SIRT2-mediated regulation of glycolytic flux: Another brick in the wall of Gary Lopaschuk's legacy in cardiac metabolism

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The heart exhibits remarkable metabolic flexibility, enabling the use of diverse substrates, including fatty acids, glucose, ketone bodies, lactate, and branched-chain amino acids, to sustain its substantial ATP demands. Substrate preference dynamically adapts to hormonal, nutritional, and physiological conditions to meet energetic demands (1). Impaired metabolic flexibility is recognized as an early feature of a wide range of cardiac diseases. For example, the diabetic heart relies predominantly on fatty acid oxidation, whereas pressure overload-induced cardiac hypertrophy is characterized by a metabolic shift toward increased glucose utilization (1). Understanding the molecular mechanisms underlying these metabolic changes has therefore become a major field of research.

Beyond transcriptional regulation, metabolic pathways are finely tuned by post-translational modifications. Among these, protein acetylation has emerged as a key regulator of cardiac metabolism (2–6). Increased protein acetylation is a characteristic of obese and diabetic animal models, such as those fed a high-fat diet (5) . This process can occur spontaneously when cellular levels of the acetyl group donor acetyl-coenzyme A (acetyl-CoA) are elevated, or it can be catalyzed by different lysine acetyltransferases. Conversely, acetylated proteins are deacetylated through two families of lysine deacetylases: histone deacetylases and sirtuins (SIRTs) (4). SIRTs have been clearly associated with the regulation of cardiac glucose and fatty acid metabolism (3, 4, 7).

Much of the recent literature has focused on acetylation within mitochondria, largely because mitochondrial conditions, specifically high concentrations of acetyl-CoA and an alkaline matrix pH, favor spontaneous non-enzymatic protein acetylation (7). Consequently, the main mitochondrial deacetylase, SIRT3, has been the subject of numerous studies. Genetic inactivation of SIRT3 has been shown to increase cardiac protein acetylation, promote fatty acid oxidation, and inhibit glucose oxidation (Fig. 1) (3). However, despite growing evidence linking acetylation to cardiac metabolic remodeling, the specific role of SIRT2, the predominant cytosolic sirtuin, in regulating cardiac glucose utilization and glycolytic flux has remained largely unexplored. In the current issue of the *American Journal of Physiology-Heart and Circulatory Physiology*, Ketema et al. sought to address this gap by exploring a potential role for SIRT2 in the regulation of cardiac glycolysis (Fig. 1) (8).

To investigate the role of SIRT2, Ketema et al. used two experimental models: isolated perfused working rat hearts and H9c2 cardiomyocytes, an immortalized clonal cell line derived from

embryonic BDX1 rat heart tissue that exhibits high glycolytic rates typical of fetal cardiomyocytes. The authors employed both pharmacological inhibition (AGK2) and siRNA-mediated knockdown of SIRT2. In addition, they examined the effects of other modulators of protein acetylation, including the SIRT1-selective inhibitor EX-527, the pan-sirtuin inhibitor nicotinamide (NAM), and the acetyltransferase inhibitor C646. A major methodological strength of the study is the direct assessment of glycolytic flux through measurement of the detritiation rate of [5-^{3}H]-glucose, providing a more accurate estimate than indirect approaches.

Ketema et al. showed that insulin stimulation increased glycolytic rates compared with unstimulated controls in isolated working rat hearts. This increase was abolished by AGK2 treatment, whereas glucose oxidation rates remained unchanged between groups. In contrast, EX-527, NAM, and C646 had no detectable effect on glycolysis, although glucose oxidation rates were generally reduced following treatment with these inhibitors.

To extend the findings obtained in isolated hearts, Ketema et al. exposed H9c2 cells to AGK2 either acutely (1 h) or chronically (24 h). AGK2 treatment significantly reduced glycolytic flux, with a more pronounced effect following chronic exposure. To determine whether this effect was attributable to SIRT2, the authors performed siRNA-mediated SIRT2 knockdown. Consistent with the pharmacological data, SIRT2 silencing similarly decreased glycolytic rates. Together, these results reveal a previously unrecognized role for SIRT2 in the regulation of cardiac glycolysis.

Intriguingly, global protein acetylation was largely unchanged by either pharmacological inhibition or SIRT2 knockdown in both H9c2 cells and isolated working rat hearts, with the exception of a NAM-induced increase. Nevertheless, SIRT2 knockdown selectively increased acetylation of key glycolytic enzymes, including glyceraldehyde-3-phosphate dehydrogenase and phosphoglycerate mutase, with a trend toward increased phosphofructokinase-1 acetylation in H9c2 cells, without altering total protein expression. In contrast, no significant changes were observed in isolated working rat hearts treated with AGK2. These results suggest that selective acetylation of specific protein targets, rather than global changes in protein acetylation, may be sufficient to modulate cardiac glycolytic activity. Furthermore, we cannot rule out the possibility that acetylation occurs in specific subcellular compartments and/or within particular organelles.

