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Arterial calcification and bone physiology: role of the bone-vascular axis

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Abstract

Bone never forms without vascular interactions. This simple statement of fact does not adequately reflect the physiological and pharmacological implications of the relationship. The vasculature is the conduit for nutrient exchange between bone and the rest of the body. The vasculature provides the sustentacular niche for development of osteoblast progenitors, and is the conduit for egress of bone marrow cell products arising, in turn, from the osteoblast-dependent hematopoietic niche. Importantly, the second most calcified structure in humans after the skeleton is the vasculature. Once considered a passive process of dead and dying cells, vascular calcification has emerged as an actively regulated form of tissue biomineralization. Skeletal morphogens and osteochondrogenic transcription factors are elaborated by cells within the vessel wall, regulating the deposition of vascular calcium. Osteotropic hormones including parathyroid hormone regulate both vascular and skeletal mineralization. Cellular, endocrine, and metabolic signals flow bidirectionally between the vasculature and bone that are necessary for both bone health and vascular health. Dysmetabolic states including diabetes, uremia, and hyperlipidemia perturb the bone-vascular axis, giving rise to devastating vascular and skeletal disease. A detailed understanding of bone-vascular interactions is needed to address the unmet clinical needs of our increasingly aged and dysmetabolic population.

I. INTRODUCTION

Bone never forms without vascular interactions[1, 2]. This obvious and simple statement of fact does not adequately reflect the physiological and pharmacological implications of the relationship. The vasculature is the conduit for nutrient exchange between bone and the rest of the body; this is relevant not only to the rapid access to the skeletal calcium “bank” needed with urgent physiological demands[3] –- be it for deposits or withdrawals -- but also the delivery of metabolic substrate to the basic multicellular unit (BMU) for bone–forming osteoblast functions[4]. The vasculature also provides the sustentacular niche for development of osteoblast progenitors[5]. Moreover, it is the conduit for egress of bone marrow cell products arising, in turn, from the osteoblast-dependent hematopoietic niche[6]. A detailed understanding of bone-vascular interactions during development and disease will be needed to address the unmet clinical needs of our increasingly aged and dysmetabolic population[7].

Remarkably, the inextricable interdependence of vascular physiology, skeletogenesis, bone remodeling, and mineral metabolism has in general escaped widespread appreciation. Arteriosclerosis – the stiffening of conduit arteries from any cause -- including medial

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calcification and fibrosis in addition to atherosclerosis -- contributes to morbidity and mortality including musculoskeletal disease. The lower extremities bear the brunt of this disease burden, best manifested in hip fracture[8], limb ischemia[9, 10], and amputation risk[9, 11, 12]. In a preclinical model of critical limb ischemia, Ignarro et al have begun to capitalize upon this physiology, coupling parathyroid hormone (PTH) - mediated bone anabolism with granulocyte colony stimulating factor (G-CSF) treatment as a strategy to mobilize endothelial progenitor cells (ePCs) and improve limb recovery[13]. In humans, Khosla, Pignolo, and colleagues have demonstrated that marrow-derived progenitors with "osteogenic signatures" portend and participate in arterial function[14, 15], valve calcification[16], and fracture repair[17]. Anabolic cross-talk between osteoblasts and endothelial cells has been manipulated by Clemens and colleagues as a successful strategy to enhance bone regeneration following injury[18, 19]. Thus, a bone-vascular axis has emerged in which the vasculature supports musculoskeletal functions -- and bone-derived cell types and endocrine/paracrine cues impact vascular health.

In this brief manuscript, we provide an overview of the intricacies of the bone-vascular axis, emphasizing interactions during disease rather than during development (the reader is referred to two outstanding reviews that detail these interactions during skeletal morphogenesis[2, 20]). We emphasize dysmetabolic states such as diabetes, dyslipidemia, and uremia that compromise vascular health and thus skeletal health via heterogeneous arteriosclerotic calcification processes[21, 22]. We recount new data demonstrating that calciotropic hormones regulate vascular calcification[23–26] – and that the dysmetabolic milieu impairs calciotropic hormone signals vital to the preservation of normal bone and vascular mineral homeostasis[27–29]. We relate the mechanisms whereby cellular, biochemical and hormonal cues elaborated by the skeleton systemically impact vascular health and vessel conduit function – and end by pointing to emerging therapeutic opportunities afforded by a better understanding of the bone-vascular axis.

II. THE CLINICAL SIGNIFICANCE OF ARTERIOSCLEROTIC CALCIFICATION

Vascular calcification has afflicted human beings for at least 5 millennia. Ötzi, the Tyrolean Ice Mummy who succumbed to homicide ~ 5300 years ago -- 500 years before Stonehenge was erected -- had significant deposits of arterial calcium in his abdominal aorta[30]. Most recently, with the enhanced longevity of modern humans the consequences of arterial calcification have become increasingly evident. Coronary artery calcium (CAC) scores identify those at greatest risk for progressive cardiovascular disease (CVD) in those with otherwise intermediate risk[31]. Tibial artery calcium (TAC) scores outperform ankle-brachial indices in portending amputation risk in patients with peripheral arterial disease (PAD)[9]. Using plain radiographs to assess patients with type II diabetes, the presence of arterial medial calcification was deemed to be a greater contributor to amputation risk than atherosclerotic calcification in this patient population[12]. The presence and extent of calcific aortic valve disease (CAVD) is the single best predictor of clinical progression in patients with asymptomatic, mild or moderate[32]- to- severe[33] calcific aortic stenosis. Using plain pelvic and femoral radiographs to phenotype vascular calcification in setting of end stage renal disease (ESRD) requiring renal replacement therapy (RRT), London and colleagues identified that arterial calcification clearly portended mortality[12]. While the five-year Kaplan-Meier survival was under 50% for ESRD patients afflicted with atherosclerotic calcification or medial calcification, the fortunate 1/3rd lacking significant arterial calcification enjoyed ~90% survival during this same period[34]. After adjustment for age, dialysis vintage, gender, ethnicity, diabetes, non-dialysis CKD status,, hypertension, tobacco use, prior parathyroid surgery, and body mass index, atherosclerotic calcification and medial calcification conveyed 5- and 16-fold increases in the relative risk for mortality compared to those without vascular calcification[12]. Very recently, using CT-based volumetric scoring of

carotid artery calcification load, intracranial carotid calcification was linked to the extent of MRI-detected CNS white matter lesions while extracranial carotid calcification was a harbinger of overt CNS infarcts[35]. Clearly, arterial calcific vasculopathy is a harbinger of cardiovascular disease.

How can arterial calcification convey such clinically significant risks? Of course, thromboembolic events and fixed reductions in vessel lumen size induce tissue ischemia, and the calcified Stary type Vb plaque[36, 37] characteristic of atherosclerotic calcification is a culprit in acute coronary syndromes[37, 38]. Aortic valve sclerosis distorts and stiffens valve leaflets, not only causing stenosis with attendant myocardial workload demand but also precluding the efficient valve leaflet coaptation that prevents regurgitant flow[39, 40]. Additionally, conduit vessel stiffening – arising from either atherosclerotic or medial mineralization[41] – impairs Windkessel physiology[42], which depends upon the rubbery elasticity of conduit vessels necessary for smooth distal tissue perfusion[43] (Figure 1). With each cardiac cycle, a portion of the kinetic energy elaborated during ventricular contraction of systole is stored as potential energy throughout the vascular tree[42]; this energy is released during diastole and helps maintain uniformity of flow in distal capillary beds during the cardiac cycle[43]. Moreover, with arterial stiffening the attendant increase in pulse wave velocity interacts with impedance mismatch along the vascular tree to elevate systolic blood pressure, increasing myocardial workload as well as end-organ barotrauma[43, 44] (Figure 1). Thus, the presence, extent and histoanatomic type of arterial calcification carries ominous predictions with respect to cardiovascular and CNS disease, mortality, and amputation risk[22].

At this point, it is useful to highlight the differences denoted when using the terms arteriosclerosis and atherosclerosis. Arteriosclerosis refers to arterial mechanical stiffening from any cause, be it from: (a) concentric medial and adventitial fibrosis with medial calcification, elastinolysis and mural thickening as occurs in type II diabetes; (b) eccentric intimal-medial atherosclerotic plaques with calcification, fibrosis, and cholesterol –laden lipoprotein deposition; or (c) neointima formation and mural fibrosis as occurs in chronic allograft vasculopathy in transplanted organs. Atherosclerosis specifically refers to the intimally oriented, eccentric, lumen-deforming processes initiating from subendothelial lipoprotein deposits with cholesterol-laden foam cell formation and subsequent matrix remodeling events that increase risk for atherothrombosis. While atherosclerosis decreases arterial compliance – i.e. causes arteriosclerosis – not all arteriosclerosis arises from atherosclerotic processes.

III. PATHOBIOLOGY OF VASCULAR CALCIFICATION

Vascular calcification was once considered only a passive process of dead and dying cells; however, data from a multitude of laboratories worldwide have clearly demonstrated that vascular calcification is an actively regulated form of extracellular matrix biomineralization[21]. Virchow's initial pathological description of "atherosclerosis" presciently identified the contributions of perturbed lipid metabolism, inflammation, and osteo-fibrogenic differentiation to the biology of vascular calcium accrual[45, 46]. Of note, vascular ossification – true ectopic bone replete with marrow elements – can be seen in up to 15% of calcified arterial lesions[47]. At the molecular level, however, the signature of active osteogenic processes are found in virtually all calcified arterial segments[48]. Studies by Linda Demer, H. Clarke Anderson, and colleagues were the first to identify osteogenic "fingerprints" in calcifying atherosclerotic and medial lesions[48]. Bone alkaline phosphatase – a highly characteristic and important ectoenzyme required for bone mineralization[49] – was localized to mineralizing arterial segments[50, 51]. Moreover, the powerful bone morphogen BMP2 was shown to be expressed in calcifying atherosclerotic plaques of human vessels[52].

