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EDITORIAL COMMENT

Metabolic Suppression of HIF-1α Contributes to Susceptibility of Ischemic Injury in Diabetic Hearts*

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Hypoxia is a potent regulator of cellular metabolism and growth. Earlier works have identified hypoxia inducible factor (HIF) signaling as the molecular pathway responsible for such adaptation (1-3). The key component of this signaling pathway consists of 2 proteins, the constitutively expressed HIF-1β (also known as aryl-hydrocarbon receptor nuclear translocator or ARNT) and a labile binding partner (HIF-1α or HIF-2α). Under normoxia, oxygen-dependent prolyl hydroxylases (PHDs) catalyze the hydroxylation of HIF-1α and HIF-2α, which is a prerequisite for their degradation (4). Additionally, asparagine hydroxylation by factor inhibiting HIF-1 (FIH1) prevents the interaction between HIF-1α and core transcription machineries (5). Both PHDs and FIH1 require oxygen, iron, and α-ketoglutarate to carry out their functions. During hypoxia, HIF-1α and HIF-2α proteins accumulate and dimerize with ARNT; this complex then translocates into the nucleus (6), where it induces expression of genes involved in glucose metabolism, mitochondrial function, cell proliferation, and viability (6-8). Thus, HIF signaling coordinates a cellular program that protects the organism from the adverse consequences of oxygen deprivation.

Downstream effectors of HIF have been extensively studied in cardiovascular disease and diabetes. HIF signaling regulates angiogenesis and vascular remodeling. Additionally, HIF-1α increases glycolytic gene expression, thereby ensuring adenosine triphosphate (ATP) production from anaerobic glycolysis (8,9). These effects have great implications in ischemic and pressure overload heart diseases (9). In addition to cardiovascular disease, altered HIF signaling has been implicated in diabetes. In patients with diabetes, the expression of ARNT is lower in the pancreas and liver and genetic deletion of this protein in these 2 organs results in diabetic phenotypes (10,11). Cardiac expression of ARNT is also reduced in the hearts of mice with genetic and diet-induced diabetes. Cardiac-specific deletion of ARNT leads to increased peroxisome proliferator-activated receptor-α expression, thereby resulting in heightened lipid uptake and oxidation. The imbalance between lipid uptake and oxidation causes lipid accumulation and spontaneous cardiomyopathy (12). This evidence demonstrates that diabetes may influence HIF signaling; at the same time, HIF signaling can modulate diabetic phenotypes.

In the heart, diabetes shifts cellular metabolism in favor of increased utilization of fatty acids (FAs) with a concomitant inhibition of glycolysis and glucose oxidation (13,14). FA-mediated suppression of glucose oxidation was first described by Randle et al. (15) in muscle and fat tissue. Increased FA oxidation results in higher levels of mitochondrial acetyl-coenzyme A and nicotinamide adenine dinucleotide hydride (NADH) and cytosolic citrate. These metabolic intermediates can allosterically inhibit 2 key glycolytic enzymes: phosphofructokinase-1 and

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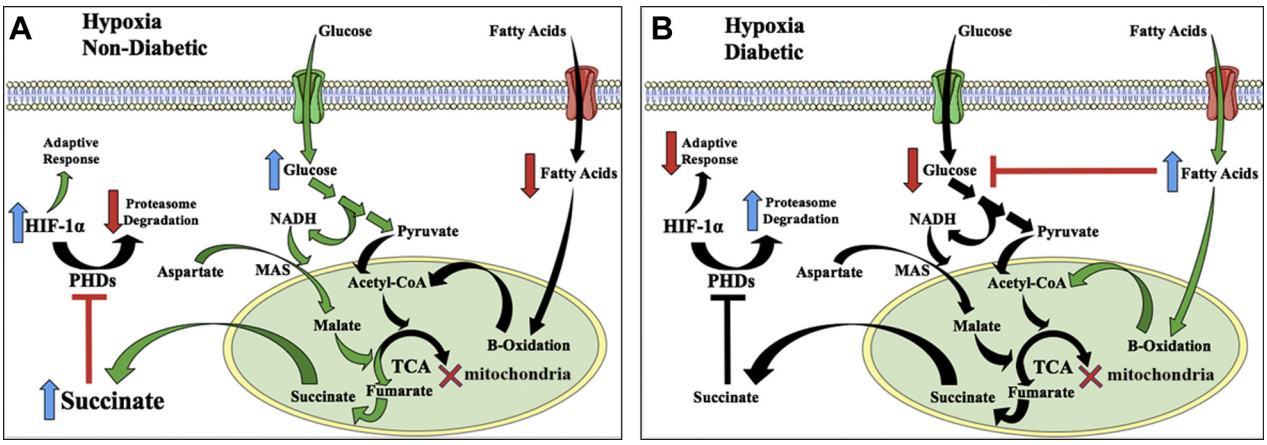
FIGURE 1 Aberrant HIF Signaling in Diabetic Hearts



