Vascular calcification, once considered a passive consequence of aging, is now recognized to be a highly regulated process akin to bone formation. Vascular calcification is prevalent across ethnicities and age groups, and observational studies show an interaction with aging in asymptomatic adults and in individuals with established coronary artery disease. Recent findings from the HORUS study have shown that the link between aging and vascular calcification is an age-old association. In this study, 137 mummies up to 4000 years old were examined with computed tomography scans. Vascular calcification was present in 47 of 137 or 34% of the mummies, and age at the time of death correlated positively with the presence of vascular calcification and the number of vascular beds with calcified vessels. In the modern era, the incidence of vascular calcification has been shown to increase with advancing age and has been reported to be <5% annually for individuals <50 years of age to >12% for individuals >80 years of age. When present, vascular calcification portends a worse clinical outcome; a meta-analysis of 218,000 patients found a 3.41-fold higher risk for cardiovascular mortality and a 3.41-fold higher risk for any cardiovascular event. Thus, understanding how aging influences the pathobiology of vascular calcification may have far-reaching implications for associated cardiovascular morbidity and mortality.

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To understand the cellular and molecular mechanisms that underlie aging-related vascular calcification, investigators have begun to focus on the vascular pathophenotype associated with Hutchinson-Gilford progeria syndrome (HGPS). This rare genetic disorder is the result of a point mutation in the LMNA gene that generates an abnormal variant of prelamin A that retains its farnesyl group and is known as progerin. The disease is characterized in part by accelerated aging with early atherosclerosis and vascular calcification. To date, the causative mechanisms for precocious vascular calcification in this disease have not been elucidated fully. In this issue of Circulation, Villa-Bellosta et al report that vascular calcification in HGPS results from decreased levels of extracellular inorganic pyrophosphate, an inhibitor of hydroxyapatite crystal formation and ectopic calcification. Using the Lmna<sup>G609G</sup> knock-in mouse model of HGPS, which expresses progerin and has pronounced aortic medial calcification, the investigators found that low levels of extracellular inorganic pyrophosphate occurred as a result of impaired synthesis and increased hydrolysis to inorganic phosphate. Pyrophosphate synthesis was impaired by substrate availability; HGPS vascular smooth muscle cells (VSMCs) generated lower levels of mitochondrial ATP than controls. They also observed a concomitant increase in the expression of tissue-nonspecific alkaline phosphatase, which hydrolyzes pyrophosphate to inorganic phosphate. The obligate role of progerin in the dysregulation of pyrophosphate metabolism was confirmed by retrovirus-mediated gene transfer to force the expression of progerin in wild-type VSMCs. Progerin-expressing VSMCs recapitulated the HGPS-VSMC phenotype with decreased pyrophosphate levels, aberrant pyrophosphate metabolism, and mineralization. Although these studies do not tell us specifically how progerin induces mitochondrial dysfunction or upregulates alkaline phosphatase expression, the findings identify pyrophosphate as a previously unrecognized mediator of vascular calcification in HGPS and may have broader applicability to our understanding of aging-related vascular calcification.