Oxylipids derived from oxidized LDL (oxLDL) were subsequently identified as potent inducers of endothelial BMP2[53, 54], TNF[55], and other macrophage-derived signals that drive mineralization of calcifying vascular cells (CVCs) in vitro and in vivo[55] (vide infra). However, in atherosclerotic mice, inhibition of arterial BMP2 signaling with matrix Gla protein (MGP) reduces vascular calcium and lesion size[56].

By histoanatomic and clinical criteria, at least 5 types of arterial calcification can be identified (Table I): atherosclerotic calcification, medial artery calcification, calcific aortic valve disease (a.k.a. calcific aortic stenosis), calcific uremic arteriolopathy (a.k.a. calciphylaxis) and the vascular calcification of ESRD (a.k.a. CKD-MBD; chronic kidney disease mineral and bone disorder). The reader is referred to recent review series for in-depth consideration[22, 57]. For the purposes of this review, only atherosclerotic calcification, medial calcification, and vascular calcification of CKD-MBD will be briefly discussed because of the immediate relevance to skeletal physiology and bone-vascular interactions.

III. A. Atherosclerotic calcification

Atherosclerotic calcification represents the prototypic lesion described by Virchow[45, 46]. In the lexicon of cardiovascular pathology this is the type Vb plaque[58] -- characterized by an eccentric, lumen deforming, outward remodeling lesion possessing a fibrous cap, cholesterol-laden macrophages and lipoprotein deposits, intensive focal inflammatory cell infiltration and localized elastinolysis[37, 38] (Figure 2). Juxtaposed mineralizing apoptotic bodies and mineralizing matrix vesicles are elaborated by neighboring vascular smooth muscle cells (VSMCs) and CVCs undergoing chondroid metaplasia [37, 38]. Stippled fibrous cap calcification is also noted, and such lesions appear to be precursors to the ruptured plaques of acute coronary syndromes[37, 38]. In bone, biomineralization occurs via either endochondral ossification or membranous ossification processes programmed by chondrocytes and osteoblasts[59–61]. Endochondral ossification is characterized by the mineralization of a chondrocyte-derived skeletal template, following chondrocyte hypertrophy, apoptosis, vascular invasion and concomitant osteoprogenitor recruitment with subsequent osteoblast-mediated bone formation. Of note, Runx2/Cbfa1 -- a master transcriptional regulator absolutely essential for both endochondral or membranous ossification processes[62] –is upregulated in the vessel walls of apoE−/− mice undergoing atherosclerotic calcification[63]. Elegant studies by Giachelli and colleagues have recently demonstrated the “trans-differentiation” of arterial VSMCs to cells of the chondrocyte and osteoblast lineage during atherosclerotic calcification[64–66] (Figure 3). Implementing SM22-Cre; Rosa26-LacZreporter; apoE−/− mice, they showed that cells originally derived from the VSMC lineage – “tagged” as blue with the Rosa locus LacZ reporter via VSMC-specific Cre activation – ended up as vascular chondrocytes and osteoblasts in the mineralizing segments of atherosclerotic plaques[65]. VSMC transdifferentiation in atherosclerotic apoE−/− mice occurred in the absence of augmented Msx2, Wnt, or Sp7/0sx signaling[65].

What then triggers osteochondrogenic “transdifferentiation” of VSMCs? Oxidative stress signaling, oxylipids, and phosphate (see CKD-MBD below) play important pathophysiological roles[67] (Figure 3). Oxysterols derived from oxidized LDL cholesterol (oxLDL) and hydrogen peroxide upregulate Runx2/Cbfa1 expression[68] and osteo/chondrogenic trans-differentiation of VSMCs[69]. Activation of bone alkaline phosphatase in these VSMCs is critical to matrix mineralization responses elicited by oxLDL and peroxide, and inhibitors of bone alkaline phosphatase limit atherosclerotic calcification by VSMCs[70]. Intriguingly, as Demer and colleagues first demonstrated, hypercholesterolemia and oxylipids derived from oxLDL also suppress bone formation[71, 72] and bone anabolic responses to PTH[29, 73]. Moreover, PTH responses were restored by administration of HDL-mimetics[29, 73]. These data implicate a metabolic milieu that can simultaneously engender atherosclerosis and osteoporosis. Thus, inflammatory oxylipids and oxLDL accumulating at

sub-intimal venues drive atherosclerotic intimal calcification by activating osteogenic BMP2 and Runx2/Cbfa1 trans-differentiation of VSMCs[67, 74]. Recruitment of calcifying vascular cells (CVCs)[75] – a mural multipotent mesenchymal cell related to the microvascular pericyte[76] – provides an additional source of osteoprogenitors[52].

III. B. Arterial medial calcification

Arterial medial calcification –sometimes called Monckebergs medial calcific sclerosis– is particularly common in the arteriosclerosis of type II diabetes[10–12, 77]. Medial calcification is a concentric process of the tunica media, associated with biomineralization initiating along mural elastin fibers[78, 79] (Figure 2). Traditional thought was that medial calcification was clinically inconsequential -- succinctly articulated in the 3rd (1984) edition of the pathology text of Robbins et al on page 518 as follows[80]: " This disorder is of relatively little clinical significance. It accounts for roentgenographic densities in the vessels of the extremities of aged individuals, but it is to be remembered that the lesions do not produce narrowing or occlusion of the vascular lumen[80]." Yet, as subsequently highlighted, medial artery calcification impairs vessel mechanics and Windkessel physiology necessary for smooth distal tissue perfusion[81] (Figure 1) – and best conveys risk for lower extremity amputation in patients with type II diabetes[12].

Similar to the membranous ossification of craniofacial bone – where osteoblast-mediated biomineralization occurs in a type I collagen –based matrix without a preceding cartilage template[61] – medial artery calcification does not involve overt chondrogenesis during disease initiation[82] (chondrogenesis is sometimes seen in advanced disease with true ectopic ossification[83]). Electron microscopy first identified bone alkaline phosphatase-positive matrix vesicles associated with fragmented elastin in human arteries afflicted with medial artery calcification[50]. Additional insights into the pathobiology of disease initiation and progression have been forthcoming from detailed study of preclinical disease models[22]. When fed high fat diets (HFD) characteristic of westernized societies, male LDLR−/− develop obesity, type II diabetes, and dyslipidemia[22] – with progressively severe medial artery calcification and subsequent atherosclerotic calcification as plaques begin to accumulate[82]. At the earliest phases of disease, arterial Msx2 and Msx1 – osteoblast homeodomain transcription factors absolutely necessary for membranous bone formation in the skull[84] – are upregulated in the aortas of these animals[85]. Immunohistochemistry and in situ hybridization demonstrates the expression of Msx2 in aortic valve interstitial myofibroblasts, arterial adventitial myofibroblasts, and a subset of cells within the tunica media[25, 48, 85]. Data from our laboratory and others have characterized adventitial myofibroblasts as pericyte-like calcifying vascular cells (CVCs) with multi-lineage mesenchymal cell potential[86] (Figure 3). As in atherosclerotic calcification, inflammation is again key[22]; diabetes with or without dyslipidemia upregulates adventitial TNF[87] production by monocytes and macrophages [55] – -- which in turn induces pro-calcific Msx2 activity and paracrine Wnt signaling cascades that promote mural calcification and fibrosis[82] (Figure 3). Along with BMP2, a powerful bone morphogen secreted by inflamed endothelial cells[88], Msx-regulated Wnt family members elaborated by myofibroblasts support the osteogenic differentiation and collagenous matrix mineralization by mesenchymal progenitors[89–91] – be it in bone [92] or within the arterial wall [25, 91]. Thus, the paracrine polypeptide milieu of osteogenic morphogens activate osteoblast gene regulatory programs in multipotent mural mesenchymal cells such as CVCs and adventitial myofibroblasts[93]. Adventitial-to-medial biological signals drive concentric involvement of arterial vessels in diabetic medial calcification – quite distinct from the eccentric atherosclerotic calcification processes organized by subintimal oxylipid deposits (Figure 2). In diabetic arteriosclerosis, neoangiogenesis arising from the vasa vasorum in the inflamed adventitial-medial junction circumferentially upregulates mural BMP-Wnt signaling, and spawns additional adventitial

myofibroblasts that can be allocated / programmed to elaborate osteogenic and fibrogenic phenotypes [94, 95] (Figure 3). Not surprisingly then, surgical stripping of arterial adventitia reduces medial calcification in preclinical disease models[96].

Of note, recent data from Rajamannan, Miller, Heistad, and colleagues have demonstrated that similar processes contribute to aortic valve calcification directed by valve interstitial cells (VICs)[97]. Activation of Msx and Wnt signaling cascades is observed in calcifying human aortic valves[98], and apoE−/− mice lacking the Wnt receptor LRP5 are protected from valve calcium accrual[99]. The behavior and molecular phenotype of the three calcifying vascular mesenchymal cell populations – CVCs, adventitial myofibroblasts, and VICs -- closely resembles that of the pericyte, the proliferative microvascular smooth muscle cell that maintains capillary integrity during neoangiogenesis[100]. Thus, as occurs in atherosclerotic calcification, medial calcification and valve calcium accrual is driven in great part by osteogenic cells derived from vascular residents[21] (Figure 3). However, exciting new data from Pignolo et al has identified a circulating CD45+ osteocalcin+ osteoprogenitor (COP) cell that elaborates BMP family members and "homes" to sites of vascular injury[16]. As initially formulated by Khosla and colleagues in their elegant studies of human bone growth and fracture repair[17], circulating osteoprogenitors may also participate in vascular mineralization processes in type II diabetes [101] as well as in true ectopic vascular ossification[102]. Therefore, strategies that inhibit vascular BMP-Wnt signaling or reduce COP cell populations may help alleviate the burden of arteriosclerotic disease in type II diabetes.

