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EDITORIAL

Raising NAD in Heart Failure

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It has long been known that cellular NAD levels are a critical regulator of metabolism and bioenergetics. The intracellular NAD pool consists of both oxidized (NAD^+) and reduced forms (NADH). NAD^+ is the main hydride acceptor in intermediary metabolism. Electrons derived from substrate catabolism are carried by NADH and used for oxidative phosphorylation and biosynthetic reactions. These reduction-oxidation reactions are not only essential for mitochondrial function and cell metabolism but also serve as important modulators of cell signaling.[1,2] NAD^+ functions as a cosubstrate for sirtuin deacylases, ADP-ribose transferases, and cyclic ADP-ribose synthases that govern posttranslational modification of proteins, DNA repair, and inflammatory responses.[2]

The cellular NAD^+ level is determined by the NAD(H) pool size (total NAD^+ and NADH concentration) as well as its reduction-oxidation state. The former is dependent on cellular NAD^+ consumption and regeneration, whereas the latter is regulated by cell metabolism and mitochondrial function (Figure). Emerging evidence suggests that derangements in the myocardial NAD pool are causally linked to metabolic remodeling and mitochondrial dysfunction in the failing heart. Stabilizing the intracellular NAD^+ level represents a promising therapeutic strategy to improve myocardial bioenergetics and cardiac function.[1,3,4] In this issue of *Circulation*, Diguet et al[5] report exciting data suggesting that supplementation with a NAD^+ precursor, nicotinamide riboside (NR), reduces cardiac dysfunction in preclinical models of heart failure.

In a genetic mouse model of dilated cardiomyopathy, induced by the deletion of serum response factor in the heart (SRF^{HKO}), Diguet et al[5] found that supplementation of NR in the diet significantly reduced left ventricular contractile dysfunction and chamber dilation. A similar, albeit moderate, effect was also observed in mice with pressure overload–induced hypertrophy and dysfunction. These observations are consistent with prior work demonstrating the beneficial effects of increasing NAD levels on cardiac hypertrophy and function in models of agonist-induced pathological hypertrophy,[6] chronic pressure overload,[7] and mitochondrial cardiomyopathy associated with Friedreich's ataxia.[7] Therefore, this study adds support to the emerging concept of increasing NAD levels as a therapeutic strategy for heart failure.

Despite the compelling evidence that expanding the intracellular NAD pool benefits the failing heart, the question of how it works remains not fully answered. Prior studies found that decreased NAD^+ availability was associated with increased protein acetylation in failing myocardium.[7–9] Those studies suggested that protein hyperacetylation was attributable to impaired NAD^+-dependent protein deacetylation by sirtuins, especially Sirt3, the mitochondria-localized sirtuin.[7,8] The benefit

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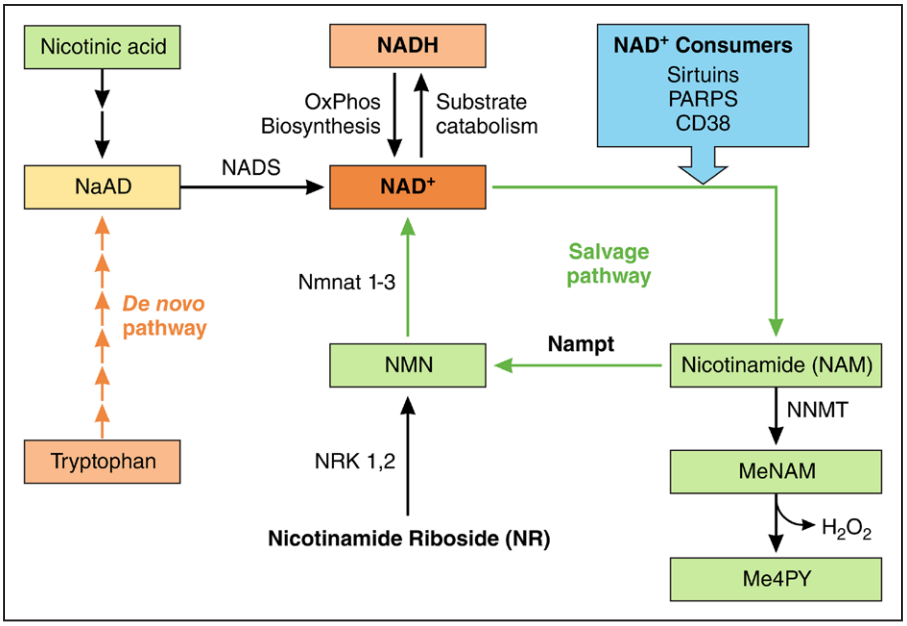

