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Unsticking the Broken Diabetic Heart: *O*-GlcNAcylation and Calcium Sensitivity

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Cardiovascular disease is the leading cause of death in patients with diabetes. However, identifying mechanisms of disease progression has proven difficult as diabetes involves dysfunction of numerous pathways including cellular signaling and mitochondrial capacity. This results in the alteration of circulating hormones and nutrients that can lead to inflammation, oxidative stress, endoplasmic reticulum stress, lipotoxicity, and glucotoxicity (1). One hallmark of diabetes is the development of hyperglycemia and one goal of patient care is to control glycemia through the modification of lifestyle and drug therapy. However, the effectiveness of antihyperglycemia drugs on the development of heart failure in individuals with diabetes is not fully understood (2). Part of this challenge results from an incomplete understanding of how glucose regulates molecular function.

Over 30 years ago, Torres and Hart (3) identified a unique posttranslational modification (PTM) by glycosylation of intracellular proteins via addition of an *O*-linked β-*N*-acetyl-D-glucosamine (*O*-GlcNAc), termed *O*-GlcNAcylation. Owing to the input of multiple metabolic pathways to generate uridine diphosphate (UDP)-GlcNAc, the substrate of *O*-GlcNAcylation, this PTM gained attention as the link between metabolism and chronic disease (4). Increases in glucose associated with diabetes lead to excess *O*-GlcNAcylation and represent a crucial link between glucose and molecular regulation. Similar to protein phosphorylation, *O*-GlcNAcylation occurs at serine and threonine residues in proteins. It has been identified on ~4,000 targets across most cellular pathways, as reviewed by Bond and Hanover (5). Unlike other PTMs that may have dozens if not hundreds of proteins modulating levels, two enzymes control *O*-GlcNAc cycling: *O*-GlcNAc transferase (OGT) for its addition and *O*-GlcNAcase (OGA) for its removal. One point of regulation comes from the few splice variants of the genes encoding these proteins that show differing subcellular localization, including the nucleus to alter gene expression (6) and the mitochondria to regulate metabolism (7). The apparent promiscuous nature of this modification has made defining specificity of regulation difficult.

In this issue of *Diabetes*, Ramirez-Correa et al. (8) identify a new mechanism of subcellular redistribution of the *O*-GlcNAcylation machinery, providing a means by which this pathway may be regulated in the diabetic heart. Examining myofilaments from the hearts of streptozotocin-induced diabetic rats, the authors found increased *O*-GlcNAcylation associated with decreased Ca^{2+} sensitivity. Using β-elimination/Michael addition with Tandem Mass Tags (TMT), they specifically map the *O*-GlcNAcylation to 63 sites on six different myofilament proteins. Removal of excess *O*-GlcNAc levels with the bacterial glycosidase *Clostridium perfringens N-acetyl-glycosidase J* (CpOGA) restores myofilament Ca^{2+} sensitivity in skinned cardiac muscles isolated from diabetic rats. However, the most novel discovery of the current study is the finding that basal levels of *O*-GlcNAc in both rodent and human myocardium localize to the Z-line and that this localization spreads toward the A-band and intensifies in streptozotocin-induced diabetes. This suggests that the increased *O*-GlcNAcylation found in pan-*O*-GlcNAc blots might be missing part of the picture. To begin to define the mechanism of this redistributed pattern, the authors used a series of immunoprecipitations and double immunoelectron microscopy to show that OGT and OGA sarcomeric distribution is inverted, altering *O*-GlcNAc levels and calcium sensitivity in the diabetic myocardium (Fig. 1). This suggests that the location of the *O*-GlcNAcylation machinery within the cell partially dictates its function and might explain some of the physiological and pathological roles of this PTM.

O-GlcNAcylation modifies a number of pathways to regulate cellular function. It therefore becomes difficult to define cause and effect. The changes in calcium signaling may also result from *O*-GlcNAc regulation of Ca^{2+} cycling either through modification of regulating transcription factors decreasing expression of sarcoendoplasmic reticulum Ca^{2+} ATPase 2a (SERCA2a) (9) or

