Inorganic Nitrite to Amplify the Benefits and toLerability of Exercise TRAINING in heart failure with preserved ejection fraction (INABLE-TRAINING)

NCT02713126

February 10, 2021

Inorganic **N**itrite to **A**mplify the **B**enefits and to**L**erability of **E**xercise **TRAINING** in heart failure with preserved ejection fraction *(INABLE-TRAINING)*

Regulatory Sponsor:	Barry A. Borlaug, MD Mayo Clinic Internal Medicine Department Cardiovascular Diseases Division 200 First Street SW Rochester, MN 55905
Funding Sponsor:	National Institutes of Health Aires Pharmaceuticals
Study Product:	Sodium Nitrite
Protocol Number: (IRBe)	16-001467
IND Number:	123333

Versions:

1 18Feb2016; Initial

2 15Sept2016: Addition of the option to down-titrate the dose of study drug for potential intolerance (from 80 to 46 mg). Correction of the number of subjects from 80 to 68 to reflect the target accrual number, not the target enrollment number

3 11 Jan 2017: Revise inclusion criteria subject population minimum age criteria from 50 years of age to 40 years of age. Addition of whole body DEXA scan and Cardiopulmonary Exercise Test (CPET) in the protocol

4 22 Mar 2017: Addition of Mayo Clinic Health System in Cannon Falls as a study site. Addition of added labs at screening, week 4, week 8 and final visit blood draws

5 21 Jun 2017: MRI/MRE substudy. Addition of SF- 36. AE description.

6 20 Sept 2017: Addition of Mayo Clinic Health Systems in Eau Claire, Mankato, Red Wing, Austin, Albert Lea and La Crosse.

7 Dec 2017: Addition of whole blood collection and BAP storage. Changed inclusion/exclusion. Added thank you note for patients.

8 10 Jun 2018: Modified protocol to change from inhaled nitrite formulation to oral formulation because of expiration/shutter of inhaled compound (decision made by manufacturer).

9 20 Sep 2018: Modified protocol to change drug dispensing and return and allow re-enrollment of phase 1 INABLE subjects.

10 16 Jan 2019: Modified protocol to update compounding site

11 14 Feb 2019: Modified protocol to change analysis plan, randomization ratio, and treatment of study drug from 10 weeks to 12 weeks because of delays in provision of study drug.

12 26 Jul 2019 Modified protocol to update compounding site and clerical errors.

13 04 Sept 2019 Modified optional MRI substudy to include gadolinium use.

14 13 Nov 2019 Modified optional MRI substudy to include hematocrit draw if not already obtained within the past 30 days and clarify timing of MRI.

15 23 Jul 2020 Due to COVID 19 will offer home-based cardiac rehabilitation program.

16 10Feb2021 Add optional muscle biopsy and add external collaborators.

Table of Contents

…….

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ITT	Intent to Treat
IND	Investigational New Drug Application
IRB	Institutional Review Board
IP	Investigational Product
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure

Study Summary

Title	Inorganic Nitrite To Amplify the Benefits and Tolerability of Exercise Training In Heart Failure With Preserved Ejection Fraction
Protocol Number	16-001467
Phase	Phase II
Methodology	This is a randomized, blinded, placebo-controlled, two-stage, parallel-group clinical trial with all subjects receiving exercise training (ET) and randomized to either inorganic sodium nitrite or matching placebo. The trial includes 2 stages. In stage I, which has been completed, patients received inhaled nitrite/placebo. In stage II, patients will receive oral nitrite/placebo.
Overall Study Duration	4 years
Subject Participation Duration	14 weeks
Single or Multi-Site	Single site
Objectives	To determine if sodium nitrite can improve the clinical responses to ET in subjects with heart failure and preserved ejection fraction (HFpEF).
Number of Subjects	100 (36 received inhaled nitrite/placebo in the completed stage I, up to another 64 will receive oral nitrite/placebo in stage II of the study)
Diagnosis and Main Inclusion Criteria	Heart failure with preserved ejection fraction (HFpEF) defined by clinical heart failure, normal EF (≥50%) and at least one of the following: • Previous hospitalization for HF with radiographic evidence (pulmonary venous hypertension, vascular congestion, interstitial edema, pleural effusion) of pulmonary congestion **or** • Catheterization documented elevated filling pressures at rest or with exercise **or** • Elevated NT-proBNP (>400 pg/ml) **or** • Echo evidence of diastolic dysfunction/elevated filling pressures manifest by medial E/e' ratio≥15 and/or left atrial enlargement **and** chronic treatment with a loop diuretic for signs or symptoms of heart failure
Study Product, Dose, Route, Regimen	Stage I tested Inhaled, nebulized sodium nitrite (AIR001) 80 mg at least 4 hours apart three times daily during waking hours (i.e. 08:00, 12:00, 16:00). Stage II is testing oral nitrite 40 mg tid capsules vs matching placebo capsules (same dosing frequency).

Duration of Administration	12 weeks
Reference therapy	Placebo
Statistical Methodology	Data will be analyzed according to ITT principles (assigned group regardless of study drug administration or compliance with ET benchmarks achieved). Primary hypotheses will be tested using a two-sample t-test on the change (baseline to 12 week. Testing will be stratified by route of administration.

1 Introduction

Heart failure with preserved ejection fraction (HFpEF) afflicts millions of older Americans and is associated with exercise intolerance, reduced quality of life (QOL), high health care costs, and increased mortality. People with HFpEF display increased cardiac filling pressures and inadequate cardiac output reserve to deliver blood to the body during exercise. Numerous lines of evidence have implicated abnormalities in nitric oxide-cyclic guanosine monophosphate (NO- cGMP) signaling as playing a key role in promoting these abnormalities. While there is currently no proven effective medical treatment for HFpEF, exercise training (ET) has been shown to improve aerobic capacity and QOL in this population.

However, ET provides benefits through peripheral effects in the muscles and vasculature, without targeting the cardiac limitations. The specific objectives of this application are to determine whether treatment with sodium nitrite (which improves the cardiac abnormalities) in addition to ET can improve exercise capacity, chronic activity levels, and QOL in people with HFpEF as compared to ET alone. The guiding hypothesis is that enhancing NO-cGMP signaling in the heart and periphery preferentially during exercise will improve functional capacity and symptoms in HFpEF above and beyond what is seen with ET alone. Conversion of nitrite (NO_{2^-}) to biologically active NO is enhanced during tissue hypoxia and acidosis, which develop during low-level exercise in HFpEF, so it is expected that this intervention will preferentially target the cardiac limitations that develop during exercise in these patients at the time of greatest need.

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.0 Summary of Protocol Changes and Rationale

Significant delays in obtaining study drug supply have forced us to make another modification to this study. In the last modification to the protocol two main changes were made: (1) we changed the route of administration of sodium nitrite from inhaled to oral, and (2) we have decreased the duration of intervention for endpoint assessment from 12 to 10 weeks. In this modification, we are changing the duration of treatment back to 12 weeks to align with what was done in the first Stage of the study, and we are changing the randomization ratio to 2:1 for nitrite relative to placebo, for the reasons enumerated below.

