Arteriosclerosis, Thrombosis, and Vascular Biology

ATVB IN FOCUS:
Nontraditional Risk Factors for Peripheral Arterial Disease: The Evidence and Underlying Mechanisms

Series Editors: Matthew Allison, Jonathan Golledge, and Janet Powell

Peripheral Vascular Calcification

Sujin Lee,* Jenny Woo,* Yabing Chen, Shipra Arya, Matthew Allison

ABSTRACT: Peripheral artery disease commonly refers to diffuse atherothrombotic disease of the arteries supplying the legs. Peripheral artery disease has been relatively understudied and has not been subject to the same intensive scrutiny and research that characterizes coronary artery disease. Moreover, the diagnosis of peripheral artery disease can be complicated by the presence of arterial calcification. Here, we provide a brief report on the current understanding of peripheral vascular calcification to include the following sections: basic mechanisms, anatomic distribution of arterial calcification, manifestations, risk factors, measurement of peripheral calcification, nonsurgical treatments, and surgical interventions.

Key Words: arteries ■ coronary artery disease ■ peripheral arterial disease ■ risk factors ■ vascular calcification

Globally, lower extremity peripheral artery disease (PAD) affects ≈113 million adults over the age of 45 years and is associated with considerable functional limitations,[1] as well as future cardiovascular disease morbidity and mortality.[2] This condition is typically diagnosed using the ankle-brachial index (ABI), which is the ratio of the highest systolic blood pressure at the ankle divided by the highest brachial artery pressure. A limitation of the ABI is artifactually elevated systolic pressures at the ankle due to arterial calcification, which can result in normal values despite the presence of significant flow-limiting disease. In this report, we provide a concise overview of the current gaps in understanding the fundamental mechanisms and therapeutic strategies related to peripheral arterial calcification, focusing on the abdominal aorta and distal vasculature.

BASIC MECHANISMS

Clinical and experimental research have revealed that vascular calcification is an actively regulated biological process.[3] Osteochondrogenic reprogramming of vascular cells, particularly vascular smooth muscle cells (VSMC), which dedifferentiate and transition into bone-like cells, is recognized as a major contributor to vascular calcification. Metabolic disorders, imbalanced mineral metabolisms, mitochondrial dysfunction,[4] and impaired DNA repair,[5] commonly associated with aging, diabetes, and chronic kidney disease (CKD), have been shown to promote vascular calcification via multifaceted cellular mechanisms, including loss of inhibitory factors for mineralization, oxidative stress, inflammation, cellular senescence, autophagy, and apoptosis.[6]

Excessive intracellular levels of reactive oxygen species, arising from impaired mitochondrial oxidative phosphorylation, reduced ATP production, and accumulation of mitochondrial DNA damage, lead to oxidative stress: a major inducer of vascular calcification. Oxidative stress decreases VSMC contractility and increases osteogenic transdifferentiation.[3,7] For example, we and others have shown that hydrogen peroxide (H_2O_2) directly promotes osteogenic differentiation and calcification of VSMC by activating protein kinase B/AKT signaling pathways, which induces the expression of Runx2 (Runt-related transcription factor 2), a master osteogenic transcription factor.[8] H_2O_2 also inhibits the phagocytosis of

Correspondence to: Matthew Allison, Division of Preventive Medicine, Department of Family Medicine, University of California San Diego, San Diego, CA. Email mallison@health.ucsd.edu
*S. Lee and J. Woo are cofirst authors.
For Sources of Funding and Disclosures, see page XXX.

Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

Nonstandard Abbreviations and Acronyms

ABI	ankle-brachial index
ALP	alkaline phosphatase
ARIC	Atherosclerosis Risk in Communities
CKD	chronic kidney disease
CT	computed tomography
Disrupt PAD III	Shockwave Medical Peripheral Lithoplasty System Study for PAD
ENPP1	ectonucleotide pyrophosphatase/phosphodiesterase 1
IC	intermittent claudication
INTACT	International Nifedipine Trial on Anti-Atherosclerotic Therapy
MAC	medial artery calcification
NF-κB	nuclear factor-κB
PAD	peripheral artery disease
Runx2	Runt-related transcription factor 2
VSMC	vascular smooth muscle cells

Highlights

- Peripheral arterial calcification arises from coordinated mechanisms involving vascular smooth muscle cell transdifferentiation, disrupted mineral metabolism, and chronic inflammation.
- The heterogeneity of peripheral calcification is shaped by anatomic, mechanical, and cellular factors, underscoring the need for a tailored approach to peripheral artery calcification diagnosis and treatment.
- Current therapies for peripheral vascular calcification are primarily supportive or disease-modifying, emphasizing the unmet need for interventions that directly prevent or reverse calcification.

extracellular vesicles, increasing calcification nodule formation.[9] Moreover, oxidative stress is linked to aging-associated epigenetic modifications, such as DNA methylation and histone acetylation, which induce VSMC phenotypic switching and calcification.[6] Autophagy, regulated by oxidative stress, is also implicated in calcification through its role in maintaining cellular proteostasis.[10]

Impaired calcium and phosphate homeostasis, as manifested by hyperphosphatemia and hypercalcemia, promotes vascular calcification in CKD, which is associated with increased oxidative stress and decreased calcification inhibitors, such as fetuin-A and pyrophosphates.[11] Elevated serum calcium and phosphate levels are associated with vascular calcification and a higher risk of cardiovascular events and mortality in the general population and CKD patients.[12–14] In this regard, higher levels of serum phosphorus were found to independently predict the incidence and prevalence of vascular calcification, comparable to traditional cardiovascular disease risk factors.[15] At the cellular level, extracellularly added calcium comparable to the levels observed in hypercalcemic subjects, with or without the addition of high phosphate, induced VSMC calcification.[16,17] Extracellular phosphorus led to intracellular phosphorus influx and VSMC calcification in culture.[18] Calcium and phosphorus induced VSMC calcification and vascular calcification in vivo were mediated by the osteogenic transcription factor Runx2, Msx2, and osterix.[16,18] In addition, increased oxidative stress, systematically or locally in aortic arteries, is a key feature of the uremic state in CKD patients and uremic rats,[19] which promoted vascular calcification and Runx2 upregulation. Consistent with the essential role of Runx2 in VSMC calcification,[7] smooth muscle cell–specific Runx2 deletion attenuated vascular calcification in a mouse model of CKD.[20]

Dysmetabolic processes in diabetes and CKD activate multiple signaling pathways (eg, protein kinase B/AKT, protein kinase Cδ, and extracellular signal–regulated kinase/mitogen-activated protein kinase),[21] which are known to converge on Runx2 upregulation via phosphorylation-dependent posttranslational modifications. These pathways enhance Runx2 transcriptional activity, a crucial step in the osteogenic transdifferentiation of VSMC and subsequent vascular calcification.

Other posttranslational modifications on Runx2 regulate its stability and transcriptional activity. For example, bone morphogenetic protein-2 induces Runx2 acetylation that enhances its activity,[22] whereas Smurf1/2 E3 ligase–mediated ubiquitination contributes to Runx2 degradation or functional alterations.[23] In addition, increased O-GlcNAcylation of Runx2 is associated with enhanced stability and transcriptional activation, promoting VSMC calcification.[24] Recently, PTM by carbonylation at Runx2 lysine residue 176 was discovered to stabilize Runx2, thereby increasing Runx2 protein levels and its transcriptional activity, and accelerating vascular calcification in CKD.[25]

Decreased calcification inhibitors, such as fetuin-A and pyrophosphate, often found in serum from CKD patients, may result in loss of inhibition on calcium and phosphate-induced signals.[26] Fetuin-A, a serum glycoprotein that binds to calcium and forms stable colloidal calciprotein particles, serves as a systemic inhibitor of mineralization,[27] whereas pyrophosphate binds to hydroxyapatite and counteracts the crystalline complex formation of phosphate with calcium to form hydroxyapatite.[28] Mechanistically, fetuin-A can be taken up by VSMC and secreted via release of matrix vesicles, thereby inhibiting matrix vesicle–initiated nucleation of calcium phosphate that leads to the development of vascular calcification.[29] Consistently, fetuin-A deficiency in

mice resulted in vascular calcification and multiple organ morbidity, although it is worth noting that calcification observed in these mice began as microcrystals on the endothelial layer as opposed to medial calcification seen in tibial artery disease in PAD.[30]