The results from this study performed with a disease model of accelerated aging to investigate vascular calcification may be extrapolated to explain this process under normal aging conditions. This is underscored by the fact that prelamin A is expressed in VSMCs as they age in the absence of the HGPS genotype. Prelamin A has been detected in blood vessels isolated from older individuals or from young patients with chronic kidney disease on dialysis who often have phenotypically chronic aged vasculature. In these vessels, prelamin A colocalizes with senescent and calcifying VSMCs. In vitro studies have demonstrated that prelamin A is increasingly expressed in presenescent cells through a mechanism involving increased oxidative stress, which itself has been implicated in the pathogenesis of vascular calcification. The role of prelamin A in modulating senescence has been established through the use of HGPS-induced pluripotent stem cells. Under basal conditions, progerin and epigenetic changes associated with premature aging are absent; however, when these cells are differentiated toward VSMCs, progerin levels increase and cells return to a senescent state. Although it is not known if senescence per se dysregulates pyrophosphate metabolism in VSMCs, it is clear that senescent VSMCs achieve a calcification phenotype. Senescent VSMCs have increased expression of the osteoblast transcription factor Runx2 and alkaline phosphatase, and they have an enhanced capacity to mineralize compared with non-senescent cells.
Evidence that dysregulation of vascular pyrophosphate metabolism is an aging-related process or that pyrophosphate levels decline with age is limited. Small observational studies of normal healthy subjects or individuals with chronic kidney disease on dialysis found only a weak inverse correlation between age and plasma pyrophosphate levels. However, abundant experimental evidence firmly links perturbations in pyrophosphate metabolism to vascular calcification. Genetic deletion of genes that regulate phosphate metabolism in mice has established that extracellular inorganic pyrophosphate plays a key role in regulating vascular calcification (reviewed elsewhere). The finding that HGPS-VSMCs synthesize less pyrophosphate owing to decreased ATP levels also implicates mitochondrial dysfunction in aging-related calcification. Mitochondrial dysfunction exists in HGPS, and proteomic analysis of murine HGPS adipocytes demonstrates modifications in the expression profile of mitochondrial proteins related to lipid metabolism, the tricarboxylic acid cycle and oxidative phosphorylation, and oxidant stress. ATP levels are also known to be decreased in HGPS fibroblasts, with levels being only 50% of that measured in control subjects. Because mitochondrial dysfunction and decreased ATP production have been demonstrated in calcifying VSMCs exposed to high levels of inorganic phosphate, it is interesting to speculate that dysregulated pyrophosphate metabolism may induce mitochondrial dysfunction. Other plausible explanations for the decrease in mitochondrial ATP generation during mitotic calcification include a decline in mitochondrial biogenesis, which occurs with advancing age, a reduction in mitochondrial mass, or an increase in mtDNA mutation.

Is dysregulation of pyrophosphate metabolism the only mechanism to explain aging-related vascular calcification? This is unlikely because other studies examining calcifying senescent VSMCs have implicated activation of the DNA damage response and acquisition of a senescent-associated secretory phenotype as the mechanism for calcification. These prelamin A–expressing VSMCs appear to regulate calcification in a paracrine manner by secreting procalcifying factors and cytokines, including interleukin-6, bone morphogenetic protein-2, and osteoprotegerin. Although pyrophosphate metabolism was not investigated in the aforementioned studies, elevated levels of inorganic phosphate are known to modulate VSMC transition to a calcifying phenotype, and hyperphosphatemia-induced nanocrystals have been shown to increase the expression of bone morphogenetic protein-2 in VSMCs. Thus, dysregulation of pyrophosphate metabolism may act in concert with the DNA damage response and other known procalcifying mechanisms to promote aging-related vascular calcification.

What can we conclude from these studies? First, it is likely that vascular prelamin A expression may emerge as a biomarker of vascular aging. This may be of importance, given the divergence between chronological and biological age in many disease states associated with vascular calcification. Second, extracellular inorganic pyrophosphate levels and indexes of phosphate metabolism should be evaluated in the examination of aging-related calcification. Whether pyrophosphate modulates other known mechanisms of vascular calcification or has predictive value for the development of calcification requires additional study. Finally, the therapeutic efficacy of exogenous pyrophosphate administration to prevent vascular calcification may be limited by the propensity for pyrophosphate to induce calcification in nonvascular tissues when in excess. This suggests that targeted therapies such as vascular gene transfer to maintain pyrophosphate levels may offer a greater therapeutic benefit to ameliorate vascular calcification with an improved side-effect profile. Although aging is inevitable, findings from the Villa-Bellosta and other similar studies will ensure that vascular calcification is not.

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References


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Vascular Calcification: An Age-Old Problem of Old Age
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