In the first Stage of the study, nitrite/placebo was administered in an inhaled formulation. However during the enrollment period, development of this product was discontinued by the manufacturer. In order to test the primary study hypotheses, we have switched from an inhaled formulation of nitrite to an oral formulation that achieves similar pharmacokinetics for Stage 2 of the study.

Since that time, the research pharmacy that was compounding our study drug (Triangle Pharmacy) has ceased production, forcing us to identify and work with a new pharmacy (RXQ) which has

introduced a very large delay because of the longer start up process, and the need for additional batch testing. After one shipment was received, RXQ Pharmacy failed to deliver further shipments of study drug and has not met production requirements, forcing us to again change the compounding pharmacy to The University of Iowa Pharmaceuticals (Iowa City, Iowa; uip.pharmacy.uiowa.edu). Potency and stability testing will be performed at University of Pittsburgh laboratories prior to release of study drug batches

We had planned to enroll another 68 patients, 34 receiving oral nitrite and 34 receiving oral placebo. However, we cannot meet this goal in two years. Our revised plan to meet our recruitment goal will be to use the data from the 17 patients receiving inhaled placebo in the first phase of the study. We will then combine these data with that from patients receiving oral placebo drug moving forward. Because we do not expect the response to inhaled and oral placebo to differ, we feel that this is scientifically justified.

Therefore, we will only require complete data from another 17 patients receiving oral placebo (for a combined total of 34 placebo patients) and another 34 patients receiving oral nitrite, for a total of 51 patients in the oral phase with complete data. Because more data is needed for patients randomized to nitrite, we will randomize patients in a 2:1 ratio moving forward of nitrite to placebo.

We have enrolled 14 patients according to the last protocol modification (on oral drug, which has been very well-tolerated). Therefore, at this time we will require complete data on another 47 patients. To allow for participant drop out and incomplete data, we will enroll up to another 60 patients to meet our goal of reaching another 47 with complete data.

1.1 Background

Heart failure (HF) is the leading cause of hospitalization among older adults in the United States. Half of people with HF have preserved ejection fraction (HFpEF), for which there is currently no proven effective treatment. People with HFpEF often have normal heart filling pressures at rest, but with exercise develop marked increases in heart filling pressures with blunted increases in cardiac output (CO). These are termed “central” (cardiac) limitations to exercise.

Exercise training (ET) has been shown to improve aerobic capacity and quality of life (QOL) in HFpEF, but the weight of evidence indicates that ET works predominantly by improving “peripheral” limitations in the muscle and blood vessels, with little direct cardiac effect. This suggests that treatments targeting central limitations that develop with exercise may allow for greater benefit from ET in HFpEF.

Recent studies have identified abnormal nitric oxide-cyclic guanosine monophosphate (NO-cGMP) signaling as a lynchpin mechanism underlying cardiac abnormalities in HFpEF. Direct NO donors such as organic nitrates have not improved chronic activity levels in HFpEF but are limited by the development of tolerance and pseudo-tolerance and by the fact that they may produce excessive hypotension in people with HFpEF. What is needed is an NO-cGMP enhancing agent that selectively targets central cardiac limitations preferentially during exercise, without untoward effects on resting cardiovascular homeostasis. Coupling of such an agent to ET may allow for synergistic benefit. Because both central and peripheral limitations will be targeted, people with HFpEF could potentially tolerate ET better, with fewer symptoms, increased daily activity levels,

and improved adherence to exercise prescription.

The specific objectives of this application are to determine whether treatment with sodium nitrite (NO_{2-}) in addition to ET can improve exercise capacity, activity levels, and QOL in people with HFpEF, as compared to ET alone. The guiding hypothesis is that enhancing NO-cGMP signaling in the heart and periphery during exercise will improve functional capacity and symptoms in HFpEF. This hypothesis is based upon work from our group showing that exercise capacity in HFpEF is limited by abnormalities in cardiac stress reserve that develop during exercise. Importantly, conversion of NO_{2-} to biologically active NO is enhanced with tissue hypoxia and acidosis developing during exercise, suggesting that NO_{2-} may preferentially target hemodynamic perturbations at the time of greatest need, with less effect at rest. Thus NO_{2-} may allow for improved tolerance and adherence to ET in HFpEF. Successful completion of this project will enable understanding of the effects enhancing NO signaling through a novel, previously unexplored pathway, to synergize with ET to improve morbidity and QOL in people with HFpEF.

Background related to Aim 1.

Exercise capacity, quantified by the peak VO_2 attained during exercise, is severely reduced in HFpEF.4, 9, 13, 14 According to the Fick principle; VO_2 is equal to the product of cardiac output (CO) and arterial venous O_2 content difference (A-V O_2diff). Haykowsky et al. have observed that reduced peak VO_2 in HFpEF is related to both low CO and impaired A-V O_2diff reserve with exercise,14 but the improvements in peak VO_2 with training are mediated exclusively by benefits in A-V O_2diff reserve.26, 27 This observation of peripheral but not central effects of training has been corroborated in HFrEF populations.28, 29 Thus, coupling an agent that

improves cardiovascular reserve to ET would be expected to allow for the greatest benefit from training, which appears to work predominantly through the periphery.

Among the potential molecular pathways underlying cardiac reserve dysfunction, abnormalities in NO-cGMP-dependent signaling have emerged as important players.9, 19, 20, 33-35 Acute increases in NO-cGMP activity are associated with systemic and pulmonary arterial and venous vaso-dilation, enabling LV and right ventricular (RV) afterload reduction, reduction in cardiac filling pressures and pulmonary pressures, improved flow-mediated dilation, and potentially enhanced LV diastolic compliance and relaxation.

In the RELAX trial, we observed that sildenafil, which increases cGMP by inhibiting its breakdown, failed to improve exercise capacity or QOL in people with HFpEF.36 This suggests that enhancing NO-cGMP through decreased breakdown is not an effective approach in this population. Alternative strategies including organic NO donors have also failed, as evidenced by the recent NEAT-HFpEF trial. However, organic nitrates are limited by tolerance 37, and are associated with greater risk of hypotension in people with HFpEF, especially at rest, which may predispose to falls and attendant morbidity in this elderly population.18 A key factor in HFpEF that complicates treatment is that the hemodynamic perturbations causing morbidity (e.g., high filling pressures) are typically present only during stress—being absent at rest (Figure 1).10, 13 To most effectively treat these derangements, the ideal therapy would become more effective during stress, without untoward effects on resting cardiovascular homeostasis.

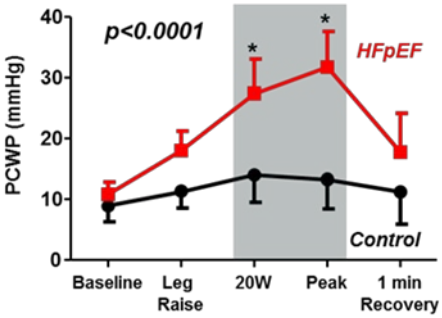


Figure 1: Pulmonary capillary wedge Pressure (PCWP) at rest and exercise.