Whether these acetylation changes drive the reduction in glycolytic flux remains unanswered. Assessing the enzymatic activity of these acetylated enzymes is necessary to establish a causal link between acetylation status and glycolytic flux. Moreover, the discrepancy between H9c2 cells and isolated hearts may reflect the greater metabolic complexity and compensatory capacity of intact cardiac tissue compared with immortalized cell lines.

Ketema et al. also examined insulin-stimulated glycolysis in H9c2 cells. While insulin significantly increased glycolytic rates compared with non-insulin-treated cells, this insulin-mediated increase was absent in both scrambled control and SIRT2 knockdown cells. Upstream insulin signaling, assessed by the phosphorylation of Akt, glycogen synthase kinase-3β, and p70 ribosomal protein S6 kinase, remained unaffected across all treatment groups. These findings preclude definitive conclusions regarding the specific contribution of SIRT2 to insulin-stimulated glycolysis in H9c2 cells. The discrepancy between preserved insulin signaling and altered glycolytic responses raises the possibility that the transfection procedure or siRNA exposure induced nonspecific metabolic changes, thereby complicating the interpretation of SIRT2-specific roles in insulin-mediated glycolysis.

More interestingly, given that pathological cardiac hypertrophy is characterized by an increased reliance on glucose for energy production, Ketema et al. evaluated the role of SIRT2 in the hypertrophic response. Phenylephrine-induced cardiomyocyte hypertrophy was significantly attenuated by AGK2 treatment in H9c2 cells. However, although AGK2 reduced glycolytic flux, this effect was no longer observed when the inhibitor was co-administered with phenylephrine. In addition, the authors found no changes in SIRT1/2 expression or protein acetylation in an *in vivo* rat model of abdominal aortic constriction-induced hypertrophy. Together, these findings raise the possibility that the effects of SIRT2 inhibition on the hypertrophic response may be dissociated from its effects on glycolytic flux. SIRT2 inhibition might modulate the structural hypertrophic response through pathways independent of its acute effects on glycolysis.

The study by Ketema et al. provides important insight into the relationship between protein acetylation, particularly of glycolytic enzymes, and the regulation of cardiac glucose metabolism. While their findings suggest that specific acetylation events may influence glycolytic flux, several questions remain, including the relevance of these modifications *in vivo*, the establishment of a causal relationship between enzyme acetylation and altered glycolytic

flux, and the underlying molecular mechanisms. Addressing these gaps will be essential to better understand how acetylation contributes to the regulation of cardiac metabolism, particularly under obese and diabetic conditions, in which increased fatty acid oxidation may contribute to elevated acetyl-CoA availability.

Importantly, the influence of acetylation on cardiac glucose metabolism likely extends beyond glycolysis itself. Previous studies have shown that increased protein acetylation in cardiomyocytes impairs glucose uptake by inhibiting the translocation of the GLUT4 transporter to the plasma membrane, thereby indirectly reducing glycolytic flux (Fig. 1) (2, 5, 6). Furthermore, acetylation likely represents only one component of the broader network of post-translational modifications involved in metabolic regulation during fat overfeeding. Indeed, increased fatty acid catabolism generates intermediates that lead to several lipid-derived post-translational modifications. For instance, acylation of the transporter CD36 has been shown to promote fatty acid utilization in the heart (9), while S-palmitoylation of the insulin-regulated aminopeptidase, a partner of the glucose transporter GLUT4, disrupts glucose uptake (10). Together, these findings highlight the complex and dynamic role of fatty acid-derived post-translational modifications in the regulation of cardiac energy metabolism.

Overall, the work of Ketema et al. reinforces the emerging role of protein acetylation in cardiac metabolic remodeling and implicates SIRT2 as a potential regulator of glycolytic metabolism. Although additional mechanistic and *in vivo* studies are required, these findings support the concept that dynamic protein acetylation contributes to the fine-tuning of cardiac substrate utilization under both physiological and pathological conditions. This study adds to the remarkable body of work established by Gary Lopaschuk and his team, a lasting legacy that will continue to shape the field of cardiac metabolism for future generations of investigators.

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DISCLOSURES

No conflict of interest, financial or otherwise, are declared by the authors.

AUTHORS CONTRIBUTIONS

E.S, S.H. and L.B. drafted, edited, revised and approved final version of manuscript.