III. C. Vascular calcification of CKD / CKD-MBD: The "perfect storm"

In 2005, KGIDO (international group on Kidney Disease: Improving Global Outcomes) codified the clinical entity CKD-MBD, the mineral and bone disorder of chronic kidney disease that encompasses the vascular calcification of CKD[103, 104]. In CKD-MBD, the clinical link between bone disease and vascular disease arising from primary perturbations in calcium phosphate homeostasis is now formally recognized. Diabetes and hypertension – two diseases that independently promote arteriosclerotic calcification – are responsible for approximately 60% of ESRD patients requiring RRT (renal replacement therapy)[105]; however, the vast majority of patients with CKD from any cause experience increased cardiovascular mortality and calcific vasculopathy[34, 106]. While antecedent diabetes, hypertension, dyslipidemia, and metabolic syndrome continue to contribute to arteriosclerosis[107], hyperphosphatemia, reduced Klotho expression, and impaired soft tissue calcification defenses are key pathophysiological components of CKD-MBD[108, 109]. Indeed, even in the setting of normal renal function, serum phosphate levels track severity and extent of coronary artery calcium[110, 111]. Hyperphosphatemia and hypercalcemia increase the elaboration of VMSC matrix vesicles -- ~100 nm diameter phosphatidylserine- and annexin-rich, bilaminate spheroids resembling the mineralizing vesicles of chondroyctes[112]. Shanahan and colleagues demonstrated that VSMCs elaborate these matrix vesicles as one mechanism to clear extracellular matrix calciprotein complexes[112, 113] (Figure 3). However, these same matrix vesicles can serve to nucleate and propagate extracellular matrix mineralization in the absence of cell-mediated pinocytotic uptake[113]. Matrix vesicle uptake by VSMCs requires serum fetuin[113], a hepatocyte-derived calcium binding protein that maintains calcium solubility in the supersaturated serum and interstitial fluid compartments[114]. Dialysis reduces serum fetuin levels as does inflammation[115]. In addition to increasing matrix vesicle release, phosphate also increases BMP2 elaboration by VSMCs and upregulates VSMC Runx2/Cbfa1 and Msx2 via SLC20A1 signaling[65, 116]. BMP2 in turn further enhances phosphate uptake and osteogenic programming of VSMCs in a feed-forward vicious cycle[117]. Thus, like oxLDL and peroxide, elevated serum phosphate can reprogram the VMSC phenotype to support osteogenic mineral deposition[66]. Furthermore,

sustained hyperphosphatemia simultaneously induces VSMC apoptosis, removing the first-line cell-mediated mechanism for clearing vascular calciprotein complexes[118]. The ensuing vascular mineral deposition is an exuberant medial calcification that is almost always superimposed on antecedent atherosclerotic and medial calcification arising from diabetes, dyslipidemia, and other causes. Finally, with the massive increase in mural extracellular matrix calcium, the second-line defenses for handling vascular extracellular matrix calcium – viz., cells of the monocyte/macrophage lineage – are recruited[119]. Vascular calcium phosphate deposition elicits innate immune responses by monocyte/macrophage cells[120], increasing the production of TNF[121] and down-stream activation of osteogenic BMP-Msx - Wnt and Runx2/Cbfa1 signaling[116, 121] – yet another, feed-forward vicious cycle in vascular mineralization. Thus, patients with CKD suffer the "perfect storm" of vascular calcification.

Perhaps not surprisingly, then, statin-based strategies focused upon LDL cholesterol reduction have generally failed to reduce cardiovascular morbidity and mortality in end stage renal disease (ESRD)[122]. This past year, a combined approach using implementing a statin with the cholesterol absorption inhibitor ezetimibe reduced overall major atherosclerotic disease endpoints including non-hemorrhagic stroke by ca. 20% -- but failed to significantly reduce myocardial infarction or associated death[123]. Strategies that emphasize phosphate binders as a primary approach to control hyperphosphatemia have met with early successes – as long as the binding is not calcium based[124–126]. As highlighted by Raggi et al, the use of calcium based phosphate binders significantly contribute to vascular calcium load in ESRD[127]. This may be directly related the impaired capacity of the uremic skeleton to rapidly handle serum calcium transients. Indeed, London demonstrated that patients with the most extensive vascular calcification exhibited the lowest levels of bone formation on histomorphometric evaluation, reflected in inappropriately normal or low PTH values[128]. As compared to sevelamer – the first phosphate binder that does not contain calcium or aluminum -- calcium based phosphate binders suppress PTH, reduce bone mass, and increase vascular calcium load in ESRD[127]. Because of the biphasic relationships between PTH, vitamin D, and vascular health in ESRD, a rigorous focus upon the role and endocrine physiology of calciotropic hormones and calcium phosphate homeostasis should provide new hope to patients afflicted with CKD[129].

IV. REGULATION OF VASCULAR CALCIFICATION BY CALCIOTROPIC HORMONES

While vascular calcification is clearly an actively regulated form of extracellular calcified matrix metabolism, remarkably few studies have been undertaken with respect to control by calciotropic hormones[130]. The prototypic calciotropic hormones are parathyroid hormone (PTH), vitamin D and its metabolites, parathyroid hormone related polypeptide (PTHrP), calcitonin, and estrogens including estradiol. Estrogen signaling via non-genotropic signaling mechanisms acutely activates endothelial nitric oxide synthase in caveolae – an acute vasodilatory response that is theoretically protective[131]. Estrogen exposure in women was shown to reduce the incidence of arterial calcification as revealed from mammography[132]. In the Rancho Bernardo Study, estrogen use in post-menopausal women was associated with reduced coronary artery calcification[133]; however, estrogen replacement therapy worsened cardiovascular endpoints in older women in the Womens Health Initiative[134] – and estrogens including low-dose oral contraceptives are associated with PAD[135]. No published studies have examined the actions of calcitonin on arteriosclerosis, but the calcitonin gene - related polypeptide, encoded by the calcitonin gene, is a vasodilator[136].

Vitamin D insufficiency is associated with PAD[137] as well as other cardiovascular diseases including congestive heart failure[138]. Additionally, vitamin D inhibits foam cell formation

and macrophage activation in patients with diabetes[139]. However, vitamin D replacement has not been shown to improve any primary cardiac endpoint – although the largest studies have not used uniform preparations of vitamin D[140]. In ESRD, a biphasic U-shaped bimodal response to calcitriol levels has clearly emerged with respect to vascular disease[141] (Figure 4a). While vitamin D insufficiency and reduced 1,25(OH2)D levels in ESRD engenders more severe secondary hyperparathyroidism, excessive 1,25(OH2)D dosing can induce low-turnover bone disease, hypoparathyroidism, and increase vascular calcium deposition. As London first established, ESRD patients with the most extensive and severe arterial calcification exhibited the lowest bone formation rates and PTH levels[128]. Vitamin D intoxication induces widespread vascular calcification and nephrocalcinosis – and is a commonly used rodent model of vascular calcification potentially relevant to the bimodal relationship in ESRD[142, 143]. The calcium sensing receptor is expressed in VSMCs as well as in parathyroid glands[144], and signaling with this agonist to control secondary hyperparathyroidism (sHPT) in uremic rat models results in reduced vascular calcium accrual[145]. Of note, in cultured VSMCs calcitriol induces calcification in part by suppressing PTHrP production and inducing alkaline phosphatase[146]. Via paracrine actions, PTHrP relaxes smooth muscle cells, inhibits fibroproliferative responses, prevents alkaline phosphatase induction and inhibits calcium deposition by signaling via the PTH/PTHrP receptor (PTH1R)[146]. Indeed, adenoviral delivery of the obligatory paracrine form of PTHrP inhibits neointima formation in a porcine model of stent-induced coronary restenosis[147, 148]. The role of VSMC PTHrP-PTH1R signals in cardiovascular arteriosclerosis has not been exhaustively studied to date in part because PTH1R-null mice die in utero due to massive cardiomyocyte apoptosis[149]. Nevertheless, the post-natal vasculopathy arising from vitamin D intoxication may involve lesioning of protective paracrine PTHrP-PTH1R signals in VSMCs in addition to hyperphosphatemia and hypercalcemia[145, 146].

This past decade, we examined the impact of PTH/PTHrP receptor signaling on arteriosclerotic calcification in the LDLR−/− model[23–25]. Bone anabolic responses that increased skeletal calcium accrual were accompanied by reductions in aortic calcium accrual[23, 24]. Additionally, arterial expression of osteogenic genes were down-regulated while skeletal expression of these same genes were increased[24, 25]. The PTH1R is highly expressed in VSMCs --- and is very susceptible to homologous desensitization upon tonic exposure to PTH or PTHrP[150]. Sustained pharmacologic vascular exposure to either PTHrP or PTH – mimicking the setting of hyperparathyroidism – induces arterial tachyphylaxis to acute PTH/PTHrP agonist administration[150]. In order to address the potential role for VSMC-autonomous role for PTH1R signaling in arteriosclerotic vascular responses, we generated and evaluated SM22-PTH1R(H223R); LDLR−/− transgenic mice[23]. In these animals, a ligand-independent constitutively active form of the PTH1R, PTH1R(H223R)[151], is expressed in VSMC from a VSMC-specific promoter[23]. As compared to non-transgenic male siblings, male SM22-PTH1R(H223R);LDLR−/− exhibited reduced aortic calcification, aortic fibrosis, and aortic oxidative stress[23]. Arteriosclerotic Wnt/beta-catenin signaling and type I collagen gene expression was concomitantly reduced[23]. Aortic wall thickness was also decreased, and ex vivo vessel mechanical compliance (distensibility) was increased. Importantly, Friedman and colleagues independently demonstrated that pulsatile administration of PTH(1–34) reduces arterial mineralization in a rat model of uremia, although fibrosis and vessel mechanics were not evaluated[26].