Inorganic nitrates such as $NaNO_2$ represent an attractive approach to treat NO-cGMP deficiency preferentially during stress in HFpEF.38-40 These molecules were previously considered as inert byproducts of endogenous NO metabolism, but recent studies have shown that NO_{2-} functions as an alternative in vivo source of NO to the classical oxygen-dependent L-arginine-NO-synthase pathway (Figure 2).

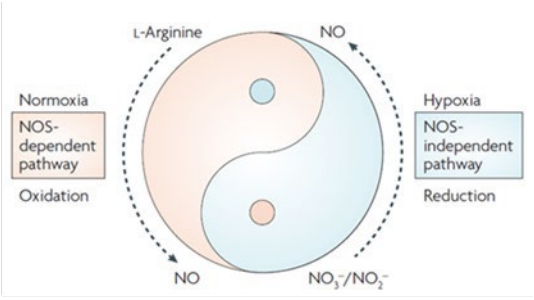


Figure 2: Two parallel sources of endogenous nitric oxide (NO) in mammals.

In contrast to direct NO donors such as organic nitrates, there is no tolerance with NO_{2-}.39 The NO_{2-} reductase activity of heme-containing proteins peaks at O_2 saturations of 40-60% but is near maximal from 20-60%, 41 meaning that bioconversion of NO_{2-} to NO is enhanced in the venous blood and tissues under conditions of physiologic duress, such as hypoxia and acidosis 38-40. In other words, NO_{2-} becomes most effective precisely at the time when it would be most needed in patients with HFpEF, without causing hypotension at rest when filling pressures, pulmonary artery pressures, and CO are normal (Figure 1). In addition, NO_{2-} may have beneficial effects on skeletal muscle bioenergetics, improving the O_2 cost of work during submaximal exercise.42, 43 Thus; NO_{2-}

represents an ideal candidate molecule to treat the central and peripheral limitations to exercise, with less risk of adverse toxicity, while complementing the effectiveness of ET to improve functional capacity in HFpEF.

Participants in this trial will undergo 12 weeks of cardiac rehabilitation for exercise training (ET) and be randomized to either nitrite or matching placebo. In Stage 1 of the trial, participants received either nebulized inhaled NaNO2 (AIR001, Aires Pharmaceuticals, Inc., San Diego, CA) or nebulized inhaled saline (placebo) throughout the training period. Study drug (80 mg) was administered utilizing the Phillips I-neb AAD nebulizer over 10-15 minutes for each dose. Study drug was administered 3 times daily during daytime hours (e.g. 08:00, 12:00 and 16:00), with one of these doses being immediately prior to onset of exercise at ET sessions throughout the course of the 12-week trial.

In Stage II of the trial, subjects will receive treatment with sodium nitrite (or matching placebo) as oral capsules at a dose of 40 mg tid, a lower nitrite dose than in Stage I. The oral formulation produces plasma nitrite levels that are similar to, but slightly less than, what is observed with intravenous administration in our previous study (plasma nitrite levels following 20 mg dose of 5.5±0.7 μM with oral vs 8.4±1.9 μM in the intravenous study).

While plasma nitrite levels are lower with oral nitrite than intravenous or inhaled, these levels are still an order of magnitude higher than what is observed with other oral formulations of nitrate/nitrite that have or are being tested in HFpEF (e.g. beetroot juice, potassium nitrate), and are similar to levels that we have observed to produce favorable hemodynamic effects in our preliminary invasive studies.

The time to maximal concentration of NO_{2^-} in plasma with the oral formulation is 30 minutes, which is longer than with inhaled (5 minutes), though plasma levels return to baseline by 3 hours following a single dose (Hughes et al. *Hypertension* 2017; electronically published ahead of press, doi: 10.1161/HYPERTENSIONAHA.117.09016). For this reason, study drug will be administered *30 minutes* prior to training in Stage 2 of the study, as opposed to immediately prior to training as was performed in Stage 1.

The principal investigator currently holds an Investigational New Drug application (IND 123333) from the FDA for use of inhaled $NaNO_2$ to treat patients with HFpEF, and is amending this IND to include the oral nitrite formulation, with permission to cross reference the existing IND (#115926) for oral nitrite held by our collaborator, Dr. Mark Gladwin.

Background related to Aim 2.

Symptoms of exercise dyspnea and fatigue in HFpEF discourage activity and thus represent a barrier to succeeding with ET and maintaining an active lifestyle during and after completing ET. By attenuating exercise-induced elevation in cardiac filling pressures, relaxing loading conditions, and improving cardiac output reserve, NO_{2^-} holds promise to reduce these symptoms and thus improve daily activity and tolerability of ET. The objectives of Aim 2 are to determine if treatment with NO_{2^-} in addition to ET improves symptoms of effort intolerance during ET and increases daily activity outside of ET. The working hypothesis is that administration of NO_{2^-} in the background of ET will improve symptoms of effort intolerance during ET and increase

average daily activity levels outside of ET. Our approach to test this hypothesis will be to quantify perceived effort and dyspnea symptoms reported during ET and evaluate whether daily activity assessed by 14-day averaged arbitrary accelerometer units (AAU14) increases more in subjects with HFpEF randomized to NO_{2^-} with ET compared to inhaled placebo with ET. The rationale for this aim is that treating symptoms of HF caused by hemodynamic perturbations will encourage/enable greater activity and tolerability of ET. After successful completion of the proposed studies for Aim 2, it is our expectation that we will have identified a new way to improve symptomatic tolerability of ET while increasing daily activity levels outside of the training environment, allowing for extension of the benefits of ET in the long term to maintain an active lifestyle and sustainable increase in long-term activity level and fitness in people with HFpEF.

The duration of ET intervention is short, generally 8-12 weeks, and the long- term goal of ET is to achieve and maintain a durable, sustained increase in activity level beyond the original intervention. Data from the HF-ACTION trial showed that despite a well-organized program with abundant resources, only 40% of participants randomized to the intervention stage actually maintained ET at the target of 90 minutes per week after 3 months.24, 25 Interventions that improve the central cardiac limitations that contribute to symptoms of effort intolerance would be expected to allow for maintenance of a more active lifestyle.

