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EDITORIAL COMMENT

Tackling Inflammation at its Source in Heart Failure

Are Mitochondria the Key?*

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Historically, medical therapy for chronic heart failure (HF) management has been developed to alleviate aberrant signaling pathways linked to HF pathophysiology. This is most successfully epitomized by targeting the neurohumoral system. Two additional hallmarks of the pathophysiology of progressive HF include reduction in high-energy phosphate (bioenergetic deficiency and/or mitochondrial dysfunction) and cardiac/systemic inflammation. To date, the efficacy in targeting these pathways in the management of HF, although showing some promise, remains less well established.

Interestingly, mitochondrial dysfunction and inflammation may be integrated defects linked to HF. The mechanism whereby mitochondrial dysfunction promotes inflammation is driven in large part by their evolutionary formation whereby mitochondria maintain vestiges of their bacterial origins. Exemplifying this, eukaryote mitochondria retain prokaryotic features including hypomethylated mitochondrial DNA, which following leakage/extrusion out of mitochondria, function as damage associated molecular patterns (DAMPs). Here, either as free cytosolic or circulating, or within extracellular vesicles, mitochondrial DNA triggers numerous immune surveillance programs including the NLRP3 inflammasome.[1] Interestingly, the NLRP3 inflammasome can be induced by solid organ, including the heart, injury, which in turn initiates myeloid cell cytokine signaling to amplify tissue infiltration of inflammatory cells and systemic inflammation.

Pertaining to this, the restoration of cellular and mitochondrial nicotinamide adenine dinucleotide (NAD^+) levels has been shown to improve mitochondrial function, cellular bioenergetics, and to blunt innate and adaptive immune cell-driven inflammation. NAD's role in energy transduction is intuitive given that it is the major cellular hydrogen carrier, a coenzyme for multiple redox enzyme reactions, and a cofactor for sirtuin deacetylase enzyme functioning. The mechanisms underlying the boosting of NAD^+ in blunting inflammation may be, in part, via metabolic modulation (immunometabolism),[1] although these and other mechanisms remain less well characterized. Nevertheless, NAD boosting strategies have become of considerable interest in degenerative conditions linked with mitochondrial dysfunction and inflammation including aging, diabetes, Parkinson disease, and HF.

NAD^+ itself can be synthesized de-novo from dietary tryptophan or vitamin B_3 derivatives including nicotinic acid (niacin) and nicotinamide (nicotinamide adenine mononucleotide [NAM]). Furthermore, NAD^+ can be recycled within cells via the NAD salvage pathway. The salvage pathway is the major NAD^+ source in most tissues in the body. Interestingly additional vitamin B_3 analogues, namely nicotinamide riboside (NR) and nicotinamide mononucleotide (NMN), which function as intermediates in this salvage pathway also increase NAD^+ levels following oral supplementation. The oral administration of these NAD precursors has begun to be explored in degenerative diseases including in models of HF.

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In experimental models of heart failure with reduced ejection fraction (HFrEF) and with preserved ejection fraction and in genetic cardiomyopathy models, NAD precursor supplementation prevents/reduces pathophysiology. The mechanisms uncovered governing these ameliorative effects are wide-ranging and include, for example, augmented mitochondrial bioenergetics and function, induction of SIRT3, and antifibrotic or antioxidant effects.[2]

The data in humans with HF are less well studied, although interestingly in a prospective population study—the Bruneck study, which employs food questionnaires—has uncovered that higher nutritional intake of NAD precursors reduced composite cardiac death (HF, myocardial infarction, and sudden death) as well as systolic blood pressure. Pivotal questions arise for directly studying NAD-boosting strategies in human HF: Are all the NAD precursors created equal? Does NAD boosting effect the heart? Can they ameliorate HF and through which mechanisms? What dosing should be used and are they safe?

The decision as to which NAD precursors to study may be influenced by their different biological effects. For example, niacin binds and activates the nicotinic acid G-protein coupled receptor (GPR109A/B). Furthermore, NR and NMN are metabolized by the gut microbiome that is proposed to elevate circulating levels of nicotinic acid and NAM. Interestingly NR and NMN do not produce flushing, precluding significant signaling through GPR109 and, in further contrast to niacin, they do not increase high-density lipoprotein cholesterol levels. Data also suggest that NR may be more readily taken up by cells than NMN is, although pharmacokinetics of uptake and metabolism may be tissue specific and require further validation.[3] Additionally, evaluation of the NAD metabolic pathway implicates, and limited biochemical data support, that NR generates a significant amount of NAM, an intermediate that is less likely to accumulate with NMN. Although high doses of NAM blunt sirtuin enzyme activity, NR has been shown to activate sirtuin enzymes, and whether changes in NAM levels may distinguish effects of NR versus NMN supplementation is a hypothetical possibility that also required validation.

