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# Regulation of metabolism by mitochondrial enzyme acetylation in cardiac ischemia-reperfusion injury

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ABSTRACT

Ischemia reperfusion injury (I/R injury) contributes significantly to morbidity and mortality following myocardial infarction (MI). Although rapid reperfusion of the ischemic myocardium was established decades ago as a highly beneficial therapy for MI, significant cell death still occurs after the onset of reperfusion. Mitochondrial dysfunction is closely associated with I/R injury, resulting in the uncontrolled production of reactive oxygen species (ROS). Considerable efforts have gone into understanding the metabolic perturbations elicited by I/R injury. Recent work has identified the critical role of reversible protein acetylation in maintaining normal mitochondrial biologic function and energy metabolism both in the normal heart and during I/R injury. Several studies have shown that modification of class I HDAC and/or Sirtuin (Sirt) activity is cardioprotective in the setting of I/R injury. A better understanding of the role of these metabolic pathways in reperfusion injury and their regulation by reversible protein acetylation presents a promising way forward in improving the treatment of cardiac reperfusion injury. Here we briefly review some of what is known about how acetylation regulates mitochondrial metabolism and how it relates to I/R injury.

## 1. Introduction

Each year, approximately 750,000 people in the United States experience a myocardial infarction (MI) [1]. For those patients fortunate enough to present to a medical clinic in a timely manner, the gold standard treatment is prompt reperfusion of the occluded artery, which is often achieved with primary percutaneous intervention (PCI). Rapid reperfusion of ischemic myocardium was established decades ago as a highly beneficial therapy for MI, but significant cell death still occurs after the onset of reperfusion. In order to understand the reasons for this, a more thorough examination of the cellular and molecular mechanisms underpinning ischemia-reperfusion (I/R) injury in the heart are required, much of which has been thoroughly reviewed [2]. Our goal here is to review the rapidly expanding field of how protein acetylation can impact cellular regulation and affect mitochondrial dysfunction in context of cardiovascular IR injury.

### 1.1. Enzymatic regulation of protein acetylation

The enzymatic regulation of protein acetylation is achieved by the balance between histone deacetylase enzymes and histone acetyltransferases. Histone modifying enzymes are categorized into three groups: readers, writers, and erasers based on their roles in reading/recognition, addition or removal of an epigenetic mark in post-translational modifications (PTMs) respectively [3] [4]. Readers are scaffolding enzymes that locate a particular modification on a histone tail, bind to it, and recruit other enzymes, such as transcriptional machinery to the modification [5]. In the case of acetylation, the reader proteins are generally bromodomain-containing proteins. The bromodomains of these enzymes form a hydrophobic cavity that allows for recognition of the acetylated lysine residues on histone tails [5]. These proteins play a significant role in the pathogenesis of multiple diseases, including cancer, inflammation, and cardiovascular disease [3,5]. The writer proteins are acetyltransferase enzymes which catalyze the addition of an acetyl moiety to the ε-amino group of lysine amino acids within histone tails [3] [6]. There are 22 known lysine acetyltransferases in human and mouse tissues, many of which have important roles in vital cellular processes [7].

The “eraser” enzymes that affect lysine acetylation are known as histone deacetylase enzymes (HDACs). The 18 known HDACs are grouped into 4 classes. Classes I, II, and IV depending on a zinc cofactor for their catalytic activity, while class III (the sirtuin family) rely on $NAD^+$ as a cofactor [8]. Class I HDACs are predominantly nuclear enzymes that have high deacetylase activity. Class IIa HDACs have about

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1000-fold lower catalytic activity than class I [9], and are thought to function mostly as scaffolding proteins that recruit other enzymes into large, multiprotein complexes that modulate transcription. Class IIb HDACs are predominantly cytosolic, with HDAC6 functioning primarily as a tubulin deacetylase [10]. Class IV, the most recently discovered of the zinc-dependent HDACs, contains only HDAC 11 [11].