In patients with HFrEF, acute administration of organic nitrates reduces filling pressures and pulmonary artery pressures but does not increase peak exercise capacity.49-51 In contrast; long-term organic nitrate therapy did improve exercise capacity in this HF subtype. The discrepancy between acute and long-term effects with organic nitrates in HFrEF has been attributed to increases in physical activity and improved fitness associated with long-term improvement in HF symptoms—a speculation that has been corroborated by increases in self-reported physical activity among subjects randomized to organic nitrates.50 However, there are important fundamental differences between HFpEF and HFrEF, 18, 52 and between organic nitrates and NO_{2^-} . People with HFpEF have a much greater drop in blood pressures with organic nitrates as compared to HFrEF, 18 which we would expect to greatly limit compliance. Further, because NO_{2^-} conversion to NO is activated by venous hypoxia and acidosis with stress, 38-40 we expect that there will be less hypotensive effect as compared to organic nitrates in the HFpEF population at steady state. Finally, in contrast to organic nitrates, NO_{2^-} is not associated with tolerance or development of endothelial dysfunction.37, 39

Externally-worn accelerometer devices provide highly quantitative, granular, high-density, patient-centric data which have been used to characterize activity and to assess the impact of interventions on activity levels in patients with chronic obstructive pulmonary disease, obesity, and arthritis. In HF, accelerometer data have been shown to reflect changes in clinical status in HF and to correlate with traditional measures of disease severity such as peak VO_2, 6-minute walk distance, QOL, and prognosis as assessed by the Seattle Heart Failure Model.53-55 Thus, accelerometer data provides an ideal metric to address the hypothesis of Aim 2.

1.2 Investigational Agent

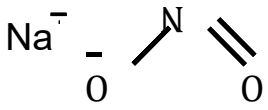
Physical, Chemical, Pharmaceutical Properties, and Formulation:

Drug Substance: The drug substance is sodium nitrite, $NaNO_2$.

Molecular Formula: $NaNO_2$

Molecular Weight: 69.0

Chemical Structure:



Nitrite exists in the body derived from both dietary intake and as a byproduct of endogenous NO metabolism. Nitrite is widely used as a food preservative and is available in much higher doses for intravenous administration as an FDA-approved treatment for cyanide toxicity.

Inhaled Nitrite (Stage 1): Inhaled, nebulized placebo or inhaled nebulized sodium nitrite was administered at 80 mg 3 times daily, at a minimum of 4 hours apart, with the first dose starting at the beginning of the active part of the day (for example, 8:00, 12:00, and 16:00). The Participants were instructed to disperse the doses by at least 4 hours and deliver them over their normal active day time. The doses do not need to be given at the same time each day if the Participant's active period varies by day, for example if the Participant arises later on the weekends.

***Oral nitrite (Stage II)*:** In Stage II of the study, nitrite will be administered using an oral formulation at dose of 40 mg po tid (taken using the same dosing frequency as during Stage I of the study, but 30 minutes prior to training rather than immediately preceding training). This produces peak concentrations at 30 minutes which return to baseline at 3 hours (Hughes et al. *Hypertension* 2017; published ahead of press, doi: 10.1161/HYPERTENSIONAHA.117.09016). Oral nitrite and matching placebo capsules will be compounded by The University of Iowa Pharmaceuticals, in Iowa City, IA who will ship to the University of Pittsburgh as specified in Dr. Mark Gladwin's IND (#115926) for ongoing stability testing (cross reference permission letter provided by Dr. Gladwin to FDA to reference CMC information), and then shipped, stored and dispensed in the Mayo Clinic Research Pharmacy.

Preliminary data administering oral nitrite 40 mg tid to (1) obese patients with metabolic syndrome for 12 weeks (n=20), and (2) patients with HFpEF and pulmonary hypertension for 10 weeks (n=5 at this time) participating in separate studies at the University of Pittsburgh has revealed an excellent safety profile, with only 1 serious adverse event that was not attributed to study drug administration (Personal communication, Dr. Mark Gladwin).

1.3 Clinical Data to Date

Preliminary Studies Related to Aim 1.

Our group has shown that exercise limitation in HFpEF is related to global impairments in cardiovascular reserve—characterized by abnormal systemic vasodilation (less reduction in arterial elastance, Ea), greater increase in pulmonary capillary wedge pressure (PCWP), blunted increases in CO relative to VO_2 (lower CO/VO_2 slope), and steeper pulmonary arterial (PA) pressure flow relationship indicating abnormal pulmonary vasodilation (n=74, Figure 3). Depressed CO reserve contributes to a net decrease in O_2 delivery to exercising muscles, increasing the reliance in muscles on O_2 extraction and anaerobic glycolysis to compensate for inadequate perfusion.

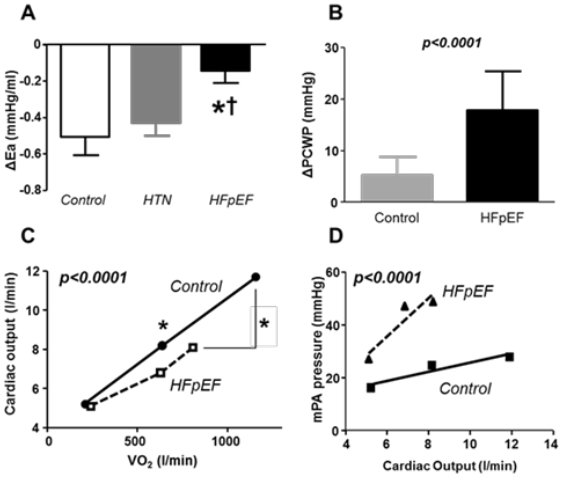


Figure 3: Compared to control populations, subjects with HFpEF display less vasodilation [A], higher filling pressures [B], impaired cardiac output reserve [C], and steeper PA pressure-flow relationships with exercise [D].

We have shown that the decrease in CO during exercise in HFpEF is coupled with lower mixed venous O_2 content and pH during submaximal exercise in patients with HFpEF compared to controls (n=74, Figure 4). In a different study, we have also observed marked reduction in coronary sinus O_2 saturation during exercise in people with HFpEF (28±9%, n=9).44 Thus, the outgoing blood traveling to the left heart from the pulmonary arteries (mixed venous) as well as the venous blood returning from the left heart (coronary sinus) becomes hypoxic and acidemic during exercise in people with HFpEF, setting the stage for greater reduction of NO_{2^-} to NO in the central circulation.

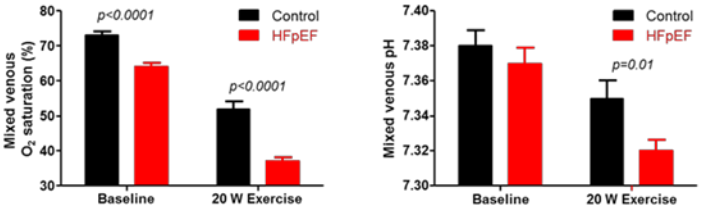


Figure 4: Compared to controls (black), subjects with HFpEF (red) display lower venous O_2 content at rest and during low-level exercise, along with greater venous acidosis (lower pH) during exercise.

Our group has recently completed a randomized, double blind, placebo controlled trial testing whether acute NO_{2^-} treatment could improve hemodynamics during submaximal exercise in HFpEF (n=29). Subjects underwent resting and exercise invasive hemodynamic assessment and were then treated with either intravenous NO_{2^-} (250 µg/kg over 5 min) or saline. After 15 minutes, repeat rest and exercise hemodynamic assessment was performed at the same workload. Compared to placebo, NO_{2^-} treated subjects displayed markedly attenuated increases in PCWP with exertion, improved exercise CO reserve, and enhanced CO/VO_2 slope (Figures 5 and 6).