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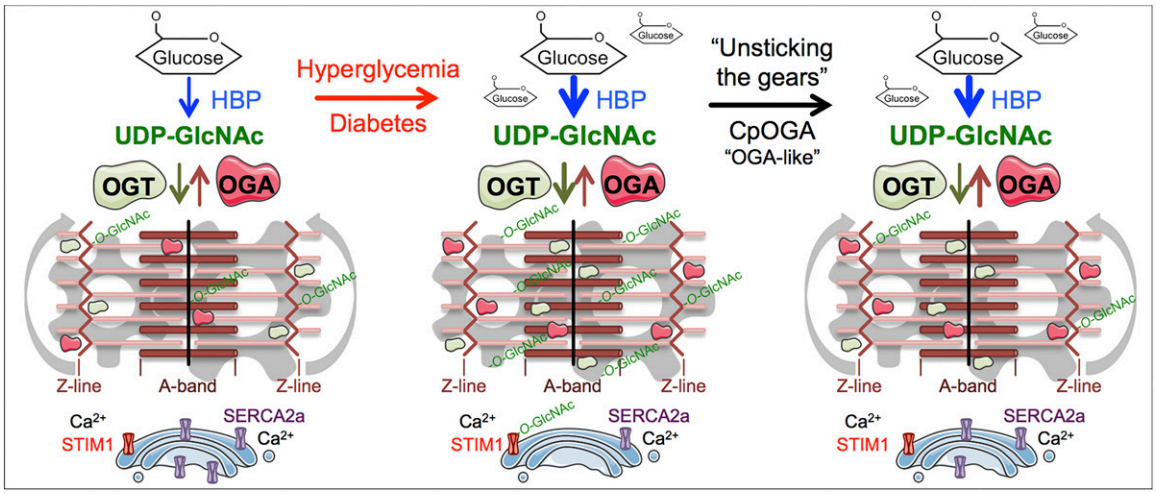

Figure 1—Schematic showing the proposed roles that increased *O*-GlcNAcylation has on calcium signaling and cardiac contractility in the diabetic heart. Under euglycemic conditions, some *O*-GlcNAcylation occurs near the Z-line of the contractile machinery associated with normal function of the heart. In the hyperglycemic states seen in diabetes, excess glucose is shunted from metabolic pathways to the hexosamine biosynthesis pathway (HBP) fueling OGT and increasing *O*-GlcNAcylation, leading to disruption of calcium regulatory proteins (SERCA2a and STIM1), *O*-GlcNAc spreading toward the A-band, and OGT/OGA redistribution resulting in decreased Ca^{2+} sensitivity associated with cardiac dysfunction. Removing the excess and aberrant *O*-GlcNAc through the expression of an OGA-like protein (CpOGA) restores calcium sensitivity and potentially rescues cardiac function. Figure was produced using images modified from Servier Medical Art (www.servier.com).

impaired store-operated Ca^{2+} entry (SOCE) through direct modification of stromal interaction molecule 1 (STIM1) (10). It is also possible that in vivo removal of *O*-GlcNAcylation might have undesired effects on proteins in other areas of the cell not related to Ca^{2+} sensitivity. The optimistic view is that, if targeted appropriately, removal of specific *O*-GlcNAc modifications may restore normal function (Fig. 1). Physiological interventions, such as exercise, are sufficient to remove excess *O*-GlcNAcylation in the diabetic heart (11), but whether this regulation is mediated by a similar redistribution of OGA/OGT or *O*-GlcNAcylation of myofilaments has yet to be explored.

Where do we go from here? As proteomic approaches are fine-tuned and more investigators start quantifying and mapping specific *O*-GlcNAcylation sites, there is a promise of defining new functions of this regulatory pathway (12). Currently, the majority of studies looking at *O*-GlcNAcylation rely on immunoprecipitation and pan-GlcNAc antibodies. Although these techniques still produce useful information, some question of their specificity has been raised (13). Additionally, there is the question of the adaptive potential of *O*-GlcNAcylation in response to ischemic stress (14) or in enhancing cardiac stem cell survival (15). Together, these different findings suggest that we are only scratching the surface for the potential that *O*-GlcNAcylation has in regulating cellular function. Despite the complex nature of diabetes and the discrepancies noted above, one thing does hold true and that is that glucose may be key to explaining the dysfunction seen in the diabetic heart. In fact, increasing levels of a single glucose transporter, GLUT1, can alter *O*-GlcNAcylation of Ca^{2+} cycling proteins (16). Additionally, not all the changes that occur as a result of *O*-GlcNAcylation have an immediate impact on the cell. Recently, it was shown that OGT interacts with the ten-eleven translocation methylcytosine dioxygenase (TET) family of proteins (17,18). These enzymes are involved in DNA hydroxymethylation and modification of the epigenetic code (19). This partnership links *O*-GlcNAcylation to long-lasting changes in gene expression, raising the possibility that any therapies designed to make immediate changes in *O*-GlcNAc levels can have effects months or even years down the road.

Importantly, the study by Ramirez-Correa et al. (8) identifies a new mechanism mediating the regulation of *O*-GlcNAcylation through subcellular redistribution of OGT/OGA and functional consequences that have immediate therapeutic potential to improve cardiac contractility. Unsticking the glucose-ridden diabetic heart fixes individual pieces of its function and supports the concept that we are on the brink of targeting therapies to improve cardiovascular outcomes in patients with diabetes.

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