Pyridine Nucleotide Regulation of Cardiac Intermediary Metabolism

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Abstract: The pyridine nucleotides NAD\(^+\) and NADP\(^+\) play a pivotal role in regulating intermediary metabolism in the heart. The intracellular NAD\(^+\)/NADH ratio controls flux through various dehydrogenase enzymes involved in both anaerobic and aerobic metabolism and also regulates posttranslational protein modification. The intracellular NADP\(^+\)/NADPH ratio controls flux through the pentose phosphate pathway (PPP) and the polyol pathway, while also regulating ion channel function and oxidative stress. Not only does the NAD\(^+\)/NADH ratio regulate the rates of ATP production, it can also modify energy substrate preference. For instance, in many forms of heart disease a greater contribution from fatty acids for oxidative energy metabolism increases fatty acid \(\beta\)-oxidation–derived NADH, which can activate pyruvate dehydrogenase (PDH) kinase isoforms that inhibit PDH and subsequent glucose oxidation. As such, novel therapies that overcome fatty acid \(\beta\)-oxidation–induced inhibition of PDH improve cardiac efficiency and subsequent function during ischemia/reperfusion and in heart failure. Furthermore, recent studies have implicated a pivotal role for increased PPP-derived NADPH in mediating oxidative stress observed in heart failure. In this article, we review the multiple actions of NAD\(^+\)/NADH and NADP\(^+\)/NADPH in regulating intermediary metabolism in the heart. A better understanding of the roles of NAD\(^+\)/NADH and NADP\(^+\)/NADPH in cellular physiology and pathology could potentially be used to exploit pyridine nucleotide modification in the treatment of a number of different forms of heart disease. (Circ Res. 2012;111:628-641.)

Key Words: nicotinamide adenine dinucleotide ■ nicotinamide adenine dinucleotide phosphate ■ pyruvate dehydrogenase ■ glucose oxidation ■ pentose phosphate pathway ■ oxidative stress

The pyridine nucleotides, nicotinamide adenine dinucleotide (NAD\(^+)\) and nicotinamide adenine dinucleotide phosphate (NADP\(^+)\), are critical coenzymes present in cells that have a key role in regulating both catabolic and anabolic cellular metabolic processes. The important role of NAD\(^+\) and NADP\(^+\) as key electron carriers in oxidoreductase reactions has been well characterized. However, their involvement in many signaling pathways has also emerged.\(^1\) This includes the involvement of NAD\(^+\) as a substrate for deacetylation reactions (such as those carried out via the...
sirtuin [SIRT] family of enzymes), the role of NADH and NADP⁺ in poly (ADP ribose) polymerase reactions, the involvement of NADPH and NADPH oxidase in free radical production, the role of NADP⁺/NADPH in the thioredoxin pathway, and their regulation of glucose flux through the polyol pathway. Furthermore, pyridine nucleotides can also aid in the regulation of ion channels that can transduce signaling pathways (such as those regulated via changes in Ca²⁺ homeostasis). Although these numerous biological actions for pyridine nucleotides all play various important roles in cellular physiology, in this review we will primarily focus on the key role of NAD⁺ and NADP⁺ in intermediary metabolism and how alterations in the NAD⁺/NADH and NADP⁺/NADPH ratios influence energy metabolism and subsequently affect contractile function of the heart.

**Bioynthesis of NAD⁺/NADP⁺**

NAD⁺ is a dinucleotide coenzyme, whose 2 nucleotides are joined together via their respective phosphate groups, with 1 nucleotide containing an adenine base and the other containing nicotinamide. Three precursors are essential for the biosynthesis of cellular NAD⁺/NADP⁺, the amino acid tryptophan, nicotinic acid (also known as vitamin B3), and the amminated form of nicotinic acid, nicotinamide. Nicotinamide is converted to nicotinamide mononucleotide via the enzymatic activity of nicotinamide phosphoribosyl transferase, the rate-limiting enzyme of NAD⁺ biosynthesis. Nicotinamide mononucleotide is subsequently converted into NAD⁺ via the ATP-requiring nicotinamide mononucleotide adenyltransferase (Figure 1). If dietary tryptophan and niacin are utilized, a much larger number of enzymatic reactions, including those of the kynurenine pathway, are required for the biosynthesis of NAD⁺ (Figure 1). Ultimately, NAD⁺ can be converted into NADP⁺ via the ATP-requiring NAD⁺ kinase. Interestingly, whereas the vast majority of cellular NAD⁺/NADH is compartmentalized in mitochondria, pyridine nucleotides are impermeable to the inner mitochondrial membrane, and, to date, both a mitochondrial nicotinamide mononucleotide adenyltransferase and NAD⁺ kinase have not been identified. It has been proposed that NAD⁺ may be able to enter the mitochondria via transient opening of the permeability transition pore, although this is in contrast to the widely held notion that this pore is normally closed in a closed state and only opens in pathophysiological conditions such as ischemia/reperfusion.

Degradation of NAD⁺ occurs primarily via NAD⁺ glycohydrolase, though cleavage via nucleotide pyrophosphatases may also play a role. Although intracellular NAD⁺/NADH and NADP⁺/NADPH ratios are potent regulators of cataobolic and anabolic intermediary metabolism, respectively, the healthy heart itself is not continually degrading and resynthesizing NAD⁺/NADP⁺ de novo, but rather its intracellular levels remain rather constant as these important coenzymes are continually recycled through various metabolic enzymes depending on the redox state of the cell. However, in the diseased myocardium, changes in intracellular NAD⁺/NADH and NADP⁺/NADPH concentrations can influence intermediary metabolism, which may subsequently affect cardiac function and will be discussed in greater detail in this review.