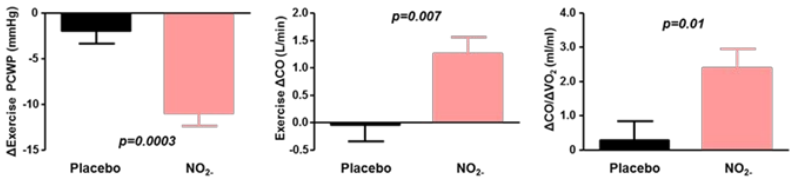


Figure 5: Compared to placebo (black), HFpEF subjects treated with acute NO_{2^-} infusion (pink) experienced 4-fold lower PCWP during submaximal exercise, coupled with greater enhancement in exercise cardiac output and improved CO/VO_2 slope.

Enhanced CO reserve in NO_{2-} treated subjects was entirely due to improved stroke volume reserve (+16±19 vs +1±14 ml, p=0.02), as there was no effect on exercise heart rate (p=0.9). Acute NO_{2-} infusion reduced the steepness of the PA pressure-flow relationship from 10.0 (IQR, 4.9 to 24) mmHg*min/L to 4.1 (IQR 2.5 to 5.0) mmHg*min/L (p<0.001), revealing a significant reduction in right ventricular loading with stress.

Notably, there was no significant effect of intravenous NO_{2-} on blood pressure at rest or during exercise compared to placebo. The stroke volume increase observed, coupled with lower PCWP and no change in blood pressure strongly suggests a positive direct myocardial effect of acute NO_{2-}. In contrast to some prior studies, 41, 42 there was no reduction in the O_2 cost of exercise; rather the proportional increase in VO_2 during submaximal exercise (20 Watts) relative to the increase prior to study drug was 106% (IQR, 98 to 136%) with NO_{2-} as compared to 90% with placebo (IQR, 84 to 111%; p<0.01 compared to NO_{2-}).

Plasma NO_{2-} levels increased to 8.4±1.9 µM at rest in subjects receiving intravenous NO_{2-} (p=0.004 vs baseline, n=4). After 5 minutes of exercise, NO_{2-} levels decreased to 3.4±0.4 µM (p<0.01 compared to pre-exercise). The calculated half-life from these data is 3.9±0.6 min, which is 10-fold faster than previously reported kinetics for NO_{2-} in humans at rest (30-40 min). This indicates active NO_{2-} consumption during exercise, and thus greater reduction of NO_{2-} to NO during stress.

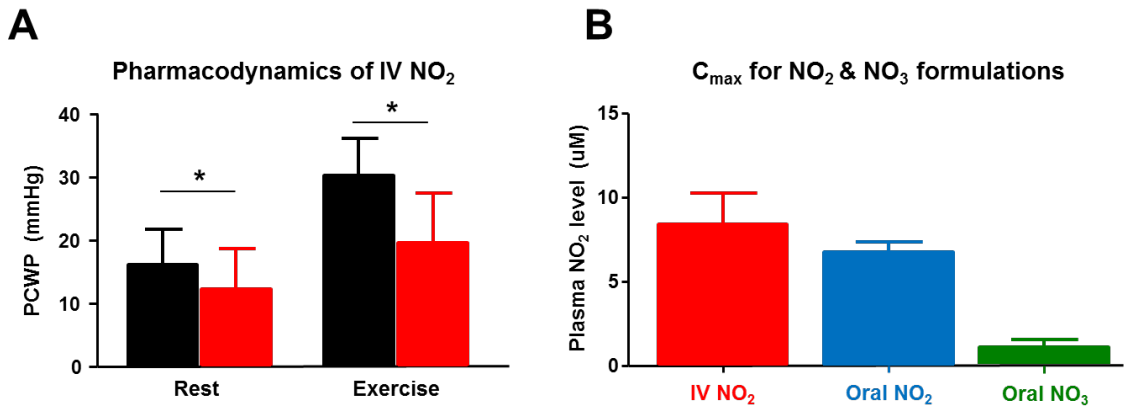


Figure 6: [A] Intravenous nitrite (NO_2) administration (50 mcg/kg/min, red) causes mild reductions in resting pulmonary capillary wedge pressure (PCWP) and dramatic reductions in exercise PCWP (**p<0.0001*). [B] Plasma NO_2 levels 30 minutes following an oral dose of 40 mg NO_2 (blue) are similar to levels observed immediately following IV NO_2 (red) in the experiments represented in panel A, and are significantly higher than NO_2 levels with 12 mmol oral nitrate (NO_3), as is being tested in other trials.

We have recently demonstrated equivalent hemodynamic benefits in a pilot study using inhaled NO_{2-} (n=30, data from the first 4 participants receiving open label drug shown). A single inhaled dose of 80 mg NO_{2-} produces similar plasma NO_{2-} levels to the IV formulation (9.3±0.9 µM) and equivalent reductions in exercise PCWP (29±2 to 19±4 mmHg, p=0.01). While the effects of oral nitrite on rest and exercise PCWP have not yet been assessed, preliminary data from Hughan and Gladwin et al has revealed that plasma nitrite levels 30 minutes following a single oral dose of 40 mg sodium nitrite (as will be used in this study) produces similar NO_{2-} levels as we observed with the intravenous formulation (Figure 6).

As shown by Hughan et al (*Hypertension* 2017; electronically published ahead of press, doi: 10.1161/HYPERTENSIONAHA.117.09016), the oral formulation of nitrite (20 mg) produces NO_{2-} levels that are similar to but slightly lower than what is observed with parenteral administration or the 40 mg single dose (5.5±0.7 μM, Figure 7). However, even the 20 mg oral nitrite dose produces plasma levels that fall within the range where we have observed favorable hemodynamic effects based upon our preliminary invasive studies (Figures 5, 6). Furthermore, the oral formulation used in Stage II of the study will also be easier for patients to administer as compared to the nebulized formulation which was cumbersome and time- consuming for patients to use.

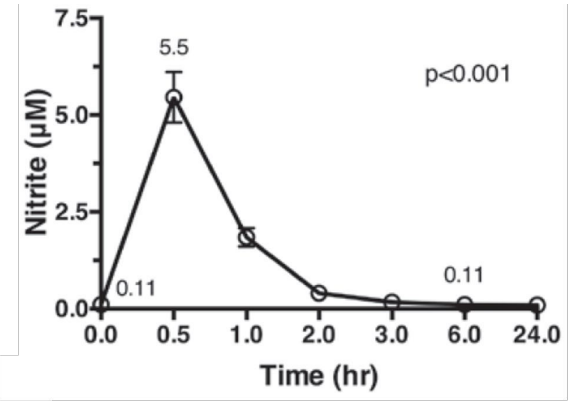


Figure 7: Pharmacokinetics of oral sodium nitrite following a 20 mg dose (from Hughan et al. *Hypertension* 2017).

No episodes of hypotension were observed using directly measured pressure with the inhaled nitrite. As compared to inhaled placebo, AIR001 (80 mg) had no effect on mean BP (0±9 vs 1±3 mmHg, p=0.8) and no effect on heart rate (-1±5 vs -2±4 bpm, p=0.7).