Figure. NAD+ biosynthesis, consumption and salvage pathways.
The intracellular NAD+ pool consists of both oxidized (NAD+) and reduced (NADH) forms. Electrons derived from substrate catabolism are carried by NADH and used for oxidative phosphorylation and biosynthetic reactions. NAD+ also functions as a cosubstrate for sirtuins, ADP-ribose transferases, and cyclic ADP-ribose synthases (CD38). Eukaryotes synthesize NAD+ from the amino acid tryptophan via the de novo pathway, or NAD+ can be salvaged from nicotinamide (NAM) and converted into nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (Nampt). NMN can also be generated by the phosphorylation of nicotinamide riboside (NR) by nicotinamide riboside kinase (Nrk). Me4PY indicates N1-methyl-4-pyridone-5-carboxamide; MeNAM, methyl-nicotinamide; NaAD, nicotinic acid adenine dinucleotide; NADS, NAD synthase; Nmnat, nicotinamide mononucleotide adenyltransferases; NNMT, nicotinamide N-methyltransferase; OxPhos, oxidative phosphorylation; and PARPS, poly(ADP-ribose) polymerases.

of increasing NAD+ levels was accompanied by a reduction in protein acetylation. However, changes in protein acetylation in the failing heart involve numerous acetylation sites on a large number of proteins. It is challenging to determine the functional significance of specific acetylation sites. Increased lysine acetylation on proteins involved in mitochondrial energy transduction pathways, including fatty acid oxidation, tricarboxylic acid cycle, and electron transport chain, has been identified in failing hearts.[7,9] One study suggested that increased protein acetylation increased the sensitivity of the mitochondrial permeability transition pore, which could be normalized by elevating NAD+ levels.[7] In contrast, the current study by Diguet et al[5] did not find evidence of altered protein acetylation in the failing hearts of SRF^{HKO} mice. In their hands, NR supplementation slightly increased, rather than decreased, global protein acetylation in the heart. The change was similar in control and SRF^{HKO} hearts, suggesting an effect independent of heart failure. Thus, these results argue against a role for protein deacetylation in mediating the benefits of NR supplementation.

Diguet et al[5] propose that the benefit observed in their study is likely caused by improved energy metabolism. This finding is logical because NAD+ levels and the associated reduction-oxidation state are powerful regulators of energy metabolism.[1,10] However, the study provided limited experimental evidence, which was largely indirect. The authors found that activities of citrate synthase and ATP-citrate lyase were increased by NR supplementation in SRF^{HKO} hearts. Moreover, acetylation of several nuclear proteins (eg, FoxO1 and p53) was elevated. These observations led to speculation that NR supplementation enhanced acetyl-coenzyme A (acetyl-CoA) generation, which resulted in increased protein acetylation in the nucleus and cytoplasm. This finding is intriguing and somewhat counterintuitive because protein hyperacetylation has been found in the failing hearts of animal models and patients. It is not clear why in this case promoting protein acetylation and cytosolic acetyl-CoA levels would benefit the failing heart. The authors also found that incubation of neonatal rat cardiomyocytes with NR did not increase oxygen consumption or ATP production but increased glycolysis. This is again confusing and difficult to reconcile with the notion of improved energy metabolism triggered by NR. Multiple possibilities can be considered for these observations. The effects of NR on energy metabolism need further investigation by assessing cardiac energetics and mitochondrial function. Additional studies comparing models and stages of heart failure, and ultimately trials in human patients, will likely shed light on common underlying mechanisms.