Pyrophosphate inhibited high inorganic phosphate-induced VSMC calcification, possibly via reducing the formation of hydroxyapatite nanocrystals.[31] Mice deficient in ENPP1 (ectonucleotide pyrophosphatase phosphodiesterase 1), an enzyme that synthesizes extracellular pyrophosphate, exhibited low plasma pyrophosphate concentrations that led to rapid development of severe aortic calcification.[28] A recent study found that oral pyrophosphate protects Abcc6$^{-/-}$ mice against vascular calcification induced by CKD.[32] Of note, the beneficial effects of bisphosphonates, nonhydrolysable analogs of pyrophosphates, on vascular calcification in humans seem to be limited to disease status and study populations.[11]

Early vascular aging, characterized by premature arterial stiffening and medial vascular calcification, is often seen as a distinctive pathological process in CKD.[33,34] Multiple molecular pathways associated with early vascular aging have also been linked to Runx2 upregulation in VSMC. For instance, elevated oxidative stress and inflammation during vascular aging can enhance Runx2 activity through NF-κB (nuclear factor-κB), BMP2, and Wnt/β-catenin signaling cascades.[35–37] The senescence-associated secretory phenotype of senescent VSMC, a hallmark of vascular aging, releases proinflammatory cytokines, such as IL (interleukin)-6 and IL-8, along with matrix metalloproteinases, which further stimulate Runx2 expression and extracellular matrix remodeling.[37] Concurrently, VSMC exposed to vascular aging release procalcific, medin-enriched extracellular vesicles that promote extracellular matrix reorganization and vascular calcification.[6,38,39]

Beyond the role of VSMCs, several circulating and residential vascular cells have been shown to exhibit calcifying potential, including adventitial myofibroblasts, valvular interstitial cells, endothelial cells (via mesenchymal transition), pericytes, and vascular progenitor cells.[40–42] Upregulation of Runx2 is associated with calcification of multiple vascular cells.[8] In addition, adaptive cellular events and intercellular crosstalk may accelerate vascular calcification. For instance, angiogenesis has been postulated to promote vascular calcification by serving as a conduit for circulating progenitor cells or pericytes and producing endothelial cells–derived cytokines, inflammatory, and angiogenic factors that directly and indirectly affect osteogenesis of vascular cells.[42] Furthermore, the crosstalk between immune cells and vascular cells has also been documented in vascular calcification.[43] Monocytes/macrophages promote VSMC calcification in a coculture system,[44,45] whereas calcifying VSMC induces migration and differentiation of macrophage/monocyte differentiation into osteoclasts, which may further contribute to vascular calcification in atherosclerosis.[46]

RISK FACTORS

Atherosclerosis is the predominant cause of PAD and is associated with traditional risk factors, including hypertension, hyperlipidemia, diabetes, and smoking.[47] In particular, metabolic risk factors, such as impaired glucose tolerance and impaired fasting glucose, are associated with increased incidence of cardiovascular disease, where affected patients are more likely to have subclinical coronary atherosclerosis,[48] as well as worse clinical outcomes after lower extremity interventions.[49]

Medial artery calcification (MAC) is most commonly associated with increasing age, diabetes, and CKD.[50] These risk factors reflect the underlying mechanisms that occur in MAC, including hyperphosphatemia seen in CKD, hyperglycemia, and the production of advanced glycation end products present in diabetes, and age-related increase in oxidative stress and DNA damage; all of which drive VSMC osteogenic phenotype switch, calcium phosphate deposition, and extracellular matrix remodeling seen in vascular calcification.[51]

A major traditional risk factor underlying both intimal and medial arterial calcification (MAC) is hypertension. At a mechanistic level, increased pressure in the arterial wall due to hypertension increases VSMC growth in large vessels while causing a cascade of abnormalities in small vessels, including volume dysregulation and enhanced vasoconstriction, leading to adaptive changes in ion transport and electrolyte metabolism at the cellular level that enhance VSMC proliferation and hypertrophy.[52] Furthermore, hypertension-related vascular remodeling results in arteriosclerosis with the renin-angiotensin system driving the VSMC osteogenic phenotype switch as demonstrated in preclinical models.

Efforts to improve cardiovascular disease risk assessment have included the evaluation of nontraditional risk factors in risk assessment models. CKD patients in particular have an elevated risk for PAD, as demonstrated in the ARIC (Atherosclerosis Risk in Communities) study that found an 82% higher age-, sex-, and race-adjusted risk for PAD in CKD patients, compared with those with normal kidney function.[53] Among patients with CKD, those with increased inflammatory markers, including C-reactive protein, white blood cell count, fibrinogen, myeloperoxidase, and increased calcification markers, including ALP (alkaline phosphatase), had an elevated risk for incident PAD.[54] These biomarkers reflect the osteogenic changes that occur to the vasculature in hyperphosphatemia, where calcium deposition in the vasculature can induce inflammation that, in turn, can trigger the upregulation of osteogenic transcription factors in VSMCs, underlying the vicious cycle of vascular

calcification that is a hallmark of patients with CKD.[55] Furthermore, calciprotein particles, which are circulating calcium and phosphate nanoparticles associated with CKD, are internalized by VSMCs and evoke transdifferentiation into osteogenic phenotype via mechanisms including inflammatory signaling and oxidative stress.[56] These particles induce the expression and secretion of proinflammatory cytokines, including IL-1β, IL-6, IL-8, and TNF-α (tumor necrosis factor-α), thus demonstrating a direct link between calcification and elevated circulating inflammatory markers. However, studies have yet to show direct in vivo imaging or tracing of labeled calciprotein particles in the arterial wall. Such evidence would have significant implications for calciprotein particles being procalcific cargo carriers rather than passive precipitates of calcium-phosphate imbalance in CKD, thereby shifting the calcification model from that of pure extracellular mineral nucleation to a combined intra/extracellular model.

Similarly, diabetes accelerates vascular calcification in PAD through a complex interplay of metabolic, inflammatory, and cellular dysfunction. Advanced glycation end products are well-established byproducts of chronic hyperglycemia that bind to receptors on VSMCS to promote oxidative stress and inflammation, as well as calcification signaling.[57,58] Diabetes induces a proinflammatory state that includes inflammatory cytokines (IL-6 and TNFα and reactive oxygen species to promote osteogenic differentiation of VSMCs.[59] More recently, hyperglycemia has been shown to disrupt extracellular pyrophosphate metabolism by decreasing ENPP1 activity and promoting the breakdown of ATP to inorganic phosphate instead of pyrophosphate, thereby decreasing the pyrophosphate to phosphate ratio and leading to increased calcification.[60] The novel connection between impaired pyrophosphate metabolism and VSMC calcification in diabetes underscores shared mechanisms driving vascular calcification across diverse etiologies.

GENETIC DISORDERS OF VASCULAR CALCIFICATION

Beyond the aforementioned risk factors, genetic factors may confer an increased risk for developing premature vascular calcification, including Mendelian disorders, such as generalized arterial calcification of infancy and arterial calcification due to deficiency of CD73. These are rare monogenic disorders of severe vascular calcification associated with genetic mutations that result in dysfunction of enzymes in the extracellular ATP-adenosine conversion pathway. In generalized arterial calcification of infancy, mutations in ENPP1 result in extensive calcification of large- and medium-sized vessels, including the lower extremities, and have a 55% mortality rate before 6 months of age due to strokes and cardiac events.[61] ENPP1 encodes an enzyme that hydrolyzes ATP to AMP and inorganic pyrophosphate, both of which are endogenous inhibitors of calcification.[62] ENPP1's role in vascular calcification is underscored by its identification as a locus in the largest multiancestry genome-wide association study meta-analysis of CAC to date.[63] ENPP1 polymorphisms are also found in diabetes, cardiovascular disease, and stroke[64] further implicating ENPP1 as an important regulator of vascular calcification.

Arterial calcification due to deficiency of CD73 is a rare adult-onset disorder of vascular calcification, where mutations in CD73 result in dysfunction of an enzyme that converts AMP to adenosine and inorganic phosphate. The reduction in extracellular adenosine levels leads to increased alkaline phosphatase activity (ALPL), which degrades pyrophosphate and consequently increases calcification.[65] Patients with arterial calcification due to deficiency of CD73 develop lower extremity claudication with diffuse circumferential calcification often seen in the femoral-popliteal arteries.[65] Interestingly, calcific femoral-popliteal arteries from patients without arterial calcification due to deficiency of CD73 also exhibit decreased CD73 compared with noncalcific arteries, highlighting the significance of CD73 as a potential therapeutic target in PAD.[66]

ANATOMIC DISTRIBUTION OF CALCIFICATION

In patients with PAD, the anatomic distribution of lower extremity arterial disease is typically segmented into the aortoiliac, femoral-popliteal, and tibial arteries. These patients often present with fibrocalcific plaque of atherosclerosis.[67] Fibrocalcific plaque formation shows a predilection for bifurcation sites and tortuous vessels.