**NAD⁺/NADH Regulation of Intermediary Energy Substrate Metabolism**

The NAD⁺/NADH redox pair functions to regulate flux through a number of pyridine nucleotide-linked dehydrogenase (ie, oxidoreductase) enzymes involved in the intermediary metabolism of both carbohydrates and fatty acids. A high ratio of NAD⁺/NADH favors substrate oxidation and the redox pair functions as an important component of electron transfer during oxidative catalobic metabolism. As an enzymatic substrate undergoes oxidation (ie, dehydrogenation), 2 hydrogen atoms are released, and NAD⁺ is reduced to NADH following the general reaction mechanism: substrate-H₂ + NAD⁺ → substrate + NADH + H⁺. This reaction contributes to regulating the catalobic metabolism of energy substrates in both the cytosolic and mitochondrial compartments, with the reduced pyridine nucleotide (ie, NADH) ultimately functioning as a soluble carrier, delivering electrons to the mitochondrial electron transport chain (ETC), for utilization in the synthesis of ATP via oxidative phosphorylation.

**NAD⁺/NADH Regulation of Glycolysis**

In the cytosolic compartment, the NAD⁺/NADH redox pair regulates flux through glycolysis. The glycolytic enzymes, glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and lactate dehydrogenase (LDH) utilize the NAD⁺/NADH redox pair as a cofactor. NADH must be continually reoxidized to...
to ensure flux through glycolysis is not restricted at the level of GAPDH. This is accomplished by several mechanisms. In the anaerobic setting (ie, in the absence of oxygen), NADH generated via GAPDH (during the oxidation of glyceraldehyde 3-phosphate to 1,3 bisphosphoglycerate) is reoxidized to NAD$^+$ by LDH (during the reduction of pyruvate to lactate). As such, in the anaerobic setting the coordinated flux through both GAPDH and LDH continually regenerates NAD$^+$, thereby ensuring glycolytic flux.

In the aerobic setting, the reoxidation of cytosolic NADH is coupled to the reduction of molecular oxygen in the mitochondrial ETC. However, as the inner mitochondrial
The membrane is impermeable to NAD\textsuperscript{+}/NADH, “shuttle” mechanisms are necessary for transporting NADH into the mitochondria and subsequently regenerating cytosolic NAD\textsuperscript{+}. In cardiac muscle, the predominant mechanism responsible is represented by the malate-aspartate shuttle (Figure 2A). In the cytosolic compartment, malate dehydrogenase (1) couples the regeneration of NAD\textsuperscript{+} to the reduction of oxaloacetate, and in the process generates malate. Malate gains access to the mitochondrial matrix via the malate-\(\alpha\)-ketoglutarate (\(\alpha\)-KG) transporter located in the inner mitochondrial membrane in exchange for \(\alpha\)-KG. In the mitochondrial matrix, the oxidation of malate to oxaloacetate via malate dehydrogenase (2) in the TCA cycle is coupled to the reduction of NAD\textsuperscript{+} to NADH, which serves as a reducing equivalent for the mitochondrial ETC. Aspartate aminotransferase in the cytosol (3) and mitochondrial matrix (4) is responsible for the interconversion of \(\alpha\)-KG to oxaloacetate and vice versa, respectively, allowing the shuttle to continue delivering cytosolic NADH to the mitochondria. The glycerol 3-phosphate shuttle operates via the reduction of cytosolic dihydroxyacetone phosphate to glycerol 3-phosphate by the NAD\textsuperscript{+}/NADH-linked cytosolic (c) glycerol 3-phosphate dehydrogenase, which regenerates NAD\textsuperscript{+} from NADH. Glycerol 3-phosphate is able to diffuse through the outer mitochondrial membrane and is subsequently oxidized back to dihydroxyacetone phosphate as FAD is reduced to FADH\textsubscript{2} via mitochondrial (m) glycerol 3-phosphate dehydrogenase.

**Figure 2. Malate-aspartate and glycerol 3-phosphate shuttles.** A, In the cytosolic compartment, malate dehydrogenase (1) couples the regeneration of NAD\textsuperscript{+} to the reduction of oxaloacetate, and in the process generates malate. Malate gains access to the mitochondrial matrix via the malate-\(\alpha\)-ketoglutarate (\(\alpha\)-KG) transporter located in the inner mitochondrial membrane in exchange for \(\alpha\)-KG. In the mitochondrial matrix, the oxidation of malate to oxaloacetate via malate dehydrogenase (2) in the TCA cycle is coupled to the reduction of NAD\textsuperscript{+} to NADH, which serves as a reducing equivalent for the mitochondrial ETC. Aspartate aminotransferase in the cytosol (3) and mitochondrial matrix (4) is responsible for the interconversion of \(\alpha\)-KG to oxaloacetate and vice versa, respectively, allowing the shuttle to continue delivering cytosolic NADH to the mitochondria. B, The glycerol 3-phosphate shuttle operates via the reduction of cytosolic dihydroxyacetone phosphate to glycerol 3-phosphate by the NAD\textsuperscript{+}/NADH-linked cytosolic (c) glycerol 3-phosphate dehydrogenase, which regenerates NAD\textsuperscript{+} from NADH. Glycerol 3-phosphate is able to diffuse through the outer mitochondrial membrane and is subsequently oxidized back to dihydroxyacetone phosphate as FAD is reduced to FADH\textsubscript{2} via mitochondrial (m) glycerol 3-phosphate dehydrogenase.