While the canonical substrates for HDACs are the lysine tails of histones, HDACs are also known to deacetylate a multitude of other proteins [12]. As more is discovered about the cardiac acetylome, it is becoming increasingly apparent that modifying the acetylation state of an enzyme can result in dramatic changes in the properties of the affected enzyme. Properties that can be affected by acetylation include enzymatic activity [13], DNA binding [14], subsequent post-translation modifications [15], protein stability [16], protein-protein interactions [17], and intracellular localization of a given protein [18].

The widespread effects of protein acetylation, combined with the large number of proteins that are reversibly acetylated, has engendered proteomic analyses aimed at decoding the “acetylome” in multiple disease states [19]. The first global acetylome analysis was carried out by Choudhary et al. and reported in *Science* in 2009 [6]. In this work, the authors used tryptic digests from an acute myeloid leukemia cell line to probe for acetylated peptides that changed in intensity in response to pharmacological HDAC inhibition using either suberoylanilide hydroxamic acid (SAHA) or MS-275. They reported 3600 lysine acetylation sites on 1750 proteins and found that acetylation sites preferentially mapped to proteins involved in diverse vital cellular processes. This was the first major investigation into non-histone lysine acetylation as a post-translational protein modification with diverse functional consequences. Since this initial work, investigations into the acetylome have begun in many fields, including cancer [20], hepatitis [21], metabolism [22], circadian biology [23], neurobiology [24], colitis [25], diabetes [26], autism [27], Parkinson's [28], and cardiovascular biology [29,30], among others. These studies have greatly enhanced our understanding of the critical role of protein acetylation in cellular signaling both in healthy and pathological states. Interestingly, many of these studies traced a large number of critical acetylation sites to the mitochondrion, where acetylation appears to have a dramatic regulatory effect, both mediated by deacetylase activity and nutrient availability [31]. While the field of acetylomics has greatly increased our knowledge of the effects of acetylation, much more remains to be discovered.

### *1.2. Lysine acetylation regulates metabolism at multiple levels within the cell*

In recent years it has become apparent that both histone and non-histone acetylation patterns are critical to many vital cellular processes in the heart and other organs. As the field has progressed, evidence has accumulated for acetylation-based regulation of cellular metabolism at the transcriptional level within the nucleus, as well as through post-translational enzyme modification within the cytoplasm and especially within the mitochondrial matrix.

In 2008, Olson's group generated two HDAC3 knockout mouse models; a global knockout and an αMHC-Cre cardiac-restricted knockout [32]. While the global HDAC3 knockout exhibited embryonic lethality, the cardiac-specific HDAC3 knockout mice were able to survive for as long as 4 months. The investigators identified “massive cardiac hypertrophy” in the conditional HDAC3 knockout mice and attributed this to metabolic dysfunction. Microarray analysis of the conditional HDAC3 KO mice revealed upregulation of genes associated with fatty acid oxidation, carbohydrate oxidation, protein catabolism, and electron transport chain proteins, among others [32]. Interestingly, ChIP analyses of the promoter regions of peroxisome proliferator-activated receptor alpha (PPARα) responsive genes revealed the presence of HDAC3 on these promoter regions in wild-type mice with a corresponding absence in the conditional knockout. The investigators concluded that HDAC3 exists in a complex with PPARα within the nucleus and acts as a corepressor of the transcription of PPARα responsive genes, affecting the metabolic state of the heart. Another study utilized an MCK-Cre model to knock out HDAC3; this model differing from the previously discussed knockout in that it knocked HDAC3 out of both cardiac and skeletal muscle, but did so only after birth, since MCK is not fully active until after birth [33]. Interestingly, these mice were able to survive much longer than the αMHC-Cre HDAC3 KO mice, surviving over a year on normal chow. However, when fed high-fat chow the MCK-Cre HDAC3 KO mice experienced severe cardiac hypertrophy and death within weeks, providing further evidence for a critical role of HDAC3 in cardiac energy homeostasis and response to the nutritional environment [33]. Contrastingly, knocking HDAC3 out of osteoprogenitor cells results in leaner mice with lower fasting glucose levels [34]. These mice also retain insulin sensitivity and are resistant to hepatic steatosis in response to a prolonged high fat diet [34], opening up the fascinating possibility that the HDAC3 might regulate the metabolic response to the nutritional environment differently in different tissue types.

