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Minireview

Dietary nitrate's effects on exercise performance in heart failure with reduced ejection fraction (HFrEF)☆

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ABSTRACT

Heart failure with reduced ejection fraction (HFrEF) is a deadly and disabling disease. A key derangement contributing to impaired exercise performance in HFrEF is decreased nitric oxide (NO) bioavailability. Scientists recently discovered the inorganic nitrate pathway for increasing NO. This has advantages over organic nitrates and NO synthase production of NO. Small studies using beetroot juice as a source of inorganic nitrate demonstrate its power to improve exercise performance in HFrEF. A larger-scale trial is now underway to determine if inorganic nitrate may be a new arrow for physicians' quiver of HFrEF treatments.

1. Introduction

Heart failure (HF), specifically heart failure with reduced ejection fraction (HFrEF), is a major public health problem. HFrEF is not only a deadly but also a disabling disease. This disability is thought to relate, at least in part, to impaired nitric oxide (NO) bioavailability. Recent discoveries regarding the inorganic nitrate pathway as a novel source of NO for the body have led to a burgeoning field of research leveraging this pathway for the treatment of diseases, including HFrEF. This pathway has particular advantages over other mechanisms for the production of NO. Moreover, most preliminary studies show that inorganic nitrate can increase both muscle power and aerobic exercise performance, which should improve patients' ability to perform their activities of daily living. Continued research is ongoing to determine if inorganic nitrate may be a novel, effective treatment for HFrEF.

2. Significance of heart failure with reduced ejection fraction (HFrEF)

Heart failure is a public health problem of epidemic proportion. It affects ~6 million people in the U.S. and ~23 million worldwide [1,2]. Unfortunately, these numbers are increasing. Adults over age 40 years now have a 1 in 5 chance of developing HF in their lifetime. Moreover, HF costs ~$39.1 billion annually in the U.S. alone. The human cost is great as well: 40% of patients die within 1 years of diagnosis and 5 y survival is only 35% [1]. In addition, those who live with HF often endure significant disability and decreased quality of life. Given the costly and disabling nature of HF and its tremendous impact on quality of life, any improvement in exercise performance would be of enormous benefit to patients with this disease.

HFrEF is a particularly disabling disease in part because it impairs patients' capacity to perform aerobic exercise and increases ventilatory effort, resulting in dyspnea. Indeed, the main classification systems used to evaluate HFrEF stratify the severity of disease based on the physical limitations it imposes. For example, as the New York Heart

Abbreviations: HF, heart failure; HFrEF, heart failure with reduced ejection fraction; cGMP, cyclic guanine monophosphate; sGC, soluble guanylyl cyclase; NO, nitric oxide; NO_3^-, nitrate; NO_2^-, nitrite; NOS, nitric oxide synthase; ROS, reactive oxygen species; ALDH-2, aldehyde dehydrogenase; PDE5, phosphodiesterase 5; PKG, cGMP-dependent protein kinase; SERCA, sarcoendoplasmic reticulum calcium transport ATPase

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Association class of HF increases (from I to IV), the limitations in aerobic exercise increase. Demonstrating impaired aerobic exercise tolerance in HF is an important component of declaring a patient "disabled" due to HF. Moreover, impaired aerobic capacity during exercise is one of the key tests used to evaluate HFrEF patients for cardiac transplantation and to predict mortality [3,4]. The lower the peak oxygen consumption ($\dot{V}O_{2peak}$) in HF patients, the worse the prognosis and the worse the disability.