Pyruvate represents the predominant end product of glycolysis under aerobic conditions in cardiac muscle and functions as the immediate precursor for carbohydrate derived acetyl-CoA entering the TCA cycle. After entry into the mitochondrial matrix via facilitated transport, pyruvate undergoes irreversible oxidative decarboxylation. This committed step in the intermediary metabolism of pyruvate is dependent on...
the activity of the pyruvate dehydrogenase (PDH) complex, which consists of PDH itself, PDH kinase (PDHK), and PDH phosphatase enzymes. PDH catalyzes the irreversible oxidation of pyruvate, coupled to the reduction of NAD+, and therefore contributes to generating NADH in the mitochondrial matrix, which is utilized for oxidative phosphorylation. NADH also plays a central role in regulating flux through PDH via product-inhibition and thus contributes to the reciprocal relationship between carbohydrate oxidation and fatty acid β-oxidation described by the Randle cycle. The mechanisms underlying the Randle cycle can become important contributors to the alterations in energy substrate metabolism observed in the ischemic and ischemic-reperfused myocardium as well as in a number of other cardiomyopathies including cardiac insulin-resistance and heart failure. As such, these mechanisms also represent viable pharmacological targets to limit myocardial injury in a variety of pathologies.

**NAD+/NADH Regulation of Fatty Acid β-Oxidation**

Mitochondrial fatty acid β-oxidation is the biochemical process that progressively liberates acetyl-CoA from long-chain acyl-CoA molecules (see Lopeschuk et al25 for detailed review of myocardial fatty acid β-oxidation). A series of 4 enzymatic reactions comprises the β-oxidation spiral: (1) acetyl-CoA dehydrogenase, (2) enoyl-CoA hydratase, (3) 3-OH acyl-CoA dehydrogenase, and (4) 3-keto acyl-CoA thiolase. Of these, 3-OH acyl-CoA dehydrogenase is a NAD-linked enzyme that couples the generation of NADH to the oxidation of a 3-OH acyl-CoA moiety, to yield the respective 3-keto acyl-CoA moiety. Flux through this step of fatty acid β-oxidation is subject to regulation by mitochondrial redox status, which itself can be influenced by the rates of both carbohydrate and fatty acid β-oxidation. In line with this, pharmacologically stimulating the oxidation of NADH via stimulation of complex I of the ETC increases mitochondrial fatty acid β-oxidation.

**NAD+/NADH Regulation of the TCA Cycle**

The TCA cycle is a series of mitochondrial enzymatic reactions that represents a metabolic hub where acetyl-CoA derived from the oxidative metabolism of both carbohydrates and fatty acids converges. The cycle is divided into 2 spans: (1) acetyl-CoA to α-ketoglutarate and (2) α-ketoglutarate to oxaloacetate. The TCA cycle oxidizes acetyl-CoA in a cumulative manner liberating CO2 and concomitantly generating reducing equivalents in the form of NADH (and FADH2). The NAD+/NADH ratio is an important regulator of flux through the TCA cycle. Increases in ATP demand drive oxidative phosphorylation and thus increase the NAD+/NADH ratio. This in turn stimulates the activity of several NAD+/NADH-linked enzymes of the cycle, including isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase, thereby increasing TCA cycle activity. Isocitrate dehydrogenase occupies the first span of the cycle, whereas α-ketoglutarate occupies the second span of the cycle, with each enzyme coupling the irreversible oxidation of isocitrate, and α-ketoglutarate, respectively, to the generation of NADH. Mitochondrial NAD+/NADH-linked malate dehydrogenase occupies the second span of the cycle, and couples the reversible oxidation of malate to the generation of NADH. This NADH is then used by the ETC for ATP production. Oxidation of NADH by fumarate coupled to ATP synthesis is also a possible mechanism for producing additional glycolytic ATP.

**NAD+/NADH Regulation of the Mitochondrial ETC**

The mitochondrial ETC couples the oxidation of reducing equivalents NADH (and FADH2) to the generation of a proton motive force across the inner mitochondrial membrane, which provides the potential energy to drive the synthesis of ATP via ATP synthase (ie, oxidative phosphorylation). Five major intrinsic components, each organized as multisubunit complexes comprise the mammalian electron transport chain: (1) complex I (NADH:ubiquinone oxidoreductase), (2) complex II (succinate:ubiquinone oxidoreductase), (3) complex III (cytochrome c), (4) complex IV (cytochrome c oxidase), and (5) complex V (ATP synthase). Electron transfer at complexes I, III, and IV contributes to the translocation of protons across the inner mitochondrial membrane. The oxidation of NADH occurs at complex I and is coupled to the reduction of lipid soluble ubiquinone, and the translocation of 4 protons per pair of electrons is transferred.