While knockout studies offer important and interesting insight into the activity of the protein product of the knocked-out gene, it is critically important to perform pharmacological studies to assess the effects of pharmacological inhibition of the protein target and gauge the translational potential of the enzyme as a drug target. To this end, Galmozzi et al. utilized the HDAC inhibitor SAHA to examine the transcriptional effects of HDAC inhibition on metabolic function both *in vitro* and *in vivo* [35]. In cultured myotubes, SAHA increased the expression of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1-α), the so-called “master regulator” of mitochondrial biogenesis. In the myotubes, the increase in PGC1-α expression correlated with increased mitochondrial biogenesis and increased oxygen consumption. Importantly, mice treated with SAHA exhibited reduced body weight and reduced circulating glucose and insulin levels secondary to enhanced oxidative metabolism in the adipose tissue and skeletal muscle [35]. The link between class I HDACs, PPAR/PGC1α signaling, and oxidative metabolism is just beginning to be elucidated, but has been addressed in at least two other recent studies focusing on diabetes [36,37]. Taken together, these lines of evidence demonstrate an essential role for class I HDACs in regulating the transcriptional metabolic program in the heart and modifying the metabolic response to the nutrient environment that the heart is subjected to. However, the complexity of the nuclear regulation of genes involved in metabolism is just beginning to be grasped. More recent work has identified eight different types of acylation that can be used to modify histones, including propionylation, succinylation, and β-hydroxybutyration, among others [38]. These non-acetyl histone acylations affect the transcriptional regulation of genes involved in vital cellular processes, including metabolism [38]. Much more work is needed to determine the effects of these acylation events and their roles in cardiovascular physiology and pathology.

Outside of the nucleus, direct post-translational acetylation of the enzymatic machinery involved in substrate metabolism has been implicated in the regulation of nearly every metabolic pathway. Recent work has identified four lysine acetylation marks on glyceraldehyde 3-phosphate dehydrogenase (GAPDH) that are critical for regulating the rate of glycolysis within hepatocytes [39]. Utilizing K-R mutations to mimic the deacetylated state of all four acetylation sites resulted in reduced glycolytic flux and a corresponding increase in gluconeogenesis. Subsequent experiments identified a reduction in GAPDH acetylation in obese and diabetic (db/db) mice [39]. It remains to be determined whether GAPDH is a target of class I HDACs.

Recent advances in immunoaffinity purification and proteomic analyses have made it possible to examine the global acetylome of tissues with impressive accuracy [40]. In 2010, Zhao et al. conducted a proteomic analysis that identified acetylation of nearly every enzyme in the pathways responsible for glycolysis, fatty acid oxidation, gluconeogenesis, the citric acid cycle, glycogen metabolism, and urea

metabolism in liver homogenates [41]. Since then, the acetylomics field has exploded, leading to many other studies identifying the acetylation-based regulation of metabolic enzymes [42]. Importantly, follow-up work by multiple groups has begun to link the acetylation state of the metabolic enzyme machinery to various cardiac pathologies.

During heart failure, the rate of fatty acid oxidation decreases, while glycolysis and ketone body utilization increase, contributing to the dysfunctional metabolic phenotype [43]. Part of this effect is transcriptional; in late-stage heart failure there is a dramatic down-regulation of the genes involved in fatty acid uptake and fatty acid β-oxidation. Interestingly, these transcriptional changes are not seen in early, compensated heart failure [44]. However, it has recently begun to be appreciated that part of the metabolic dysregulation is due to hyperacetylation of mitochondrial enzymes, which occurs even in early stage, compensated heart failure and increases as the heart failure worsens [44]. In 2016, Horton et al. performed acetylproteomics on heart homogenates taken from wild type mice and compared them to hearts from mice subjected to transverse aortic constriction (TAC) as a model of heart failure [45]. This study revealed a global increase in mitochondrial protein acetylation in the failing hearts, including proteins involved in fatty acid β-oxidation, the tricarboxylic acid (TCA) cycle, and electron transport chain complexes. Further, a significant increase in succinate dehydrogenase A (SDHA) acetylation was observed in the heart failure samples, which corresponded with a decrease in SDHA activity, slowing the flow of electrons through complex II of the electron transport chain [45]. Acetylation of SDHA could have major consequences in cardiac I/R injury, since SDHA oxidizes succinate, which, as mentioned previously, accumulates in ischemia and contributes dramatically to ROS production in reperfusion [46].

