Vascular calcification is increased in patients with diabetes mellitus and is associated with increased morbidity and mortality rates compared with patients with diabetes mellitus without calcification. Despite its considerable clinical significance, little is known about the molecular pathways through which vascular calcification is triggered by diabetes mellitus pathology, although several diabetes mellitus–associated factors, including high glucose, could conceivably play important roles in the pathogenesis. Therefore, a greater understanding of the mechanisms through which diabetes mellitus may induce calcification is required to develop effective strategies to interrupt this process.

The pathogenesis of vascular calcification is a highly complex, active, cell-regulated process, with several cell types including smooth muscle cells, macrophages, and circulating bone marrow–derived cells playing a role. Mechanistic studies of vascular calcification have largely focused on calcium and phosphate homeostasis, the release of calciifying vesicles, changes in the extracellular matrix, loss of inhibition, and cellular differentiation, along with several other molecular processes. Each of these processes has been explored further in detail. For example, the switching of vascular smooth muscle cells to osteoblast-like cells capable of generating a mineralized matrix has been associated with the release of calciifying vesicles, bone morphogenetic proteins, inflammation, and oxidative stress associated with Runx2 activity and AKT signaling. Understanding the connections between these pathways, along with those to diabetes mellitus–associated complications, could aid in the development of anticalcification therapeutics.

Hyperglycemia is a major complication associated with diabetes mellitus, with chronic hyperglycemia being tied to vascular complications in both patients with type 1 and type 2 diabetes mellitus. However, how hyperglycemia is related to the development of vascular calcification in patients with diabetes mellitus is currently unknown. One potential connection between hyperglycemia and vascular calcification may lie in the post-translational modification, O-GlcNAcylation. O-GlcNAcylation is the glycosylation process through which N-acetylglucosamine (O-GlcNAc) gets added to serine and threonine residues of proteins. Recently, this process has been associated with signaling, transcription, and chronic disease pathologies, including diabetes mellitus, because such O-GlcNAcylation shares similarities to the process of protein phosphorylation, although its regulation differs from the process of phosphorylation, which is regulated by many kinases and phosphatases. Instead O-GlcNAcylation involves a tight regulation controlled by 2 molecules: one that adds O-GlcNAc onto proteins, N-acetylglucosaminyl-transferase, and one that removes the modification, N-acetylglucosaminidase. Of interest, O-GlcNAcylation has been shown to stimulate chondrogenesis and osteogenesis and correlates with the transcriptional activity of the osteogenesis regulator, Runx2. Given the association between osteogenesis and vascular calcification and the involvement of O-GlcNAcylation in diabetes mellitus, exploring the role of O-GlcNAcylation in the development of diabetes mellitus–induced calcification may provide important mechanistic findings.

In this issue of Circulation Research, Heath et al build on previous studies exploring the development of vascular calcification in diabetes mellitus. Specifically, they provide novel mechanistic insight that identifies O-GlcNAcylation of AKT

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on its T430 and T479 amino acids as a potential regulator of diabetes mellitus–induced calcification. The in vitro analysis of this post-translational modification hints at a pathway in which the phosphorylation of AKT on S473 may be regulated by AKT T430/T479 O-GlcNAcylation. This work concludes that phosphorylated AKT may result in a signaling cascade that leads to increased Runx2 transcription and activity, thereby inducing vascular calcification (Figure 1).

Using streptozotocin-injected mice, a drug-induced mouse model of type 1 diabetes mellitus, Heath et al.\textsuperscript{20} found a strong increase in vascular O-GlcNAcylation after the onset of increased blood glucose levels, along with a significant increase in aortic calcium content and vascular stiffness. In an effort to gain mechanistic understanding of the connection between O-GlcNAcylation and the development of vascular calcification, they used a mouse vascular smooth muscle cell culture model. When these cells were cultured in osteogenic differentiation media along with an inhibitor of N-acetylglucosaminidase, Thiamet-G, or with shRNA knockdown of the N-acetylglucosaminidase enzyme, they developed a mineralized matrix. Suggesting blocking the removal of O-GlcNAc may promote a mineralization process. Supporting this in vitro finding in vivo, Thiamet-G administration to streptozotocin diabetic mice was found to further enhance the level of vascular calcium accumulation. Taken together, these results suggest that O-GlcNAcylation may promote vascular calcification in smooth muscle cells and diabetic mice.

On a mechanistic level, when the AKT T430/T479 amino acids were mutated such that they could no longer be post-translationally modified by O-GlcNAcylation, constitutively active AKT phosphorylation at S473 and cellular calcium content were reduced to control levels. In addition, AKT binding to a component of the mammalian target of rapamycin (mTOR) complex 2, Rictor, was substantially reduced by these mutations. With these findings, it can be postulated that O-GlcNAcylation may aid in the binding of AKT to the mTOR complex 2, leading to AKT phosphorylation at S473. The role of mTOR in this pathway was further confirmed by blocking O-GlcNAcylation–driven calcification in smooth muscle cells through rapamycin treatment. Altogether, these findings point to the AKT T430/T479 amino acids as crucial regulators of an O-GlcNAcylation–driven AKT signaling pathway that leads to calcium accumulation. Although the full mechanistic pathway connecting O-GlcNAcylation to the development of vascular calcification remains to be found, this work helps elucidate a few potential key regulators and signals involved. It also suggests O-GlcNAcylation of AKT as a potential target in the treatment of diabetes mellitus–induced calcification.

Additional analysis including in vivo imaging of vascular calcification and the use of additional model systems could strengthen the interpretations of this study. Although the in vitro analysis in the work by Heath et al.\textsuperscript{20} confirmed calcification through the use of alizarin red staining, the in vivo analysis measured it indirectly. Increased calcium content and vascular stiffness measurements suggest medial calcification; however, some form of imaging would be needed to confirm this. For example, vascular calcifications in diabetic animals can be clearly seen through the use of a near-infrared fluorescent imaging probe (red) in apolipoprotein E-deficient (apoE\textsuperscript{−/−}) diabetic (streptozotocin [STZ]+) mice and controls (STZ−). Ao indicates aorta; AV, aortic valve; and FT, fibrous trigone.
agent (Figure 2). Imaging could also provide a means to more closely follow and evaluate changes in the development of diabetes mellitus–induced calcification when used in combination with modulators of O-GlcNAcylation of AKT.

Exploring this pathway in additional diabetic animal models, including those of type 2 diabetes mellitus, could be of importance, especially because type 2 diabetes mellitus is a known risk factor for cardiovascular disease, with insulin resistance correlating with increased vascular calcification.21 Studying O-GlcNAcylation of AKT in animal models of type 2 diabetes mellitus such as Ldlr−/− diabetic mice could help expand the clinical relevance of the findings by Heath et al.20 It could also potentially discriminate mechanisms involved in the development of medial versus intimal calcification, helping to identify therapies most likely to benefit specific patient populations.

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References

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