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The Nitrate-Nitrite-NO Pathway and Its Implications for Heart Failure and Preserved Ejection Fraction

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Abstract The pathogenesis of exercise intolerance in patients with heart failure and preserved ejection fraction (HFpEF) is likely multifactorial. In addition to cardiac abnormalities (diastolic dysfunction, abnormal contractile reserve, chronotropic incompetence), several peripheral abnormalities are likely to be involved. These include abnormal pulsatile hemodynamics, abnormal arterial vasodilatory responses to exercise, and abnormal peripheral O_2 delivery, extraction, and utilization. The nitrate-nitrite-NO pathway is emerging as a potential target to modify key physiologic abnormalities, including late systolic left ventricular (LV) load from arterial wave reflections (which has deleterious short- and long-term consequences for the LV), arterial vasodilatory reserve, muscle O_2 delivery, and skeletal muscle mitochondrial function. In a recently completed randomized trial, the administration of a single dose of exogenous inorganic nitrate has been shown to exert various salutary arterial hemodynamic effects, ultimately leading to enhanced aerobic capacity in patients with HFpEF. These effects have the potential for both immediate improvements in exercise tolerance and for long-term "disease-modifying" effects. In this review, we provide an overview of key mechanistic contributors to exercise intolerance in HFpEF, and of the potential therapeutic role of drugs that target the nitrate-nitrite-NO pathway.

Keywords Heart failure with preserved ejection fraction · Inorganic nitrate · Wave reflections · Afterload · Mitochondria · Therapy

Introduction

Heart failure (HF) affects ~2 % of the western population [1] and remains the most common cause of hospitalization in adults >65 years of age [1]. Approximately half of patients with HF have HF with preserved ejection fraction (HFpEF) [1–13]. Multiple therapies that provide substantial clinical benefit are available in HF with reduced ejection fraction (HFrEF) [14]. In contrast, effective pharmacologic interventions that improve outcomes in patients with HFpEF are not yet available, and are urgently needed [15]. Novel therapeutic approaches should be based on the pathophysiology of the disease, which unfortunately remains incompletely understood. Increasing evidence suggests a role for reduced nitric oxide bioavailability in this condition [16, 17].

Unfortunately, conventional methods of increasing NO through modulation of the soluble guanylyl cyclase system have been unsuccessful [18]. However, the nitrate-nitrite-NO pathway has recently emerged as another therapeutic target for increasing NO signaling, particularly in the presence of hypoxia and acidosis (i.e., in skeletal muscle during exercise). A normoxic mechanism of NO release in conduit arteries has also been reported, which may reduce pulsatile load to the heart. The nitrate-nitrite-NO pathway has the potential target to modify key physiologic abnormalities (late systolic LV load from arterial wave reflections, arterial vasodilator reserve, muscle O_2 delivery and utilization, and mitochondrial

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function), which may lead to both immediate improvements in exercise tolerance and long-term "disease-modifying" effects.

In this review, we provide an overview of peripheral and cardiac abnormalities in HFpEF and the potential role of the nitrate-nitrite-NO pathway as a therapeutic target in HFpEF.

Peripheral Mechanisms of Exercise Intolerance in HFpEF and Role of the Nitrate-Nitrite-NO Pathway

Exercise intolerance is the hallmark of HFpEF [19–22]. The pathogenesis of exercise intolerance in HFpEF remains incompletely understood. The early pathophysiologic paradigm was that increases in left ventricular (LV) filling pressure during exercise were not accompanied by increases in end-diastolic volume, failing to recruit the Frank-Starling mechanism and to augment stroke volume [23]. Diastolic dysfunction, with increased diastolic stiffness and impaired relaxation, was proposed to be the key contributor to the pathophysiology of this disease [24]. Studies also reported the presence of abnormal contractile reserve and chronotropic incompetence, leading to an abnormal cardiac output reserve [25–27]. However, abnormalities in stroke volume reserve [25–27] chronotropic incompetence [28] or cardiac output reserve [26] have not been consistently found in this population (reviewed in [29••]).

In addition to cardiac abnormalities, HFpEF appears to be associated with key "peripheral" abnormalities, which contribute to exercise intolerance. These include increased arterial stiffness and wave reflections [30], impaired vasodilatory reserve during exercise [31], abnormalities in skeletal muscle fiber type and capillary density [32••], and mitochondrial dysfunction [26].