The regulation of the ETC by NAD+/NADH appears to differ, depending on the model system studied. Early studies in isolated mitochondrial preparations suggest that a key regulator of ETC activity is the matrix concentration of ADP, with increasing ADP concentrations increasing the flux of protons via the F0/F1 ATP synthase downstream of the increased generation of NADH in the TCA cycle and via the catabolic degradation of energy substrates. However, in the intact myocardium, numerous studies demonstrate that the concentrations of cytosolic and mitochondrial energetic intermediates including ATP, ADP, PCr, NADH, and NAD+ are not altered in response to increases in ATP demand induced by increases in cardiac work. These issues, along with difficulties in measuring actual intramitochondrial levels of the metabolites, make it difficult to discern by what mechanism(s) the supply and delivery of reducing equivalents to the ETC regulates ETC activity in the intact heart.

**NADP+/NADPH Regulation of Intermediary Energy Substrate Metabolism**

The biological role of NADP+/NADPH is different from that of NAD+/NADH. Whereas NAD+/NADH primarily regulates flux through a number of dehydrogenase enzymes involved in the intermediary metabolism of both carbohydrates and fatty acids that provide energy, NADP+/NADPH is primarily used in anabolic biosynthetic reactions such as fatty acid synthesis and thus utilizes significant amounts of energy. A large fraction of the cellular requirement for NADPH is met via enzymatic machinery of the pentose phosphate pathway (PPP). Both glucose-6-phosphate (G6P) dehydrogenase and 6-phosphogluconate dehydrogenase of the PPP facilitate reduction of NADP+ to yield NADPH. In addition, NADPH can be generated in the cell via the enzymatic activity of the malic enzyme, which decarboxylates malate to pyruvate while subsequently reducing NADP+. An isoform of NADP+ isocitrate dehydrogenase...
Present in both the cytosol and mitochondria can also produce NADPH. Recent studies suggest that the inner mitochondrial membrane NADPH transhydrogenase may also play a key role in the regulation of cellular NADPH content. NADPH serves a number of essential cellular functions, including (1) de novo fatty acid synthesis, whereby it acts as a cofactor for both \(\beta\)-ketoacyl acyl carrier protein (ACP) reductase and enoyl ACP reductase, (2) the regulation of oxidative stress, where it acts as a cofactor for both glutathione reductase and thioredoxin reductase, producing reduced glutathione and reduced thioredoxin to deal with oxidative stress induced by toxins such as hydrogen peroxide, and (3) cholesterol biosynthesis, where it acts as a cofactor for 3-hydroxy-3-methylglutaryl CoA reductase, the rate-limiting enzyme of the mevalonate pathway.

In contrast to its function in alleviating oxidative stress via the glutathione reductase pathway, NADPH may also contribute to oxidative stress in atherosclerosis, ischemic heart disease, and heart failure serving as a cofactor for NADPH oxidase-mediated increases in superoxide production.

The clinical utility of inhibiting NADPH oxidase for the treatment of cardiovascular disease is the topic of in-depth discussion in a separate review in this thematic series.

**PPP in the Heart**

The PPP (Figure 3) consists of both an irreversible oxidative phase, where G6P is sequentially metabolized into ribulose 5-phosphate, and a reversible nonoxidative phase where ribulose 5-phosphate is sequentially metabolized into either fructose-6-phosphate or glyceraldehyde-3-phosphate as it re-enters the glycolytic pathway (illustration: Ben Smith).

**Figure 3. The pentose phosphate pathway (PPP).** The PPP contains 2 phases. The first phase encompasses the irreversible oxidative phase (depicted in the light green box) that produces NADPH via G6P dehydrogenase and 6-phosphogluconate dehydrogenase. The second phase involves the reversible nonoxidative phase (depicted in the light blue box) that takes ribulose-5-phosphate and converts it into either fructose-6-phosphate or glyceraldehyde-3-phosphate as it re-enters the glycolytic pathway (illustration: Ben Smith).
NADPH for de novo fatty acid synthesis in lipogenic tissues such as liver and adipose tissue. However, malic enzyme also operates in reverse, where it carboxylates pyruvate into malate. This malate is subsequently transported into the mitochondrial matrix in exchange for α-ketoglutarate to serve as an anaplerotic substrate to replenish TCA cycle intermediates.53,54 Interestingly, recent studies have shown that myocardial malic enzyme mRNA expression is increased following aortic banding–induced cardiac hypertrophy.55,56 It has been postulated that although such a mechanism may maintain TCA cycle intermediates at normal levels, carboxylation of pyruvate through malic enzyme is less energetically efficient versus its normal route of entry into the mitochondria through oxidative decarboxylation via PDH.56 Indeed, enhancing PDH activity in aortic banded hearts decreased malate levels and improved contractile function, findings associated with a restoration of the hypertrophy-induced reduction in intramyocardial triacylglycerol (TAG) content.55 Such observations suggest that by limiting the amount of pyruvate available to the malic enzyme, NADPH is readily available for de novo fatty acid synthesis, explaining why TAG content was restored to normal levels. However, the heart is not believed to synthesize a large amount of TAG through de novo type I fatty acid synthesis,43 with most of the intramyocardial TAG pool thought to arise from incorporating fatty acids taken up by the heart into the glycerol-3-phosphate lipogenic pathway.57–60 As such, these previous results may suggest a reverse Randle cycle–like effect, where increased rates of glucose oxidation spare fatty acids from mitochondrial β-oxidation, and thereby partition fatty acids into the myocardial TAG pool. Future studies will need to readdress these discrepancies, determining whether the NADPH-requiring β-ketoacyl ACP and enoyl ACP reductases are expressed at levels high enough to regulate a significant flux of fatty acid synthesis via this route. This is supported by recent studies demonstrating a novel role for fatty acid synthase in the heart with regard to Ca2+ signaling during stress and the observation of significant rates of de novo fatty acid synthesis from [14C]acetate in the heart.61