Intimal calcification, which is associated with atherosclerosis, is characterized by lipid accumulation, inflammation, fibrosis, and development of focal plaques, whereas medial calcification is associated with hydroxyapatite deposition, which directly increases arterial stiffness and is commonly seen in patients with diabetes and ESRD. Although intimal calcification typically occurs in advanced atherosclerotic lesions, often promoted by localizing biomechanical factors,[68] MAC occurs early in the course of the disease along elastic lamellae. Medial calcification is hypothesized to be the major contributor of arterial calcification and stiffness in PAD.[69]

The hemodynamic effects on plaque formation are evidenced by the high incidence of athero-calcific plaque in the superficial femoral artery in the adductor canal region and the popliteal artery above the knee.[70] In the adductor canal, the superficial femoral artery is surrounded by tight muscles before it curves to cross the sharp border of the aponeurosis and dives into surrounding soft, fatty tissue.[71] Similarly, the location of the popliteal artery

at the knee flexure may cause repeated trauma to the vessel, inducing structural change in the arterial wall. By contrast, tibial vessels, which have the highest incidence of vascular calcification in the lower extremities (33%), more commonly demonstrate MAC.[72] MAC is characterized by calcium phosphate deposition in the medial layer of the vessel wall and is frequently seen in patients with increased age, diabetes, or CKD.

The anatomic distribution of vascular calcification is also categorized based on the size of the affected vessels. Microvascular calcification (ie, vessel diameter of ≈100 µm) is a hallmark of calciphylaxis, which is a rare and highly morbid disease of accelerated arteriolar calcification.[73] Calciphylaxis is commonly found in patients with CKD but is also associated with diabetes, hyperparathyroidism, vitamin D or K deficiencies, and female sex. Calciphylaxis lesions predominantly appear in the abdomen and lower extremities, with 1 single-center study reporting up to 80% of lesions occurring in the lower extremities.[74]

MEASUREMENT OF PERIPHERAL CALCIFICATION

The cornerstone of diagnosing PAD is the physical examination, with attention to diminished or absent pulses, trophic skin changes, and ulceration. The ABI and toe-brachial index are commonly used to quantify the potential degree of obstructive disease, with the systolic pressure at the ankle or toe, respectively, divided by the higher of the 2 brachial systolic pressures used to quantify the reduction in blood flow to the lower extremities. However, studies have shown the limitations of the ABI. In one study, 43% of patients with PAD who presented with symptoms and exhibited ≥50% stenosis on duplex ultrasound received normal or inconclusive resting ABIs.[75] Moreover, patients with diabetes, CKD, coronary artery disease, or tissue loss have been observed to have vessel incompressibility, which can lead to the artificial elevation of the ABI, and thus a missed diagnosis.[75] Pulse volume recordings and duplex ultrasound offer additional insight into hemodynamic significance and anatomic location of stenoses or occlusions, with ultrasound able to visualize plaque morphology and detect calcification to a limited extent.

Certain patterns of calcification can help distinguish medial from intimal calcification. In surface ultrasound, MAC is recognized on longitudinal views by echogenic abluminal bands and typically smooth endothelial interfaces. An x-ray railroad parallel track appearance is pathognomonic of MAC versus eccentric plaque-related calcium is usually intimal calcification. Computed tomography (CT) detects calcium as high-density signals, but conventional CT cannot differentiate between MAC and intimal calcification. Newer CT techniques such as dual-energy CT and micro-CT may be able to differentiate the 2, whereas invasive methods of intravascular ultrasound or optical coherence tomography can distinguish between the 2 types of calcification.[76]

Peripheral arterial calcification scoring is a method to evaluate PAD and overcome the limitations of vessel incompressibility. These scoring systems are applied through the use of imaging modalities such as X-ray and CT, ultrasound, magnetic resonance imaging, magnetic resonance angiography, and catheter angiography.[77] Intravascular imaging, including intravascular ultrasound, can identify intimal versus medial calcification,[50] but it is invasive. Magnetic resonance imaging has been noted to be less sensitive for evaluating arterial calcification,[78] whereas CT imaging also does not show microcalcification or distinguish between medial versus intimal calcification.

Current calcification scoring systems include the MAC score and adaptations of the Agatston score for CT imaging of lower limbs.[79] The MAC score, applied via foot radiographs, assigns 1 point per vascular segment with calcification exceeding 2 cm in the dorsalis pedis, plantar, and metatarsal arteries, or >1 cm in the hallux and nonhallux digital arteries, up to a maximum of 5.[80] Pedal MAC scores have similarly been used in patients with chronic limb-threatening ischemia.[81] CT-based approaches extend Agatston scoring to lower limb arteries, including the aortoiliac, femoral-popliteal, and tibial segments.[82–84] Vessel-by-vessel calcium scoring has also been done via CT imaging of the femoral-popliteal, anterior tibial, tibioperoneal trunk, posterior tibial, and peroneal arteries.[85]

Current national guidelines[69] recommend duplex ultrasound, CT angiography, magnetic resonance angiography, and catheter angiography. Duplex ultrasound is listed as a modality with the least risk, if trained technical and medical personnel are present. CT angiography and magnetic resonance angiography were mentioned to provide greater detail though there are risks of radiation or contrast exposure. Catheter angiography was mentioned to have a higher level of risk and was only to be restricted to more severe cases of PAD.

MANIFESTATIONS

PAD can be clinically categorized into 2 levels of increasing severity: (1) intermittent claudication, where patients experience cramping lower extremity pain with activity and (2) chronic limb-threatening ischemia, where patients experience rest pain in their foot, tissue loss, or gangrene.

Based on the symptoms of patients with PAD, the presence of flow-limiting lesions can be localized. For instance, in the aortoiliac system, which comprises the infrarenal aorta as well as the common iliac, external, and

internal iliac arteries, patients may present with buttock or lower extremity claudication, impotence, and absence of femoral pulses (ie, Leriche syndrome). Similarly, stenosis in the femoral-popliteal arteries or the tibial and pedal arteries manifests as ischemic symptoms in the calf or foot just distal to the location of a hemodynamically significant lesion.

Arterial calcification reduces vessel compliance, causes arterial wall stiffness, and alters blood flow dynamics.[86] In larger arteries, medial arterial stiffness increases pulse pressure and cardiac afterload, both of which have been associated with left ventricular hypertrophy and heart failure. In smaller arteries, arterial calcification has been associated with stasis and a diminished autoregulatory response, which may lead to reduced perfusion.[87] Moreover, calcified arteries exhibit poor arterial compliance, decreased vasodilatory response, and an impaired ability to recover from ischemic events.[88]

NONSURGICAL TREATMENTS

Several mechanism-driven therapeutic strategies have been tested in preclinical models to evaluate their potential to alleviate vascular calcification. For instance, antioxidant therapies such as resveratrol have shown efficacy in mitigating oxidative stress–induced Runx2 activation and vascular calcification.[89] Inhibiting Wnt signaling, exemplified by the use of niclosamide, has been shown to effectively suppress the expression of osteogenic genes, including *Runx2*, and reduce vascular calcification.[90] Glucose-lowering agents like sodium-glucose cotransporter-2 inhibitors not only exhibit high efficacy in glycemic control but also directly attenuate vascular calcification by promoting Runx2 proteasomal degradation,[91] supporting the use of sodium-glucose cotransporter-2 inhibitors as a compelling therapeutic option in diabetes-associated vascular calcification.

Calcium channel blockers are antihypertensive agents that inhibit voltage-dependent calcium channels in the plasma membrane and thus decrease intracellular calcium content. Preclinical studies have demonstrated a reduction in vascular calcification in animal models that received calcium channel blockers. However, clinical trials studying other calcium channel blockers have failed to demonstrate a beneficial effect on vascular calcification.[92,93] For example, the INTACT trial (International Nifedipine Trial on Anti-Atherosclerotic Therapy) investigated the effect of nifedipine on CAC progression and found no effect on the progression of existing lesions.[94]

In a vitamin D model of calcification in rabbits, calcified arteries demonstrated an upregulation in angiotensin 1 receptor, whereas treatment with angiotensin receptor blockers prevented vascular calcification.[95] Similarly, a vitamin D model of vascular calcification in rats demonstrated an association between increased arterial calcification and increased levels of angiotensin II and aldosterone in the tissue, where treatment with captopril and spironolactone decreased calcification.[96] Notably, pharmacological inhibition of the renin-angiotensin axis in preclinical studies has demonstrated a decrease in the osteogenic phenotype switch of VSMCs in vitro and in vivo, although clinical trials in humans have yet to demonstrate a consistent improvement in vascular calcification.