This study also contributes valuable and much needed information on NAD+ biosynthesis and metabolism in heart failure. Eukaryotes synthesize NAD+ from the amino

acid tryptophan via the de novo pathway, and NAD^+ can be salvaged from nicotinamide after it is consumed by NAD^+-dependent enzymes.[10,11] In mammalian cells, the salvage pathway is considered the primary mechanism to maintain a continuous supply of NAD^+. In this pathway, nicotinamide is converted into nicotinamide mononucleotide (NMN) by nicotinamide phosphoribosyltransferase (Nampt). NMN can also be generated by the phosphorylation of NR by nicotinamide riboside kinase 1 or 2. Adenylylation of NMN by nicotinamide mononucleotide adenylyl transferases forms NAD^+ (Figure). The basis for decreased NAD^+ levels in the failing heart likely involves multiple mechanisms. Impaired biosynthesis or salvage of NAD^+, overactivation of NAD^+-consuming enzymes, and altered intermediary and mitochondrial metabolism have all been proposed.[6,7] The study by Diguet et al[5] revealed that the Nampt was uniformly downregulated in the failing hearts of humans and mice, whereas nicotinamide riboside kinase 2 was upregulated. They suggest that decreased NAD^+ salvage via the Nampt reaction in the failing heart is an important mechanism for the decreased NAD^+ pool, for which the upregulation of nicotinamide riboside kinase 2 failed to compensate in the absence of exogenous NR. Therefore, they advocated for NR supplementation as the strategy of choice for restoring NAD^+ levels. Although the hypothesis is plausible, it is not mutually exclusive of the alternative strategies, such as stimulating Nampt activity or directly providing NMN, both of which have shown efficacy in protection against a variety of disorders associated with depletion of intracellular NAD^+, including heart failure, neurodegeneration, and aging.[7,12,13] Diguet et al[5] found substantial increases of Methyl-nicotinamide (MeNAM) and N1-Methyl-4-pyridone-5-carboxamide (Me4PY) after NR supplementation, although nicotinamide levels were unchanged. The finding seems to indicate that feeding the NAD pool with NR without salvage nicotinamide may lead to overflow of downstream metabolites of nicotinamide (Figure). It is important to note that the conversion of MeNAM to Me4PY is accompanied by reactive oxygen species generation. The biological significance of these reactions has not been fully investigated, but it is important for therapeutic application and should be addressed in future studies.

Despite the discrepancies in the mechanistic insight, results from the present study corroborate prior reports that targeting NAD^+ levels is a promising therapeutic strategy for heart failure. The translational potential is further enhanced by the fact that multiple compounds are available as NAD^+ precursors or Nampt activators. Although no information is currently available on the pharmacokinetics or tolerability of these compounds in patients, similar information from healthy volunteers has been reported recently. The coauthors of the study by Diguet et al[5] published dynamic changes of the NAD^+ metabolome in human subjects after a single dose of NR.[14] Another recent study has described the pharmacokinetics of NR and its effect on blood NAD^+ levels in healthy volunteers after 9 days of treatment.[15] These studies are extremely valuable for moving forward, but much more is needed. For example, it is unknown whether similar changes in NAD^+ and its metabolites occur in the heart as that detected in the blood. Moreover, pharmacokinetics and safety information in patients with heart failure are required before efficacy issues can be addressed. It is also worth mentioning that all the preclinical studies reported so far used a prevention approach (ie, the NAD^+ pool was expanded at the same time as heart failure induction). For clinical application, it would be more appropriate to treat the subject when cardiac dysfunction has already developed. This type of study will advance our understanding for both mechanisms and efficacy in a scenario relevant for therapy and, thus, is critical for translation.

ARTICLE INFORMATION

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