Another metabolic pathway that contains hyperacetylated enzymes in heart failure is fatty acid β-oxidation [43]. Sankaralingam et al. demonstrated that induction of heart failure by constriction of the abdominal aorta in obese mice resulted in an increase in acetylation of long chain acyl-CoA dehydrogenase (LCAD) [47]. They further demonstrated that this increase in acetylation increases the rate of fatty acid β-oxidation, correlating to an increase in the expression of the acetyltransferase GCN5-like protein 1 (GCN5L1). In contrast, other studies have observed that increased acetylation of fatty acid β-oxidation enzymes leads to a decrease in their activity, which has been ascribed to changes in Sirt3 activity [48,49]. These conflicting data raise the intriguing possibility that the acetylation state of individual lysine sites might separately, and oppositely, regulate the enzymatic function of fatty acid β-oxidation enzymes.

Much less is known about the acetylation state of enzymes within the mitochondrial matrix in cardiac I/R injury.

## 2. Mitochondrial dysfunction in cardiac ischemia-reperfusion injury

Ischemic injury to the cell begins, unsurprisingly, with the occlusion of a major artery supplying oxygen and nutrients to a field of tissue. This creates an imbalance between myocardial oxygen supply and demand which then translates into angina and myocardium tissue death if not promptly recognized and treated in an acute setting. Upon the initiation of ischemia at the cellular level, the mitochondria rapidly deplete what little oxygen is left, switching to glycolysis in an attempt to maintain ATP production [50]. The end product of glycolysis is pyruvate, which under normal circumstances enters the TCA cycle and eventually provides ATP through oxidative phosphorylation. However, ischemia leads to pyruvate accumulation shunning the oxidative phosphorylation. Excess pyruvate is converted to lactate by lactate dehydrogenase, returning some of the accumulating NADH to $NAD^+$. Lactate accumulation readily drives up the $H^+$ concentration within the cell, lowering the pH. The intracellular acidification that occurs during ischemia has both beneficial and detrimental consequences for the myocyte. For unknown reasons, the lowered pH prevents mitochondrial transition pore (mPTP) opening from occurring. Prevention of mPTP opening during ischemia prevents mitochondrial depolarization and metabolic collapse, promoting survival of the myocyte. However, accumulation of $H^+$ during ischemia drives the import of $Na^+$ through the $H^+/Na^+$ exchanger in the plasma membrane of the myocyte, resulting in secondary accumulation of $Na^+$ within the myocyte, which further drives $Ca^{++}$ entry into the cell through the $Na^+/Ca^{++}$ transporter, resulting in calcium accumulation within the cell. The higher cytosolic calcium concentration results in an influx of calcium into the mitochondrial matrix.

With the onset of reperfusion, the built-up lactic acid is washed out and physiological pH is restored in the intracellular compartment of the myocyte. Problematically, this releases the pH-based inhibition of the mPTP opening. Simultaneously, the restoration of physiological levels of oxygen to the cell results in a burst of reactive oxygen species (ROS), resulting in significant damage by protein and lipid oxidation. This burst of ROS, combined with calcium overload from pH-driven ionic disturbances caused by ischemia, triggers opening of the mPTP, which allows the non-specific passage of any molecule smaller than 1.5 kDa across the mitochondrial membrane [51]. Mitochondrial permeability transition then results in collapse of the mitochondrial membrane potential, mitochondrial swelling, and the initiation of cell death.