Peak O_2 uptake (VO_2) during exercise, the most widely accepted index of aerobic capacity, is consistently reduced in HFpEF [23, 25–27, 33••]. Peripheral O_2 utilization requires not only an adequate cardiac output during exercise but also adequate flow redistribution, characterized by blood flow being preferentially directed to exercising skeletal muscle, in order to achieve an adequate matching of perfusion with metabolic demands. This preferential flow distribution is dependent on the exercise-induced reduction in the local resistance of skeletal muscle arterioles (i.e., exercise-induced vasodilation). Therefore, the normal exercise-induced vasodilation in exercising muscle serves two important roles: (1) to reduce the "total" (i.e., systemic) vascular resistance during exercise, which reduces LV afterload, promoting a greater cardiac output for any given contractile state; and (2) to preferentially divert flow to exercising muscle, promoting matching between metabolic needs and oxygenated blood delivery, thus allowing the "periphery" to optimally "utilize" the cardiac output to maximize oxygen consumption.

In several studies, compared to age-matched hypertensive subjects without HF [28, 33••], or age- and comorbidity-matched controls [27], patients with HFpEF demonstrated blunted exercise-induced reductions in systemic vascular resistance, indicating reduced vasodilatory reserve [33••]. This abnormal vasodilatory reserve leads to excessive LV afterload on one hand, and implies abnormal peripheral oxygen delivery on the other. The peripheral ability to redistribute flow to working muscle is a key component of the normal response to exercise [34••], and depends on the vasodilatory response in locomotive muscle, allowing it to effectively "compete" for the available cardiac output [34••]. Given the increase in metabolic demands imposed by active skeletal muscle contraction, vasodilation occurs as an adaptive response to maintain a balance between O_2 supply and demand [34••, 35]. Skeletal muscle vasodilation during exercise allows the local vasculature to overcome humoral and reflex-mediated vasoconstriction [34••]. Impaired vascular responses within skeletal muscle can have dramatic consequences for O_2 extraction, creating a marked imbalance between O_2 delivery and requirement in muscle that results in a larger O_2 deficit, accentuated intracellular metabolic perturbations, and enhanced glycolysis even at low levels of activity (reviewed in [34••]).

The vasodilator response in exercising muscle is dependent on both endothelium and endothelium-independent pathways [34••]. As will be discussed below, nitrite-derived NO is an endothelium-independent mediator of hypoxic vasodilation [35–37] and increased muscle blood flow during exercise [38, 39••].

While addressing exercise intolerance in HFpEF is a key therapeutic goal, there is also a need to address long-term underlying abnormalities that contribute to chronic LV remodeling and the long-term course of the disease. A large body of evidence now indicates that late systolic pulsatile load from wave reflections (which are increased in HFpEF [30]) have adverse long-term consequences on LV remodeling and function [40••, 41••, 42]. The pulse wave generated by the LV travels forward in arteries and is partially reflected at sites of impedance mismatch (i.e., bifurcations, points of change in arterial size or wall stiffness, predominantly in middle-sized conduit arteries) [42–44]. Wave reflections travel back to the heart, merging into a discrete reflected wave that arrives while the LV is still ejecting blood in mid-to-late systole [42, 45]. Wave reflections increase the late systolic workload of the LV and profoundly impact the LV loading sequence (late relative to early systolic load) [43, 44, 46–48]. Experimental animal data demonstrate that late systolic load from wave reflections leads to LV hypertrophy and fibrosis [40••]. These causal findings are supported by human data demonstrating an association between reflected wave amplitude an LV hypertrophy in the general population [49] and between reductions in reflection magnitude and the regression of LV mass during antihypertensive therapy (independent of blood pressure

reduction) [50]. Experimental studies also demonstrate that late systolic load promotes abnormal relaxation [41••]. In support of these causal findings, wave reflections have been shown to be independently associated with diastolic dysfunction in clinical [51, 52] and population-based cohorts [53••]. Similarly, recent data implicate wave reflection magnitude and late systolic load as a strong predictor of incident heart failure in the general population [48, 54••], strongly supporting animal and human mechanistic findings from previous studies and demonstrating the relevance of late systolic load in humans. Nitrate-nitrite-pathway-derived NO production has been shown to favorably impact late systolic load [55•, 56••]. Therefore, in addition to their exercise-enhancing mechanistic effects, interventions that enhance the nitrate-nitrite-NO pathway exert peripheral arterial effects with a potential for chronic "disease-modifying" benefits in HFpEF.