(A) In normal hearts under hypoxia, succinate levels increase dramatically because of increased glycolysis and MAS activity. Increased succinate levels inhibit PHD activity, thereby facilitating HIF-1α stabilization. **(B)** In diabetic hearts under hypoxia, the aberrant increase in fatty acid metabolism inhibits glycolysis. Reduced NADH influx into mitochondria through MAS blunts the increase in succinate during hypoxia, resulting in the inability to stabilize HIF-1α. CoA = coenzyme A; HIF = hypoxia inducible factor; MAS = malate-aspartate shuttle; NADH = nicotinamide adenine dinucleotide hydride; PHD = prolyl-hydroxylase; TCA = tricarboxylic acid.
Figure adapted from Servier Medical Art (28).

pyruvate dehydrogenase (16). The inhibition of phosphofructokinase-1 in turn leads to accumulation of glucose-6-phosphate, which inhibits hexokinase (16); therefore, overall glycolytic flux is reduced. Although diabetic hearts are still able to generate adequate amounts of ATP through FA oxidation under normoxia, they are less capable of producing energy under hypoxia because of reduced anaerobic glycolysis. These defects may contribute to the worse outcome of patients with diabetes and acute myocardial infarction and explain why cardiovascular complications are the primary cause of death in patients with type 2 diabetes (17,18). Nevertheless, precisely how the metabolic derangement controls cellular response to hypoxia beyond energy production remains to be answered.

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The article by Dodd et al. (19) in this issue of *JACC: Basic to Translational Science* brings a novel insight into the susceptibility of diabetic hearts to ischemic injury by demonstrating that they fail to accumulate HIF-1α under hypoxia through a proteasome-dependent mechanism. Under hypoxia, the heart normally accumulates high levels of succinate; however, this increase is attenuated in the diabetic heart (**Figure 1**) (20). Succinate is a potent repressor of PHDs, and a reduced succinate to α-ketoglutarate ratio allows for increased activity of PHDs under hypoxia (21). In cell culture, supplementation of insulin-resistant cells with dimethyl fumarate (which can be converted to succinate) restores the HIF-1α protein accumulation under hypoxia. A similar effect was achieved through treating cells with DMOG, a PHD inhibitor. Additionally, administration of DMOG to diabetic rats results in better functional recovery after cardiac ischemia/reperfusion in an ex vivo heart perfusion system.

More important, the authors mechanistically link increased FA oxidation to the failure of succinate accumulation in diabetic hearts under hypoxia. In hypoxia, the forward flow of electron transport chain is inhibited. Anaerobic glycolysis thereby becomes a vital source of ATP production, generating NADH as a byproduct. However, if the electron equivalents cannot be used, excessive cytosolic NADH would bring anaerobic glycolysis to a halt. In addition to lactate production, malate/aspartate shuttle allows for the transport of electron equivalents into the mitochondria, thus restoring cytosolic $NADH/NAD^+$ ratio. Increased mitochondrial malate and fumarate in this situation can drive succinate dehydrogenase in reverse and result in succinate accumulation (**Figure 1**) (20). Supplementation of cell culture media with FA forces cultured cells to use FA, which results in inhibition of glycolysis and reduced HIF-1α accumulation. Importantly, the authors demonstrated that both palmitate and oleate have similar inhibitory effects; therefore, the change in cellular