Decreased skeletal muscle power (speed × force of contraction) also impairs quality of life and predicts increased mortality. Most typical daily activities require muscle power, as opposed to aerobic capacity. Getting out of bed, rising from a chair, opening a jar, lifting groceries, picking up a child, and climbing stairs are all activities that require power. Although it is well-known that HFrEF impairs aerobic capacity, it is less widely appreciated that HFrEF also decreases muscle strength and power [5,6]. HFrEF patients are less powerful even when compared to equally-sedentary but healthy individuals with comparable limb muscle mass, indicating that the muscular deficits in HFrEF are not simply due to physical inactivity or muscle atrophy [6]. Instead, recent studies have demonstrated that the muscle dysfunction found in HF patients is characterised by derangements at the molecular level [7–10]. In HF, decreased muscle power also predicts increased mortality [11]. In one study of > 100 HF patients, decreased skeletal muscle power was a more powerful predictor of mortality than $\dot{V}O_{2peak}$ (Fig. 1) [12]. Even in young healthy adults, lower muscle power predicts increased mortality. In a study of > 1 million young men, all-cause mortality was a striking 122.3 per 100,000 person-years in the weakest versus 5.6 per 100,000 person-years in the strongest men [13]. Clearly, muscle power is an extremely important target for treatment in HF, yet is one that is not presently addressed by any standard medications or therapies.

3. NO deficiency — a key derangement in HFrEF

Numerous factors account for the decline in exercise performance in HFrEF patients. These include, but are not limited to, increased skeletal muscle breakdown, increased oxidative stress, inflammation, and hypoperfusion. An excellent review of the many mechanisms that affect skeletal muscle function in heart failure is provided by Schulze and Toth in, Heart Failure, A Companion to Braunwald's Heart Disease [14]. It is beyond the scope of this mini-review to detail all of these factors; instead, we will focus on one key molecular factor contributing to these derangements — low NO bioavailability [15]. The evidence for decreased NO bioavailability is manifold. Breath NO levels are lower in

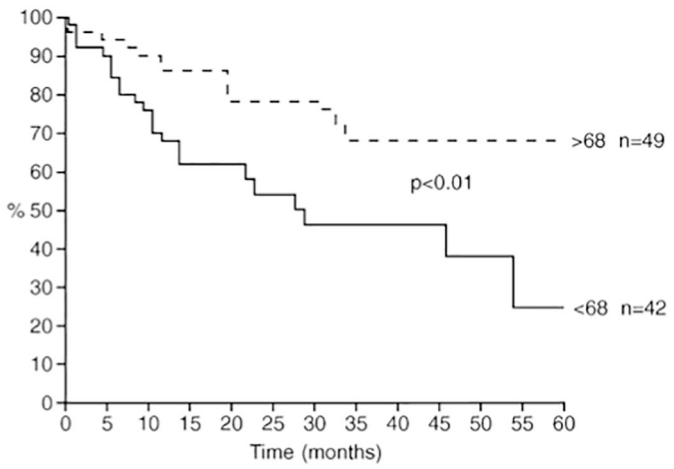

Fig. 1. Muscle power as a predictor of survival in patients with HFrEF [12]. Kaplan Meier lifetime analysis of survival stratified by peak torque index of the knee flexor muscles at a cut-off point of 68 N-m × 100 per kg body weight.

patients with HFrEF compared with healthy individuals [16]. Decreased plasma levels of nitrosothiols and cyclic guanosine monophosphate (cGMP) — a key mediator of NO effects — also indicate low NO bioavailability in HFrEF [17]. NO stimulates guanylyl cyclase (sGC) to increase cGMP production, which has opposite effects in smooth and skeletal muscle. In smooth muscle (e.g., in the arterial wall), NO causes vasodilation via stimulation of sGC and hence increased cGMP. In skeletal muscle, NO stimulation of sGC and upregulation of cGMP increases force of contraction [18] and the capacity for mitochondrial fatty acid oxidation [19]. To be sure, there are other pathways by which NO affects muscle. It is evident that NO deficiency could cause a decrease in cGMP and consequent impairments in vasodilation and aerobic exercise capacity, as well as decreased muscle power. Indeed, patients with HFrEF have impaired endothelial function, as was demonstrated by human cardiac catheterization studies in the 1990s [17]. Importantly, this impaired endothelial function in HFrEF is independently associated with an increased incidence of HF hospitalization, cardiac transplantation, and death [20].