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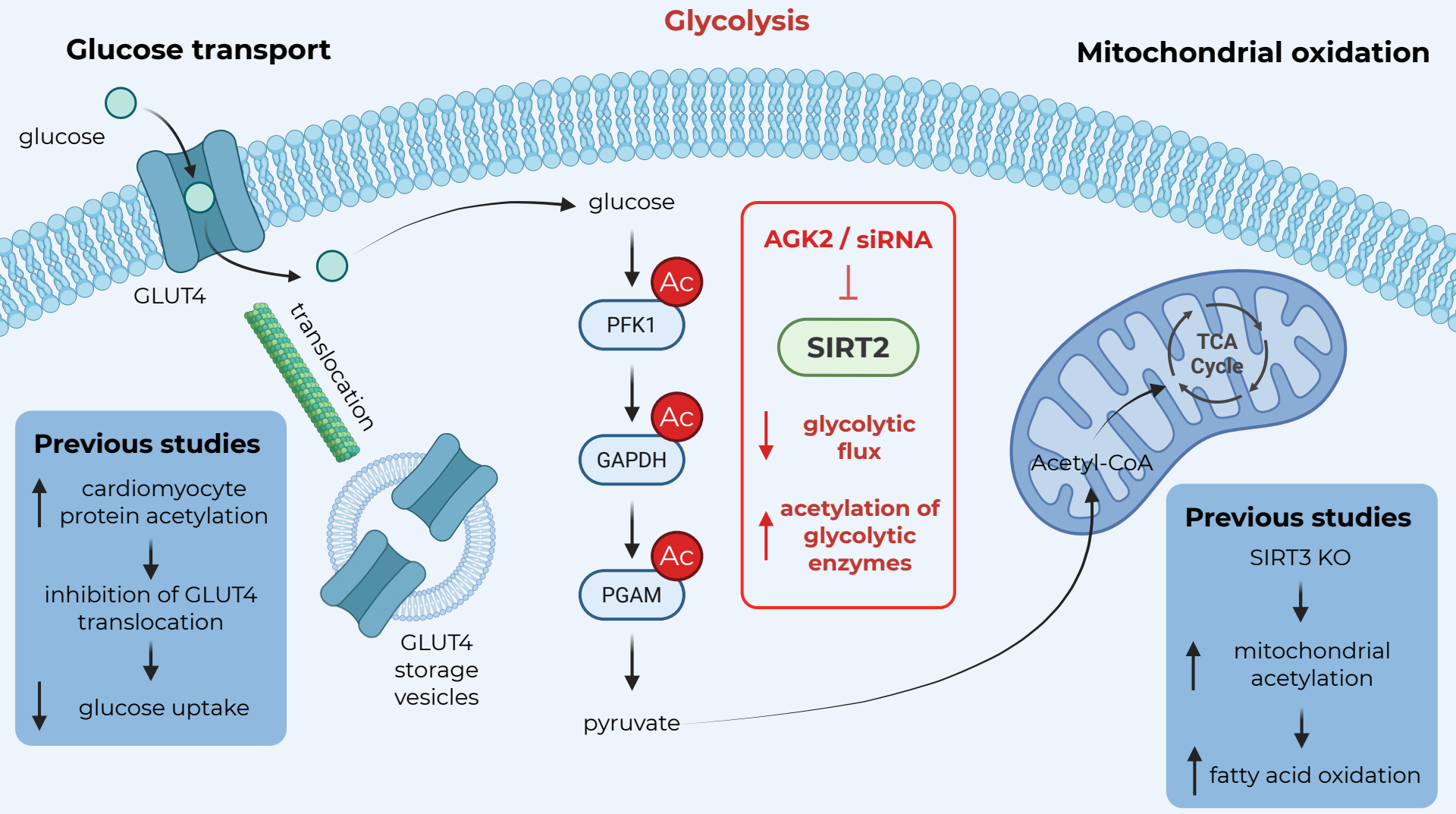
FIGURE LEGEND

Figure 1. Schematic of the multifaceted mechanisms involved in acetylation-mediated regulation of cardiac glucose metabolism. Abbreviations: Ac, acetylated; Acetyl-CoA, acetyl-coenzyme A; ATP, adenosine triphosphate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PFK1, phosphofructokinase-1; PGAM, phosphoglycerate mutase; siRNA, small interfering RNA; SIRT2, sirtuin 2; SIRT3, sirtuin 3; TCA cycle, tricarboxylic acid cycle. Figure created using a licensed version of BioRender.com.



TAKE HOME MESSAGE SIRT2 emerges as a potential regulator of cardiac glycolytic metabolism through acetylation of glycolytic enzymes, linking post-translational modifications to cardiac flexibility.