HHS Public Access

Author manuscript

Circ Res. Author manuscript; available in PMC 2022 May 28.

Published in final edited form as:

Circ Res. 2021 May 28; 128(11): 1642–1645. doi:10.1161/CIRCRESAHA.121.319308.

NAD Repletion Therapy: A Silver Bullet for HFpEF?

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Keywords

mitochondria; heart failure; nicotinamide riboside; protein acetylation

Heart failure is clinically classified according to LV ejection fraction as preserved (HFpEF) or reduced (HFrEF). These sub-types, while not entirely distinct, reflect different underlying pathogenic mechanisms, and treatments that are effective in HFrEF often fail to impart similar benefits in HFpEF. Because HFrEF is easier to recognize clinically and model experimentally, it has received the lion's share of attention, culminating in a number of effective approaches for its treatment. In contrast, there remains no evidence-based therapy that prolongs survival in HFpEF patients, at a time when its incidence is outpacing that of HFrEF.

Ongoing clinical research is defining distinct phenotypic clusters (so-called phenomes) of HFpEF that include hypertension, aging, diabetes, obesity, renal dysfunction, hypertrophy and diastolic dysfunction. Hampering the development of mechanism-based therapies has been the lack of animal models that faithfully recapitulate the multifactorial causes and phenotypic heterogeneity of clinical HFpEF. In this context, the Hill laboratory has addressed this challenge by developing a reproducible experimental model that mimics key features of HFpEF.[1] Specifically, they produced a murine model that incorporates metabolic and mechanical stressors, by combining high-fat diet (HFD) with pharmacological inhibition of nitric oxide synthase using L-NAME. This "two-hit" model recapitulates many features of human HFpEF, including LV hypertrophy, diastolic dysfunction, oxidative stress and fibrosis. Importantly, this model also manifests extra-cardiac pathogenic alterations that are common in clinical HFpEF, including vascular dysfunction (hypertension) and impaired metabolism (obesity and glucose intolerance).[1] As such, it provides a platform for discovering disease mechanisms and testing next-generation therapeutic approaches in proof-of-concept studies.

NAD and the quest for a silver bullet

In this issue of *Circ Res*,[2] the authors identify NAD depletion as a central feature of HFpEF, while also providing evidence of the therapeutic efficacy of NAD repletion. NAD is a critical cofactor that is synthesized *de-novo* or via a salvage pathway from its nicotinamide-

Disclosures: none

derived precursors. Oxidized (NAD+) and reduced (NADH) forms of NAD control cellular redox status, bioenergetics, substrate metabolism, mitochondrial biogenesis and dynamics. Because NAD content and the ratio of its oxidized-to-reduced forms regulate metabolic flux, ensuring homeostasis within a physiological range is critical in diverse cardiac, metabolic and neurologic disease models.[3]

The extent of NAD depletion, the efficacy of NAD repletion, and whether this approach reverses diastolic dysfunction in advanced HFpEF, have remained largely unknown. Tong *et al.*[2] report that NAD content was reduced in HFpEF and repleted with oral nicotinamide riboside (NR) therapy. Despite ongoing stress (HFD+L-NAME), they also showed that NR improved diastolic function, LV hypertrophy, exercise capacity and pulmonary congestion after 8-weeks of treatment. Their findings coincide with another recent study, which documented the efficacy of a second NAD precursor (nicotinamide), in ameliorating impaired myocyte relaxation and diastolic dysfunction in rodent models, including aging (2-year-old C57BL/6J mice), hypertension (*Dahl* salt-sensitive rats), and cardiometabolic syndrome (ZDF rats).[4] Together, these studies provide support for the strategy of reversing HFpEF-related cardiac dysfunction with NAD repletion therapy.