The mechanisms by which NO bioavailability is reduced in HFrEF include both decreased production and enhanced degradation of NO [17]. In HFrEF, the activity of the endothelial isoform of NO synthase, eNOS, is decreased [17], while the levels of reactive oxygen species (ROS) that degrade NO are increased [21]. ROS levels are higher, at least in part, due to decreased antioxidant defenses [17]. Moreover, this increased oxidative stress in the left ventricle is correlated with the severity of HFrEF [22]. Studies in animal models further support the idea that decreased NO bioavailability is pathophysiologically linked to HF, rather than simply associative. These murine studies demonstrate a protective effect of enhanced production of NO (via eNOS overexpression) against HF development. Consistent with this premise that eNOS is beneficial, animal models that are deficient in eNOS are more susceptible to HF development, left ventricular hypertrophy, and hypertension [17]. Thus, increasing NO bioavailability in HFrEF patients is an attractive target for the amelioration of HFrEF symptoms.

4. Nitric oxide (NO) production: the main metabolic pathways

There are 3 main pathways for increasing NO production, as shown in Fig. 2. The pharmacologic, organic nitrate pathway has the longest history in Western medicine, with drugs such as nitroglycerin (glyceryl trinitrate) used for over 150 years to ameliorate angina and HFrEF symptoms due to its vasodilatory effects. However, it wasn't until almost 100 years later that scientists discovered that NO was the primary molecule responsible for the vasodilatory effects. Nitroglycerin generates NO through mitochondrial aldehyde dehydrogenase (ALDH-2) [23] by generating the intermediary products nitrate (NO_3^-) and 1,2-glyceryl dinitrate; the NO_3^- in the mitochondria is subsequently reduced to NO and/or converted to S-nitrosothiol. An important disadvantage of this pathway is that prolonged organic NO_3^- treatment often induces tolerance (i.e., impaired vasodilation response to nitroglycerin treatment) and cross-tolerance (i.e., impaired endothelium-dependent vasodilation), with oxidative stress playing an important role. Data suggest that inactivation of a key enzyme in the processing of nitroglycerin — mitochondrial ALDH-2 — by reactive oxygen species (ROS) is central to tolerance and cross-tolerance [24]. Chronic nitroglycerin treatment can also lead to supersensitivity to vasoconstrictive molecules through chronic activation of protein kinase C [23]. Different organic nitrates have varying propensities for inducing tolerance [25], and their use may be limited by side effects such as severe headaches, hypotension, and rebound vasoconstriction, a phenomenon that occurs following nitrate withdrawal and which may be attributable to coronary vasoconstriction.

A second pathway by which the body can synthesize NO is the endogenous nitric oxide synthase (NOS) pathway (Fig. 2) discovered by Furchgott, Ignarro, and Murad, who were awarded the 1998 Nobel Prize in Physiology or Medicine [26] "*for their discoveries concerning*