The Tian and O'Brien laboratories at the University of Washington have begun to tackle these same of questions where they selected NR as an NAD^+-boosting agent to study. Their initial pharmacokinetic study in healthy volunteers investigated the effect of up-titrating NR levels from 250 mg daily to 1,000 mg twice daily in 8 healthy volunteers over a 9-day period. In that acute study, they identified no adverse effects of NR and showed that whole blood NAD^+ levels rose significantly, albeit to varying degrees in all volunteers and that over this duration, there were stable steady-state increases in whole blood NAD^+. In a follow-up small intervention study in 4 hospitalized subjects with stage D HFrEF, using NR up to 1,000 mg twice daily for 3-6 days resulted in no obvious adverse effects. Here, too, NR increased whole blood NAD^+ levels and showed that NR improved peripheral blood mononuclear cell (PBMC) mitochondrial respiratory capacity and blunted transcript levels encoding cytokines linked with the NLRP3 inflammasome and encoding interleukin (IL)-6. They went on to show in PBMCs from a larger cohort of hospitalized subjects with HFrEF that their mitochondrial respiratory capacity was blunted and that transcript levels of these same cytokines were increased when compared to healthy control subjects. Furthermore, in elegant in vitro experiments they demonstrated that that could replicate the NR ameliorative effect to restore mitochondrial respiration and blunt inflammation in primary PBMCs exposed to mitochondrial DAMPs extracted from human HF tissue. Interestingly, they also showed integrated feed-forward mechanisms whereby mitochondrial reactive oxygen species evoked inflammation, and IL-6 in turn blunted mitochondrial respiratory function. These studies support the hypothesis that cardiac damage linked to HF propagate systemic inflammation via DAMP-initiated activation of the NLRP3 inflammasome. In this issue of *JACC: Basic to Translational Science*, Wang et al[4] have expanded on these findings in 30 ambulatory subjects with stable HFrEF. Subjects were randomized to 1,000 mg NR twice daily or matching placebo for 12 weeks. The new findings confirm the following: that NR was very well tolerated; there was a wide interindividual variability in the increase in whole blood NAD^+ levels; and that steady-state NAD^+ levels was achieved within 4 weeks and remained stable thereafter. Furthermore, and consistent with the effects of NAD boosting, the degree of improvement in PBMC mitochondrial respiration and the blunting of NLRP3 transcript levels were, respectively, positively or inversely correlated relative to increase in NAD^+ levels. The correlations with other cytokines were less robust, suggesting that the blunting of the NLRP3 inflammasome is an early myeloid cell signature of NAD boosting. It was also interesting to note that NR did not change the expression of IL-10, a pivotal anti-inflammatory cytokine. Finally, it should be noted that this study was not powered for efficacy, although Wang et al[4] did explore echo parameters, a 6-minute walk test, and the Minnesota Living With Heart Failure Questionnaire score. None of the

prespecified surrogate parameters were altered in this pilot short-term study. These investigators have an ongoing study to measure cardiac NAD^+ levels and mitochondrial integrity and function in myocardial tissue from subjects with HFrEF being pre-emptively supplemented with NR prior to tissue-biopsy at the time of left ventricular assist device implantation (NRII [Mechanistic Studies of Nicotinamide Riboside in Human Heart Failure]; NCT04528004).

Moving forward, many questions remain about NAD boosting in HF, as alluded to in this editorial. Although, these preliminary data support that this approach is safe, direct cardiac mechanisms need to be understood prior to initiating larger longer-term efficacy powered studies. Studies powered to explore changes in cardiac systolic/diastolic dysfunction using tissue Doppler and advanced-imaging in selected patients with HF who exhibit systemic inflammation with elevated C-reactive protein, among other things, would be of great interest. Additionally, evaluation of the hypothesis that cardiac-initiated mitochondrial DAMPs trigger systemic inflammation is warranted. This concept could be explored by measuring the levels of circulating free, or extra-vesicular-contained mitochondrial DNA, in various types and stages of HF and then determine whether NAD boosting alleviates these DAMPs. If this is proven, targeting mitochondrial fidelity and inflammation concurrently would uncover a novel approach in the management of chronic HF. Furthermore, restoring mitochondrial function and integrity to alleviate mitochondrial DAMP extrusion may restrain the initiation of cardiac inflammation at its source, thereby precluding the use of broad anti-inflammatory or immune-modulatory therapies with their potential unwanted systemic adverse consequences.[5] Although more needs to be done, this work conceptually creates a crack in the door into novel therapeutic approaches for the management of HF.

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