidneys, cardiac valves, media of arteries; acts locally in VSMCs to bind BMP2 and thus limit mineralization.
Osteopontin (OPN)	A phosphoprotein expressed in mineralized tissue; inhibits mineralization of VSMCs in vivo when full length and phosphorylated but <u>when cleaved</u> facilitates vascular mineralization.
Osteoprotegerin (OPG)	A decoy receptor for RANKL expressed in many cells and tissues, especially the arterial media; may inhibit ALP activity.

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