NADP+/NADPH and the Thioredoxin System in the Heart

Thioredoxin-1 is a ubiquitously expressed 12-kDa peptide, the active site of which contains a conserved dithiol (Cys-Gly-Pro-Cys), and functions as a protein disulfide dehydrogenase.62,63 The thioredoxin-2 isofrom is localized to mitochondria and is highly expressed in metabolically active tissues including the heart, liver, and brain.62,63 The thioredoxin isoforms function as intracellular reducing agents and are involved in regulating reduced thiols in redox-sensitive proteins, with thioredoxin being oxidized in the process. NADPH is utilized to regenerate reduced thioredoxin in a reaction catalyzed by thioredoxin reductases. As such, several factors, including the expression and activity of the thioredoxin isoforms, the expression and activity of thioredoxin reductases, and the intracellular NADP+/NADPH ratio,62,63 are important regulators of the intracellular redox state. Recent evidence implicates alterations in thioredoxin function as important factors accompanying various cardiac pathophysiological states including cardiac hypertrophy and the progression to heart failure (see below).

Pyridine Nucleotides and Bioenergetics in Cardiovascular Disease

As previously alluded to, cellular redox status is an important regulator of energy fuel metabolism, and many enzymes involved in controlling redox status are under regulation of NAD+/NADH and NADP+/NADPH ratios.64,65 Changes in intracellular NAD+/NADH and NADP+/NADPH content can take place in the diseased myocardium (for overview, see Figures 4 and 5), with these changes influencing intermediary metabolism in the heart and impacting cardiac function.

NAD+/NADH Regulation of GAPDH and LDH During Myocardial Ischemia

The limiting oxygen supplies during myocardial ischemia prevent the oxidation of NADH by the complexes of the mitochondrial ETC, and thus NADH builds up in the cytosol. During the initial stages of ischemia, this buildup of NADH inhibits GAPDH and subsequent anaerobic glycolysis. As mentioned previously, for glycolysis to proceed, the accumulated NADH is oxidized back to NAD+ via the enzymatic activity of LDH (Figure 4). Depending on the severity of ischemia, this accumulated lactate can be transported out of the cardiac myocyte via the monocarboxylic acid transporter.66 If the ischemia becomes too severe, then the accumulated lactate cannot be washed out. This is of particular interest because accumulated lactate in the heart has been proposed to be responsible for mediating anginal pain via its activation of sustained electric currents through acid sensing ion channel 3.67 As lactate continues to accumulate, this will also contribute to the inhibition of GAPDH and glycolysis will not be able to proceed until reperfusion of the ischemic heart is established.68 Furthermore, a classic study by Neely and Grotjohann indicated that glycolytic by-products such as lactate, protons, and NADH may result in irreversible damage to the myocardium during ischemia.69 Another study by Cross et al suggests that it is the accumulation of lactate, an increased lactate/pyruvate ratio, and subsequent increase in the NADH/NAD+ ratio that plays a major role in causing myocardial ischemic damage.70 Thus, it has been proposed that increasing glycolytic rates during ischemia can be protective, possibly via providing an anaerobic source of ATP in close association with the sarcosomal membrane, which may aid in the maintenance of ionic homeostasis.71 Increased glycolytic rates in the heart have also been shown to delay the development of contracture during ischemia.72,73 In contrast, an increase in glycolytic rates during ischemia that is not accompanied via a similar increase in flux through glucose oxidation will increase proton production from uncoupled glucose metabolism, which can be just as damaging to the ischemic heart via reducing cardiac efficiency74,75 (see below). In addition, ischemic preconditioning, which is one of the most universally accepted forms of cardioprotection, exacerbates the development of contracture during ischemia.76,77 However, preconditioning inhibits glycolysis and proton production attributable to uncoupled glucose metabolism.78 Hence, the contribution of glycolysis to ischemic
injury is equivocal and remains to be completely elucidated, while using contracture as an index of ischemic injury has limitations.

**Increased Aldose Reductase Activity and Flux Through the Polyol Pathway During Ischemia and Reperfusion**

Another factor contributing to the elevated cytosolic NADH/NAD⁺ ratio and inhibition of GAPDH during myocardial ischemia is increased activity of aldose reductase (AR) and sorbitol dehydrogenase, subsequently increasing flux of glucose through the polyol pathway. Indeed, a number of studies in isolated hearts from both rats and mice have demonstrated increased AR activity in response to low-flow ischemia, whereas sorbitol dehydrogenase activity is increased in hearts in response to global ischemia. Inhibition of AR with zopolrestat in both normal and spontaneous acute diabetic Bio-Bred (BB/W) rats improved recovery of function and diminished the decline in intracellular ATP content. Moreover, this impaired recovery of LVDP was associated with reduced glucose oxidation rates, myocardial ATP content, and an elevated lactate/pyruvate ratio. However, inhibition of AR in these mice restored glucose oxidation rates and lowered the cytosolic NADH/NAD⁺ ratio, as indicated by a decrease in the lactate/pyruvate ratio, while improving the recovery of LVDP and decreasing creatine kinase release following severe low-flow ischemia.