The Nitrate-Nitrite-NO Activation Pathway

NO formation occurs via two known pathways in mammals (Fig. 1): (1) NO synthases (NOS) catalyze the formation of NO from L-arginine and O_2 [57]; (2) circulating nitrate (previously considered an inert product of NO metabolism) can be converted to NO through the nitrate-nitrite-NO pathway, which is largely independent of NOS [39••, 57–61].

Under normal conditions, the nitrate-nitrite-NO pathway appears to be dependent primarily on dietary inorganic nitrate intake. Vegetables (leafy green vegetables and beetroot, in particular) [57, 61–64] are the main source of inorganic nitrate in the diet (>80 %). Nitrate is also directly absorbed in the gastrointestinal tract (without requiring conversion to nitrite), with high bioavailability (>90 %) [65] and circulates in plasma with a half-life of ~6–8 h, in contrast to nitrite, which has a short half-life (~30–40 min) [66–68]. Approximately, 25 % of circulating nitrate is taken up by the salivary glands, where it is reduced to nitrite by the oral flora; the remaining ~75 % of circulating nitrate is excreted by the kidneys [69, 70]. Oral cavity commensal bacteria reduce nitrate to nitrite, which has a high oral bioavailability (>90 %) [61, 63, 64]. Nitrite present in the blood stream is reduced directly to NO, a reaction catalyzed by several molecules, particularly deoxygenated myoglobin [36, 71–73], and deoxygenated hemoglobin [74], but also xanthine oxidoreductase [75], mitochondrial respiratory chain enzymes [76], aldehyde oxidase [77], carbonic anhydrase [78], vitamin C [79], polyphenols [80, 81], and even endothelial NO synthase [82, 83]. Importantly, the conversion of nitrite to NO occurs much more rapidly in the context of hypoxia [84•] and acidosis [85•].

In sharp contrast to NO production from the nitrate-nitrite-NO pathway, which is enhanced in the presence of hypoxia, NO production via the classic L-arginine pathway is strongly inhibited by hypoxia [83], given its reliance on molecular oxygen (Fig. 1). Exercising muscle is characterized by a low pO_2 [39••, 61], which favors the formation of NO from circulating nitrite. Deoxyhemoglobin supports the reduction of nitrite to NO [86••], which plays a key role in modulating small resistance vessels (particularly of skeletal muscle), where O_2 extraction from the circulation to the tissues is most marked. In these tissues, the O_2 saturation of hemoglobin approaches the P_{50} (the O_2 concentration at which half the hem is saturated), an optimum balance point between the greater reductive potential of hem in the R (oxy) state tetramer and the number of un-ligated deoxy-hem sites necessary for nitrite binding (which are more plentiful in the T-state tetramer). This favors conversion rates of nitrite to NO and hence vasodilatation [87,