Given the similarities in the underlying mechanism of vascular calcification with that of bone formation, bisphosphonates have been evaluated as a potential therapy. Preclinical studies have demonstrated a dose-dependent response to bisphosphonates in the reduction of vascular calcification in a rabbit model of atheroarteriosclerosis.[97] Large human studies have demonstrated that bisphosphonate use in elderly women is associated with decreased vascular calcification.[98] However, the majority of clinical studies report a lack of anticalcifying effects of bisphosphonates in conventional doses.[99] SNF472, the hexasodium salt of phytic acid, is a novel calcification inhibitor that binds to hydroxyapatite crystals to prevent further calcium deposition in the arterial wall.[100] Clinical trials have demonstrated a reduction in calciphylaxis-related events and a survival benefit of SNF472 administration in patients with calciphylaxis.[101]

Although numerous mechanism-driven therapies have demonstrated promise in preclinical models for attenuating vascular calcification, their translation into effective, targeted treatments in clinical settings remains limited. Many of these interventions exert indirect effects on vascular calcification by influencing upstream molecular pathways or systemic metabolic states. Moreover, agents like calcium channel blockers and bisphosphonates, despite showing efficacy in animal models, have yielded inconsistent or negligible results in clinical trials, often failing to halt the progression of existing calcific lesions. Novel compounds such as SNF472 show potential in acute, high-risk contexts like calciphylaxis, yet their applicability to more common, progressive forms of vascular calcification is not well established. As such, current therapies remain largely supportive or disease-modifying rather than directly calcification-targeting, underscoring the need for strategies capable of reversing or preventing chronic, progressive vascular calcification.

SURGICAL INTERVENTIONS

Calcification presents a major barrier to the success of endovascular interventions in PAD, as it impairs vessel compliance, hinders device delivery, and increases the risk of procedural complications. Several advanced techniques have been proposed to address this challenge, including subintimal recanalization, retrograde tibial or pedal access, direct puncture of calcified plaque, and the direct extravascular calcium interruption procedure.[102–104] Intravenous lithotripsy (IVL) is considered the gold standard for heavily calcified lesions, which utilizes a balloon

Table. Summary of Diagnostics and Clinical Management of Peripheral Artery Calcification

Domain	Key concepts	Evidence-based guidelines
Risk factors	Diabetes	Diabetes, CKD, and aging are the strongest predictors of medial arterial calcification in peripheral arteries.[108]
	CKD	
	Aging	
	Hyperphosphatemia, hypercalcemia	
	Inflammation and oxidative stress	
	Genetic predisposition	
Clinical manifestation	Associated with limb ischemia, impaired wound healing, and amputation risk	Higher medial artery calcification has been shown to correlate with lower toe pressures and higher WIfI scores, indicating more severe ischemia and poor wound healing.[109]
	Increased arterial stiffness and pulse pressure	
Diagnostic approaches	ABI >1.40 (noncompressible arteries)+TBI<0.7 demonstrating decreased perfusion	TBI <0.70 suggests PAD in patients with high ABI. CT calcium scoring is being investigated for prognostic value in PAD and awaits further validation through large-scale prospective studies.
	WIfI scores	
	Plain X-ray: Linear vessel wall calcification	
	Duplex ultrasound: Acoustic shadowing from calcified segments	
	CT scan/CTA: Quantitative calcium scoring	
Management strategies	No specific therapy to reverse calcification	Emphasis on prevention and modulation of progression. SNF472 (calcification inhibitor) in phase II trials.[100]
	Risk factor control: Optimize diabetes, phosphate balance, PTH levels in CKD	
	Statins: Limited effect on calcification progression	
	Sodium thiosulfate, SNF472, etidronate under investigation for calcification inhibition	
Revascularization considerations	Calcification may limit success of endovascular interventions (eg, poor balloon expansion, stent underdeployment)	Device choice is critical in severe PAC. Preprocedural imaging (CT) often needed for planning.
	Use of IVL or atherectomy may be required	
Prognosis and outcomes	Peripheral artery calcification is associated with worse limb outcomes (eg, nonhealing ulcers, amputations) and cardiovascular events	Peripheral calcification is an independent predictor of major adverse limb and cardiovascular events.[50,110]
	Higher mortality in patients with coexisting medial calcification and PAD	

ABI indicates ankle-brachial index; CKD, chronic kidney disease; CT, computed tomography; CTA, computed tomography angiography; IVL, intravascular lithotripsy; PAD, peripheral artery disease; TBI, toe-brachial index; and WIfI, wound, ischemia, and foot infection.

catheter with integrated sonic pressure wave generators to fracture calcium deposits, enabling better vessel expansion. The Disrupt PAD III trial (Shockwave Medical Peripheral Lithoplasty System Study for PAD) demonstrated that IVL followed by drug-coated balloon angioplasty significantly outperformed standard percutaneous transluminal angioplasty in patients with severely calcified femoropopliteal lesions, showing higher procedural success and primary patency at 1 year, with fewer major dissections and reduced need for provisional stenting.[105] Similarly, a meta-analysis of 9 studies involving 681 patients found that IVL reduced diameter stenosis by over 59% with a low incidence of complications, confirming its efficacy across a broad patient population.[106]

Atherectomy, which physically removes plaque using specialized cutting or rotational devices, has also shown benefit in treating dense, calcified lesions. In pooled patient-level analyses, directional atherectomy achieved substantial acute luminal gain and reduced residual stenosis, though it may carry a higher embolic risk compared with IVL.[107] In addition, cutting and scoring balloons have been used to facilitate controlled plaque modification by creating linear fractures in the calcified lesion, improving the efficacy of subsequent balloon angioplasty. These devices are particularly useful when calcium is eccentric or superficial and may be used in conjunction with IVL or atherectomy in hybrid strategies.

In practice, the selection of technique depends on lesion morphology, distribution of calcium, and operator expertise, but evidence increasingly supports the use of IVL and atherectomy-alone or in combination for optimizing outcomes in the most challenging calcified PAD cases (Table).

CONCLUSIONS

Arterial calcification in the lower extremities among patients with significant obstructive disease creates diagnostic and therapeutic challenges. Because calcification typically occurs in those with diabetes or CKD, these patients are particularly vulnerable to worse clinical outcomes such as tissue loss, gangrene, and subsequent repetitive amputation. As such, there is a dire need to further elucidate the mechanisms of peripheral vascular calcification, as well as the traditional and nontraditional risk factors, to optimize risk stratification and treatment strategies for these patients.

ARTICLE INFORMATION

Affiliations

Division of Vascular Surgery and Endovascular Therapy, Heart and Vascular Institute, Mass General Brigham, Boston (S.L.). Division of Vascular Surgery, Department of Surgery (J.W.) and Division of Vascular Surgery, Department of Surgery (S.A.), Stanford University, Palo Alto, CA. Department of Pathology and Laboratory Medicine, Oregon Health Sciences University and Research Department, Portland Veterans Affairs Medical Center (Y.C.). Division of Preventive Medicine, Department of Family Medicine, University of California San Diego (M.A.).

Acknowledgments

The impetus for this work was provided by Dr Janet Powell, to who we pay tribute in her memory.

Sources of Funding

The original research of Y. Chen is supported by the National Institutes of Health (NIH) HL146103, HL158097, HL167201, and AG082839; the United States Department of Veterans Affairs BX005800, BX004426, BX006321, and CX002706; and American Heart Association (AHA) 23TPA1066040. The original research of S. Arya is supported by the NIH UG3HL181184, the United States Department of Veterans Affairs I01HX003215 and AHA 25TPA1479692.

Disclosures

None.