The anoxic, nutrient-depleted, acidotic intracellular milieu during ischemia results in the accumulation of NADH and $FADH_2$ reducing equivalents, inhibition of fatty acid β-oxidation and the TCA cycle, shutdown of the electron transport chain, and the activation of glycolysis. During this time, the $F_1F_0$-ATPase runs in reverse in an attempt to maintain the mitochondrial membrane potential, utilizing the ATP being generated from glycolysis [52]. Interestingly, a reduction in the activity of the electron transport chain has been shown to reduce ROS burst and partially prevent opening of the mPTP, resulting in improved recovery from I/R injury [53]. Taken together, these studies offer a strong argument that a reduction in oxidative phosphorylation in very early reperfusion may aid the recovery of the cardiac myocytes from injury, ostensibly by reducing the ROS burden experienced within the mitochondria.

### 2.1. Acetylation in ischemia/reperfusion injury

In 2011, Shinmura et al. demonstrated that caloric restriction protected mice against I/R injury, and that this cardioprotection correlated with a decrease in acetylation of electron transport chain complexes, which correlated with decreases ROS production [54]. Another, more recent study examined the effect of I/R injury in $Sirt3^{+/-}$ mouse hearts [55]. This study identified a greater extent of I/R injury *ex vivo* in the $Sirt3^{+/-}$ mouse hearts when compared to wild-type hearts. The authors go on to show evidence that mitochondrial proteins are increased in acetylation in the $Sirt3^{+/-}$ hearts, and that this correlates with decreased activity of complex I and MnSOD [55]. These two sets of data would seem to suggest that increased mitochondrial enzyme acetylation is deleterious in cardiac I/R injury, but controversy exists surrounding the role of Sirt3 in I/R injury. Most recently, $Sirt3^{-/-}$ was determined to slow baseline metabolic activity in the heart, but wild-type and $Sirt3^{-/-}$ mice showed no difference in recovery from I/R injury [56].

The type of major proteomics studies that have proven extremely useful in advancing our understanding of the acetylome in heart failure have yet to be applied to models of I/R injury. The existing research on mitochondrial protein acetylation in I/R injury mostly derives from small studies of Sirt3 modification, which are limited in scope and contain major limitations to their interpretation. Given the plethora of acetylated proteins within the mitochondria (one study estimated 65% of mitochondrial proteins contain a lysine acetylation mark [43] more research into the mitochondrial acetylome and its changes in response to I/R injury are of great importance. Table 1 gives a list of mitochondrial enzymes affected by acetylation in various cardiac pathological models.

**Table 1**
Regulation of mitochondrial enzymes activity in heart through acetylation.

| Mitochondrial enzyme acetylation | Biological function | Effect of acetylation in heart | Model | Reference |
|---|---|---|---|---|
| Aconitase | TCA cycle | Increased activity | Isolated murine heart | [57] |
| Acetyl-CoA synthetase 2 | Acetate metabolism | Decreased activity | | [58,59] |
| Complex I | ETC | Decreased /N.C. (?) | IR in cardiomyoblast cells, *Ex-vivo* IR, HF | [60] [55] [48,56] [61] |
| Complex II Succinate dehydrogenase | ETC | Decreased activity | *Ex-vivo* IR | [61,62] [63] |
| Complex III | ETC | N.C. (?) | IR, HF | [61,64] |
| Complex IV | ETC | N.C. (?) | IR, HF | [64] [61] |
| Complex V | OXPHOS | Decreased activity | HF | [65] |
| Forkhead box protein O3 (FOXO3a) | Transcription | Decreased activity | Cardiac hypertrophy | [66] |
| Glutamate dehydrogenase | Amino acid catabolism | Decreased activity | | [67,68] |
| Isocitrate dehydrogenase | TCA cycle; antioxidant system | Increased activity | Porcine heart | [69] |
| Ku70 | DNA repair | ? | Murine cardiomyocytes | [70] |
| Liver kinase B1 (LKB1) | AMPK Pathway | Decreased activity | Cardiac hypertrophy | [71,72] |
| Long chain acetyl Co-A dehydrogenase | Fatty acid metabolism | Decreased activity | Heart failure, Cardiac hypertrophy | [49,73] [64] |
| Malate dehydrogenase | TCA Cycle | No effect | Purified bovine heart | [69] |
| MnSOD (SOD2) | Mitochondrial antioxidant system | Decreased activity | Isolated cardiomyocytes, cardiac hypertrophy | [66] [55,61,65,74] |
| MnSOD & Catalase | | Upregulated with HDACi treatment | *Ex-vivo* IR | [75] |
| Mitochondrial ribosomal protein L10 (MRPL10) | Protein synthesis | Increased activity | | [76] |
| Optic atrophy 1 (OPA1) | Mitochondrial biogenesis | Decreased activity | | [77] |
| Oxoguanine glycosylase | Base Excision Repair | Decreased activity | Cardiomyocytes | [78] |
| Pyruvate dehydrogenase | TCA cycle | Decreased activity | Human cardiomyocytes | [79,80] [81] |

