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Can Nitrites AMP_k up Sirt-ainty to Treat HFpEF?

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Heart failure with preserved ejection fraction (HFpEF) remains a common yet vexing clinical syndrome, with no approved therapies despite prevalence, morbidity, and mortality on par with those of heart failure with reduced ejection fraction (HFrEF).¹ Therapies and devices proven effective in HFrEF have repeatedly fallen short in their application to HFpEF. ¹ This is no longer surprising, as we increasingly recognize HFpEF as a syndrome characterized by multiple mechanisms and multi-organ engagement. Involved are not just the myocardium, but also the systemic and pulmonary vasculature, kidneys, skeletal muscle, and metabolic state.² Multiple co-morbidities, including hypertension, obesity, and diabetes mellitus, conspire to increase fluid retention and left ventricular (LV) filling pressures, often with ventricular hypertrophy and diastolic dysfunction. In the pulmonary vasculature, elevated LV filling pressure augments pulsatile loading on the right ventricle (RV)³; this, combined with passive elevations in pressure and pulmonary vasculature remodeling, can lead to pulmonary hypertension in about half the patients.¹ Meanwhile, microvascular and metabolic dysfunction in the peripheral skeletal muscle contribute to sarcopenia and exercise intolerance.¹ These deficiencies collectively lead to the breathlessness, marked exertional intolerance, and fluid retention that characterize HFpEF.

While success in battling this multi-faceted syndrome has been elusive to date, there are potential avenues that offer hope. One is nitric oxide (NO) and its downstream signaling mediators, cyclic guanosine monophosphate (cGMP) and protein kinase G, which regulate vascular tone, inflammation, metabolism, and cardiovascular protection.⁴ NO is synthesized in the vasculature by the homodimer endothelial nitric oxide synthase (eNOS), which in the presence of tetrahydrobiopterin (BH4) converts L-arginine into L-citrulline and NO. NO can modify protein function by direct post-translational modification (S-nitrosylation), or activate its sensor protein soluble guanylate cyclase (sGC) to produce cGMP. The latter in turn stimulates protein kinase G (PKG, also known as cyclic GMP-dependent kinase, cGK1) to trigger many of its protective effects.¹ Both NO and cGMP levels appear deficient in LV

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myocardium and pulmonary vessels of HFpEF patients,^{2,5} and so research has sought to potentiate these pathways. This includes supplementing NO synthesis with L-arginine or BH4, stimulating sGC-cGMP synthesis (e.g. vericiguat), or attenuating NO-stimulated cGMP hydrolysis by inhibiting phosphodiesterase-5 (PDE5).⁴ This last approach showed promise in animal models of cardiac hypertrophy and ischemia,⁶ though a clinical trial in HFpEF proved disappointing.⁷ However, PDE5 inhibition can work only if (a) enough cGMP is being produced, and (b) if not, PDE5 upregulation is to blame. Neither appear true for HFpEF.⁵ Rather, systemic vascular inflammation and excessive reactive oxygen species (ROS) are proposed to create NO deficiency and impair sGC responsiveness to NO,⁴ playing a major role in myocardial, renal, pulmonary, and skeletal muscle dysfunction.^{2,8} This is particularly problematic for a PDE5 inhibition strategy, as this enzyme predominately targets cGMP generated via an NO-signaling pathway⁹.

If HFpEF is indeed a state of diffuse NO/ROS imbalance and net NO deficiency, leveraging alternative cGMP activation or NO-generation strategies maybe more effective. One avenue currently being tested in patients is valsartan-sacubitril (LCZ696) which combines an angiotensin receptor blocker with a neprilysin inhibitor, the latter suppressing proteolysis of natriuretic peptides (NP) that may enhance cGMP signaling independent of NO.¹⁰ Another avenue to augment NP signaling is suppression of phosphodiesterase-9, which was recently shown to hydrolyze NP-coupled cGMP (unlike PDE5), and be upregulated in clinical HFpEF.⁹

Still another approach is enhancing nitrate (NO_3^-) or nitrite (NO_2^-) to stimulate the NO pathway. Administration of either can generate NO-signaling independent of eNOS, though their metabolism differs, and while prior efforts with nitrate have been disappointing, new work with nitrite is more favorable. Dietary nitrate comes from leafy green vegetables requiring reduction by oral commensal bacteria to become nitrite. Nitrite is also generated by intravascular NO oxidation and is more biologically active than nitrate and can be converted into NO by reduction.⁴ Nitrite activity is enhanced by hypoxic/acidic conditions, which has made it particularly intriguing for countering conditions where vascular flow is limiting. Both nitrates and nitrites are currently being evaluated in preclinical and clinical studies. Dietary nitrate partially reverses features of metabolic syndrome in eNOS-deficient mice⁴ and improves exercise capacity in a small human HFpEF cohort.¹¹ Borlaug *et al*¹² infused intravenous sodium nitrite in HFpEF patients and reported a fall in exercise-induced elevation of pulmonary artery wedge pressure and increased cardiac output in a randomized, double-blind, placebo-controlled study. Nitrite infusion also enhances circulating cGMP and arteriolar dilation in normoxic conditions.¹³ Both nitrites and nitrates also show hemodynamic benefits in PH.⁴ However, their effects on skeletal muscle which could be important in HFpEF are less well known. Furthermore, the mechanisms by which nitrate-nitrite-NO act, and whether they overlap with traditional NO-dependent pathways, remain inadequately understood.