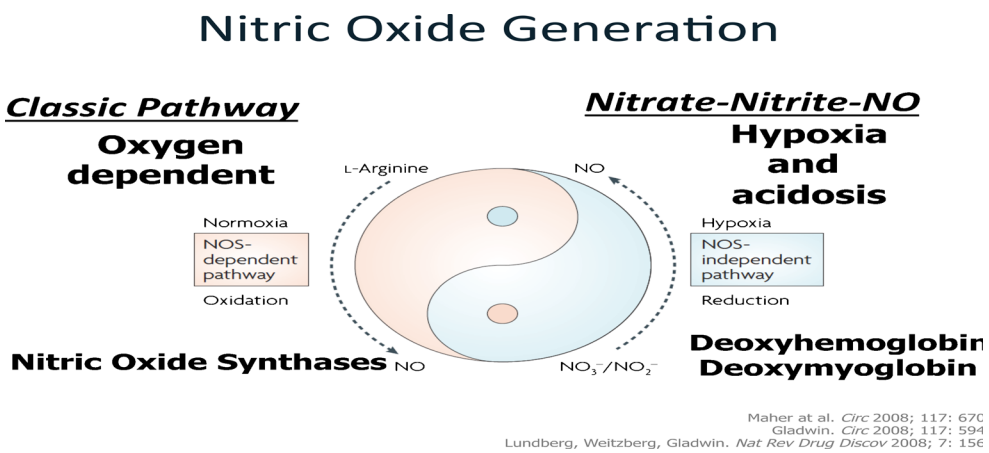

Fig. 1 NO generation via nitric oxide synthase (NOS) or through the nitrate-nitrite-NO pathway. NOS requires molecular oxygen for conversion of L-arginine to NO and citrulline. In the settings of hypoxia, NOS-mediated NO generation is very inefficient [57]. On the other hand, the activation of nitrate/nitrite to NO occurs to a greater extent in the setting of ischemia and acidosis [84•, 85•], and operates largely through NOS-independent mechanisms and is chiefly driven by deoxyhemoglobin [86••, 153]. This is ideal for HFpEF because is generates NO "when" and "where" it is needed. Reprinted by permission from Macmillan Publishers Ltd: *Nature Reviews Drug Discovery*. Lundberg, Weitzberg, Gladwin, 2008 [57]

88]. NO by deoxymyoglobin also enhances blood flow to skeletal muscle and matches O_2 supply to increased metabolic demands under hypoxic conditions [36]. Xanthine oxidoreductase also converts nitrite to NO when O_2 levels are low [75, 89]. In summary, the endogenous nitrate-nitrite pathway is a physiological effector of hypoxic vasodilation via NO release, which is independent of the L-arginine pathway and largely independent of NO synthase.

NO generation via the nitrate-nitrite-NO pathway is also preferentially activated in the presence of acidosis [85•]. This mechanism may enhance the selectivity for vasodilation in tissues in which local PO_2 is insufficient to support aerobic metabolism, in which lactate production increases, with a resultant fall in pH [39••]. The role of this pathway in regulating flow to predominantly aerobic vs. anaerobic fibers requires further research.

Inorganic Nitrate as Therapeutic Approach in HFpEF

The arterial hemodynamic characteristics of HFpEF patients (stiff arteries with wide pulse pressure [30], reduced exercise-induced vasodilation [27, 28, 33••, 90], and enhanced wave reflections [30]) dictate a set of "ideal" characteristics for a vasoactive intervention in this condition, namely, one that (1) does not significantly reduce mean arterial pressure at rest, avoiding hypotension; (2) enhances exercise-induced vasodilation to reduce LV afterload during exercise; (3) has selectivity for enhancing local vasodilation in hypoxic/acidotic environments, in order to match blood flow to metabolic demands (i.e., directing blood flow to exercising muscle); and (4) reduces wave reflections and late systolic load, which likely contributes to LV diastolic dysfunction and chronic maladaptive remodeling [40••, 41••, 42, 50–52, 53••]. Inorganic nitrate (or nitrite) precisely satisfies these characteristics.

Inorganic nitrate-nitrite-derived NO exerts weak microvascular vasodilatory effects in the absence of hypoxia but acts as a potent arterial vasodilator in the presence of local hypoxemia [39••]. Concentrations of nitrite (for which nitrate is a precursor) as high as 100–1000 μmol/L are typically required to induce relaxation of arterial rings in vitro [84•, 91]. Similarly, a lack of vasodilator activity was seen with a concentration of 200 μmol/L of nitrite in the forearms of healthy volunteers [92]. This situation is, however, different under hypoxic conditions. Maher et al. studied 40 volunteers and demonstrated that, during normoxia, forearm arterial blood flow during local infusions of nitrite at incremental doses (from 40 nmol/min to 7.84 μmol/min) increased by a maximum of 64 % during normoxia, but it increased by as much as 121 % during hypoxia [84•]. The nitrate-nitrite pathway thus seems ideal for enhancing vasodilation, O_2 extraction, and oxygen utilization during exercise. Indeed, inorganic nitrate improves muscle blood flow during exercise [38, 39••]. Interestingly, in addition to the predominantly hypoxic microvascular dilation (required to reduce local resistance and increase blood flow), normoxic activation of nitrite in conduit vessels has recently been demonstrated [55•]. This normoxic nitrite activation is likely responsible for the reduction in indices of wave reflections recently reported with inorganic nitrate in HFpEF and in normal volunteers [55•, 56••].

As described below, recent data support the promise of inorganic nitrate in HFpEF, via promotion of exercise-induced vasodilation (ultimately leading to enhanced aerobic capacity), in the absence of blood pressure reductions at rest, while at the same time reducing LV late systolic load [56••]. This represents an optimal "tailored" approach to abnormal arterial hemodynamics in HFpEF.