Our in vivo data with the SM22-PTH1R(H223R) transgene demonstrate that sustained VSMC PTH1R activation actually reduces vascular oxidative stress and pro-calcific and pro-fibrotic signals that drive arteriosclerotic disease[23]. Recall that dysmetabolic states associated with macrovascular disease -- such as diabetes, dyslipidemia, and uremia -- induce tissue resistance to PTH1R activation[27, 29, 152]. These findings converge to prompt a major shift in our thinking as concerns the pathobiology of arteriosclerosis in type II

diabetes and other dysmetabolic states, including primary and secondary hyperparathyroidism (pHPT and sHPT, respectively). How so? The negative impact of dysmetabolic states (dyslipidemia, diabetes, uremia) on PTH1R signaling may become to be viewed in part as acquired insufficiencies of endocrine paracrine PTHrP actions that help preserve vascular health. The increase in carotid IMT associated with pHPT[153] might be viewed in part as being the consequence of homologous vascular desensitization[150, 154] occurring in response to (a) tonically elevated PTH levels; and (b) impaired capacity of paracrine VSMC PTHrP production to restrain proliferative[155] and calcific[146] arteriosclerotic responses (Figure 4b). However, via its bone anabolic actions PTH signaling upregulates circulating intact OPN[24] – an inhibitor of vascular mineralization – and supports the hematopoietic niche including cellular elements such as ePCs that program vascular healing responses. Furthermore, PTH upregulates the expression of matrix Gla protein (MGP)[156, 157], an important negative regulator of matrix mineralization and BMP2/4 signaling in the vasculature[56]. Whether MGP participates in PTH inhibition of vascular myofibroblast BMP2 signaling[25] remains to be evaluated. The relative contributions of direct vs. indirect actions of PTH1R on vascular health are undergoing additional scrutiny and evaluation.

V. THE IMPACT OF ARTERIOSCLEROSIS ON SKELETAL HEALTH AND FUNCTION

Atherosclerosis, calcification, mural hypertrophy and fibrosis, and elastin matrix senescence cause arteriosclerosis, the age-associated vascular stiffening that impairs Windkessel physiology necessary for smooth distal tissue perfusion. With aging, vascular remodeling processes can increase wall thickness and regionally reduce conduit artery lumen patency, thus worsening arterial compliance and its clinical impact[43]. In the musculoskeletal system, lower extremities experience the brunt of arteriosclerotic disease. Claudication and amputation are the most salient manifestations that reduce mobility and increase morbidity, but hip fracture is also increased with peripheral arterial disease (PAD) [8]. Multiple studies have now established that the presence and extent of arteriosclerotic calcification conveys lower extremity amputation risk. Medial arterial calcification of type II diabetes conveys a 3-fold increased risk for amputation[12]. Recently, implementing CT scanning and the Agatston method to quantify tibial artery calcification (TAC), Guzman established that TAC scoring outperforms ankle-brachial indices (ABI) in predicting lower extremity amputation risk[9]. Applying the conventional ABI method to characterize peripheral artery disease (PAD), Cummings and colleagues showed that PAD increased the rate of femoral neck bone loss and increased the risk for hip fracture in men[9]. They concluded that “Further research should examine the biological mechanisms underlying the association between reduced limb blood flow and fractures.” [9] The increasing prevalence of type II diabetes and associated PAD will contribute to the future arteriosclerotic disease burden in our society.

Atherosclerotic deposits can and do impact bone marrow arteries. However, the physiological responses of bone to arteriosclerosis are multifactorial. In 1985, Brenneise and Squier examined the relationships between atherosclerotic disease and blood flow in rhesus monkeys maintained on high fat atherogenic diets[158]. These diets induced extensive calcified atherosclerotic carotid plaques and as well as atherosclerotic changes in arteries perfusing skeletal muscle. However, atherogenic diets failed to induce significant atherosclerotic changes in the principal nutrient arteries of maxillary and mandibular bone --- and did not alter osseous lumen area, intraosseous vessel wall thickness, or vessel lumen area / tissue area[158]. Nevertheless, atherosclerosis reduced osseous blood flow by 80% in the anterior mandible, posterior mandible, and maxilla[158]. How can this occur? These seminal findings indicated that macrovascular Windkessel function and endothelial function necessary for regulating bone tissue perfusion are severely impaired with atherosclerosis and

arteriosclerosis[81]. Recently, we described and characterized a novel murine model of arteriosclerotic vascular stiffening -- the osteopontin (OPN)-null mouse on the LDLR-deficient background. Even in the absence of atherogenic diets, male OPN−/−;LDLR−/− mice exhibit aortic adventitial fibrosis and vascular stiffening. Using fluorescence microsphere perfusion assays, we demonstrated significantly reduced lower extremity bone (femur) blood flow in arteriosclerotic OPN−/−;LDLR−/− animals[159]. By contrast, blood flow to the kidneys was not significantly altered as assessed in this assay. Thus, arteriosclerosis and atherosclerosis impair skeletal blood flow[158, 159].

At this point it should be noted that in healthy young long bone, diaphyseal blood flow is primarily centrifugal, with flow originating from marrow compartment (supplied via nutrient artery) to trabeculae and bone cortex[160] (Figure 5). However, with aging flow becomes increasingly centripetal, with the greatest extent of diaphyseal cortex being perfused by periosteal arteries[161]. This age-dependent change in the “vector” of blood flow may become more exaggerated due to the enhanced sensitivity of nutrient artery to vasoconstrictors as compared to vasodilators[162]. With aging the vasodilator response in bone nutrient arteries becomes increasingly reduced[163]. Blood flow is a critical determinant of the bone formation rate at the BMU within the adult skeleton[4]. The interactions between age-dependent changes in this cortical bone flow vector and arteriosclerotic conduit vessel stiffening have not been systematically examined. Since osteoblast-mediated bone formation is directly related to perfusion[4], these age-dependent changes are predicted to contribute to reduced skeletal anabolism in the absence of intervention.

To be sure, perfusion- and diffusion – dependent nutrient supplies must be rate-limiting contributors to the impairment of bone physiology with arteriosclerotic disease. Indeed, Prisby et al highlighted that it is that the proximity of the bone-forming multicellular unit (BMU) to the microvasculature – not the mass of the skeletal microvasculature – that is critical in maintaining bone anabolism[164]. Vascular endothelial growth factor (VEGF), a prototypic angiogenic factor produced by osteoblasts, was shown to be critically important[164]. Moreover, the sub-intimal vascular accumulation of oxidized LDL in bone impairs anabolic responses to PTH[27]. However, it has become increasingly evident that the vasculature itself provides paracrine and juxtacrine cues that regulate osteoblast function functions[165]. Ephrin B2, BMP2, RANKL, and nitric oxide are but a few of the potent osteotropic signals elaborated by endothelial cells[165–171]. Furthermore, the vasculature also provides the sustentacular niche for osteoblast progenitors[172] --- and is the conduit for egress of bone marrow – derived formed elements from the osteoblast-regulated hematopoietic niche[173]. By coupling enhanced PTH-mediated bone anabolism with granulocyte colony stimulating factor (G-CSF) treatment as a strategy to mobilize endothelial progenitor cells (ePCs), Ignarro has demonstrated the capacity to implement endocrine pharmacotherapy to enhance ischemic limb recovery in mice[13]. Thus, multiple bone-vascular interactions may contribute to impaired skeletal anabolism with arteriosclerosis. The important preclinical “proof of principle” provided by Ignarro et al[13] supplies compelling pharmacologic and physiological evidence for an emerging bone-vascular axis that regulates cardiovascular and skeletal health.

VI. SKELETAL FUNCTION AND VASCULAR HEALTH: THE BONE-VASCULAR AXIS

In the preceding sections, we’ve emphasized the impact of vascular disease on skeletal function. However, in the past decade it has became clear that bone is in fact an endocrine organ[174], capable of elaborating phosphaturic hormones such as DMP1 and FGF23 as relevant to the pathobiology of vascular disease[175]. FGF23 (fibroblast growth factor 23) is an osteocyte-derived hormone that enhances phosphate excretion by the kidney via down-

