Aortic calcification increasingly afflicts our aging and dysmetabolic population. In the past decade, multiple studies from preclinical and clinical investigators have identified that active osteogenic/chondrogenic mechanisms contribute to aortic calcium load. Cellular and matrix signals control vascular calcium phosphate mineral deposition via processes resembling membranous and endochondral bone formation. Clinical consequences also have begun to emerge. Atherosclerotic calcification of the aortic arch portends ischemic cerebrovascular events in women, although mitral annulus calcification may be a better predictor. Medial artery calcification, a nonobstructive form of aortofemoral calcium accumulation, is a characteristic vascular pathology seen in patients with type II diabetes or chronic renal failure. Medial artery calcification not only is a predictor of cardiovascular mortality but also increases the risk for lower extremity amputation; vascular stiffening perturbs normal aortofemoral Windkessel physiology, thus compromising distal tissue perfusion while increasing afterload, pulse pressure, and myocardial workload. Aortic valve calcium accumulation assessed by echocardiography is a strong predictor of disease progression in patients with initially asymptomatic aortic stenosis. In end-stage renal disease patients on dialysis, the presence of aortic valve calcification is a particularly ominous risk factor for cardiovascular mortality. All forms of aortic and valve calcification are increased along the metabolic syndrome continuum. The detailed analysis by Katz et al just demonstrated that aged, dyslipidemic LDLr−/− ApoE0/100 mice develop hemodynamically significant calcific aortic stenosis with concomitant left ventricular hypertrophy and reduced ejection fraction. Likewise, our understanding of molecular pathobiology has advanced in recent years. Inflammatory regulators that induce oxidative stress, activate metalloproteinase-dependent matrix turnover, enhance mural angiogenesis, promote arterial BMP–Msx2–Wnt signaling, and deplete tissue pyrophosphate levels have emerged, revealing many potential therapeutic targets. Yet, a major limitation in the field has been the inability to spatially resolve and potentially quantify the dynamics of vascular matrix metabolism and mineralization from the earliest stages of aortic disease initiation. In this issue of Circulation, Aikawa and colleagues find an innovative way to address this unmet scientific need by applying multimodality optical and MRI molecular imaging to the calcifying vasculopathy of apoE-deficient mice. In this model, the apoE−/− mouse forms significant atheromas with profound aortic chondroid metaplasia and endochondral mineralization when fed high-fat diets typical of Westernized societies. Multimodality nanoprobes for vascular cell adhesion molecule-1 induction, visualized by both magnetic resonance imaging and near-infrared fluorescence microscopy, detected endothelial inflammation as a very early feature of aortic value disease in apoE-deficient mice. This is very reassuring because endothelial activation and T-cell and monocyte recruitment are critical components in the progression of human calcifying aortic valve disease. Perhaps even more exciting data arise from the novel probes used that resolve vascular gelatinase enzyme activity (the Figure). Using GelSense680, a probe that fluoresces when enzymatically cleaved, the authors demonstrate that both macrophages and valve myofibroblasts contribute to matrix metalloproteinase (MMP)-2 and MMP-9 activity. Metalloproteinases—necessary for matrix remodeling, angiogenesis, and bone formation in the skeleton—have recently emerged as critical components in the pathobiology of aortic calcification and aneurysm. Qin et al demonstrated that periadventitial administration of MMP-2 and MMP-9 inhibitors abrogated aortic medial calcification in a nicotine plus vitamin D vascular injury model. Thus, should the aortic valve interstitial myofibroblast and the

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migratory aortic adventitial myofibroblast similarly contribute to vascular remodeling and calcification responses, the ability to spatially resolve and quantify vascular myofibroblast MMP activity during disease initiation, progression, and treatment could have a significant impact on aortic disease evaluation and management.

Finally, implementing OsteoSense750, a novel bisphosphonate conjugated to a near-infrared fluor, the authors can identify the very earliest phases of calcium deposition in the aortic leaflet. Like all bisphosphonates, this noncleavable pyrophosphate analog avidly binds calcium and thus accumulates at sites of active biomineralization. Using the Vector Red substrate for alkaline phosphatase (ALP), allowing colorimetric (and visual fluorescence) imaging of ALP activity in frozen sections, the authors spatially placed ALP activity at valve interstitial myofibroblasts that score positive with OsteoSense750 and smooth muscle cell α-actin. This is of particular importance because ALP not only is a very early marker of osteogenic differentiation but also degrades tissue matrix metabolism, with effects on aortic biomechanics and membrane lipid transport, encountered in idiopathic infantile arterial calcification, type II diabetes, and T2DM.