Organic vs. Inorganic Nitrate/Nitrite

While both organic and inorganic nitrate/nitrite ultimately both act by increasing nitric oxide bioavailability, important differences between the two classes of drugs lead to substantial differences in their action. Regarding their similarities, both classes of agent lead to venodilation [84•, 93] (Table 1). Both agents have the potential to decrease the impact of coronary stenoses on the myocardium [93, 94]. Yet despite these important similarities, many differences between these two classes exist, as will be described below.

Ingestion and Activation of Inorganic Nitrate/Nitrite vs. Organic Nitrates

Because of its reliance on the "enterosalivary" circuit, plasma levels of nitrate peak approximately 1–2 h after ingestion, whereas plasma levels of nitrite peak later at around 2–4 h after ingestion [95]. The activation of nitrate to nitrite is reliant upon bacteria within the oral cavity and subsequent swallowing of the formed nitrite; therefore, factors that disrupt these processes will lead to lower plasma nitrite concentrations. These include the use of antibiotics, antiseptic mouthwash, or expectoration [96–100]. Importantly, inorganic nitrate/nitrite is not subject to significant first-pass hepatic metabolism [66, 70].

Organic nitrates, on the other hand, do not require bacterial reduction for their activation and are not available from the diet. Commonly used organic nitrates, such as nitroglycerin and isosorbide dinitrate, are subject to extensive first-pass metabolism in the liver making their oral bioavailability more variable [70, 101], though this is the not the case for isosorbide mononitrate [102]. Once ingested, organic nitrates require activation in the cytochrome P450 system leading to nitric oxide release [70].

Table 1 Key differences between inorganic and organic nitrate

	Organic nitrates	Inorganic nitrate
Chemical structure	Esters of nitric acid ($RONO_2$), where R-represents an organic residue. They are thus small non-polar hydrocarbon chains attached to a nitrooxy-radical ($-ONO_2$)	Salts of nitric acid (nitrate anion bonded to metal cation, such as Na or K)
NO release	Tonic	Released preferentially during hypoxia and acidosis ("when" and "where" it is needed)
Tolerance	Yes	No
Headaches	Yes, often pronounced	No
Currently used by clinicians	Yes, variably	No
Oral bioavailability	Variable, due to hepatic first-pass metabolism	High oral bioavailability. Do not undergo first-pass metabolism
Metabolism and elimination	Cytochrome P450 system, ALDH-2	Via the nitrate-nitrite pathway: - Enterosalivary circulation (reductase activity of bacteria on tongue generates nitrite) - Nitrite is metabolized to NO via deoxyhemoglobin, deoxymyoglobin, xanthine oxidase and other enzymes - Ultimately excreted by the kidneys
Published or ongoing trials in HFpEF	Nitrate's effect on activity tolerance in HFPEF (NEAT HFpEF): phase IIb cross-over trial of isosorbide mononitrate in HFpEF [154] NCT01516346: phase IIa parallel-arm trial testing the effect of prolonged therapy (24 weeks) with isosorbide dinitrate ± hydralazine on late systolic LV load (primary endpoint), LV mass, fibrosis, and diastolic function	Published: phase IIa single-dose inorganic nitrate trial, demonstrating improvements in aerobic capacity, exercise vasodilatory, and cardiac output reserve, late systolic load. Possible improvement in mitochondrial oxidative function [56••]. Ongoing, single dose: phase IIa trials with single-dose inhaled and IV nitrite (NCT02262078, NCT01932606) Ongoing, sustained administration: - Phase IIa dose-finding pharmacokinetics/ pharmacodynamics study with KNO_3 (NCT02256345) - KNO_3CK OUT HFPEF: phase IIb cross-over RCT with KNO3 for 6 weeks

Alternative activation via mitochondrial aldehyde dehydrogenase has also been demonstrated [70, 103].

Tolerance

While the pathophysiology is not fully understood, a major limitation in the use of organic nitrates is the induction of tolerance over a short time period, leading to reduced efficacy of the agent [104]. Consequently, nitrates must be dose intermittently, allowing for periods where the subject is relatively untreated [93, 105]. Rebound ischemia has been demonstrated following acute nitrate withdrawal [93, 106], suggesting that even this strategy is not without its limitations.