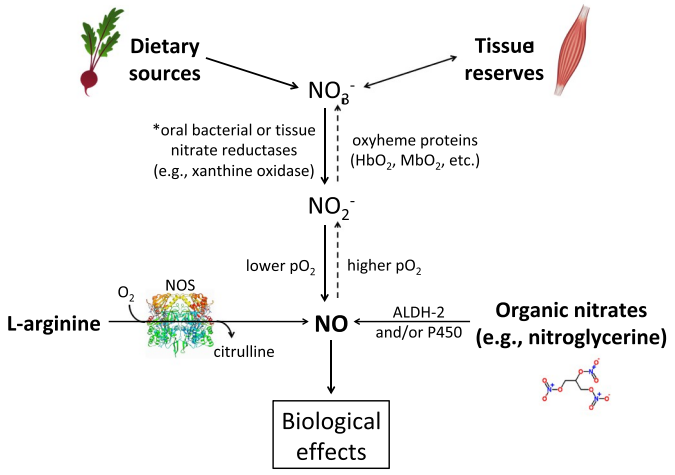

Fig. 2. The main pathways for nitric oxide (NO) production. The dietary pathway (starting at upper left) utilizes inorganic nitrates and is facilitated by lower pO_2 and pH. Ingestion of nitrate-containing foods, especially high-nitrate foods such as beetroot are the source of the inorganic nitrate (NO_3^-) start this pathway. NO_3^- is reduced to nitrite (NO_2^-) by reductases or acidic conditions and facilitated by oxyheme proteins. Then NO_2 is reduced to NO under the appropriate conditions. Importantly, skeletal muscle can serve as a 'reservoir' for nitrate [30]. As shown by the dashed arrows, this pathway can also 'run in reverse' with NO being used to create NO_2^- and then NO_3^- given the appropriate conditions.

*Note: NO_3^- can be taken up from the circulation into the salivary glands and go through this reduction pathway again in what is known as 'the enterosalivary pathway.'

The endogenous pathway (lower left) uses NO synthase and oxygen to create citrulline and NO. An abbreviated depiction of the organic nitrate pathway (lower right) shows the production of NO derived from pharmacologic sources, such as nitroglycerin.

ALDH-2 = aldehyde dehydrogenase, P450 = cytochrome P450.

nitric oxide as a signaling molecule in the cardiovascular system" (Nobelprize.org). In this endogenous pathway, NO is synthesized enzymatically from the conditionally essential amino acid L-arginine, oxygen, and NADPH by three NOS isoforms: neuronal NOS (nNOS or NOS1), inducible NOS (iNOS or NOS2), and endothelial NOS (eNOS or NOS3). nNOS and eNOS are calcium dependent. In addition to NO, L-citrulline is produced and can be recycled to generate de novo arginine. Co-factors necessary in this process include tetrahydrobiopterin (BH4), flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), and heme. There are limitations to this pathway, however. First, it requires oxygen. Thus, NO production by NOS enzymes decreases with ischemic duration, as shown by Giraldez et al. [27]. Second, NOS activity diminishes as pH decreases, with a marked lowering at pH < 7.0. Not only do abnormal/diseased tissues have a pH in this range, but also healthy skeletal muscle that is engaged in vigorous exercise experiences pH decreases into this range [28]. Interestingly, the third NO production pathway works best in ischemic and/or acidic conditions, and thus is a perfect complement to the NOS pathway.

The third NO metabolic pathway is the dietary or inorganic nitrate pathway (also referred to as the enterosalivary pathway). Ironically, though this pathway likely has existed for millennia, it wasn't discovered until ~1994. There is a suggestion, however, that the effects of this dietary nitrate pathway were known in ancient China, where it was noticed that KNO_3 was useful for "treating symptoms… such as acute heart pains" [29]. In this 'enterosalivary' pathway, NO is produced through sequential reduction of inorganic dietary nitrate [29] independently of NOS, beginning with inorganic NO_3^- from dietary sources (e.g., beets and dark green leafy vegetables). Reduction to nitrite (NO_2^-) is facilitated by bacterial nitrate oxidoreductases from commensal bacteria in the oral cavity. The very low pH of the stomach also favors the chemical reduction of NO_3^- to NO_2^-, as well as the reduction of NO_2^- to NO. The intestine then absorbs the anions, which are taken up by the circulation [29]. Once in the circulation, the skeletal muscle may take up nitrate and serve as an important 'reservoir' of nitrate (Fig. 2) [30]. Further acidic reduction of NO_2^- to NO occurs in tissues, catalyzed by deoxy-hemoglobin, deoxy-myoglobin, xanthine oxidoreductase, or other nitrate reductases. Heavily exercising skeletal muscle is also a prominent tissue for the final conversion to NO because the metabolic conditions are appropriate (i.e., low pH and low pO_2) [28]. Of note, this pathway can also move in reverse with decreasing acidity and abundant oxygen (e.g., nitrite can be made from NO). Recent data from Omar et al. show that this hypoxia-driven reduction of NO_2^- to NO can occur in small resistance arterioles, thus facilitating tissue perfusion [31]. Thus, this pathway is especially beneficial in hypoxia and ischemia. Although an estimated 75% of circulating nitrate is excreted by the kidneys or exhaled as NO, there is active re-uptake of approximately 25% of circulating nitrate by the salivary glands [32], which then secrete NO_3^-, thus re-starting the enterosalivary cycle of nitrate reduction in the oral cavity. The fact that the body actively recycles nitrate in this pathway underscores its biological importance. Indeed, estimates suggest that a significant fraction of the body's NO is derived from this exogenous pathway. It is also interesting to note that Omar et al. [31] showed that supraphysiologic/near-physiologic levels of inorganic NO_2^- can be reduced to NO in normoxic – but not hypoxic or hyperoxic – conditions in conduit arteries. In short, the inorganic NO_3^- pathway for NO generation is complex, likely has been operative in mankind for millennia, and functions in conditions not conducive to NO production by NOS. There are also several other specific advantages of this inorganic nitrate pathway as a source for NO generation over the other two pathways.