In this issue of *Circulation*, Lai and colleagues¹⁴ elucidate new mechanisms of nitrite pharmacotherapy that may prove relevant for skeletal muscle and pulmonary defects in HFpEF. The authors first examined skeletal muscle in obese ZSF1 rats (double leptin receptor deficient), which have a number of features of HFpEF, including obesity, metabolic

syndrome, diabetic nephropathy, and diastolic ventricular dysfunction. They found nitrite improves hyperglycemia by stimulating the energy sensor, 5'-adenosine monophosphate kinase (AMPK) in skeletal muscle. Consistent with prior work,¹⁵ AMPK in turn upregulates membrane translocation of the glucose transporter (GLUT4) in an insulin-independent manner. Nitrite activation of AMPK had been previously reported in the setting of myocardial ischemia/reperfusion¹⁶ where it was shown to depend on mitochondrial ROS due to inhibition of dynamin-related protein 1 and thus mitochondrial fission. In the new study, AMPK activation also required mitochondrial ROS, but the authors found this required activation of sirtuin-3 (SIRT3), a mitochondrial deacetylase linked to stress response and metabolic signaling (Figure).¹⁴ In addition to obese ZSF1 rat skeletal muscle, nitrite dependent SIRT3-AMPK signaling was found in *in vitro* human skeletal muscle cell culture, SIRT3-knockout mice, and muscle biopsies from nitrite treated obese human subjects. Metformin, a compound that activates AMPK and is commonly used in diabetes mellitus, had similar effects on SIRT3-AMPK signaling.

The authors also examined PH in HFpEF, combining the obese ZSF1 rat with Sugen (SU5416, a vascular endothelial growth factor (VEGF)-receptor blocker)-induced PH. This model displayed PH and RV hypertrophy in obese ZSF1 rats, without further alterations to systemic mean pressure, LV filling pressure, or LV ejection fraction.¹⁷ Early nitrite or metformin administration prevented PH in this model, and in lungs, nitrite again stimulated AMPK in the medial layer of the pulmonary vasculature. Therapeutic benefit of nitrite or metformin worked in young but not older obese ZSF1-PH rats. The cause for this is unclear but important to resolve, as most HFpEF patients with or without PH are elderly.

This new study provides novel links between nitrite, AMPK, and SIRT3. Since SIRT3 is known to protect cardiomyocytes from aging, oxidative stress, and cardiac hypertrophy,¹⁸ the findings by Lai and colleagues of a nitrite-SIRT3 link in both skeletal muscle and pulmonary vasculature, two major systems that are also defective in HFpEF, makes these findings of particular interest. While intriguing, the mechanism linking ROS and SIRT3 activation remains unclear, as prior studies have shown that SIRT3 activation suppresses ROS¹⁸ and that cardiotoxins such as doxorubicin that enhance mitochondrial ROS in turn blunt SIRT3 expression.¹⁹ Here both would seem to go in the same direction. However, a requirement of ROS for nitrite efficacy does fit well with proposed HFpEF pathophysiology. Whether nitrite also engages any NO and/or cGMP signaling remains to be determined. Another interesting aspect of the study was the lack of a dose-dependent benefit; instead, improvements in hyperglycemia and AMPK phosphorylation in obese ZSF1 were blunted at the higher nitrite dose. This is reminiscent of the recently published NEAT-HFPEF study,²⁰ which found HFpEF treated with isosorbide mononitrate (Imdur) had no benefit in exercise tolerance and showed a trend towards a dose-dependent *decrease* in activity levels, especially at the highest dose of Imdur (120 mg/day). The causes for this inverse dose response, and whether this may contribute to a decline in efficacy in rats, needs to be better understood, as it suggests getting the right dosing in individual patients may be required for benefit.

While Lai *et al* present the ZSF1 ($\pm$ PH) as a HFpEF model, it is important to note where it falls short. Neither model displayed intravascular or whole body fluid overload, diastolic LV

pressures were not pathologically elevated, and exertional intolerance and higher mortality rates were not reported. HFpEF is not obesity, DM, diastolic dysfunction – nor necessarily their combination. These are syndrome features that many individuals also have but without HF symptoms. We have not yet generated an experimental model that has these features and also really has HF. Nonetheless, the models reflect key properties of HFpEF, and the finding of similar SIRT3 pathway activation in rat, mice, and human models supports significance.

Following a period of disappointing clinical trials, most of them targeting the renin-angiotensin-aldosterone system, interest in HFpEF has re-intensified with new work targeting alternative pathways that could augment intrinsic protective pathways. The new work presented here linking nitrite to SIRT3-AMPK sheds new light on mechanisms that may well be important to HFpEF with or without PH. Understanding these pathways deepens our appreciation that HFpEF is indeed a systemic syndrome, and pleiotropic therapies that can favorably impact multiple organ systems may prove more fruitful than previous efforts that focused primarily on the heart. Ongoing translational studies in humans will be needed to determine if nitrite indeed provides benefit to patients with a disease that sorely needs effective treatment.

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Figure. Nitrite and its proposed interaction with Sirtuin-3/AMPK

cGMP, cyclic guanosine monophosphate; PKG, protein kinase G; ROS, reactive oxygen species; p-AMPK, phosphorylated 5'-adenosine monophosphate (AMP) kinase; GLUT4, glucose membrane transporter.