regulation of proximal tubule sodium phosphate symporters Slc34A1/NaPi-2a and Slc34A3/NaPi-2c.[176] Hyperphosphatemia and vascular calcification were observed in FGF23 null mice -- mitigated by simultaneous reductions in the vitamin D 1-alpha-hydroxylase CYP27B1[177]. Of note, FGF23 directly suppresses CYP27B1[178]. Thus, the vascular toxicity arising from deficiency in this osteoblast- and osteocyte- derived hormone arises from endogenous calcitriol intoxication with hyperphosphatemia[179]. With declining renal function, post-prandial elevations in FGF23 are amongst the earliest changes observed[180], and likely serve as a bone-derived defense mechanism for limiting the toxicities of hyperphosphatemia in conjunction with PTH[175]. With progressive uremia, end-organ responses to FGF23 are diminished due to reduced Klotho co-factor expression by the kidney and parathyroid glands [181–185]. Of note, both FGF23/Klotho[185, 186] and DMP1[187] signaling have been demonstrated to exert direct, beneficial vascular actions. Thus, the kidney emerges as a particularly important intermediary in the bone-vascular axis via hormonally regulated phosphate excretion and Klotho production (Figure 6). Osteopontin (OPN), a very potent inhibitor of matrix mineralization in its phosphorylated form[188], is secreted into the circulation by skeletal osteoblasts and increases in response to bone anabolic stimuli such as PTH[24]. Since the osteoblast-derived OPN is highly phosphorylated[189] and stable to proteolysis[190], this circulating pool of OPN may serve as an important defense as well to vascular mineral accrual as first posited by Giachelli[188]. Studies by Ducy and Karsenty have highlighted the important role of bone-derived osteocalcin (OCN) as a Gla-dependent hormone controlling beta-cell longevity[191]. With respect to arteriosclerosis, the capacity of OCN to support adiponectin production by adipocytes may be of particular importance[191]. Adiponectin suppresses the inflammatory responses elicited by TNF[192, 193] – an important stimulus for vascular calcification as highlighted above[82]. Moreover, adiponectin – null mice develop arterial calcification that is mitigated by adenoviral – mediated vascular restoration[194]. Recently, Monsonego-Ornan have provided new evidence that OCN may in also directly enhance osteochondrogenic mineralization of VSMCs[143]; this has yet to be confirmed in atherosclerotic disease models. Nevertheless, given that bone marrow-derived cell types can either promote or limit vascular calcium accrual, two arms of the bone-vascular axis clearly emerge -- one being cell-mediated via the paracrine actions of osteoblasts on hematopoietic niche and cell egress, and the other being humoral via osteoblast- and osteocyte-derived endocrine signals that regulate renal phosphate excretion, PTH production, and vascular proximity to the BMU (Figure 6).

Thus, with respect to diseases of the bone-vascular axis, three relationships can now be envisioned (Figure 7). Most certainly, metabolic milieu and genetics can independently cause arteriosclerotic disease and bone disease. Additionally, primary changes in arteriosclerotic vessel functions arising from milieu and genetics can cause bone disease via alterations in perfusion. However, given emerging nature of the bone-vascular axis, primary disease changes in bone and bone marrow function arising from milieu and genetics may cause vascular disease. Under this latter and more novel view, a healthy skeleton and marrow is a good thing for maintenance of vascular health. The relative contributions of bone-derived cellular vs. endocrine signals to changes in vascular physiology may be particularly important with uremia, aging, and diabetes[195]. A better understanding of how bone-derived endocrine cues and marrow-derived cell types interact to regulated vascular health is clearly necessary.

VIII. CONCLUSIONS AND FUTURE DIRECTIONS

Preclinical and clinical studies performed over the past two decades have converged to highlight the presence of and critical role for a bone –vascular regulatory axis in human health. Not unlike the famous legend of the three blind men describing the elephant, the perspectives of experts in endocrinology, cardiology, developmental biology, orthopedics,

biochemistry, genetics, pathology, engineering and hematology often emphasize different features of the bone-vascular axis. Fortunately, the picture emerging from the assembly of these multiple viewpoints is providing an increasingly sharper image. Calciotropic hormones have direct and indirect vasculotropic actions deserving consideration; both excesses[196] and insufficiencies[128] in calciotropic hormones such as PTH engender vascular disease. The inability to tightly regulate calcium phosphate homeostasis results in vascular toxicity. Cellular and endocrine signals arising from bone and marrow impact vascular physiology and function – and are maintained in part by PTH-dependent signals[13, 182, 197]. Age- and disease-dependent changes in vascular physiology impact bone health and fracture risk. Inflammation and oxidative stress / oxylipid signals reciprocally regulate bone and vascular mineralization – and impair PTH/PTHrP receptor signaling important to bone and vascular health[29, 67, 198]. The kidney is an important intermediary in the bone-vascular axis via hormonally regulated phosphate expression and Klotho expression[182, 199]. Declining renal function and tissue resistance to PTH/PTHrP receptor signaling represent key features in the perturbation of the bone-vascular axis with disease (Figure 6). Endocrine regulation of the bone-vascular axis is feasible; for example, strategies that modulate PTH/PTHrP receptor signaling, end organ responsiveness, and calcium phosphate homeostasis offer opportunities to improve bone health and preserve vascular health in patients with diabetes, dyslipidemia, and uremia PAD[13, 23, 29, 127, 200, 201].

On a cautionary note, however, our understanding of the relationships between bone and vascular mineral homeostasis is rudimentary. As clinicians, we are coming to appreciate the reciprocal relationships between bone mass / osteoporosis and atherosclerosis as detected by vascular calcification[202–205] – but the endocrine regulation of this relationship may change with age. For example, aminobisphosphonate therapy for osteoporosis decreases the risk for aortic valve and thoracic aorta calcification in women > 75 years old – but INCREASES risk in women under age 55[206]. Furthermore, although helpful to bone in some contexts, oral calcium supplementation in those with renal insufficiency[127] and advanced age[207, 208] may have down-side impacts on cardiovascular health. As our understanding of the bone-vascular axis continues to improve, so too will our capacity to meet the clinical needs of our patients afflicted with musculoskeletal and vascular diseases.

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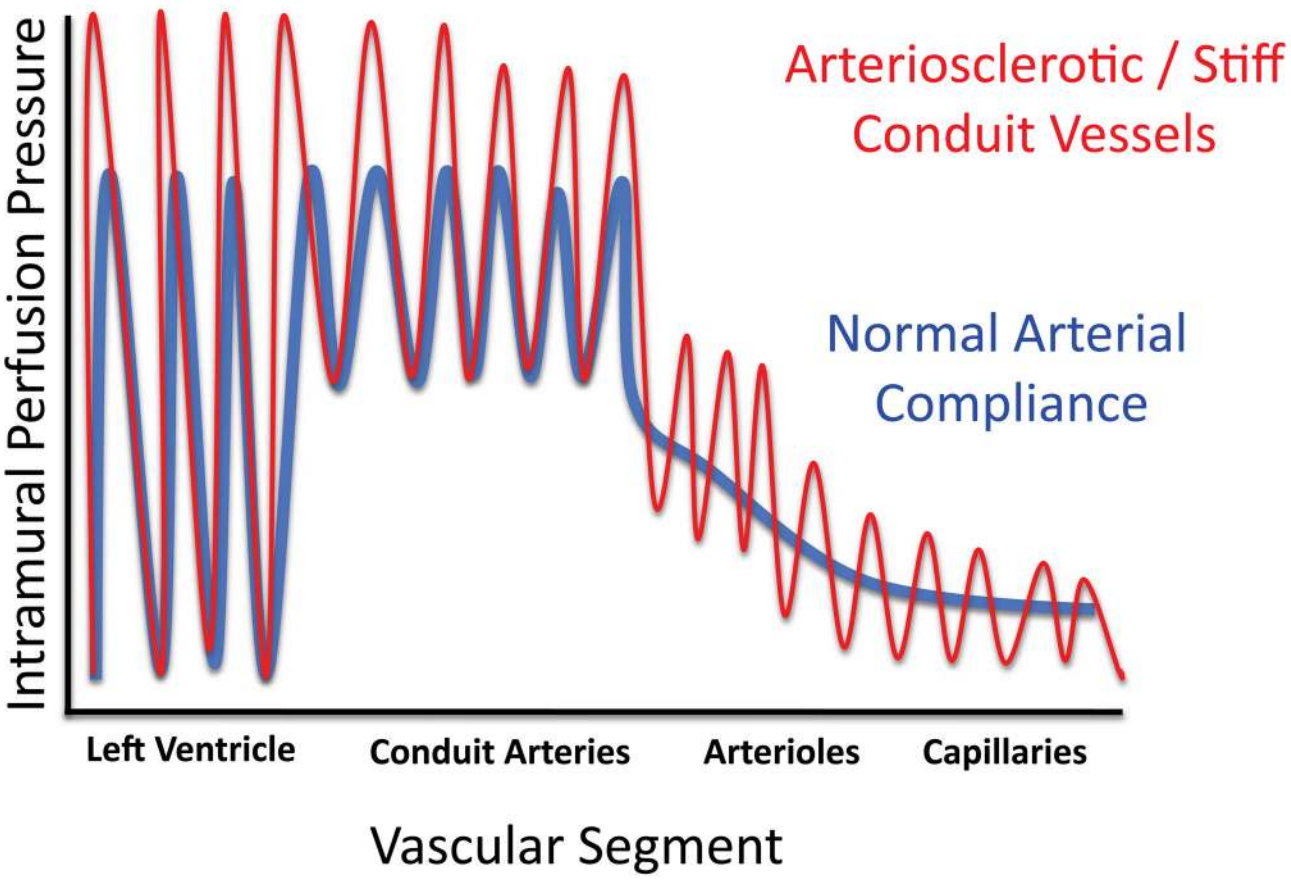

Figure 1. Consequences of arterial stiffening and impaired Windkessel physiology

During systole, some kinetic energy is stored as potential energy in the elastic conduit arteries. This permits not only coronary perfusion but also smooth distal capillary perfusion during diastole (blue tracing). With arteriosclerotic stiffening (red tracing), less potential energy is stored during systole, giving rise to impaired, pulsatile and erratic flow during diastole (2/3rds of the cardiac cycle)[209]. Systolic blood pressure is also increased. See O'Rourke and Hashimoto[81] for an excellent review and additional details.