5. Advantages of the inorganic nitrate pathway as a source of NO for the cardiovascular system

The exogenous inorganic nitrate pathway for generation of NO has distinct advantages over the NOS pathway and over organic nitrate drugs (see Table 1). As mentioned above, the inorganic nitrate pathway functions best in ischemic and acidic conditions, particularly at the arteriolar level [31], thus facilitating NO-related effects such as vasodilation and skeletal muscle contraction, particularly in stressed or diseased tissues. The NOS pathway, by contrast, is O_2-dependent and therefore does not function well under these conditions. The inorganic pathway also has a slower onset of action than some organic nitrates, which may contribute to fewer reports of hypotension, flushing, and severe headaches [33–36]. There are also reports that inorganic nitrate treatment lessens mitochondrial oxidative stress [37], whereas organic nitrates, such as nitroglycerin, increase it [23]. As mentioned above, this increase in oxidative stress and other mechanisms cause tolerance with continuous use of organic nitrates such as nitroglycerin. Importantly, there are data from studies of continuous infusion of inorganic nitrite in nonhuman primates [38] and in humans [39] showing that there is no tolerance to inorganic nitrite. A recent study by Andrew Murray's group demonstrated another benefit of the inorganic pathway: dietary inorganic nitrate increases arginine availability for the NOS pathway by suppressing cardiac arginase expression and increasing tissue L-arginine in both normoxic and hypoxic conditions [37]. Recent data from Chirinos et al. in patients with HF with preserved ejection fraction also show that inorganic nitrate reduces the left ventricular load late in systole, which is caused by reflected waves from the arterial tree. Inorganic nitrate administration shifts the reflected wave later into diastole, thereby improving coronary perfusion pressure [40]. Table 1 also includes advantages of inorganic nitrate therapy over sildenafil and other phosphodiesterase 5 (PDE5) inhibitors; these drugs also increase cGMP, induce vasodilation, and have other cGMP-mediated effects, but do not increase NO. Thus, there are several advantages of the inorganic nitrate pathway over other pathways for NO production, and

Table 1
Comparison of the inorganic NO_3^- pathway with other sources of NO and/or cGMP.

Other sources of NO/cGMP	Advantage of inorganic NO_3^-
L-Arginine[a]	• Not dependent on NO synthase (NOS) • Functions well in acidic tissues • Functions well in ischemic tissues; does not require molecular oxygen
Organic, pharmacologic nitrates (e.g., nitroglycerin)[b]	• Does not cause tolerance • Does not increase reactive oxygen species (ROS) • May be less likely to cause hypotension[c] • May be less likely to cause flushing or headache[c] • Decreases left ventricular late systolic load • Does not cause cross-tolerance with nitroglycerin [39]
Phosphodiesterase 5 inhibitors (e.g., sildenafil)	• May be less likely to cause hypotension[c] • May be less likely to cause flushing or headache[c] • Less likely to cause retinal dysfunction and vision changes through inhibition of PDE6

