Can Nitrite $\text{AMP}_k$ Up Sirt-ainty to Treat Heart Failure With Preserved Ejection Fraction?

Steven Hsu, MD; David A. Kass, MD

Heart failure with preserved ejection fraction (HFpEF) remains a common yet vexing clinical syndrome, with no approved therapies despite prevalence, morbidity, and mortality on a par with those of heart failure with reduced ejection fraction.1 Therapies and devices proven effective in heart failure with reduced ejection fraction have repeatedly fallen short in their application to HFpEF.1 This is no longer surprising, because we increasingly recognize HFpEF as a syndrome characterized by multiple mechanisms and multiorgan engagement. Involved are not just the myocardium, but also the systemic and pulmonary vasculature, kidneys, skeletal muscle, and metabolic state.2 Multiple comorbidities, 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 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.

Although success in battling this multifaceted 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, which, in the presence of tetrahydrobiopterin, converts l-arginine into l-citrulline and NO. NO can modify protein function by direct posttranslational modification (S-nitrosylation), or activate its sensor protein soluble guanylate cyclase to produce cGMP. The latter in turn stimulates protein kinase G (also known as cGMP-dependent kinase) to trigger many of its protective effects.6 Both NO and cGMP levels appear deficient in LV 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 tetrahydribopterin, stimulating soluble guanylate cyclase–cGMP synthesis (eg, 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 although a clinical trial in HFpEF proved disappointing.7 However, PDE5 inhibition can work only if (1) enough cGMP is being produced, and (2) if not, PDE5 upregulation is to blame. Neither appear true for HFpEF.4 Rather, systemic vascular inflammation and excessive reactive oxygen species (ROS) are proposed to create NO deficiency and impair soluble guanylate cyclase 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, because this enzyme predominately targets cGMP generated via an NO-signaling pathway.9

If HFpEF is indeed a state of diffuse NO/ROS imbalance and net NO deficiency, leveraging alternative cGMP activation or NO-generation strategies may be more effective. One avenue currently being tested in patients is valsartan-sacubitril (LCZ696) that combines an angiotensin receptor blocker with a neprilysin inhibitor, the latter suppressing proteolysis of natriuretic peptides that may enhance cGMP signaling independent of NO.10 Another avenue to augment natriuretic peptide signaling is suppression of phosphodiesterase-9, which was recently shown to hydrolyze natriuretic peptide–coupled cGMP (unlike PDE5) and to be upregulated in clinical HFpEF.11

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 endothelial nitric oxide synthase, although their metabolism differs; and, whereas previous 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 counteracting conditions where vascular flow is limiting. Both nitrites and nitrates are currently being evaluated in preclinical and clinical studies. Dietary nitrate partially reverses the features of metabolic syndrome in endothelial nitric oxide synthase–deficient mice and improves exercise capacity in a small human HFpEF cohort.12 Borlaug et al13 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. Both nitrites and nitrates also show hemodynamic benefits in pulmonary hypertension (PH). However, their effects on skeletal muscle that 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 colleagues 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 that nitrite improves hyperglycemia by stimulating the energy sensor, 5′-adenosine monophosphate kinase (AMPK) in skeletal muscle. Consistent with previous work, AMPK in turn upregulates membrane translocation of the glucose transporter in an insulin-independent manner. Nitrite activation of AMPK had been previously reported in the setting of myocardial ischemia/reperfusion where it was shown to depend on mitochondrial ROS because of the 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).

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 treating 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–receptor blocker)–induced PH. This model displayed PH and right ventricular hypertrophy in obese ZSF1 rats, without further alterations to systemic mean pressure, LV filling pressure, or LV ejection fraction. 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. The 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, because most HFpEF patients with or without PH are elderly.

This new study provides novel links between nitrite, AMPK, and SIRT3. Because SIRT3 is known to protect cardiomyocytes from aging, oxidative stress, and cardiac hypertrophy, the findings by Lai and colleagues of a nitrite-SIRT3 link in both skeletal muscle and pulmonary vasculature, major systems that are also defective in HFpEF, makes these findings of particular interest. Although intriguing, the mechanism linking ROS and SIRT3 activation remains unclear, because previous studies have shown that SIRT3 activation suppresses ROS and that cardiotoxins such as doxorubicin that enhance mitochondrial ROS in turn blunt SIRT3 expression. 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 NO signaling, with or without cGMP involvement, remains to be determined. Another interesting aspect of the study is the lack of a dose-dependent benefit; instead, improvements in hyperglycemia and AMPK phosphorylation, observed at lower nitrite doses in obese ZSF1, were blunted at the higher dose. This is reminiscent of the recently published Nitrate’s Effect on Activity Tolerance in Heart Failure With Preserved Ejection Fraction (NEAT-HFPEF) study, which found that HFpEF treated with isosorbide mononitrate (Imdur) had no benefit in exercise tolerance and showed a trend toward a dose-dependent decrease in activity levels, especially at the highest dose of Imdur (120 mg/d). The causes for this inverse dose response, and whether this may contribute to a decline in efficacy in rats, needs to be better understood, because it suggests nailing the right dosing in individual patients may be required for benefit.

**Figure.** Nitrite and its proposed interaction with Sirtuin-3/AMPK. cGMP indicates cyclic guanosine monophosphate; GLUT4, glucose membrane transporter; p-AMPK, phosphorylated 5′-adenosine monophosphate (AMP) kinase; PKG, protein kinase G; and ROS, reactive oxygen species.
Although Lai et al present the ZSF1 (± 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, diabetes mellitus, diastolic dysfunction – or necessarily their combination. These are syndrome features that many individuals also have but without heart failure symptoms. We have not yet generated an experimental model that has these features and also really has heart failure. 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 intensified with new work targeting alternative pathways that could augment intrinsic protective pathways. The new findings 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 pleotropic therapies that can favorably impact multiple organ systems may prove more fruitful than previous efforts that focused primarily on heart failure symptoms. We have not yet generated an experimental model that has these features and also really has heart failure. Nonetheless, the models reflect key properties of HFpEF, and the finding of similar SIRT3 pathway activation in rat, mice, and human models supports significance.

Disclosures

None.

References


KEY WORDS: Editorials | glucose | heart failure | nitrites | protein kinases | signal transduction | sirtuins | stroke volume

Downloaded from http://circ.ahajournals.org/ by guest on September 23, 2017
Can Nitrite $AMP_k$ Up Sirt-ainty to Treat Heart Failure With Preserved Ejection Fraction?
Steven Hsu and David A. Kass

_Circulation_. 2016;133:692-694; originally published online January 26, 2016;
doi: 10.1161/CIRCULATIONAHA.116.021409
_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/133/8/692

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published
in _Circulation_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located,
click Request Permissions in the middle column of the Web page under Services. Further information about
this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation_ is online at:
http://circ.ahajournals.org//subscriptions/