The available information suggests that inorganic nitrate and inorganic nitrite do not induce tolerance. In a study of chronic (8–11 weeks) nitrate administration in hypertensive rats, blood pressure response remained preserved over the duration of therapy [107]. Similarly, inorganic nitrate supplementation with beetroot juice led to sustained reductions in blood pressure in hypertensive adults over a month of therapy [108]. In addition to blood pressure, studies in healthy volunteers have demonstrated important ergogenic benefits to inorganic nitrate [38, 109–116], which persist through at least 2 weeks of therapy [117]. Moreover, 2 weeks of inorganic nitrate led to improved hemodynamic responses to exercise in healthy volunteers [118].

Similar data are available for inorganic nitrite, again suggesting that tolerance does not develop following chronic administration. In a primate study, repeated daily bolus administration of intravenous nitrite, on a background of continuous nitrite infusion, continued to reduce blood pressure over 14 days [67]. In a study of two-kidney-one-clip hypertensive rats, 4 weeks of oral sodium nitrite persistently reduced blood pressure [119]. Finally, a study of oral nitrite supplementation in spontaneously hypertensive rats demonstrated a continuous blood pressure response over 1 year of therapy [120].

These data combine to demonstrate that while organic nitrate therapy is associated with tolerance if given

continuously, the same phenomena does not occur with inorganic nitrate/nitrite.

Endothelial Dysfunction

Through related pathways as induced with the development of tolerance [106], the administration of organic nitrates, whether intravenous, transdermal, or orally, has been shown to worsen endothelial function [106, 121–124]. As endothelial dysfunction is thought to be a contributing factor to the development of HFpEF [16, 17], avoiding further compromise in endothelial function and nitric oxide bioavailability may be an important goal. Conversely, inorganic nitrate/nitrite do not worsen endothelial function [125, 126], with some reports demonstrating an improvement in endothelial function following the administration of nitrate [100, 108, 118, 127, 128] or nitrite [94].

Adverse Effects of Organic Nitrates vs. Inorganic Nitrate/Nitrite

Headache is a common side effect following the administration of organic nitrate [101], and can limit compliance with the medication. In the African-American Heart Failure Trial, nearly 50 % of subjects randomized to the organic nitrate-containing arm complained of headache, with approximately one-third developing dizziness [129]. Moreover, hypotension can be seen, and may rarely result in syncope [101].

Inorganic nitrate is very well tolerated, with no limiting side effects consistently reported in the literature with its oral use [108]. When administered in the form of beetroot juice, red urine and stools can be observed [100, 108]. The hypotensive effects of nitrate are dose-dependent and fairly mild, averaging −4.4/−1.1 mmHg in a recent meta-analysis [65], though age may modify the blood pressure response [130].

In a recent single-dose cross-over trial in HFpEF, we did not observe any vasoactive symptoms (such as headache) or any change in blood pressure following nitrate ingestion. This suggests that resting hypotension is an unlikely adverse effect of inorganic nitrate in this population [130]. Importantly, the lack of a blood pressure response to nitrate has also been shown in both elderly [130, 131] and diabetic individuals [126], further suggesting that there may be important differences in nitrate effects in different patient populations.

Inorganic nitrite may have more pronounced hypotensive effects than inorganic nitrate. Even low-dose infusions of nitrite can decrease mean arterial pressure significantly [39••], with more prolonged infusions and higher doses leading to more pronounced hypotensive effects [66–68]. Moreover, as with organic nitrates, a significant proportion of subjects given oral inorganic nitrite developed headache and dizziness, with greater incidence noted with higher doses [66, 132].

Another consideration is the short half-life of nitrite, which may lead to nitrite level swings during intermittent administration [133]. For oral formulations without enteric coating, the production of NO from nitrite in the acidic environment of the stomach may lead to the production of large amounts of NO soon after administration, which may explain some of the adverse effects seen with this formulation [134]. The side effect profile of inhaled sodium nitrite may differ from the profile of the oral formulations, but it needs to be better defined in the HFpEF patient population.

Methemoglobinemia is an uncommon but well-recognized risk of nitrite therapy [66–68]. However, recent studies in PAD suggest that, at dose that can exert adequate vasoactive effects, the risk of clinically significant methemoglobinemia is very low [132, 135, 136]. Importantly, in the only study of chronic (10 weeks) sodium nitrite supplementation, methemoglobin levels did not increase over 3 % of total hemoglobin [132], thus remaining in ranges that are generally considered safe [136]. Prior studies of intravenous sodium nitrite, however, have led to more significant increases in methemoglobin levels [68]. Methemoglobinemia is unlikely to be an important side effect of organic nitrate therapy, although studies are underway in HFpEF, which will provide better information about the incidence of side effects at different doses in patients with HFpEF (see below).