With oral nitrite, which will be used in Stage II of this study, there is a modest vasodilator effect leading to reduction in mean arterial pressure (Figure 8) that peaks between 15 and 45 minutes following single dose administration (Hughan et al. *Hypertension* 2017; published ahead of press, doi: 10.1161/HYPERTENSIONAHA.117.09016 and Hughan, Gladwin et al, unpublished data from separate trial of patients with obesity and metabolic syndrome, n=20).

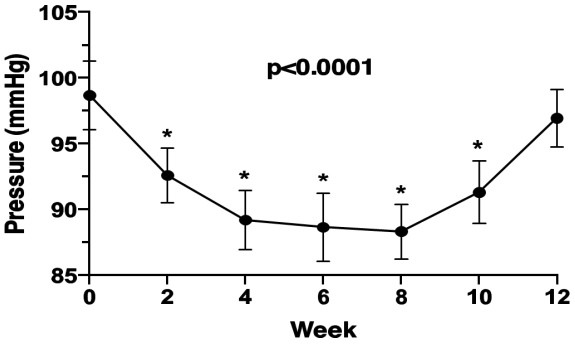


Figure 8: Changes in mean arterial pressures (MAP) with oral nitrite, 40 mg tid, assessed biweekly in obese patients with metabolic syndrome (n=20, unpublished data, Hughan et al).

This reduction in blood pressure noted with oral nitrite in these studies was not associated with development of reflex tachycardia. In the study of Hughan et al. testing oral nitrite at a dose of 40 mg tid, no patient developed symptomatic hypotension.

In our acute invasive studies, NO_{2-} infusion and inhalation did not affect the peripheral components of VO_2, evidenced by no change in the increases in arterial-venous O_2 difference (A-V O_2diff) with stress (p=0.8). Because ET has been shown to improve peak VO_2 through benefits on A-V O_2diff, but not through cardiac effects, we hypothesize based upon our preliminary findings with both intravenous and inhaled NO_{2-} during submaximal exercise that

sustained, longer-term treatment will allow for even greater synergistic benefit with ET in subjects with HFpEF.

Our group has recently completed an invasive hemodynamic study (n=106) where echocardiography was performed at rest and during maximal effort exercise simultaneously with directly measured intracardiac and vascular pressures. PA systolic pressures (PASP) estimated by echocardiography were strongly correlated with directly measured pressures at rest and during exercise, while significant but less robust correlations were observed between PCWP and the E/e' ratio during cycle ergometry. These data demonstrate the feasibility and validity of exercise echocardiography to assess hemodynamic reserve in our group.

Preliminary Studies related to Aim 2.

We have shown that patients with HFpEF develop marked increases in cardiac filling pressures, PA pressures, and inadequate CO reserve during exercise, and that each of these abnormalities can be improved during submaximal exercise by acute infusion of NO_2- (please refer to preliminary studies related to Aim 1, above). In addition, our group has shown that in subjects with HFpEF, the degree of NO-cGMP impairment, assessed by the reactive hyperemic index response to upper stage cuff occlusion is directly related to more severe symptoms of dyspnea and fatigue during matched submaximal exercise (Figure 9, n=31).9 Thus, enhancing NO-cGMP signaling with NO_2- may improve symptoms of exercise intolerance both by improving central pressures and cardiac output as well as by improving microvascular endothelial function, and in so doing, improve daily activity levels.

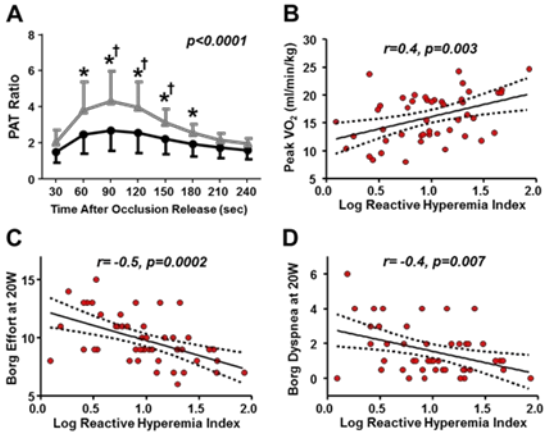


Figure 9: [A] Compared to controls (gray), subjects with HFpEF (black) display less flow mediated dilation (FMD) after upper arm cuff occlusion release. Worsening FMD (lower reactive hyperemia index) is associated with lower peak VO_2 [B] and more severe symptoms of fatigue [C] and dyspnea [D] during low-level exercise (20W).

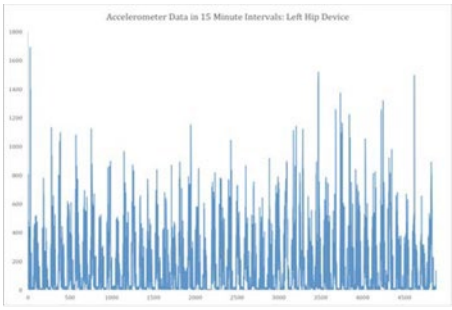


Figure 10: Example data from a HFpEF subject showing averaged arbitrary accelerometry units (Y axis) vs time (X axis) over a duration of 42 days.

Accelerometry is the primary endpoint for a recently-completed NHLBI-sponsored trial in which Mayo was the lead center, testing whether isosorbide mononitrate improves daily activity tolerance in people with HFpEF in a double-blind crossover trial. Our coordinating center has been the leading enroller in this trial, and we have acquired high quality accelerometer data from all participants enrolled (Figure 10). Subjects have reported no difficulties maintaining the devices on throughout the course of the day and initial analysis of data quality reveals very little missing data.

1.4 Dose Rationale and Risk/Benefits

Nitrite delivery by intravenous, inhaled and oral routes of administration has been shown to be safe, and the plasma nitrite levels achieved with each route have been shown by our group and others to cause favorable effects on cardiac hemodynamics that we expect to improve both symptoms and exercise capacity in patients with HFpEF, as will be tested in this study.

Intravenous nitrite administration reduces pulmonary capillary wedge pressures (PCWP) at rest (-3 mmHg, $p<0.0001$) and particularly during exercise (adjusted exercise values 19±5 mm Hg vs. 28±6 mm Hg; $p=0.0003$). Similar effects are observed on pulmonary artery (PA) pressures, and there was a greater increase in cardiac output reserve (all $p<0.01$). Plasma nitrite levels achieved with the intravenous formulation were 8.39±1.88 μM (Borlaug et al. *J Am Coll Cardiol* 2015; 66:1672–82). While our group has shown that nitrite reduces arterial stiffness (*J Am Coll Cardiol.* 2017;70(2):136-148.), there was no symptomatic hypotension or methemoglobinemia with intravenous nitrite in this study, and no adverse events.