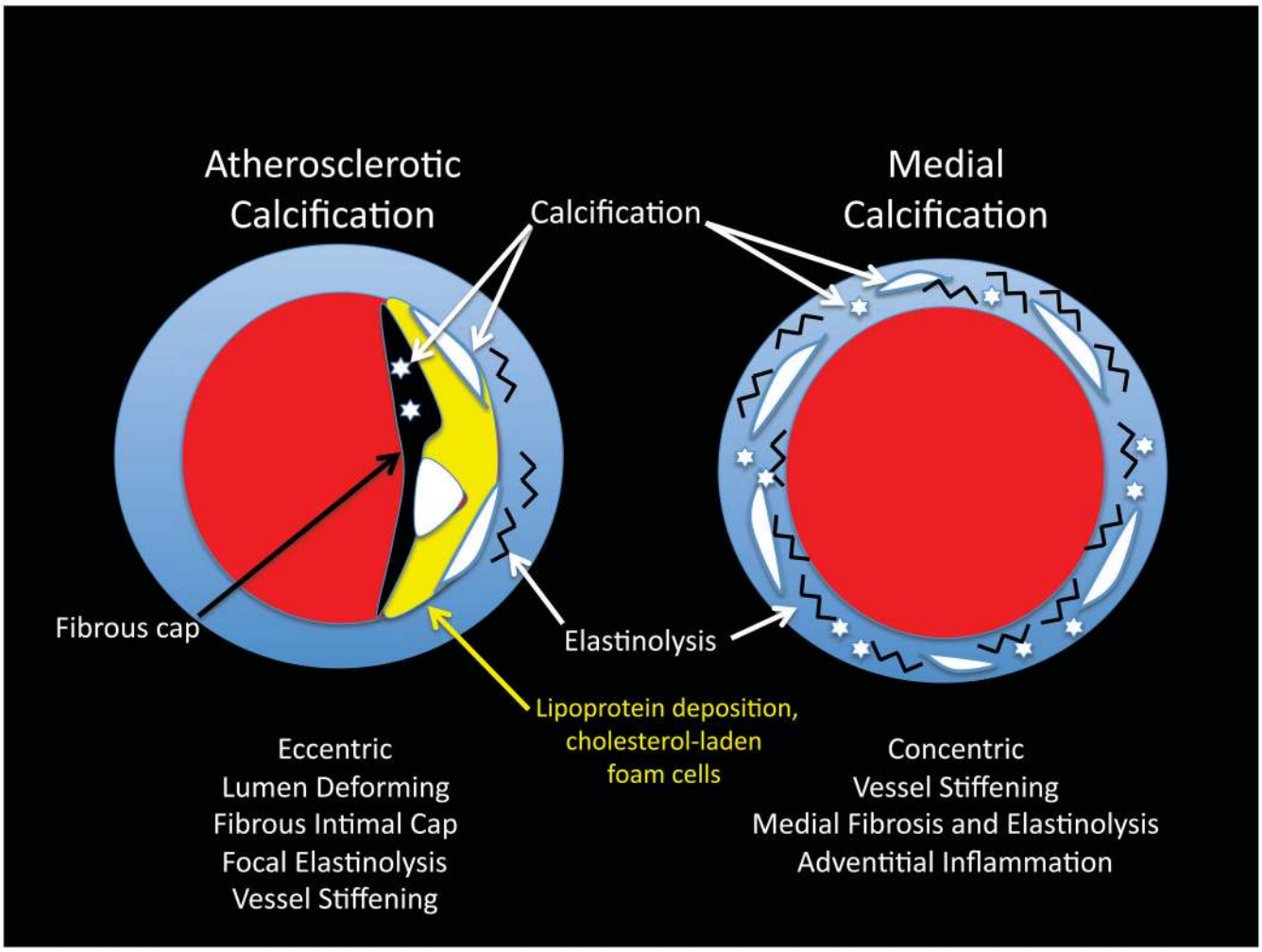

Figure 2. Atherosclerotic vs. Medial Arterial Calcification

Both atherosclerotic calcification and medial calcification stiffen arterial conduit vessels, impairing Windkessel physiology. The eccentric remodeling of atherosclerotic calcification also reduces lumen diameter, and predisposes to acute thrombosis.

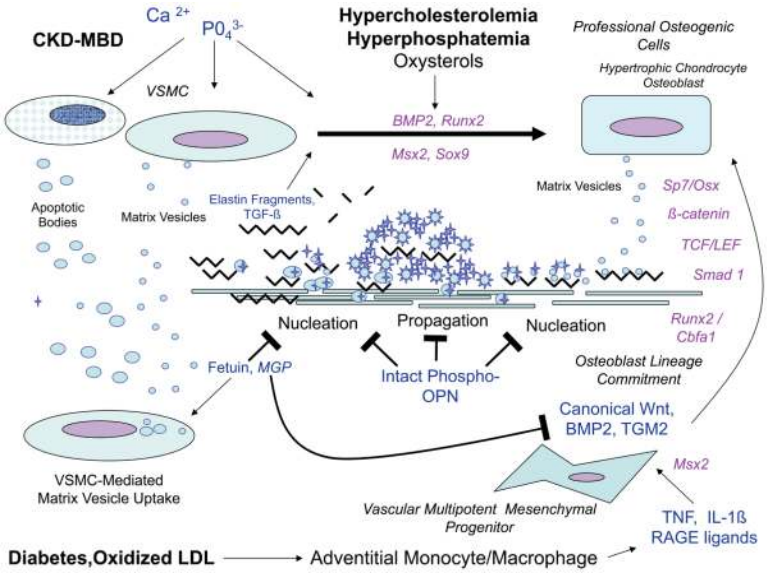

Figure 3. Vascular osteogenic cell origins, functions and phenotypes in arterial calcification

Vascular mineralization is regulated by processes overlapping yet distinct form those that control skeletal bone formation. Osteogenic progenitors can arise from "transdifferentiation" of VSMCs, or osteogenic lineage allocation of multipotent mesenchymal progenitors. Health VSMCs also play an important role in limiting vascular calcium accrual via fetuin- and MGP- dependent pinocytotic uptake of matrix vesicles. Metabolic and inflammatory insults induce vascular changes that impair normal VSMC function/viability and induce osteogenic differentiation of vascular mesenchymal cells. Not shown are the circulating osteoprogenitors that may contribute to the "vascular ossification" – true bone formation replete with marrow elements – that can be seen in ~15% of specimens. Extracellular factors are lettered in blue, while intracellular transcriptional regulators are colored lavender. See text for details, adapted from Mizobuchi, Towler, and Slatopolsky[108]. TGM2, tissue transglutaminase[210, 211].