Flux of glucose through the polyol pathway is unique in that its 2 sequential enzymes, (AR and sorbitol dehydrogenase) affect both NADP⁺/NADPH and NAD⁺/NADH ratios. Although AR inhibition has been clearly demonstrated to influence the cytosolic NAD⁺/NADH ratio and subsequent glycolytic rates, AR inhibition may also influence oxidative stress during ischemia via increasing the intracellular NADPH/NADP⁺ ratio. As such, treatment of isolated mouse hearts during reperfusion following global ischemia with the AR inhibitor, SNK-860, reduced thiobarbituric acid reactive substances, suggesting that AR inhibition also reduces lipid peroxidation and subsequent oxidative stress, though intracellular NADPH levels were not determined in this study.

![Figure 4. Effects of ischemia on cytosolic and mitochondrial NADH. Ischemia is characterized by diminished coronary flow and hence oxygen delivery. As oxygen functions as the terminal electron acceptor in the mitochondrial ETC, ischemia is accompanied by the accumulation of reducing equivalents including NADH in the mitochondria. In the cytosolic compartment, (1) NADH generated at the GAPDH reaction of glycolysis is reoxidized to NAD⁺ and coupled to the reduction of pyruvate to lactate, as under ischemic conditions neither glycolytically derived pyruvate nor NADH readily enter the mitochondria. Upon reperfusion (re-introduction of coronary flow and oxygen delivery), reducing equivalents are again readily reoxidized by the mitochondrial ETC, and the contribution of lactate dehydrogenase to the regeneration of cytosolic NADH decreases. Instead, (2) fatty acid β-oxidation rates quickly recover, and enhanced NADH generation via fatty acid β-oxidation activates PDHK isoforms to inhibit PDH, (3) thereby reducing glucose oxidation-derived NADH.](image-url)
Fatty Acid β-Oxidation–Derived NADH and Inhibition of PDH and Glucose Oxidation During Ischemia and Reperfusion

During myocardial ischemia, mitochondrial NAD⁺ decreases and NADH increases, resulting in a dramatic increase in the NADH/NAD⁺ ratio. Although overall oxidative metabolism decreases during myocardial ischemia, of the remaining residual oxidative metabolism, the majority is accounted for via fatty acid β-oxidation. As described in the Randle cycle section, fatty acid β-oxidation–derived NADH is able to inhibit PDH via product inhibition (Figure 4) and thus limit glucose oxidation, which reduces the efficiency of contractile function during ischemia. Moreover, an increased NADH/NAD⁺ ratio has been shown to activate PDHK, which will phosphorylate and contribute to decreased PDH activity.

Our working hypothesis continues to revolve around the concept that overcoming this fatty acid β-oxidation–induced inhibition of glucose oxidation in the ischemic heart will improve the efficiency of contractile function, possibly via increasing the coupling of glycolysis and glucose oxidation, which decreases proton production. Indeed, a previous study in our laboratory demonstrated in a model of low-flow ischemia that stimulating residual oxidative metabolism to ward glucose improved myocardial efficiency and subsequent contractile function. We have also shown that inhibiting fatty acid β-oxidation during demand-induced ischemia in pigs reduces myocardial lactate accumulation and improves LV contractile work.

On reperfusion of the ischemic heart, fatty acid β-oxidation rates quickly recover to preischemic rates and limit the recovery of glucose oxidation rates once again via the Randle cycle. A number of studies have also shown that overcoming this fatty acid–induced inhibition of glucose oxidation during reperfusion can also limit injury and subsequently improve the recovery of cardiac function. Indeed, novel malonyl CoA decarboxylase (MCD) inhibitors reduce myocardial fatty acid β-oxidation rates, resulting in a secondary increase in glucose oxidation rates that improves the recovery of cardiac function in isolated working rat hearts subjected to reperfusion after a global no-flow ischemic insult. In addition, isolated working hearts from mice deficient for MCD exhibit a dramatic increase in glucose oxidation rates and subsequent recovery of cardiac function when subjected to an identical ischemia/reperfusion protocol. Similar findings have been shown in an in vivo model of ischemia/reperfusion injury, as MCD−/− mice demonstrate a significant reduction in infarct size 24 hours after a 30-minute ischemic insult induced via temporary ligation of the left anterior descending coronary artery. Furthermore, we hypothesize that the reduction in infarct size is due to enhanced PDH activity, as MCD−/− mice have elevated PDH activity in tissue isolated from the ischemic area at risk, and mice deficient for PDHK4, which also demonstrate increased myocardial glucose oxidation rates, exhibit a reduction in infarct size after left anterior descending coronary artery ligation.

Glucose Oxidation–Derived NADH and Smooth Muscle Cell Apoptosis in Pulmonary Arterial Hypertension

In pulmonary arterial hypertension (PAH), it has been proposed that increased endoplasmic reticulum stress and uncontrolled proliferation of pulmonary artery smooth muscle cells...
are key factors mediating disease progression.93–95 Interestingly, PDH activity is reduced in pulmonary artery smooth muscle cells from PAH patients,96 whereas stimulating PDH activity and subsequent glucose oxidation with dichloroacetate (DCA) has been shown to induce pulmonary artery smooth muscle cell apoptosis and reverse the development of PAH in monocrotaline-treated rats.97 Furthermore, MCD/−/− mice, which have increased glucose oxidation rates due to increased malonyl-CoA–induced inhibition of fatty acid β-oxidation,98,99 are also protected against the development of PAH induced by chronic hypoxia.99 Similar findings were obtained in wild-type mice treated with the fatty acid β-oxidation inhibitor, trimetazidine, which we have previously shown to increase glucose oxidation rates secondary to its inhibition of 3-keto acyl-CoA thiolase.100,101 The proposed mechanism for the beneficial effects of increasing PDH activity in PAH involves increased glucose oxidation-derived NADH, which increases superoxide production via complex I of the ETC, ultimately activating a series of downstream signaling events that promote apoptosis of pulmonary artery smooth muscle cells.102 In contrast, fatty acid β-oxidation–derived NADH should also increase complex I-mediated superoxide production, and the observation that fatty acid β-oxidation inhibitors promote pulmonary artery smooth muscle apoptosis99 suggests that alternative mechanisms may also partake in their protective effects against PAH. These alternative mechanisms may be related to an interruption of the Warburg effect and the unique metabolic requirements for doubling cellular biomass (for review, see Vander Heiden et al103).