## 3. HDAC inhibitors protect the heart from I/R injury

In recent years, HDAC inhibitors have come under intense scrutiny as a possible treatment strategy in ischemia reperfusion injury. Zhao et al. first investigated the effects of pharmacological HDAC inhibition in an *ex vivo* model of cardiac I/R injury in 2007 [82]. Here, the authors examined models of both early and delayed pharmacological preconditioning. For the early preconditioning, the pan-HDAC inhibitor trichostatin A was administered to isolated mouse hearts in 5 min cycles immediately prior to I/R injury. For the delayed preconditioning model, TSA was administered to mice 24 h before heart excision, then hearts were subjected to Langendorff perfusion and I/R injury. In both models, TSA significantly reduced the amount of infarct injury and improved left ventricular function following I/R. These changes correlated with increased phosphorylation and activity of p38 mitogen activated protein kinase (p38 MAPK) in the presence of HDAC inhibition [82]. Later work by the same group would identify NF-κB as an essential mediator of HDAC-inhibition cardioprotection in the delayed preconditioning model [83]. These authors then went on to test the effect of HDAC inhibition in their delayed preconditioning model on Akt and MKK3 knockout mice. The authors found that knocking out Akt or MKK3 abrogated the effect of HDAC inhibition-mediated preconditioning, identifying Akt and MKK3 as essential mediators of pharmacological preconditioning with TSA, implicating HDACs in modulating the activity of the reperfusion-induced salvage kinase (RISK) pathway [84].

All of these studies utilized the “pan-HDAC” inhibitor TSA, which inhibits class I and class IIb HDACs at low concentrations. For true “pan-HDAC” inhibition, much higher concentrations must be used. To better understand the role of individual HDAC classes in I/R injury, we set out to determine whether the effects of class I HDAC inhibition or class IIb HDAC inhibition were primarily responsible for HDAC-inhibition mediated preconditioning [85]. To do this, we treated rats with TSA, the class I selective HDAC inhibitor MS-275, or the class IIb HDAC inhibitor Tubastatin A. Rats were treated with each drug or the vehicle DMSO 24 h prior to I/R, and again 1 h prior to I/R to ensure adequate HDAC inhibition. We found that hearts from the MS-275 treated rats were much more resistant to I/R injury than the hearts from TSA treated rats. Tubastatin A conferred no protection against I/R injury at all, suggesting that class I HDACs are predominantly detrimental to the heart in early reperfusion. In investigating the mechanism by which MS-275 preconditioned the heart, we discovered that class I HDAC inhibition resulted in an upregulation of mitochondrial superoxide dismutase (SOD2) and catalase, both antioxidants which likely mitigated ROS-induced damage during reperfusion [85]. While interesting, all of the aforementioned studies examined HDAC inhibition in the setting of pretreatment, which is not very useful for the real-world treatment of I/R injury, where heart attacks are highly unpredictable.

In 2008, Granger et al. advanced the knowledge of HDAC activity in I/R injury in two important ways: by examining HDAC inhibition in the reperfusion phase of I/R injury and by utilizing an *in vivo* model [86]. The investigators administered TSA at three different time points; one hour before I/R, 45 min into the reperfusion phase, and 12 h post-reperfusion. After 48 h, they harvested the hearts and examined the extent of infarction within the ischemic field. The authors concluded that HDAC activity increases as a result of I/R injury, and that TSA administration significantly rescues myocardium from infarction when administered either 1 h prior to I/R injury or 45 min into reperfusion. TSA administered 12 h into reperfusion had no effect on infarct area. Utilizing isolated cardiac myocytes, the authors went on to identify decreases in HIF1-α and VEGF expression, to which they ascribe the beneficial effects of TSA treatment [86].