[a] The substrate used for NOS-related production of NO.
[b] Not all pharmacologic nitrates are prone to these same disadvantages or to the same degree (see review by Munzel et al. [52]).
[c] These symptoms were not observed in our preliminary studies of HFrEF patients treated with 11.2 mmol of inorganic nitrate in the form of beetroot juice [34,35,41], and inorganic nitrate does not cause significant cerebrovascular dilation in HF with preserved ejection fraction [40].

eventually, cGMP amplification. These advantages make inorganic nitrate an attractive potential therapeutic agent for increasing NO, especially in patients who have reduced NO bioavailability – such as those with HFrEF.

6. Inorganic nitrate effects on exercise performance in patients with HFrEF

Preliminary data show that inorganic nitrate improves muscle power and NO bioavailability. In one small, double-blind, placebo-controlled study, 11.2 mmol of inorganic nitrate, given in the form of concentrated beetroot juice, increased quadriceps muscle power within 2 h after a single dose [34]. Peak muscle power was increased by ~11% at the highest movement velocity tested. The inorganic nitrate consumption was accompanied by a ~20 fold increase in plasma nitrate levels, as well as a 35–50% increase in breath NO levels [34]. Calculated maximal velocity of contraction and maximal power were also significantly increased after ingestion of dietary inorganic nitrate (13 and 12%, respectively) [34]. The improvement in muscle power seen after a single dose of inorganic nitrate is comparable to that which would be expected to result from ~2 to 3 months of resistance exercise training [34]. Based on a study by Toth et al. [6], in which patients with HFrEF were shown to have less powerful muscle function (even after correcting for muscle mass and other variables), the improvements observed after inorganic nitrate treatment would have erased ~1/3 of the HF-related deficit in muscle power [34]. This improvement in muscle power after inorganic nitrate therapy contrasts with the lack of an effect that standard HF therapies (i.e., beta-adrenergic antagonists, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and aldosterone antagonists) have on muscle power [34]. It is unclear whether inorganic nitrate can also increase myocardial contractile power, as it does in skeletal muscle. Nevertheless, an improvement in skeletal muscle function alone should help patients with HFrEF perform many of their activities of daily living because many activities rely on muscle power.

Results from studies on the effect of inorganic nitrate on aerobic exercise capacity in patients with HFrEF are slightly more mixed. Coggan et al. showed in a small, double-blind, placebo-controlled study that dietary inorganic nitrate markedly increased plasma nitrate, plasma nitrite, and breath NO levels (by 1469 ± 245%, 105 ± 34%, and 60 ± 18%, respectively) [41]. Simultaneous with this increase in NO bioavailability, $\dot{V}O_{2peak}$ increased by 8 ± 2% and time to fatigue increased by 7 ± 3%. Exercise efficiency, however, did not change [41]. Although this increase in $\dot{V}O_{2peak}$ is small, based on outcomes studies, this change in $\dot{V}O_{2peak}$ should translate into a ~10% decrease in the annual risk of death or transplant [41,42].

The improvement in $\dot{V}O_{2peak}$ seen in the study by Coggan et al. correlates well with results of a study by Kerly et al., in which a single dose of inorganic nitrate enhanced incremental shuttle walk test performance in patients with HFrEF by ~18% as compared with placebo [43]. Of note, these positive effects on aerobic performance also correlate with a study of inorganic nitrate's effects in HF patients with preserved ejection fraction [36]. A recent study by Hirai et al. in male patients, most of whom had ischemic cardiomyopathy, however, did not show an improvement in $\dot{V}O_{2peak}$ after repeated dosing of inorganic nitrate [44]. Differences in HFrEF etiology, other patient characteristics, or nitrate dosing regimens between studies may account for the differences in $\dot{V}O_{2peak}$ results. Although the source of inorganic nitrates was beetroot juice in both the Coggan [41] and Hirai [44] studies, the inorganic nitrate dose that was ingested 2 h before exercise testing was 11.2 mmol in the Coggan study and 6.45 mmol in the Hirai study. The difference in $\dot{V}O_{2peak}$ results does not appear to be due to differences in HFrEF severity or ejection fraction.