**Increased Flux Through the PPP in Heart Failure**

As mentioned previously, metabolic rates through the PPP in the adult heart are thought to be minimal, due to low activity of G6P dehydrogenase, the rate-limiting enzyme of the oxidative PPP.51,52 In contrast, recent studies have suggested that flux through the PPP in the heart increases as the heart hypertrophies and progresses to heart failure104,105 (Figure 5). Gupta et al demonstrated a significant increase in G6P dehydrogenase activity in myocardium from dogs with heart failure, which was associated with increased NADPH levels and subsequent NADPH oxidase–mediated reactive oxygen species (ROS) production. Interestingly, inhibition of G6P dehydrogenase decreases superoxide production in failing hearts. Although these findings suggest that increased flux through the PPP is detrimental to the failing heart, no functional data have yet been provided demonstrating potential improvement in response to inhibition of G6P dehydrogenase. On the other hand, Dahl salt-sensitive rats fed a high salt diet to induce the development of congestive heart failure also exhibited increased flux through G6P dehydrogenase and the PPP, but treatment with DCA increased flux through the PPP further and actually protected against congestive heart failure development.105 However, DCA is a well-characterized stimulator of PDH and subsequent glucose oxidation rates, and it is possible that the beneficial effects of DCA involve improved glucose oxidation and cardiac energetics, which has been postulated to be a novel therapeutic approach for the treatment of heart failure.25,74,106 Indeed, aortic-banded hypertrophic hearts have increased anaplerotic flux through malic enzyme that contributes toward their reduced contractile function, an effect prevented via treatment with DCA.55 DCA-induced flux of pyruvate through PDH versus malic enzyme increases the bioavailability of NADPH, which, combined with a Randle cycle–like effect to spare fatty acids from mitochondrial fatty acid β-oxidation, may contribute to increased lipogenesis and normalized TAG content in hypertrophic hearts. Thus, increased NADPH bioavailability via this route and not the PPP may reduce oxidative stress and account for the beneficial effects of DCA against congestive heart failure observed via Kato et al.105

Additional work supporting a protective role for G6P dehydrogenase in cardiac function is seen after inhibition of G6P dehydrogenase in adult cardiac myocytes, which results in reduced contractile function that can be prevented via adenoviral overexpression of G6P dehydrogenase.107 Furthermore, whole-body G6P dehydrogenase–deficient mice exhibit increased septal wall thickening, adverse LV structural remodeling, and reduced fractional shortening as they age in comparison to their wild-type littermates. Of interest is that it has also been suggested that humans deficient for G6P dehydrogenase have a reduced risk of coronary heart disease, although this study was correlational in nature and it cannot be attributed to a specific effect on the myocardium. Further studies looking at G6P dehydrogenase in the myocardium implicate it as having a causal effect in the development of heart failure, as its protein expression and activity were increased in hearts from obese Zucker rats before the development of LV dysfunction.109 Similar findings have been obtained in humans, whereby increased G6P dehydrogenase–derived NADPH has been proposed to fuel superoxide production through NADPH oxidase in surgically discarded LV biopsies from patients with congestive heart failure.110 Furthermore, increased activity of G6P dehydrogenase was observed in a model of right ventricular failure in young goats.111

Despite the discrepancies in the contribution of PPP flux to overall myocardial intermediary metabolism, the metabolic rate through the PPP is only a fraction of overall glucose uptake (≈50 nmol/g dry weight per minute versus 5–10 μmol/g dry weight per minute).112 Moreover, even if G6P dehydrogenase activity is elevated 2- to 3-fold in the failing heart, overall glucose uptake increases to ≈15 μmol/g dry weight per minute, indicating that the contribution of the PPP to overall glucose metabolism is an even smaller percentage than that in the normal heart. Nonetheless, as ROS may be key mediators of signal transduction pathways, even a 2- to 3-fold increase in NADPH production can ultimately lead to profound changes in intracellular signaling, potentially having very important effects on the myocardium in cardiovascular disease. It is clear from these recent studies that flux through the PPP plays a role in the diseased myocardium; however, the underlying cause that induces an increase in PPP flux in the failing heart remains to be determined.