Sirtuin-dependent and independent effects of NAD+ repletion

Mechanisms underlying the therapeutic effects of NAD repletion are complex. Tong *et al.*[2] highlight the potential importance of NAD+-mediated Sirt3-dependent deacetylation of mitochondrial proteins. Sirt3 is localized in the mitochondria and they found decreased Sirt3 content and increased mitochondrial protein acetylation in HFpEF, including those involved in fatty acid oxidation, such as VLCAD. In mitochondria, Sirt3-dependent lysine deacetylation of target proteins regulates fundamental bioenergetic processes, substrate metabolism, mitochondrial dynamics, and the unfolded protein response. Sirt3 dysregulation has been implicated in cardiac hypertrophy, fibrosis and oxidative stress, all hallmark features of HFpEF. Interestingly, Tong *et al.*[2] found that NR-mediated NAD repletion successfully improved myocardial function in HFpEF and reversed VLCAD hyperacetylation without correcting overall bulk mitochondrial protein acetylation. It is worth noting that the premise of protein hyperacetylation as a causal event in the pathogenesis of HF has been questioned recently by findings in a mouse model, which develops extreme hyperacetylation of its mitochondrial proteome without exhibiting altered myocardial bioenergetics or a predisposition to HF.[5] In addition, the stoichiometry of protein acetylation warrants consideration. Although a relatively minor increase in the percent of residues with activating modifications can significantly augment enzymatic activity, the result of minor increases in inhibitory modifications is less predictable. Also, the impact of acetylation depends on its interaction with other post-translational protein modifications. Thus, additional investigation is needed to elucidate the role of acetylation/deacetylation in regulating fatty acid oxidation and other bioenergetic processes in this and other models of HFpEF.

Although Tong *et al.* focus on Sirt3, and mitochondria contain much of the cardiomyocyte NAD pool, cytosolic sirtuins have also been implicated in HFpEF pathophysiology and may in part mediate the therapeutic efficacy of NAD precursor therapy. Nicotinamide improved

cardiomyocyte passive stiffness and calcium-dependent relaxation through deacetylation of Titin and SERCA2a, likely via a Sirt1-dependent mechanism.[4] A clinical corollate of Sirt1-related benefits was reported in HFpEF patients who underwent exercise training for cardiac rehabilitation, and demonstrated concomitant increases in NAD+ and Sirt1 coincided with improved metabolic status and decreased oxidized LDL.[6]

Systemic effects of NAD+ repletion on cardiac function

NAD precursor therapy exerts a multiplicity of systemic actions, which likely play a major role in determining its ultimate efficacy as a HFpEF therapeutic, by impacting blood pressure, obesity, inflammation, and both skeletal muscle and liver metabolism. As the authors note, their “two-hit” model exhibited whole-body characteristics that are common in HFpEF patients, including hypertension, obesity, and altered metabolism. They also found that NR treatment improved glucose tolerance despite no apparent change in food intake, obesity, or hypertension. Recognizing the pleiotropic effects of *in vivo* NR administration, they treated a separate cohort of HFpEF mice with P7C3-A20, an agent that restores NAD^+ levels by activating NAMPT, a key enzyme in the salvage pathway. Unlike NR, P73C3-A20 did not impact glucose tolerance but still reversed diastolic dysfunction. Although NAMPT activators no doubt have other systemic actions, the latter results corroborated the NR findings in this model. Nonetheless, further elucidating the systemic *in vivo* actions of each NAD repletion strategy is critically important, particularly focusing on the concentrations of circulating substrates and hormones, vascular function, inflammation and metabolism in skeletal muscle and liver, which may also vary between various strategies.