Imaging the pro-osteogenic vascular matrix metabolism cascade. Recent studies of preclinical disease models and human aortic pathological specimens have suggested that a sequence of active osteogenic processes contributes to the pathobiology of aortic calcified matrix accumulation (see elsewhere for detailed reviews). Aikawa et al. have implemented novel multimodality molecular imaging techniques with near-infrared fluorescence microscopy and MRI detection to temporally and spatially resolve many critical components in aortic valve matrix metabolism in the apoE−/− mouse model. Coreregistration of ALP activity and calcium phosphate deposition with osteogenic gene regulatory programs and MMP activity in smooth muscle α-actin+ cells definitively establishes that vascular myofibroblasts actively participate in aortic calcification at the earliest stages of disease. See text for details.

In contrast, other well-mineralized areas were visualized by both methods, viz, as chondroid metaplasia of the aortic tunica media occurs via endochondral bone-like mineralization. Thus, the OsteoSense750 light-based calcium imaging strategy is profoundly more sensitive, capable of detecting the earliest phases of matrix mineralization likely occurring just as aortic valve tissue PPI levels begin to decline with ALP induction (the Figure).

The utility of these imaging technologies in development of novel therapeutics in preclinical disease models is readily apparent. As strategies attacking the pathobiology of vascular mineralization and matrix turnover are developed (the Figure), in vivo experiments that establish pharmacokinetic-pharmacodynamic responses and doses required to prevent or reverse aortic calcification are now possible. For example, the effects of exogenous metalloproteinase inhibitors or reactive oxygen scavengers could be evaluated for effects on vascular matrix metabolism, with effects on aortic biomechanics and load-independent cardiac performance subsequently assessed.

Given the utility of LDLr−/− and LDLr+/− ApoB100/100 mice as models of metabolic syndrome, type II diabetes, uremic aortic calcification, and calcific aortic stenosis, future studies will no doubt encompass these excellent animal models of aortic disease pathobiology. Because superoxide induction coincides with valve calcification and activates vascular pro-MMP-9, visualization and manipulation of oxidative stresses vis-à-vis the matrix metabolism responses visualized will add much to our understanding of the aortic injury response. Moreover, because the ratio of tissue PPI to
Pi (inorganic phosphate) is critical to the control of mineral deposition,\(^\text{20}\) assays imaging vascular ENPP1 and ENPP2 (autotoxin) enzyme activity can be envisioned and may reveal further metabolic differences between aortic tissues at early and later stages in the disease progression cascade (the Figure). Thus, the work of Aikawa et al\(^\text{13}\) has begun to address critical, unmet scientific needs in imaging aortic matrix metabolism in preclinical models.

The near-infrared spectral characteristics of the optical imaging agents used avoid background tissue autofluorescence (eg, elastin), and pioneering work in cancer imaging has clearly demonstrated clinical feasibility in humans. However, optical imaging, while quantitative with terrific resolution that permits detailed anatomic coregistration, suffers from extinction and reflection phenomena that attenuate the signal.\(^\text{14}\) To detect and follow vascular metabolic changes from the very earliest stages of aortic valve disease in humans, completely noninvasive metabolic imaging techniques will likely be required. As demonstrated, vascular magnetic resonance imaging holds tremendous promise for early detection of valve inflammation.\(^\text{13,14}\) For optical imaging of aortic valve metabolism, however, specialized endovascular imaging catheters will likely be necessary for studies in humans.\(^\text{14}\) Noninvasive carotid arterial optical imaging should become possible; fluorescence tomography holds promise for imaging optical signals to tissue depths of \(\leq 10\) cm, depending on interposed tissue characteristics.\(^\text{14}\) Thus, technological hurdles exist that currently limit translation of near-infrared fluorescence microscopic metabolic optical imaging to comprehensive human aortic valve evaluation with aging, metabolic syndrome risks, diabetes, and uremia. All in all, however, the innovative study of Aikawa et al\(^\text{13}\) is a most wonderful thing to see.

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