7. Mechanisms of action of NO relating to improved exercise performance in HFrEF

There are several NO-mediated effects on muscle contractile function [18]. Because the net effect of inorganic nitrate ingestion in patients with HFrEF is an improvement in muscle contractile function, the NO-related pathways that increase muscle contractile function must 'win' over the NO-related pathways that would decrease muscle function. As mentioned above, NO increases sGC activity, thereby increasing cGMP. This leads to an increase in the speed of muscle shortening, and hence an increase in power [18]. Increased NO availability can also result in nitrosylation of the ryanodine receptor on the sarcoplasmic reticulum, which opens this channel [45]. This may result in an increase in calcium release by the sarcoplasmic reticulum and subsequent enhancement of both maximal muscle contraction velocity and power. Other NO-related effects (e.g., nitrosylation of troponin I [46] or myosin [47]) that would be expected to have a negative effect on muscle contractile function appear to be weaker than these positive effects. The sum of these pleiotropic NO-related effects in vivo in patients with HFrEF appears to result in increased muscle power, which should translate into improved functional capacity.

Several NO-mediated mechanisms also likely contribute to the general improvement in aerobic capacity after inorganic nitrate administration. Enhanced efficiency of mitochondrial oxidative phosphorylation is one mechanism implicated in the reduced oxygen costs of exercise [48–50]. The increase in $\dot{V}O_{2peak}$ described by Coggan et al. in

patients with HFrEF likely resulted from an increase in cardiac output and/or aterio-venous O_2 difference at peak exercise [41]. In that study, peak diastolic blood pressure trended lower and heart rate trended higher after inorganic nitrate ingestion, suggesting a higher cardiac output accompanying lower peripheral vascular resistance [41]. Since NO is known to be the original "endothelium relaxing factor", as coined by Furchgott, it is reasonable to conclude that the arteriolar vasodilating effects of NO may contribute to an improvement in cardiac output and, hence, $\dot{V}O_{2peak}$. Studies of infusions of inorganic nitrite in patients with HFrEF corroborate this, as they show that increased forearm blood flow increases in the infused arm [39]. There are also data from animal models of NOS manipulation and in vitro studies that indicate that NO from NOS may also have direct, positive cardiac lusitropic, inotropic, and/or chronotropic effects distinct from responses to vasodilation. These effects were detailed in a review by Massion et al. [51]. Data from patients with heart failure with preserved ejection fraction show that inorganic nitrate, in contrast to organic nitrates, can also lessen late systolic pulsatile left ventricular load. Whether one or all of these direct cardiac and pulsatile load effects are active in patients with HFrEF remains unclear.

8. Summary – conclusions

NO is a powerful radical with pleiotropic effects. It is of particular therapeutic interest in the treatment of HFrEF because NO bioavailability is low in HFrEF patients and is thought to be a key mediator of HF pathophysiology. There are three main pathways that can be leveraged to increase NO bioavailability. The last to be discovered, the inorganic nitrate pathway, has several advantages over the endogenous and organic pharmacologic pathways, as well as over PDE5 inhibitors. Inorganic nitrates have been shown in most small clinical studies to improve exercise performance – both muscle power and aerobic exercise performance. A larger study, the INIX-HF trial (NCT02797184), is now underway to perform the studies necessary and sufficient to set up a multicenter study of the effectiveness of inorganic nitrate for the improvement of exercise performance and quality of life in patients with HFrEF.

Conflicts of interest

The authors have no conflicts of interest to declare that are related to this topic.

Transparency document

The Transparency document associated with this article can be found, in online version.

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