Distinguishing between causal and coincidental metabolic remodeling

Tong *et al.*[2] demonstrate that HFpEF mice had PDK4 upregulation and impaired mitochondrial pyruvate oxidation capacity, both signatures of HFD and metabolic disease. However, NR treatment did not improve these abnormalities, suggesting that impaired carbohydrate oxidation is not a causal determinant of their HFpEF cardiac phenotype. In contrast, therapy was associated with a reduction in VLCAD hyperacetylation and improvement in palmitoyl-carnitine oxidation capacity in isolated mitochondria from HFpEF mice. These parameters showed a good association with functional recovery, but the extent to which they are causal and whether the mitochondrial abnormalities translate into altered metabolism in the intact heart will require further examination. Rates of metabolism are multi-determined by circulating substrates and hormones, tissue perfusion, and hemodynamic load. In addition, a comprehensive understanding of HFpEF will also require additional investigation into the metabolism of ketones, amino acids and additional fatty acid species, as well as a systematic analysis of cardiac bioenergetics.

NAD depletion appears to be a common finding in experimental and clinical studies of heart failure regardless of etiology. For example, Tong *et al.*[2] showed a similar reduction in NAMPT transcripts in myocardial samples from HFpEF and HFrEF patients. A treatment that potentially addresses a common pathogenic mechanism is appealing from a clinical standpoint. However, the parallel reduction in NAD content also indicates that NAD depletion is not a differentiating feature of HFpEF vs. HFrEF. Rather, it suggests

that other mechanisms underlie these distinct phenotypes. Conceivably, the degree of NAD depletion in specific subcellular microdomains and the localization and function of NAD-dependent proteins might also differentiate these distinct phenotypes. Moreover, mechanisms underlying NAD depletion that focus on upstream pathways will be important to sort out. For one, AMPK which controls substrate and energy metabolism, increases cellular NAD+ levels and enhances Sirt1 activity in skeletal muscle.[7] To what extent alterations in a cardiac AMPK-NAD-Sirt1 axis is involved in HFpEF remains to be elucidated. Moreover, mechanisms responsible for NAMPT down-regulation including miRNA regulated transcription warrant examination.

Conclusions

As with any good study, we are left with unanswered questions that need to be addressed as we consider the potential clinical utility of NAD repletion therapy. Will findings in mice translate to large animals and ultimately humans? We are just learning about the clinical pharmacokinetics and diverse physiological effects of NAD replacement treatment. In older men, NR has had limited impact thus far on insulin sensitivity,[8] a beneficial effect which was readily observed in rodents. Do the effects of NR treatment depend on HFpEF phenotype? The benefit of NR in these young male mice with HFpEF was striking, but female mice were less susceptible to HFpEF in this model[9] and were not included in this treatment study. Whether women and older patients with HFpEF will respond to NAD replacement will need to be addressed, although recent clinical research is encouraging in suggesting improvement in insulin-sensitivity in post-menopausal women treated with nicotinamide mononucleotide.[10]

NAD-targeted therapy is gaining momentum with ongoing clinical trials testing NAD precursors in a variety of conditions. This is driven by emerging experimental work as well as the premise that as commercially available nutritional supplements, they will be well-tolerated. Initial short-term studies with NAD precursors are encouraging in this regard, but the adverse experience with the use of vitamin therapy in lung cancer prevention is a reminder that long-term studies will be needed to confirm safety of NAD precursors in HFpEF.

While awaiting the results of the ongoing trial of nicotinamide in HFrEF (ClinicalTrials.gov) and the authors' trial of NR in HFpEF, additional investigation is needed to answer mechanistic questions about the systemic nature of this disease and its response to NAD-targeted therapeutics. How do the vascular, inflammatory and systemic metabolic components interact to promote HFpEF? How do the individual components of this HFpEF model (HFD and L-NAME alone) interact, are they simply additive, synergistic or unique? What are the metabolic, bioenergetic and molecular alterations in this and other HFpEF models?

Clinical HFpEF is a complex systemic disorder rooted in multiple pathogenic mechanisms that converge to produce clinically diverse phenotypes. It remains a very challenging problem, but with a multi-disciplinary approach and collaboration between scientists and clinical investigators, initial progress is finally being made in pursuit of effective therapies.

Acknowledgments

Sources of funding

Supported in part by NIH R01 HL148008, R01 HL149344, R01 HL137259

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