Figure 4A

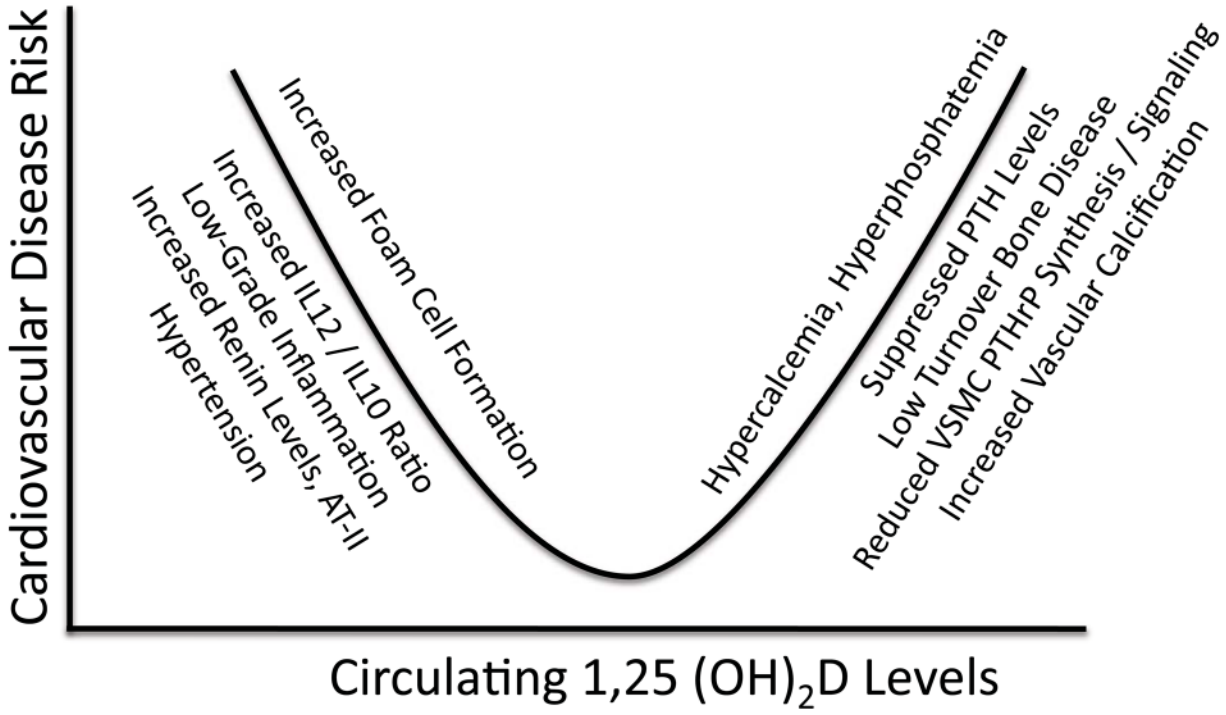

Figure 4B

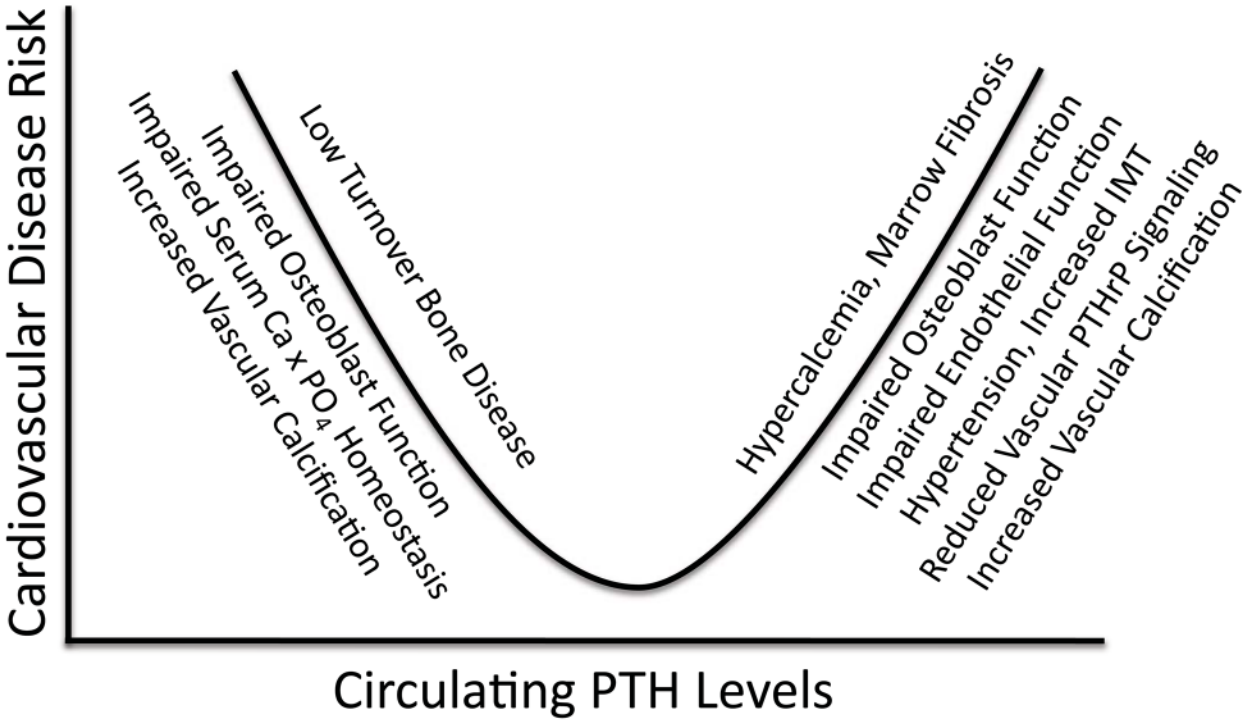

Figure 4. The biphasic relationship between cardiovascular disease and calciotropic hormones

As in all key endocrine systems, there also is a "sweet spot" that represents the optimal set point for calciotropic hormones levels and vascular health. Panel A, both calcitriol excess and deficiency have been associated with cardiovascular disease[141, 212], recently confirmed in children with CKD[213]. Panel B, similar cardiovascular problems arise with either both excesses[196] and insufficiencies[127, 128] in PTH. See text for details.

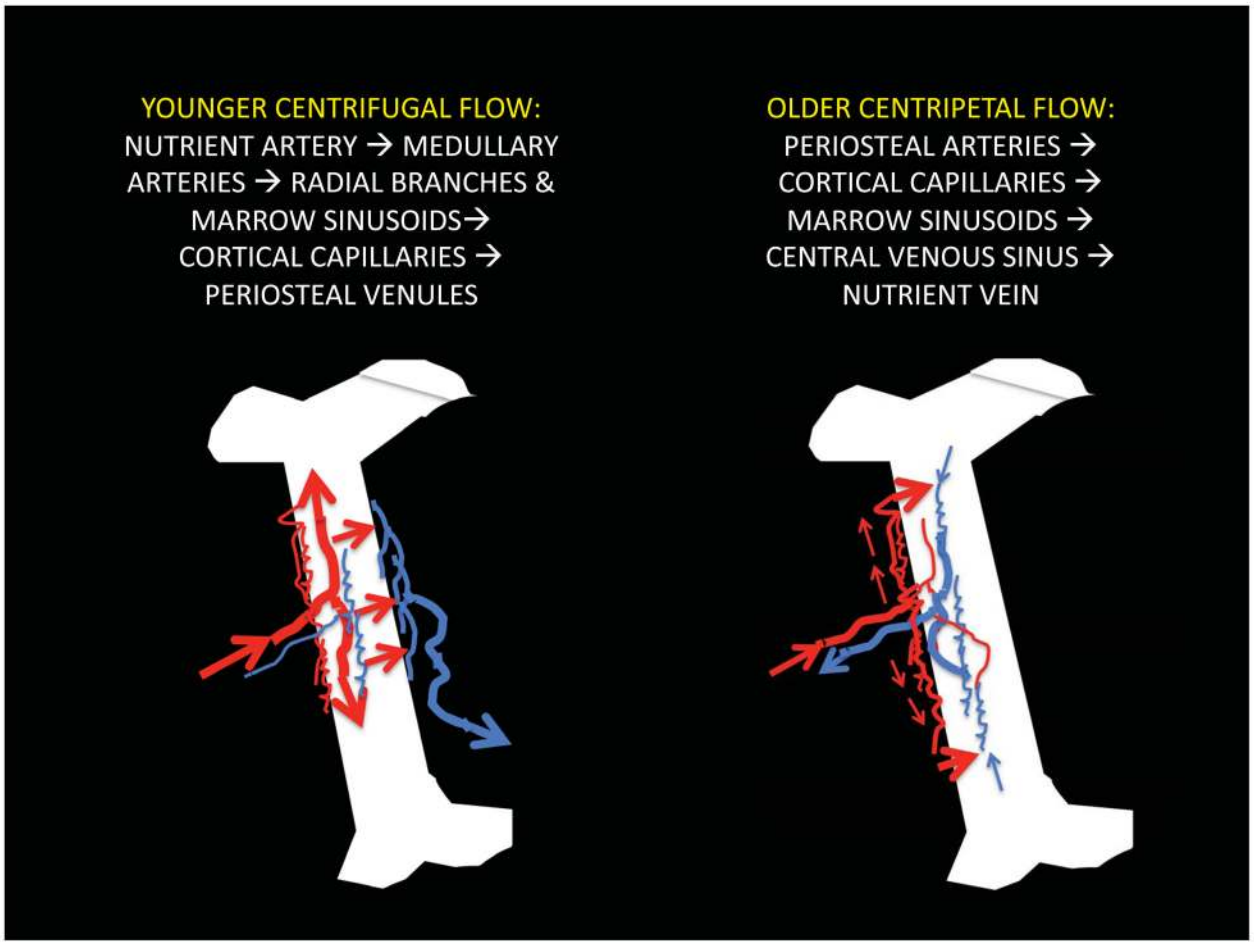

Figure 5. Age-dependent changes in cortical blood flow of long bones

Left panel, healthy cancellous bone has a marrow flow of about 20 cc/min/100 grams via the nutrient, ascending and descending medullary arteries; this helps maintain a relatively high intramedullary pressure that drives centrifugal flow through cortical bone (~ 5 cc/min/gram)[160]. Right panel, with aging [161] and arteriosclerosis[158, 159] perfusion is altered, with blood supply to the aging cortex provided primarily from periosteal conduit vessels[161]. Age- and exercise - related changes in vasodilatation responses of nutrient arteries may impact the extent of centrifugal vs. centripetal cortical blood flow[163, 214] since the nutrient arteries are more responsive to vasocontrictors[162].