Cancer and Inorganic Nitrate

There has been a concern regarding whether a high nitrate diet may predispose to gastric cancer in humans through conversion to nitrite in the stomach and the generation of *N*-nitroso compounds [137–139]. This is a controversial issue derived from animal studies [140], although the relationship between nitrate intake and cancer has not been demonstrated in humans [59, 117, 141]. In fact, many studies show either no relationship or even an inverse relationship between a high intake of nitrate and the occurrence of gastric cancer [137, 142–146]. The Joint FAO/WHO Expert Committee on Food reviewed all the available evidence, but failed to establish a definite link between nitrate intake and risk of developing cancer [147, 148]. Furthermore, The World Cancer Research Fund/American Institute of Cancer Research found no evidence linking ingestion of vegetables which are known to be high in nitrate with the development of cancer [149].

Effects of Inorganic Nitrate in HFpEF

We recently performed a randomized double-blinded cross-over trial [56••] of a single dose of 12.9 mmol of inorganic nitrate (nitrate-rich beet root juice, NR-BRJ) vs. a nitrate-

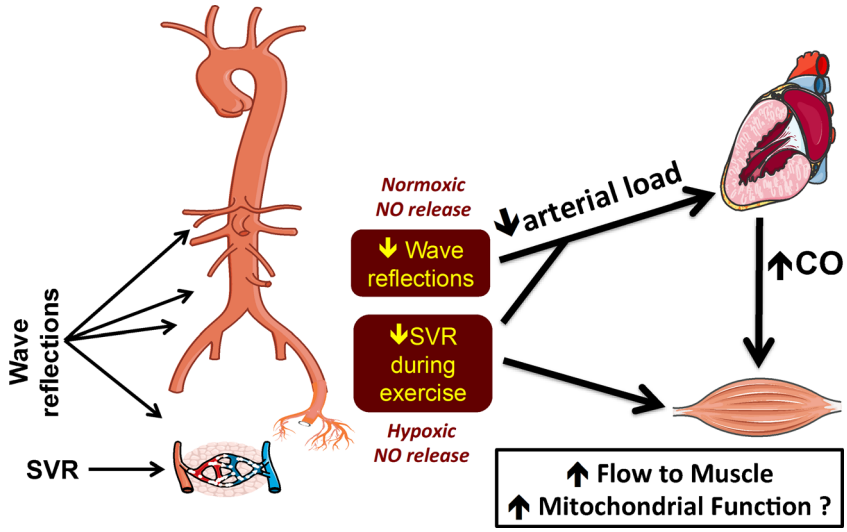

Fig. 2 Systemic peripheral mechanisms by which inorganic nitrate enhanced aerobic capacity in HFpEF

depleted placebo (PB) in stable subjects with symptomatic HFpEF [56••]. Serum NO metabolites were markedly greater after NO_3^- supplementation (326.0 vs. 10.0 μM; $P=0.0003$). Nitrate supplementation resulted in a greater peak VO_2 (12.6 ±3.7 vs. 11.6±3.1 mL O_2/kg/min; $P=0.005$) and total work performed (55.6±35.3 vs. 49.2±28.9 kJ; $P=0.04$). Ventilatory threshold (a marker of the cellular anaerobic threshold) was also greater following NO_3^- supplementation (7.6±1.8 vs. 7.0±1.4 mL O_2/kg/min; $P=0.03$). Because total work performed and O_2 consumption increased in tandem, exercise efficiency was not different between the interventions. Interestingly, the later effect is different than the effect seen in athletes and younger subjects, among whom exercise efficiency increases [38, 95, 110, 114, 115, 117], demonstrating the importance of characterizing specific adaptations to exercise in this particular patient population.