REFERENCES

1. GBD 2019 Peripheral Artery Disease Collaborators. Global burden of peripheral artery disease and its risk factors, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet Glob Health*. 2023;11:e1553–e1565. doi: 10.1016/S2214-109X(23)00355-8
2. Enos WF, Holmes RH, Beyer J. Landmark article, July 18, 1953: coronary disease among United States soldiers killed in action in Korea. Preliminary report. By William F. Enos, Robert H. Holmes and James Beyer. *JAMA*. 1986;256:2859–2862. doi: 10.1001/jama.256.20.2859
3. Chen X, He Y, Fu W, Sahebkar A, Tan Y, Xu S, Li H. Histone deacetylases (HDACs) and atherosclerosis: a mechanistic and pharmacological review. *Front Cell Dev Biol*. 2020;8:581015. doi: 10.3389/fcell.2020.581015
4. Tyrrell DJ, Blin MG, Song J, Wood SC, Zhang M, Beard DA, Goldstein DR. Age-associated mitochondrial dysfunction accelerates atherogenesis. *Circ Res*. 2020;126:298–314. doi: 10.1161/CIRCRESAHA.119.315644
5. Uryga A, Gray K, Bennett M. DNA damage and repair in vascular disease. *Annu Rev Physiol*. 2016;78:45–66. doi: 10.1146/annurev-physiol-021115-105127
6. Sutton NR, Malhotra R, St Hilaire C, Aikawa E, Blumenthal RS, Gackenbach G, Goyal P, Johnson A, Nigwekar SU, Shanahan CM, et al. Molecular mechanisms of vascular health: insights from vascular aging and calcification. *Arterioscler Thromb Vasc Biol*. 2023;43:15–29. doi: 10.1161/ATVBAHA.122.317332
7. Byon CH, Javed A, Dai Q, Kappes JC, Clemens TL, Darley-Usmar VM, McDonald JM, Chen Y. Oxidative stress induces vascular calcification through modulation of the osteogenic transcription factor Runx2 by AKT signaling. *J Biol Chem*. 2008;283:15319–15327. doi: 10.1074/jbc.M800021200
8. Chen Y, Zhao X, Wu H. Transcriptional programming in arteriosclerotic disease: a multifaceted function of the Runx2 (runt-related transcription factor 2). *Arterioscler Thromb Vasc Biol*. 2021;41:20–34. doi: 10.1161/ATVBAHA.120.313791
9. Suzuki H, Hayakawa M, Kobayashi K, Takiguchi H, Abiko Y. H_2O_2-derived free radicals treated fibronectin substratum reduces the bone nodule formation of rat calvarial osteoblast. *Mech Ageing Dev*. 1997;98:113–125. doi: 10.1016/s0047-6374(97)00077-8
10. Dai XY, Zhao MM, Cai Y, Guan QC, Zhao Y, Guan Y, Kong W, Zhu WG, Xu MJ, Wang X. Phosphate-induced autophagy counteracts vascular calcification by reducing matrix vesicle release. *Kidney Int*. 2013;83:1042–1051. doi: 10.1038/ki.2012.482
11. Byon CH, Chen Y. Molecular mechanisms of vascular calcification in chronic kidney disease: the link between bone and the vasculature. *Curr Osteoporos Rep*. 2015;13:206–215. doi: 10.1007/s11914-015-0270-3
12. Larsson TE, Olauson H, Hagström E, Ingelsson E, Arnlöv J, Lind L, Sundström J. Conjoint effects of serum calcium and phosphate on risk of total, cardiovascular, and noncardiovascular mortality in the community. *Arterioscler Thromb Vasc Biol*. 2010;30:333–339. doi: 10.1161/ATVBAHA.109.196675
13. Adeney KL, Siscovick DS, Ix JH, Seliger SL, Shlipak MG, Jenny NS, Kestenbaum BR. Association of serum phosphate with vascular and valvular calcification in moderate CKD. *J Am Soc Nephrol*. 2009;20:381–387. doi: 10.1681/ASN.2008040349
14. West SL, Swan VJ, Jamal SA. Effects of calcium on cardiovascular events in patients with kidney disease and in a healthy population. *Clin J Am Soc Nephrol*. 2010;5(Suppl 1):S41–S47. doi: 10.2215/CJN.05860809
15. Tuttle KR, Short RA. Longitudinal relationships among coronary artery calcification, serum phosphorus, and kidney function. *Clin J Am Soc Nephrol*. 2009;4:1968–1973. doi: 10.2215/CJN.01250209
16. Reynolds JL, Joannides AJ, Skepper JN, McNair R, Schurgers LJ, Proudfoot D, Jahnen-Dechent W, Weissberg PL, Shanahan CM. Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. *Clin J Am Soc Nephrol*. 2004;15:2857–2867. doi: 10.1097/01.ASN.0000141960.01035.28
17. Yang H, Curinga G, Giachelli CM. Elevated extracellular calcium levels induce smooth muscle cell matrix mineralization in vitro. *Kidney Int*. 2004;66:2293–2299. doi: 10.1111/j.1523-1755.2004.66015.x
18. 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. doi: 10.1161/01.RES.0000216409.20863.e7
19. Yamada S, Taniguchi M, Tokumoto M, Toyonaga J, Fujisaki K, Suehiro T, Noguchi H, Iida M, Tsuruya K, Kitazono T. The antioxidant tempol ameliorates arterial medial calcification in uremic rats: important role of oxidative stress in the pathogenesis of vascular calcification in chronic kidney disease. *J Bone Miner Res*. 2011;27:474–485. doi: 10.1002/jbmr.539
20. Cobb AM, Yusoff S, Hayward R, Ahmad S, Sun M, Verhulst A, D'Haese PC, Shanahan CM. Runx2 (Runt-related transcription factor 2) links the DNA damage response to osteogenic reprogramming and apoptosis of vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol*. 2021;41:1339–1357. doi: 10.1161/ATVBAHA.120.315206
21. Heath JM, Sun Y, Yuan K, Bradley WE, Litovsky S, Dell'Italia LJ, Chatham JC, Wu H, Chen Y. Activation of AKT by O-linked N-acetylglucosamine induces vascular calcification in diabetes mellitus. *Circ Res*. 2014;114:1094–1102. doi: 10.1161/CIRCRESAHA.114.302968
22. Jeon EJ, Lee KY, Choi NS, Lee MH, Kim HN, Jin YH, Ryoo HM, Choi JY, Yoshida M, Nishino N, et al. Bone morphogenetic protein-2 stimulates Runx2 acetylation. *J Biol Chem*. 2006;281:16502–16511. doi: 10.1074/jbc.M512494200
23. Choi YH, Kim YJ, Jeong HM, Jin YH, Yeo CY, Lee KY. Akt enhances Runx2 protein stability by regulating Smurf2 function during osteoblast differentiation. *FEBS J*. 2014;281:3656–3666. doi: 10.1111/febs.12887
24. Zhang W, Sun Y, Yang Y, Chen Y. Impaired intracellular calcium homeostasis enhances protein O-GlcNAcylation and promotes vascular calcification and stiffness in diabetes. *Redox Biol*. 2023;63:102720. doi: 10.1016/j.redox.2023.102720
25. Zhai X, Cao S, Wang J, Qiao B, Liu X, Hua R, Zhao M, Sun S, Han Y, Wu S, et al. Carbonylation of Runx2 at K176 by 4-hydroxynonenal accelerates vascular calcification. *Circulation*. 2024;149:1752–1769. doi: 10.1161/CIRCULATIONAHA.123.065830
26. Chen Y, Zhao X, Wu H. Arterial stiffness. *Arterioscler Thromb Vasc Biol*. 2020;40:1078–1093. doi: 10.1161/ATVBAHA.120.313131
27. Schäfer C, Heiss A, Schwarz A, Westenfeld R, Ketteler M, Floege J, Muller-Esterl W, Schinke T, Jahnen-Dechent W. The serum protein α2–Heremans-Schmid glycoprotein/fetuin-A is a systemically acting inhibitor of ectopic calcification. *J Clin Invest*. 2003;112:357–366. doi: 10.1172/JCI17202
28. Lomashvili KA, Narisawa S, Millan JL, O'Neill WC. Vascular calcification is dependent on plasma levels of pyrophosphate. *Kidney Int*. 2014;85:1351–1356. doi: 10.1038/ki.2013.521
29. Kapustin AN, Chatrou MLL, Drozdov I, Zheng Y, Davidson SM, Soong D, Furmanik M, Sanchis P, De Rosales RTM, Alvarez-Hernandez D, et al. Vascular smooth muscle cell calcification is mediated by regulated exosome secretion. *Circ Res*. 2015;116:1312–1323. doi: 10.1161/CIRCRESAHA.116.305012
30. Herrmann M, Babler A, Moshkova I, Gremse F, Kiessling F, Kusebauch U, Nelea V, Kramann R, Moritz RL, McKee MD, et al. Lumenal calcification and microvasculopathy in fetuin-A-deficient mice lead to multiple organ morbidity. *PLoS One*. 2020;15:e0228503. doi: 10.1371/journal.pone.0228503
31. Prosdocimo DA, Wyler SC, Romani AM, O'Neill WC, Dubyak GR. Regulation of vascular smooth muscle cell calcification by extracellular pyrophosphate homeostasis: synergistic modulation by cyclic AMP and hyperphosphatemia. *Am J Physiol Cell Physiol*. 2010;298:C702–C713. doi: 10.1152/ajpcell.00419.2009
32. Bouderlique E, Kervadec J, Tang E, Zaworski J, Coudert A, Rubera I, Duranton C, Khan E, Haymann JP, Leftheriotis G, et al. Oral pyrophosphate protects Abcc6$^{-/-}$ mice against vascular calcification induced by chronic kidney disease. *J Mol Med (Berl)*. 2024;102:1217–1227. doi: 10.1007/s00109-024-02468-y
33. Dai L, Schurgers LJ, Shiels PG, Stenvinkel P. Early vascular ageing in chronic kidney disease: impact of inflammation, vitamin K, senescence and genomic damage. *Nephrol Dial Transplant*. 2020;35(Suppl 2):ii31–ii37. doi: 10.1093/ndt/gfaa006