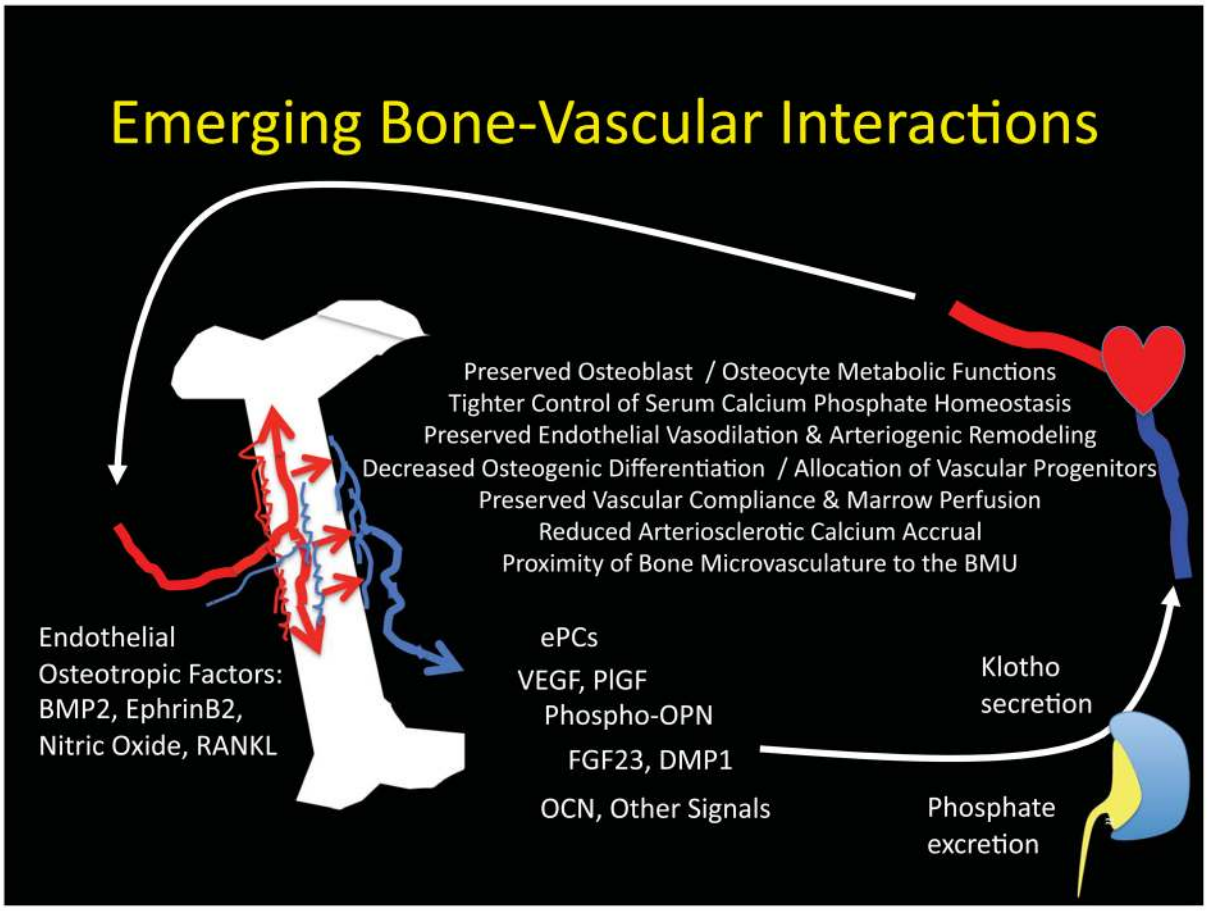

Figure 6. Clinical promises and pitfalls of the emerging bone-vascular axis

A bidirectional endocrine / metabolic relationship exists between bone and the vasculature that mutually benefits bone and vascular health. The kidney is an important intermediary via regulation of phosphate excretion[182] and the elaboration of Klotho[109, 199]. Importantly, PTH/PTHrP receptor signaling (a) maintains bone formation, sustains hematopoietic niche function[215] and ePC mass[13]; (b) promotes intact osteoblast OPN[24] and osteocyte FGF23[197, 199] secretion; (c) supports renal Klotho production[199]; and (d) suppresses aortic osteo-fibrogenic Wnt/β-catenin signaling[23, 146] and vascular calcium accrual[23, 26]. PTH/PTHrP receptor signaling also reduces aortic[23] and skeletal[216] oxidative stress, and maintains the proximity of the microvasculature to the BMU during bone formation[164]. Declining renal function and tissue resistance to PTH/PTHrP receptor signaling represent key features in the perturbation of the bone-vascular axis with disease. P1GF, placental growth factor. RANKL, receptor activator for NF-KappaB Ligand. BMU, basic multicellular unit. See text for details.

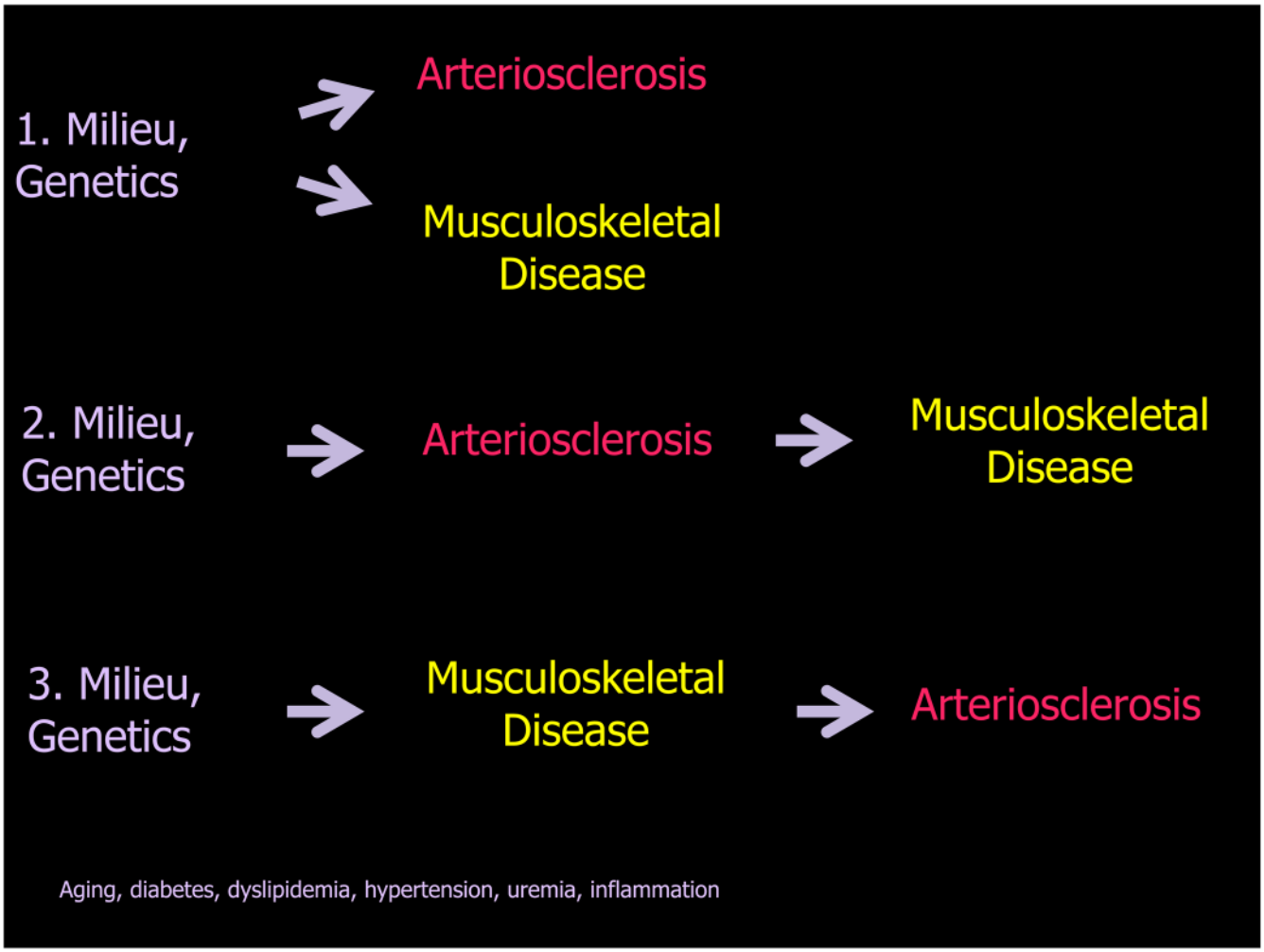

Figure 7. Metabolic milieu, genetics, arteriosclerosis, and musculoskeletal disease

Oxylipids simultaneously drive arteriosclerotic calcification, suppress bone formation, and increase osteoclastogenesis; parallel progression arteriosclerosis and musculoskeletal disease ensues (schema 1). However, via vessel stiffening and reductions in endothelium-dependent control of bone perfusion, arteriosclerosis can negatively impact bone anabolic responses necessary for skeletal homeostasis and fracture repair (schema 2). Finally, since osteoblasts, osteocytes, and a bone marrow elaborate cellular elements and hormonal cues that prevent arteriosclerotic remodeling and preserve vascular health, primary disease of bone may give rise to or at least exacerbate arteriosclerotic disease (schema 3).

TABLE I

COMMON HISTOANATOMIC TYPES OF VASCULAR CALCIFICATION

TYPE	CHARACTERISTICS/SETTINGS	PATHOBIOLOGY
Calcific aortic valve disease (CAVD) a.k.a. senile calcific aortic stenosis	Calcification of aortic valve leaflets Advanced age, bicuspid aortic valvfe, hypercholesterolemia, metabolic syndrome, diabetes, hypertension,	Fibrofatty expansion of valve fibrosa, splitting of elastic laminae, oxylipid deposition. Both osteogenic mineralization and amorphous calcium phosphate nodules deposited. True ectopic ossification-- woven bone formation -- in ~13% of specimens, but ectopic osteogenic interstitial cells (VICs) and circulating osteo-progenitors contribute to valve osteogenic cell populations.
Atherosclerotic intimal calcification (AIC)	Calcification of atherosclerotic plaques; eccentric, lumen-deforming, oriented by patchy intimal lipoprotein deposits. Same risk factors as CAVD.	Focal fibrofatty atherosclerotic plaque calcification with VSMC chondroid metaplasia and endochondral mineralization observed at base of lesions. Calcifying vascular cells (CVCs) related to pericytes also contribute. Driven by oxylipids from oxLDL. Lipid core and fibrous microcalcifications, focal elastinolysis noted.
Arterial medial calcification (AMC, sometimes MAC) a.k.a. Mönckeberg's medial calcific sclerosis	Calcification of the arterial tunica media; concentric, extensive, almost confluent. Common in type II diabetes, autonomic neuropathy, end stage renal disease / CKD5	Circumferential calcification of the tunica media with lipidaceous matrix vesicles, elastinolysis, and early expression of membranous mineralization programs (later endochondral). Inflammation-driven adventitial – medial BMP-Wnt signaling directs initial osteogenic programming of vascular multipotent mesenchymal progenitors (pericytes, CVCs).
Vascular calcification of chronic kidney disease a.k.a. chronic kidney disease - mineral & bone disorder (CKD-MBD)	CKD5 with any of the above. Major perturbations in calcium phosphate homeostasis, & reductions in serum fetuin, pyrophosphate.	VSMC apoptosis and osteochondrogenic metaplasia driven by hyperphosphatemia, worsened by iatrogenic hypoparathyroidism and low-turnover bone disease. Occurs most often in settings of antecedent medial and atherosclerotic disease processes that initiated prior to uremia
Calcific uremic arteriolopathy (CUA), a.k.a. calciphylaxis	CKD4 or CKD5 with dermal arteriolar medial calcification and dermal fat necrosis, usually of pannus, buttocks, or lower extremities. Usually on warfarin.	Arteriolar (generally < 50 micron diameter) medial calcification with fibroproliferative occlusion leads to tissue necrosis. Dermal fat, lung, and mesentery most significantly affected. Warfarin impairs matrix Gla protein (MGP) gamma carboxylation and functions, including BMP2/4 inhibition & fetuin-dependent clearance of mineralizing matrix vesicles.