Xie et al., carried out the most recent *in vivo* investigation of HDAC inhibition in I/R injury, utilizing both mice and rabbits, determining functional outcomes by echocardiography in addition to studies of infarct area [87]. This work utilized the pan-HDAC inhibitor SAHA, administered at the onset of reperfusion to mimic the most clinically relevant treatment setting. The authors found substantial improvement of LV function 24 h after I/R in the SAHA treated animals, which correlated with reduced infarct sizes in SAHA treated animals. The authors went on to identify increased autophagic flux in the border zone of infarcted hearts as a possible mechanism for SAHA mediated post-conditioning [87].

Our group recently discovered that HDAC1 is present in the mitochondria of cardiac myocytes, but not endothelial cells or fibroblasts; the first known observation of mitochondrial HDAC1 in mammalian tissue [88]. We subsequently developed a mitochondria-targeted class I HDAC inhibitor. Because our inhibitor is concentrated in the mitochondria it can be administered in dose 200–300-fold below the $K_i$ for

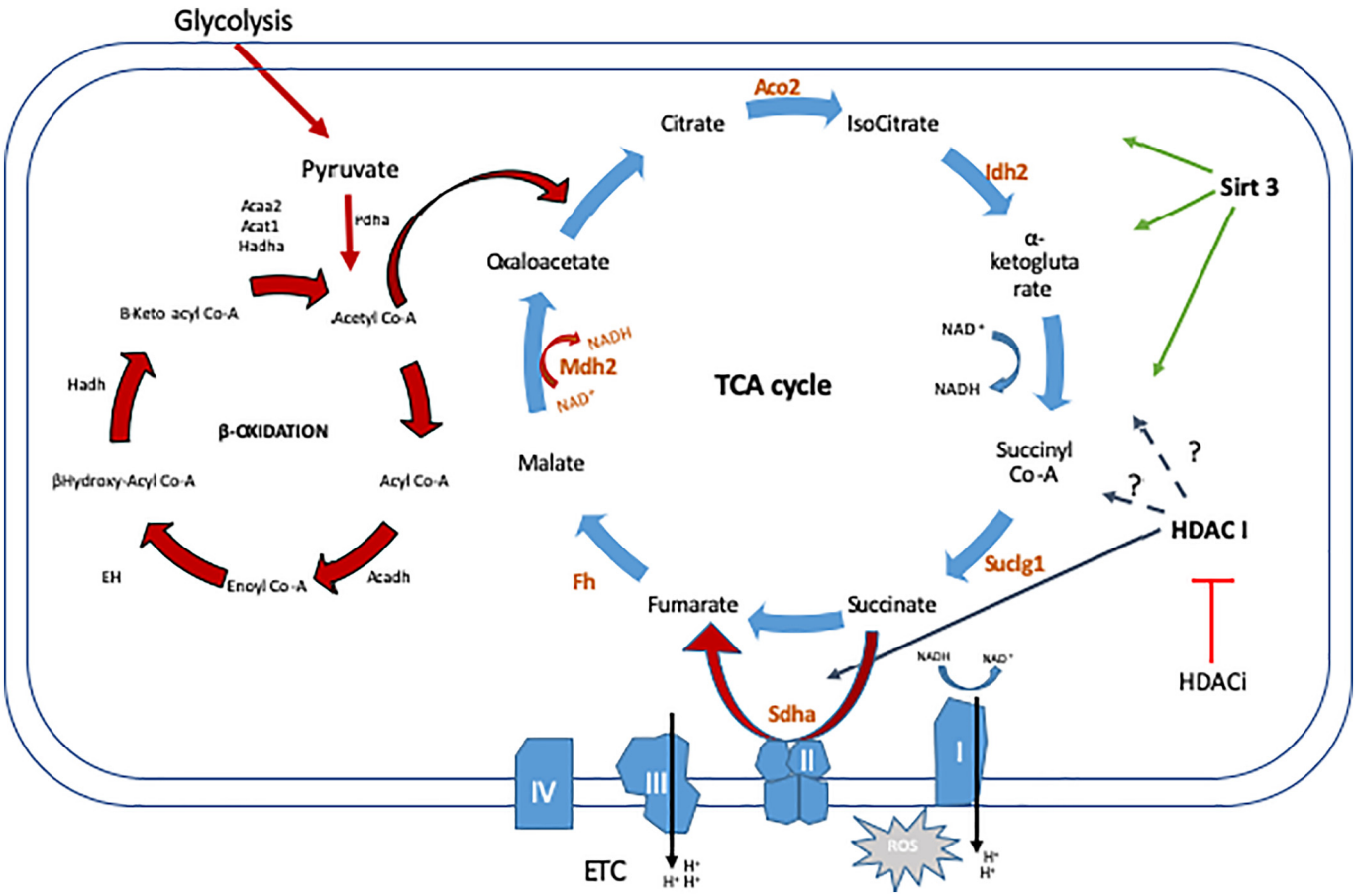


**Fig. 1.** Targets of acetylation/deacetylation in metabolic pathways known to affect I/R injury. Sirt 3 is known to deacetylate many mitochondrial enzymes that can contribute to I/R injury. Increased Sirt 3 activity is beneficial in cardiac disease whereas inhibition of class I HDAC activity is protective. Therefore, it appears that Sirt 3 and the mitochondrial HDAC1 may target the deacetylation of unique sets of lysine's in the mitochondria. The acetylation of some of those lysine's affecting pathway activity beneficial for cardioprotection in I/R and some contributing to I/R injury.

nuclear and cytoplasmic HDAC1. We observed that inhibition of the mitochondrial HDAC1 during early reperfusion significantly improves cardiac function and greater tissue viability in the first hour following I/R injury. Interestingly, we see that the mitochondrial-targeted HDACi protective effect appears to be mediated by a reduction in oxidative metabolism and subsequent ROS generation in early reperfusion. Because previous work has demonstrated that robust SDHA activity is a major contributor of ROS production we examined whether SDHA is a specific target of mitochondrial HDAC1 [46,89]. Our data shows that HDAC1 binds to SDHA and that inhibition of HDAC1 in