metabolism is independent of the saturation of FA species. Additionally, the authors use a FA uptake inhibitor in their in vitro insulin resistance model to demonstrate that the metabolic rewiring and the failure of HIF-1α to accumulate depend on FA utilization rather than changes in the insulin signaling pathway. Taken together, they present a pathway that increased FA utilization (probably from substrate abundance) in diabetes results in glycolysis suppression, reduced transport of electron equivalents into mitochondria during hypoxia, reduced succinate accumulation, and ultimately failure of HIF-1α to accumulate (**Figure 1**).

This paper elegantly demonstrates the diabetes-mediated rewiring of cellular metabolism and response to hypoxia and provides the molecular mechanism for the authors' (22,23) previous observation of changes in tricarboxylic acid cycle metabolites in diabetic hearts. However, the identified molecular mechanism can play a role beyond regulation of hypoxic adaptation of diabetic hearts. Although diabetic hearts under hypoxia failed to accumulate succinate because of reduced NADH production through glycolysis, the inhibition of glycolysis also occurs under normoxia (16); therefore, it would be of great interest to profile succinate and α-ketoglutarate levels in these hearts. Multiple cellular enzyme families require oxygen and use α-ketoglutarate and iron as cofactors. These include the prolyl hydroxylase family, the Jumonji-C domain containing histone demethylase family, and the TET deoxyribonucleic acid (DNA) hydroxylase family (which affects subsequent DNA demethylation) (24). Succinate is one of the products of these enzymatic reactions, and increased ratio of succinate over α-ketoglutarate can inhibit the activity of these enzymes (21). If normoxic diabetic hearts still have reduced succinate levels, both TET DNA hydroxylases and Jumonji-C domain histone demethylases can be hyperactivated, which could result in global epigenetic changes. Profiling the locus where DNA and histone methylation are altered in this setting may shed further insights to the pathogenesis of diabetic heart disease.

Although Dodd et al. (19) described a molecular pathway that could potentially be targeted for treating ischemic complications in diabetic patients, translating the findings into clinical practice require more careful consideration. The in vitro findings in this manuscript would argue for the use of cell-permeable succinate or fumarate as a therapeutic agent; however, pharmacological increase of succinate level poses a potential threat. Chouchani et al. (20) demonstrated that succinate accumulation is required for the cardiac ischemia/reperfusion injury through increased reverse electron transport chain upon reperfusion. Therefore, novel therapy aiming at stabilizing HIF proteins in diabetic hearts should function downstream of succinate and should preferably directly target the PHDs.

Additionally, this manuscript demonstrates the utility of DMOG as a preventive agent for cardiac ischemia/reperfusion injury in diabetic hearts; however, the effect of DMOG administration during ischemic events remains to be determined. As a result, patients at risk will have to receive chronic HIF hydroxylase suppression. Currently, PHD inhibitors are used to treat certain forms of anemia because HIF stabilization promotes renal production of erythropoietin and increases erythropoiesis (25). Therefore, chronic administration of the drug (that may be needed to prevent ischemic injury) may result in erythrocytosis, which is also associated with adverse cardiac events (26). Additionally, inhibition of PHD via tricarboxylic acid cycle metabolites (similar to what the authors proposed) has been implicated in several cancers (27). Thus, unless we can achieve organ-specific delivery of PHD inhibitors, patients should be monitored closely for cancer and adverse cardiac events.

Overall, Dodd et al. (19) identified increased FA oxidation in diabetes as the root cause of failure to accumulate HIF-1α under hypoxia. Reduced NADH production from anaerobic glycolysis is mechanistically linked to reduced accumulation of mitochondrial succinate, which in turn leads to PHD hyperactivation. This manuscript is one of the first to implicate such a mechanism in cardiac pathophysiology. Although the authors performed proof-of-principle experiments to rescue the disease phenotype, more detailed studies are needed to better target the molecular pathways without causing untoward consequences.

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