34. Shanahan CM. Mechanisms of vascular calcification in CKD-evidence for premature ageing? *Nat Rev Nephrol*. 2013;9:661–670. doi: 10.1038/nrneph.2013.176
35. El Assar M, Angulo J, Rodríguez-Mañas L. Oxidative stress and vascular inflammation in aging. *Free Radic Biol Med*. 2013;65:380–401. doi: 10.1016/j.freeradbiomed.2013.07.003
36. Derwall M, Malhotra R, Lai CS, Beppu Y, Aikawa E, Seehra JS, Zapol WM, Bloch KD, Yu PB. Inhibition of bone morphogenetic protein signaling reduces vascular calcification and atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2012;32:613–622. doi: 10.1161/ATVBAHA.111.242594
37. Ortiz-Montero P, Londoño-Vallejo A, Vernot JP. Senescence-associated IL-6 and IL-8 cytokines induce a self- and cross-reinforced senescence/inflammatory milieu strengthening tumorigenic capabilities in the MCF-7 breast cancer cell line. *Cell Commun Signal*. 2017;15:17. doi: 10.1186/s12964-017-0172-3
38. Whitehead M, Yusoff S, Ahmad S, Schmidt L, Mayr M, Madine J, Middleton D, Shanahan CM. Vascular smooth muscle cell senescence accelerates medin aggregation via small extracellular vesicle secretion and extracellular matrix reorganization. *Aging Cell*. 2023;22:e13746. doi: 10.1111/acel.13746
39. Duer M, Cobb AM, Shanahan CM. DNA damage response: a molecular lynchpin in the pathobiology of arteriosclerotic calcification. *Arterioscler Thromb Vasc Biol*. 2020;40:e193–e202. doi: 10.1161/ATVBAHA.120.313792
40. Parhami F, Morrow AD, Balucan J, Leitinger N, Watson AD, Tintut Y, Berliner JA, Demer LL. Lipid oxidation products have opposite effects on calcifying vascular cell and bone cell differentiation. A possible explanation for the paradox of arterial calcification in osteoporotic patients. *Arterioscler Thromb Vasc Biol*. 1997;17:680–687. doi: 10.1161/01.atv.17.4.680
41. Yao Y, Jumabay M, Ly A, Radparvar M, Cubberly MR, Bostrom KI. A role for the endothelium in vascular calcification. *Circ Res*. 2013;113:495–504. doi: 10.1161/CIRCRESAHA.113.301792
42. Collett GD, Canfield AE. Angiogenesis and pericytes in the initiation of ectopic calcification. *Circ Res*. 2005;96:930–938. doi: 10.1161/01.RES.0000163634.51301.0d
43. Zhao Y, Huang Z, Gao L, Ma H, Chang R. Osteopontin/SPP1: a potential mediator between immune cells and vascular calcification. *Front Immunol*. 2024;15:1395596. doi: 10.3389/fimmu.2024.1395596
44. Tintut Y, Patel J, Territo M, Saini T, Parhami F, Demer LL. Monocyte/macrophage regulation of vascular calcification in vitro. *Circulation*. 2002;105:650–655. doi: 10.1161/hc0502.102969
45. Deuell KA, Callegari A, Giachelli CM, Rosenfeld ME, Scatena M. RANKL enhances macrophage paracrine pro-calcific activity in high phosphate-treated smooth muscle cells: dependence on IL-6 and TNF-alpha. *J Vasc Res*. 2012;49:510–521. doi: 10.1159/000341216
46. Byon CH, Sun Y, Chen J, Yuan K, Mao X, Heath JM, Anderson PG, Tintut Y, Demer LL, Wang D, et al. Runx2-upregulated receptor activator of nuclear factor κB ligand in calcifying smooth muscle cells promotes migration and osteoclastic differentiation of macrophages. *Arterioscler Thromb Vasc Biol*. 2011;31:1387–1396. doi: 10.1161/ATVBAHA.110.222547
47. Selvin E, Erlinger TP. Prevalence of and risk factors for peripheral arterial disease in the United States: results from the National Health and Nutrition Examination Survey, 1999-2000. *Circulation*. 2004;110:738–743. doi: 10.1161/01.CIR.0000137913.26087.F0
48. Meigs JB, Larson MG, D'Agostino RB, Levy D, Clouse ME, Nathan DM, Wilson PWF, O'Donnell CJ. Coronary artery calcification in type 2 diabetes and insulin resistance: the Framingham Offspring Study. *Diabetes Care*. 2002;25:1313–1319. doi: 10.2337/diacare.25.8.1313
49. van Haelst ST, Haitjema S, de Vries JP, Moll FL, Pasterkamp G, den Ruijter HM, de Borst GJ. Patients with diabetes differ in atherosclerotic plaque characteristics and have worse clinical outcome after iliofemoral endarterectomy compared with patients without diabetes. *J Vasc Surg*. 2017;65:414.e5–421.e5. doi: 10.1016/j.jvs.2016.06.110
50. Dong Y, Liu Y, Cheng P, Liao H, Jiang C, Li Y, Liu S, Xu X. Lower limb arterial calcification and its clinical relevance with peripheral arterial disease. *Front Cardiovasc Med*. 2023;10:1271100. doi: 10.3389/fcvm.2023.1271100
51. Ho CY, Shanahan CM. Medial arterial calcification: an overlooked player in peripheral arterial disease. *Arterioscler Thromb Vasc Biol*. 2016;36:1475–1482. doi: 10.1161/ATVBAHA.116.306717
52. Harvey A, Montezano AC, Touyz RM. Vascular biology of ageing-Implications in hypertension. *J Mol Cell Cardiol*. 2015;83:112–121. doi: 10.1016/j.yjmcc.2015.04.011
53. Wattanakit K, Folsom AR, Selvin E, Coresh J, Hirsch AT, Weatherley BD. Kidney function and risk of peripheral arterial disease: results from the Atherosclerosis Risk in Communities (ARIC) study. *J Am Soc Nephrol*. 2007;18:629–636. doi: 10.1681/ASN.2005111204
54. Chen J, Mohler ER, Xie D, Shlipak M, Townsend RR, Appel LJ, Ojo A, Schreiber M, Nessel L, Zhang X, et al; CRIC Study Investigators. Traditional and non-traditional risk factors for incident peripheral arterial disease among patients with chronic kidney disease. *Nephrol Dial Transplant*. 2016;31:1145–1151. doi: 10.1093/ndt/gfv418
55. Zhang YX, Tang RN, Wang LT, Liu BC. Role of crosstalk between endothelial cells and smooth muscle cells in vascular calcification in chronic kidney disease. *Cell Prolif*. 2021;54:e12980. doi: 10.1111/cpr.12980
56. Kutikhin AG, Feenstra L, Kostyunin AE, Yuzhalin AE, Hillebrands JL, Krenning G. Calciprotein particles: balancing mineral homeostasis and vascular pathology. *Arterioscler Thromb Vasc Biol*. 2021;41:1607–1624. doi: 10.1161/ATVBAHA.120.315697
57. Basta G, Schmidt AM, De Caterina R. Advanced glycation end products and vascular inflammation: implications for accelerated atherosclerosis in diabetes. *Cardiovasc Res*. 2004;63:582–592. doi: 10.1016/j.cardiores.2004.05.001
58. Hangai M, Takebe N, Honma H, Sasaki A, Chida A, Nakano R, Togashi H, Nakagawa R, Oda T, Matsui M, et al. Association of advanced glycation end products with coronary artery calcification in Japanese subjects with type 2 diabetes as assessed by skin autofluorescence. *J Atheroscler Thromb*. 2016;23:1178–1187. doi: 10.5551/jat.30155
59. Turner ME, Aikawa E. Updating the paradigm: inflammation as a targetable modulator of medial vascular calcification. *Cardiovasc Res*. 2023;119:2259–2261. doi: 10.1093/cvr/cvad139
60. Flores-Roco A, Lago BM, Villa-Bellosta R. Elevated glucose levels increase vascular calcification risk by disrupting extracellular pyrophosphate metabolism. *Cardiovasc Diabetol*. 2024;23:405. doi: 10.1186/s12933-024-02502-w
61. Ferreira CR, Kintzinger K, Hackbarth ME, Botschen U, Nitschke Y, Mughal MZ, Baujat G, Schnabel D, Yuen E, Gahl WA, et al. Ectopic calcification and hypophosphatemic rickets: natural history of ENPP1 and ABCC6 deficiencies. *J Bone Miner Res*. 2021;36:2193–2202. doi: 10.1002/jbmr.4418
62. Goding JW, Grobben B, Slegers H. Physiological and pathophysiological functions of the ecto-nucleotide pyrophosphatase/phosphodiesterase family. *Biochim Biophys Acta*. 2003;1638:1–19. doi: 10.1016/s0925-4439(03)00058-9
63. Kavousi M, Bos MM, Barnes HJ, Lino Cardenas CL, Wong D, Lu H, Hodonsky CJ, Landsmeer LPL, Turner AW, Kho M, et al. Multi-ancestry genome-wide study identifies effector genes and druggable pathways for coronary artery calcification. *Nat Genet*. 2023;55:1651–1664. doi: 10.1038/s41588-023-01518-4
64. Bochenski J, Placha G, Wanic K, Malecki M, Sieradzki J, Warram JH, Krolewski AS. New polymorphism of ENPP1 (PC-1) is associated with increased risk of type 2 diabetes among obese individuals. *Diabetes*. 2006;55:2626–2630. doi: 10.2337/db06-0191
65. St Hilaire C, Ziegler SG, Markello TC, Brusco A, Groden C, Gill F, Carlson-Donohoe H, Lederman RJ, Chen MY, Yang D, et al. NT5E mutations and arterial calcifications. *N Engl J Med*. 2011;364:432–442. doi: 10.1056/NEJMoa0912923
66. Moorhead WJ, Chu CC, Cuevas RA, Callahan J, Wong R, Regan C, Boufford CK, Sur S, Liu M, Gomez D, et al. Dysregulation of FOXO1 (forkhead box O1 protein) drives calcification in arterial calcification due to deficiency of CD73 and is present in peripheral artery disease. *Arterioscler Thromb Vasc Biol*. 2020;40:1680–1694. doi: 10.1161/ATVBAHA.119.313765
67. Demer LL, Tintut Y. Vascular calcification: pathobiology of a multifaceted disease. *Circulation*. 2008;117:2938–2948. doi: 10.1161/CIRCULATIONAHA.107.743161
68. Stary HC, Chandler AB, Dinsmore RE, Fuster V, Glagov S, Insull W, Rosenfeld ME, Schwartz CJ, Wagner WD, Wissler RW. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arterioscler Thromb Vasc Biol*. 1995;15:1512–1531. doi: 10.1161/01.atv.15.9.1512
69. David Smith C, Gavin Bilmen J, Iqbal S, Robey S, Pereira M. Medial artery calcification as an indicator of diabetic peripheral vascular disease. *Foot Ankle Int*. 2008;29:185–190. doi: 10.3113/FAI.2008.0185
70. Han Y, Guan M, Zhu Z, Li D, Chen H, Yuan C, Li C, Wang W, Zhao X. Assessment of longitudinal distribution of subclinical atherosclerosis in femoral arteries by three-dimensional cardiovascular magnetic resonance vessel wall imaging. *J Cardiovasc Magn Reson*. 2018;20:60. doi: 10.1186/s12968-018-0482-7