Inhaled nitrite (Stage I): In Stage I of this trial, participants received a dose of inhaled nebulized nitrite 80 mg 3 times daily. Participants developing symptoms of intolerance could be down-titrated to 46 mg 3 times daily. The nebulizer route was difficult to use for many of the participants, and mindful of the neutral results of the INDIE trial (manuscript currently under review), the manufacturer of the inhaled nitrite compound discontinued manufacture. For this reason, we are switching to an oral route of administration of nitrite in Stage II of our study.

Oral nitrite (Stage II): Like inhaled nitrite, oral nitrite has been found to be safe, without significant adverse effects in Phase I studies. The bioavailability of oral nitrite is excellent (95%) and similar to intravenous formulations. High doses of nitrite have also been provided in several studies by oral route in the form of beet root juice. Nutraceutical preparations are currently being sold in health stores with levels of nitrite (12.7 mg per tablet) that are similar to what will be used in Stage II of our study.

As of July 31, 2017, a total of 84 subjects were enrolled in 8 clinical trials testing oral sodium nitrite conducted under Dr. Gladwin's IND# 115926 at the University of Pittsburgh. Subjects enrolled are predominantly female (57%) and Caucasian (77%). Among the 8 clinical trials, 2 clinical trials have been completed; one is a Phase 1 study aimed to establish the pharmacokinetics, metabolism and interconversion of nitrate to nitrite, nitrite to nitric oxide, and interaction with Conjugated Linoleic Acid (cLA) to form nitro-fatty acids in vivo in healthy adult normal volunteers (Hughan et al. *Hypertension* 2017; published ahead of press, doi: 10.1161/HYPERTENSIONAHA.117.09016). The other is a Phase 2 open labeled study designed to assess the safety and feasibility of oral nitrite therapy on the changes in skeletal muscle bioenergetics and physical capacity in older heart failure patients.

Five Phase 2 studies are ongoing. Of the five active studies, 2 studies are in the patient population of overweight/obese adults with metabolic syndrome and hypertension, at risk for insulin resistance and endothelial dysfunction, one in adult obese asthmatics with metabolic syndrome, and another one in older HF patients, one in adult HFpEF patients aged ≥ 18 years and another one in older HFpEF patients aged ≥ 70 years. Dr. Gladwin's group has also initiated

another Phase 1 nitrate study aimed to determine distinguishing features of the structure and function of the oral and gut microbiome in PH-HFpEF and controls.

The oral nitrite for Stage II of this study will be provided under the supervision of our collaborator, Dr. Mark Gladwin, is an internationally recognized leader in the basic and clinical science of the inorganic nitrate/nitrite/NO pathway. Dr. Gladwin has held an IND for sodium nitrite (IND # 70,411) for cardiovascular applications and currently has an approved IND for the use of sodium nitrite for lung transplant recipients (IND # 111,643). The cardiovascular IND involved the administration of sodium nitrite to 69 normal volunteers in 4 phase I-II clinical trials without observed adverse effects. This study will be performed under the IND held by the PI, allowing the FDA to cross reference the Dr. Gladwin's current IND for the oral formulation.

Dr. Gladwin has also treated 11 subjects with sickle cell disease on this IND without observed adverse effects. The lower doses of nitrite used in these investigational treatment regimens – 60-120 mg daily, or 20-40% of the intravenous dose (300mg) used in the emergency treatment of cyanide poisoning – do not produce methemoglobin levels greater than 3% and have not been associated with clinically significant hypotension. There have been no adverse events noted in the 80 treated normal human volunteers and patients with sickle cell disease.

With 20 mg oral nitrite, there are modest reductions in blood pressure with no increase in heart rate (Hughan et al. *Hypertension* 2017; electronically published ahead of press, doi: 10.1161/HYPERTENSIONAHA.117.09016). Similar to our prior studies with inhaled and intravenous nitrite, oral nitrite was observed in this study to cause a very mild and clinically insignificant rise in plasma methemoglobin levels, with no patients developing levels >3%.

The effects of oral nitrite on central hemodynamics have not yet been examined, but given the similar plasma nitrite levels observed with oral administration (Figure 7) and modest reduction in blood pressures (Figure 8), we expect reductions in pulmonary artery and pulmonary capillary wedge pressure at rest and during exercise of roughly similar magnitude to what we have observed following intravenous and inhaled preparations.

Risk from Study Drug-Overview

Sodium nitrite (NO_{2-}) has vasodilating properties, and the most significant expected risk in this population would be symptomatic hypotension. NO_{2-} is converted in vivo to nitric oxide (NO) and thus may cause vasorelaxation. We have observed that nitrite delivered by the intravenous and inhaled routes reduces arterial stiffness and vasodilates conduit vessels as well as vessels at the arteriolar level (*J Am Coll Cardiol.* 2017;70(2):136-148.), without causing excessive BP reduction or symptomatic hypotension.

The oral formulation of nitrite has been observed to reduce blood pressure. This compound has been administered to patients with obesity and metabolic syndrome, as well as HFpEF and PH as part of Dr. Gladwin's ongoing NIH-sponsored studies, and no severe adverse events or symptomatic hypotension have developed following treatment with this oral nitrite formulation (Dr. Mark Gladwin, personal communication 2018). We will carefully assess for any adverse

effects that could be related to excessive BP reduction in the study as outlined below in the protocol.

Mechanism of Action: Inorganic sodium nitrite is an intrinsic vasodilator that is converted in blood and tissues to nitric oxide (NO). Endogenous NO_{2-} represents the body's largest reservoir of NO which results in vasodilation and may improve ventricular diastolic compliance.

Dosage: AIR001 solution for nebulization. The dose of 80mg/ml is administered through a specialized nebulizer device. The oral dose of 40 mg is administered as a capsule, administered three times daily (same as the inhaled formulation).

Metabolism: AIR001 is rapidly absorbed through the alveolar-capillary interface, achieving peak concentrations in less than 5 minutes. Oral nitrite is rapidly absorbed through the alimentary tract, achieving peak levels at 30 minutes. NO_{2-} is elevated in plasma following both routes of administration and is rapidly converted to NO in areas of low oxygen tension.

Interactions and Contraindications: There are no known drug interactions. NO_{2-} is not a substrate or inhibitor for hepatic cytochrome p-450 enzymes. Nebulized inhaled nitrite (NO_{2-}) should not be used with other NO donors (e.g. organic nitrates, nitroprusside) to minimize risk of hypotension, and these are listed as exclusion criteria for safety. A drug-drug interaction study with sildenafil dosed at 20mg three times daily did not show a pharmacodynamic interaction, but in this study patients treated with phosphodiesterase inhibitors will be excluded.

Adverse Effects: The most frequent reported adverse effects of AIR001 used in Stage 1 are cough, headache, and dizziness. These effects were reported as mild to moderate intensity and rarely result in discontinuation of medication.

In a Phase 2 study of oral nitrite therapy in older heart failure patients, Gladwin and colleagues found a higher propensity for hypotension in subjects aged > 70 years with heart failure compared to healthy controls. Two research subjects enrolled in this study were withdrawn by Investigator due to hypotension. In all other studies, oral nitrite and nitrate are generally safe and well tolerated, and all AEs thought to be related to nitrite and nitrate were mild to moderate in severity, and were all resolved with or without minor treatments. No drug related SAEs have been observed in all of the protocols. In sum, the number and nature of anticipated AEs are expected.