reperfusion decreases SDHA activity. This opens up the possibility that preventing the deacetylation of SDHA may represent one of the mechanisms in which inhibition of the mitochondrial HDAC1 decreases ROS and protects myocyte viability and preserves ventricular function (Fig. 1). The precise mechanism behind this interesting phenomenon remains unclear and is the subject of ongoing research in our laboratory.

### *3.1. The opposing effects of sirtuin and HDAC activity*

Given the aforementioned studies, HDAC inhibitors appear to have a promising future role in the treatment of multiple cardiac disease states. The harmful effects of HDAC activity in various pathological cardiac conditions would seem to suggest that widespread protein deacetylation within the myocyte is deleterious to the injured heart. However, it has been repeatedly demonstrated that increased sirtuin activity is beneficial in cardiac disease [90]. This raises the tantalizing possibility that increased acetylation is neither beneficial nor detrimental in the context of the global acetylome within a cell, but rather that changes in acetylation of enzymes within specific signaling pathways alters those pathways in ways that modulate response to injury. This nuanced understanding of protein acetylation requires much more research in order to determine how the targets of HDACs and sirtuins differ, and how the acetylation of these targets in turn affects cardiac disease. This line of inquiry holds great promise in the future development of therapeutics for cardiac disease.

## 4. Conclusion

Treatment of ischemia-reperfusion injury in the heart remains a challenging clinical scenario which is currently largely limited to the physical re-establishment of blood flow to the ischemic tissue bed. Our understanding of the metabolic perturbations elicited by ischemia-reperfusion injury is in its nascency, but further understanding of the role of these metabolic pathways in reperfusion injury presents a promising way forward in improving the treatment of cardiac reperfusion injury. Critical to this will be a better understanding of the effect of acetylation on the activity of mitochondrial enzymatic machinery. Harnessing this understanding could lead to the development of promising new therapeutics that alter the metabolic response to injury and improve clinical outcomes.

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