**NAD⁺ Regulation of SIRT3 and Cardiac Hypertrophy**

Models of pathological hypertrophy are associated with a loss of cellular NAD⁺ levels, as observed in mice after aortic
banding,116 in mice continuously infused with angiotensin II,117 or in neonatal cardiac myocytes treated with phenylephrine.117 Confusion of mice with NAD+ prevented the development of cardiac hypertrophy and reduced fibrosis in response to angiotensin II, which was associated with a restoration of intramyocardial ATP content.117 Furthermore, as increased NAD+/NADH results in the activation of SIRT3,118 it was demonstrated that the development of cardiac hypertrophy was associated with increased deacetylation of LKB1 and subsequent activation of 5’AMP-activated protein kinase (AMPK), a key antihypertrophic signaling molecule and regulator of energy metabolism.119–124 Interestingly, these effects were not observed in mice deficient for SIRT3. Whether the beneficial effects of NAD+ supplementation resulted in improvements in AMPK-regulated energy metabolism have not been determined.

**Thioredoxin-1, Oxidative Stress, and Cardiac Hypertrophy**

Previous reports indicate that thioredoxin-1 is a negative regulator of cardiac hypertrophy, at least in part, by attenuating elevated levels of oxidative stress. Specifically, in transgenic mice that express dominant-negative thioredoxin-1, cardiac hypertrophy is evident under basal conditions, and the hypertrophic response to aortic banding is exaggerated and accompanied by elevated oxidative stress.6 Conversely, adenoviral-mediated overexpression of thioredoxin-1 attenuates the development of oxidative stress and cardiac myocyte hypertrophy secondary to prohypertrophic agents, including noradrenaline125 and angiotensin II.126 The overexpression of thioredoxin-2 also attenuates angiotensin II–mediated ROS formation and cardiac hypertrophy.127

The effects of thioredoxin-1 overexpression are accompanied by decreased redox-mediated activation of prohypertrophic signaling via Ras (ie, overexpression of thioredoxin-1 facilitates the reduction of critical thiols in Ras thereby decreasing its activation)125 and decreased nuclear-cytoplasmic shuttling of class II histone deacetylases (HDAC) (ie, overexpression of thioredoxin-1 facilitates the reduction of thiols in HDAC4, thereby limiting its nuclear export, allowing HDAC4 to repress the prohypertrophic program).7 In addition, thioredoxin also upregulates microRNA-98 expression to exert its antihypertrophic effects.126 These previous findings point to the importance of NADP+/NADPH and the thioredoxin system in attenuating cardiac hypertrophy. However, it should be noted that the ability of thioredoxin-1 to decrease the formation of ROS has been dissociated from its ability to attenuate cardiac hypertrophy,128,129 indicating a high level of complexity to its effects. This was demonstrated in studies involving the overexpression of thioredoxin interacting protein (TXNIP), a negative regulator of thioredoxin activity, which limited ROS formation but not cardiac myocyte hypertrophy induced by angiotensin II.129 On the other hand, previous reports also demonstrate that genetic deletion of TXNIP affects neither thioredoxin-1 activity, nor ROS formation, yet decreases cardiac hypertrophy after transverse aortic constriction, effects that are accompanied by a remodeling of the cardiac metabolic phenotype,74 specifically increased myocardial glucose uptake.128 However, this protection was not sustained, as TXNIP-deficient mice undergo adverse LV remodeling and decompensation with prolonged hemodynamic stress. Because TXNIP has been demonstrated to be a negative regulator of glucose uptake and glycolysis,130,131 these findings suggest that the enhanced glycolytic phenotype of the hypertrophied heart may initially be an adaptive response, but this metabolic phenotype becomes maladaptive in the progression to a decompensated heart failure. The thioredoxin system also appears to play an important role in myocardial ischemia-reperfusion injury,132–135 though whether this involves alterations in energy metabolism is unknown. Taken together, the body of experimental evidence indicates that the thioredoxin system, particularly thioredoxin-1, is an important antioxidant molecule that utilizes NADPH to regulate the redox status of various intracellular proteins, and exerts a variety of cardioprotective effects.

**Summary**

Alterations in intracellular NAD+/NADH and NADP+/NADPH content have significant effects on intermediary metabolism that may ultimately affect cardiac function, especially in the diseased myocardium. For example, fatty acid β-oxidation–produced NADH has potent effects on inhibiting PDH and subsequent glucose oxidation, which reduces the efficiency of contractile function in the heart during ischemia and reperfusion. As such, stimulating glucose oxidation can improve cardiac function in the ischemic heart and limit reperfusion injury. Although metabolic therapies for the heart are not directly aimed at targeting intracellular NAD+/NADH and NADP+/NADPH concentrations, changes in the content of these pyridine nucleotides may occur secondary to changes in intermediary metabolism, and how they may affect some of their more recently discovered biological functions, such as sirtuin deacetylase activity, is yet to be determined. Pharmacological targeting of these other biological functions of pyridine nucleotides and how they may impact intermediary metabolism also remains to be determined. For example, as SIRT-mediated deacetylation consumes NAD+, would this substantially alter the NAD+/NADH ratio in such a manner that activates PDHK to inhibit PDH and subsequently decrease glucose oxidation rates? Will inhibiting NADPH oxidases increase the NADPH/NADP+ ratio in a manner that diverts glucose-6-phosphate to increase flux through anaerobic glycolysis? Or can it possibly feed pyruvate through malic enzyme and increase anaplerosis even further in the hypertrophied heart, decreasing the oxidative decarboxylation of pyruvate through PDH and reducing contractile efficiency? We hope that the answers to these questions, and the links between pyridine nucleotides, the regulation of intermediary metabolism, and the control of more recently discovered cellular functions of NAD+/NADP+, will be elucidated in future studies. The findings of these studies should yield important implications for novel therapeutics designed to target the multifaceted roles of pyridine nucleotides.

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