71. Banerjee S. Superficial femoral artery is not left anterior descending artery. *Circulation*. 2016;134:901–903. doi: 10.1161/CIRCULATIONAHA.116.023690
72. Yuan C, Ni L, Zhang C, Hu X, Wu X. Vascular calcification: New insights into endothelial cells. *Microvasc Res*. 2021;134:104105. doi: 10.1016/j.mvr.2020.104105
73. Nigwekar SU, Thadhani R, Brandenburg VM. Calciphylaxis. *N Engl J Med*. 2018;379:399–400. doi: 10.1056/NEJMc1807324
74. Gallo Marin B, Aghagoli G, Hu SL, Massoud CM, Robinson-Bostom L. Calciphylaxis and kidney disease: a review. *Am J Kidney Dis*. 2023;81:232–239. doi: 10.1053/j.ajkd.2022.06.011
75. AbuRahma AF, Adams E, AbuRahma J, Mata LA, Dean LS, Caron C, Sloan J. Critical analysis and limitations of resting ankle-brachial index in the diagnosis of symptomatic peripheral arterial disease patients and the role of diabetes mellitus and chronic kidney disease. *J Vasc Surg*. 2020;71:937–945. doi: 10.1016/j.jvs.2019.05.050
76. Zhang L, Li L, Feng G, Fan T, Jiang H, Wang Z. Advances in CT techniques in vascular calcification. Review. *Front Cardiovasc Med*. 2021;8:716822. doi: 10.3389/fcvm.2021.716822
77. Procaccini L, Olivieri M, Serafini FL, Mantini C, Maffei E, Cademartiri F. *Cardiovascular Calcification*. 2021:177–204.
78. McDermott MM, Greenland P, Liu K, Guralnik JM, Celic L, Criqui MH, Chan C, Martin GJ, Schneider J, Pearce WH, et al. The ankle brachial index is associated with leg function and physical activity: the Walking and Leg Circulation Study. *Ann Intern Med*. 2002;136:873–883. doi: 10.7326/0003-4819-136-12-200206180-00008
79. Ferraresi R, Ucci A, Pizzuto A, Losurdo F, Caminiti M, Minnella D, Casini A, Clerici G, Montero-Baker M, Mills J. A novel scoring system for small artery disease and medial arterial calcification is strongly associated with major adverse limb events in patients with chronic limb-threatening ischemia. *J Endovasc Ther*. 2021;28:194–207. doi: 10.1177/1526602820966309
80. Liu IH, Wu B, Krepkiy V, Ferraresi R, Reyzelman AM, Hiramoto JS, Schneider PA, Conte MS, Vartanian SM. Pedal arterial calcification score is associated with the risk of major amputation in chronic limb-threatening ischemia. *J Vasc Surg*. 2022;75:270.e3–278.e3. doi: 10.1016/j.jvs.2021.07.235
81. Darwish M, Thananayagam T, Addous S, Davies H, Gwilym BL, Meecham L. Predictive ability of the pedal medial arterial calcification score for major adverse limb events among patients with chronic limb-threatening ischemia. *Ann Vasc Surg*. 2025;110(Pt A):385–395. doi: 10.1016/j.avsg.2024.08.017
82. Chowdhury MM, Makris GC, Tarkin JM, Joshi FR, Hayes PD, Rudd JHF, Coughlin PA. Lower limb arterial calcification (LLAC) scores in patients with symptomatic peripheral arterial disease are associated with increased cardiac mortality and morbidity. *PLoS One*. 2017;12:e0182952. doi: 10.1371/journal.pone.0182952
83. Huang CL, Wu IH, Wu YW, Hwang JJ, Wang SS, Chen WJ, Lee WJ, Yang WS. Association of lower extremity arterial calcification with amputation and mortality in patients with symptomatic peripheral artery disease. *PLoS One*. 2014;9:e90201. doi: 10.1371/journal.pone.0090201
84. Guzman RJ, Brinkley DM, Schumacher PM, Donahue RM, Beavers H, Qin X. Tibial artery calcification as a marker of amputation risk in patients with peripheral arterial disease. *J Am Coll Cardiol*. 2008;51:1967–1974. doi: 10.1016/j.jacc.2007.12.058
85. Rimmerman ET, Musini KN, Chou TH, Wynveen MK, Patel SA, Beall M, Bobbey AJ, Atway SA, Go MR, Stacy MR. Vessel-by-vessel computed tomography calcium scoring of the foot in peripheral artery disease: association with patient-level factors. *Adv Wound Care (New Rochelle)*. 2023;12:603–610. doi: 10.1089/wound.2022.0151
86. Soldatos G, Jandeleit-Dahm K, Thomson H, Formosa M, D'orsa K, Calkin AC, Cooper ME, Ahimastos AA, Kingwell BA. Large artery biomechanics and diastolic dysfunction in patients with Type 2 diabetes. *Diabet Med*. 2011;28:54–60. doi: 10.1111/j.1464-5491.2010.03146.x
87. Lanzer P, Boehm M, Sorribas V, Thiriet M, Janzen J, Zeller T, St Hilaire C, Shanahan C. Medial vascular calcification revisited: review and perspectives. *Eur Heart J*. 2014;35:1515–1525. doi: 10.1093/eurheartj/ehu163
88. Guo X, Kassab GS. Increased serum klotho with age-related aortic stiffness and peripheral vascular resistance in young and middle-aged swine. *Front Physiol*. 2020;11:591. doi: 10.3389/fphys.2020.00591
89. Lee J, Hong SW, Kim MJ, Kwon H, Park SE, Rhee EJ, Lee WY. Metformin, resveratrol, and exendin-4 inhibit high phosphate-induced vascular calcification via AMPK-RANKL signaling. *Biochem Biophys Res Commun*. 2020;530:374–380. doi: 10.1016/j.bbrc.2020.07.136
90. Tanaka T, Asano T, Okui T, Kuraoka S, Singh SA, Aikawa M, Aikawa E. Computational screening strategy for drug repurposing identified niclosamide as inhibitor of vascular calcification. *Front Cardiovasc Med*. 2021;8:826529. doi: 10.3389/fcvm.2021.826529
91. Wu S, Luo X, Chen Y, Wang Z, Liu X, Sun N, Zhao J, Luo W, Zhang J, Tong X, et al. Sodium-glucose cotransporter 2 inhibitors attenuate vascular calcification by suppressing endoplasmic reticulum protein thioredoxin domain containing 5 dependent osteogenic reprogramming. *Redox Biol*. 2024;73:103183. doi: 10.1016/j.redox.2024.103183
92. Pitt B, Byington RP, Furberg CD, Hunninghake DB, Mancini GB, Miller ME, Riley W. Effect of amlodipine on the progression of atherosclerosis and the occurrence of clinical events. PREVENT Investigators. *Circulation*. 2000;102:1503–1510. doi: 10.1161/01.cir.102.13.1503
93. Bruining N, de Winter S, Roelandt JR, Rodriguez-Granillo GA, Heller I, van Domburg RT, Hamers R, de Feijter PJ; EUROPA/PERSPECTIVE Investigators. Coronary calcium significantly affects quantitative analysis of coronary ultrasound: importance for atherosclerosis progression/regression studies. *Coron Artery Dis*. 2009;20:409–414. doi: 10.1097/MCA.0b013e32832fa9b8
94. Lichtlen PR, Hugenholtz PG, Rafflenbeul W, Hecker H, Jost S, Deckers JW. Retardation of angiographic progression of coronary artery disease by nifedipine. Results of the International Nifedipine Trial on Antiatherosclerotic Therapy (INTACT). INTACT Group Investigators. *Lancet*. 1990;335:1109–1113. doi: 10.1016/0140-6736(90)91121-p
95. Armstrong ZB, Boughner DR, Drangova M, Rogers KA. Angiotensin II type 1 receptor blocker inhibits arterial calcification in a pre-clinical model. *Cardiovasc Res*. 2011;90:165–170. doi: 10.1093/cvr/cvq391
96. Henry PD, Bentley KI. Suppression of atherogenesis in cholesterol-fed rabbit treated with nifedipine. *J Clin Invest*. 1981;68:1366–1369. doi: 10.1172/jci110384
97. Rosenblum IY, Flora L, Eisenstein R. The effect of disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP) on a rabbit model of athero-arteriosclerosis. *Atherosclerosis*. 1975;22:411–424. doi: 10.1016/0021-9150(75)90021-0
98. Elmariah S, Delaney JA, O'Brien KD, Budoff MJ, Vogel-Claussen J, Fuster V, Kronmal RA, Halperin JL. Bisphosphonate use and prevalence of valvular and vascular calcification in women MESA (The Multi-Ethnic Study of Atherosclerosis). *J Am Coll Cardiol*. 2010;56:1752–1759. doi: 10.1016/j.jacc.2010.05.050
99. Hildebrand S, Cunningham J. Is there a role for bisphosphonates in vascular calcification in chronic kidney disease? *Bone*. 2021;142:115751. doi: 10.1016/j.bone.2020.115751
100. Perelló J, Ferrer MD, Del Mar Pérez M, Kaesler N, Brandenburg VM, Behets GJ, D'Haese PC, Garg R, Isern B, Gold A, et al. Mechanism of action of SNF472, a novel calcification inhibitor to treat vascular calcification and calciphylaxis. *Br J Pharmacol*. 2020;177:4400–4415. doi: 10.1111/bph.15163
101. Sinha S, Nigwekar SU, Brandenburg V, Gould LJ, Serena TE, Moe SM, Aronoff GR, Chatoth DK, Hymes JL, Carroll KJ, et al. Hexasodium fytate for the treatment of calciphylaxis: a randomised, double-blind, phase 3, placebo-controlled trial with an open-label extension. *EClinicalMedicine*. 2024;75:102784. doi: 10.1016/j.eclinm.2024.102784
102. Ichihashi S, Sato T, Iwakoshi S, Itoh H, Kichikawa K. Technique of percutaneous direct needle puncture of calcified plaque in the superficial femoral artery or tibial artery to facilitate balloon catheter passage and balloon dilation of calcified lesions. *J Vasc Interv Radiol*. 2014;25:784–788. doi: 10.1016/j.jvir.2014.01.016
103. Kum S, Huizing E, Samarakoon LB, Lim D, Unlu C, Sato T. The direct extravascular calcium interruption arterial procedure technique for heavily calcified vessels. *J Vasc Surg Cases Innov Tech*. 2020;6:369–373. doi: 10.1016/j.jvscit.2020.04.007
104. McGuirl D, Giles KA. Retrograde tibio-pedal access for endovascular interventions for treating peripheral arterial disease. *Ann Vasc Surg*. 2024;107:136–139. doi: 10.1016/j.avsg.2023.12.105
105. Tepe G, Brodmann M, Werner M, Bachinsky W, Holden A, Zeller T, Mangalmurti S, Nolte-Ernsting C, Bertolet B, Scheinert D, et al; Disrupt PAD III Investigators. Intravascular lithotripsy for peripheral artery calcification: 30-day outcomes from the randomized disrupt PAD III trial. *JACC Cardiovasc Interv*. 2021;14:1352–1361. doi: 10.1016/j.jcin.2021.04.010
106. Wong CP, Chan LP, Au DM, Chan HWC, Chan YC. Efficacy and safety of intravascular lithotripsy in lower extremity peripheral artery disease: a systematic review and meta-analysis. *Eur J Vasc Endovasc Surg*. 2022;63:446–456. doi: 10.1016/j.ejvs.2021.10.035