Inorganic nitrate led to a greater fall in systemic vascular resistance (SVR) at peak exercise (−42 vs. −32 %; $P=0.03$). This was accompanied by a greater increase in cardiac output (change 122 vs. 89 %; $P=0.006$), increased heart rate (78 vs. 65.6; $P=0.001$) and a trend toward greater stroke volume increase (22.6 vs. 12.7 %; $P=0.13$). The aortic augmentation index (derived from radial arterial tonometry) was significantly decreased by NO_3^- supplementation (NO_3^- 132.2 vs. 141 %; $P=0.03$). There was no significant change in resting blood pressure with NO_3^- supplementation. This is in contrast to blood pressure-lowering effect in healthy volunteers and drug-naive hypertensive subjects [89, 150] and may partially be explained by recent data indicating that the BP-lowering effect of inorganic nitrate is modified by age [130] or the renal effects in pathways downstream of the AT1-receptor [151]. We also assessed local skeletal muscle mitochondrial oxidative capacity following a standardized forearm exercise protocol and demonstrated a trend toward improved skeletal muscle mitochondrial function following NR-BRJ.

In summary (Fig. 2), inorganic nitrate increased peak VO_2 and total work during a maximal exercise test. It enhanced the exercise vasodilatory reserve, without affecting SVR or blood pressure at rest. It also reduced late systolic LV load, which is implicated in diastolic dysfunction and LV remodeling. Nitrate also led to increased cardiac output during exercise, likely from reduced afterload, although direct myocardial effects might be involved. Finally, in accordance with other studies, our recent pilot data suggest an improvement in mitochondrial oxidative function [113, 152]. However, nitrate did not affect the O_2 cost of exercise, as has been demonstrated in younger and/or healthier populations [38, 109, 112, 115, 117]. Although the cross-over design allowed for adequate power to assess physiologic endpoints, this trial was limited by its small sample size, and the inclusion of a predominantly male (88 %) and African-American (82 %) population.

Other Ongoing Trials With Inorganic Nitrate and Nitrite

Based on these initial findings, we are currently finalizing a phase IIa dose–response and pharmacokinetic study (NCT02256345) of repeated nitrate supplementation in HFpEF. We are also and initiating a phase IIb cross-over randomized clinical trial of sustained administration of potassium nitrate (KNO_3). The *Effect of KNO_3 Compared to KCl on Oxygen UpTake in HFpEF* (KNO_3CK OUT HFpEF) trial will enroll 76 subjects, and will assess the effects of a 6-week period of potassium nitrate administration on aerobic capacity (primary endpoint), quality of life, late systolic LV load, myocardial function, and

various physiologic adaptations to exercise (vasodilatory response to exercise, skeletal muscle phosphocreatine recovery kinetics, muscle tissue perfusion, and spatial matching between perfusion and oxidative capacity). Each subject will be exposed to both the active drug (potassium nitrate) and control (potassium chloride) in a randomized, cross-over, blinded design. Subjects will be enrolled with explicit assurance for adequate representation of women and African-Americans.

Other phase IIa trials assessing the hemodynamic effects of single-dose inhaled-nitrite supplementation (NCT02262078) and intravenous sodium nitrite administration in the catheterization laboratory (NCT01932606) are also underway.

Conclusion

Novel therapies for HFpEF are urgently needed. As our understanding of this condition increases, evidence suggests an increasing role for peripheral abnormalities in the pathogenesis of the disease, including abnormal pulsatile load, and reduced exercise-induced vasodilation. Furthermore, given the increased arterial stiffness and preload dependence in this population, excessive reductions in systemic vascular resistance at rest need to be avoided. However, selective vasodilators, such as inorganic nitrate, which can lead to preferential vasodilation at the time of exercise and in the appropriate vascular beds, have great potential in increasing O_2 availability in the periphery and ultimately, aerobic capacity. Moreover, evidence suggests that inorganic nitrate also improves late systolic load via normoxic NO production in conduit arteries, which may ultimately exert long-term disease-modifying effects via reductions in maladaptive LV remodeling and improvements in LV function. Modulation of the inorganic nitrate/nitrite pathway thus represents a novel avenue by which to improve exercise capacity in HFpEF.

Compliance with Ethical Standards

Conflict of Interest Julio A. Chirinos has received personal fees from Brystol Myers Squibb, OPKO Healthcare, Fukuda Denshi, Microsoft and Merck, grants from National Institutes of Health, American College of Radiology Network, Fukuda Denshi, Microsoft, Brystol Myers Squibb, and non-financial support from Atcor Medical outside the submitted work. In addition, Dr. Chirinos is named as inventor in a pending University of Pennsylvania patent application for the use of inorganic nitrates/nitrites for the treatment of HFpEF.

Payman Zamani declares that he has no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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