107. Madhavan MV, Shahim B, Mena-Hurtado C, Garcia L, Crowley A, Parikh SA. Efficacy and safety of intravascular lithotripsy for the treatment of peripheral arterial disease: an individual patient-level pooled data analysis. *Catheter Cardiovasc Interv*. 2020;95:959–968. doi: 10.1002/ccd.28729
108. Lanzer P, Hannan FM, Lanzer JD, Janzen J, Raggi P, Furniss D, Schuchardt M, Thakker R, Fok PW, Saez-Rodriguez J, et al. Medical arterial calcification: JACC state-of-the-art review. *J Am Coll Cardiol*. 2021;78:1145–1165. doi: 10.1016/j.jacc.2021.06.049
109. Sundaram S, Barksdale C, Rodriguez S, Wooster MD. The impact of small artery disease (SAD) and medial arterial calcification (MAC) scores on chronic wound and amputation healing: can it tell us more? *Ann Vasc Surg*. 2025;110(Pt B):260–275. doi: 10.1016/j.avsg.2024.07.089
110. Lee S, Tanaka M, Patel S, Zacharias N, Hedgire S, Malhotra R, Dua A. Clinical utility of infrapopliteal calcium score for the evaluation of severity of peripheral artery disease. *J Vasc Interv Radiol*. 2024;35:370.e2–376.e2. doi: 10.1016/j.jvir.2023.11.023