The most frequent expected adverse effects with oral nitrite are dry mouth (infrequent), and nausea/abdominal pain/methemoglobinemia/flushing/headache/dizziness (rare).

Abuse Potential: No risk for abuse of AIR001 has been demonstrated.

Pregnancy Risk: Although nitrite exists widely in nature and human ingestion occurs daily in foodstuffs and through water sources, no human data exist on the safety of administration of nitrite during pregnancy or lactation. AIR001 and oral nitrite should not be administered to women who are pregnant or lactating. Effective contraceptive measures should be utilized by women of child-bearing potential who receive either form of nitrite. Given the ubiquitous nature

of nitrite in the environment and the short half-life of AIR001, prolonged contraceptive use after ceasing AIR001 inhalational use is unnecessary, and should be predicated on the clinical judgment of the subject's clinician.

2 Study Objectives

Aim 1: Determine whether treatment with NO_2^- in addition to ET for 12 weeks improves exercise capacity and hemodynamic reserve in HFpEF. Expired gas analysis, inert gas (C_2H_2) rebreathe, and echocardiography will be performed during rest and exercise to measure oxygen consumption (VO_2), CO, and hemodynamics before and after completion of 12 weeks of ET with NO_{2-} vs ET with inhaled placebo.

- Our **primary hypothesis** is that combination therapy with ET and NO_{2-} will be associated with greater improvement in peak VO_2 compared to ET plus placebo. Our **secondary hypotheses** are that ET and NO_{2-} will lead to greater improvement in CO reserve (CO/VO_2 slope), peak workload, and exercise hemodynamics (lower echocardiography estimated pulmonary artery pressure and E/e' ratio).

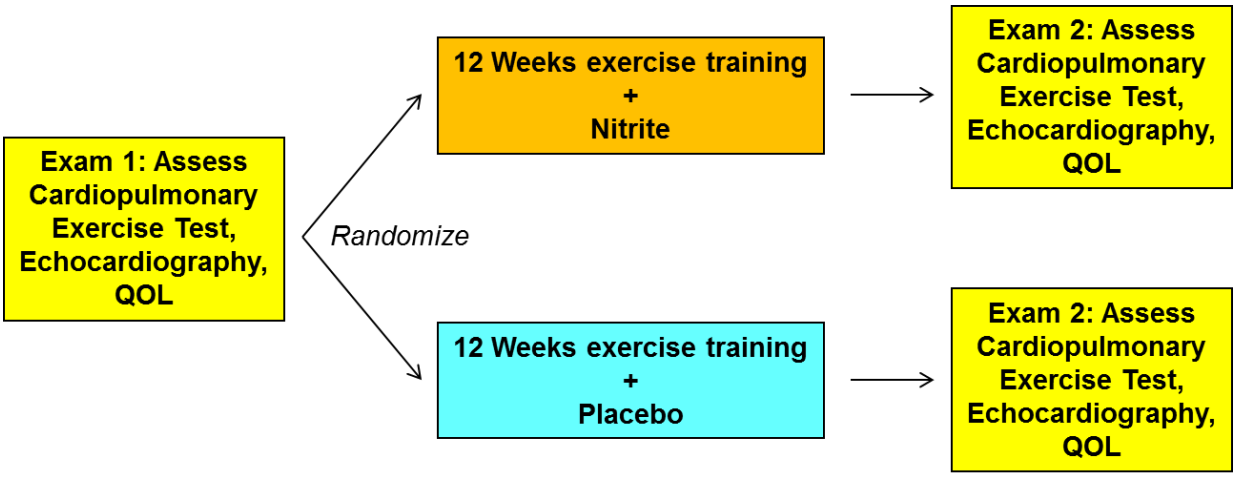
Aim 2: Determine whether treatment with NO_{2-} in addition to ET for 12 weeks increases daily activity levels and QOL, and reduces symptoms of effort intolerance during ET. Subjects will use externally worn accelerometer devices to track daily physical activity. Tolerability of ET will be assessed by Borg perceived effort and dyspnea scores. Large and small vessel endothelial function (brachial and digital arteries) and quality of life (QOL) will be assessed.

- Our **primary hypothesis** is that combination of NO_{2-} and ET will be associated with greater improvement in daily activity levels compared to ET with placebo. Our **secondary hypotheses** are that combination of NO_{2-} and ET will be associated with improvement in symptoms of dyspnea and fatigue, improved tolerability to ET, enhanced endothelial function and better QOL compared to ET with placebo.

3 Study Design

3.1 General Design

This is a Phase 2 randomized, double-blind, parallel group placebo-controlled trial testing whether inorganic sodium nitrite, as compared to placebo, can enhance the benefits from chronic exercise training (ET) in subjects with HFpEF (see flow diagram below).



The total duration of the trial is 14 weeks.

Subjects will be screened from the inpatient and outpatient heart failure services and interested qualified subjects will be offered participation. Once consent has been obtained baseline assessment of cardiovascular function and exercise capacity will be performed. All subjects will undergo 12 weeks of ET. Participants will be randomized to receive sodium nitrite 3 times daily or matching placebo 3 times daily during the study period.

In Stage I this was with inhaled medicine for the full 12 weeks of ET. In Stage II, study drug will be administered using an oral preparation for the full 12 weeks. Study subjects will wear accelerometry devices to track daily activity levels at home.

At the Screening visit, during consent, subjects will be given the option to participate in an optional MRI sub-study where they will be asked to complete 2 Magnetic Resonance Imaging (MRI) scans. One scan will be completed prior to or as close as possible to the beginning of cardiac rehabilitation, the other within 2 weeks of completing week 12 of ET (for Stage II). A hematocrit will need to be obtained prior to the MRI if not already drawn within the past 30 days. We will evaluate adipose tissue content and cardiac structure and function. We will determine whether these variables relate to exercise capacity/intolerance in participants, and evaluate how they change with ET with or without nitrite. Subjects will be excluded from this optional substudy if they have contraindications to MRI including aneurysm clips, cardiac pacemakers, internal defibrillators, severe claustrophobia and pregnancy. Women of childbearing potential will undergo a urine pregnancy test; positive test results will yield exclusion from study participation. Patients will be excluded for contraindications to gadolinium-based MRI contrast material, namely glomerular filtration rate less than or equal to 30ml/min.

The MRI scans will be performed on a clinical scanner or at the Center for Advanced Imaging Research in the Opus building. Patients will have ECG leads placed, as is done for cardiac MRI examinations. In some patients this may require shaving the chest for lead placement. Prior to scanning a peripheral intravenous (IV) line will be placed. Patients may be scanned prone and/or supine. The examination will last approximately one hour. Intravenous contrast material (gadolinium) will be used. Subjects will receive $25/scan for participating in this MRI sub-study part of the main study.

Also at the screening visit, during consent, subjects will be given the option to participate in an optional muscle biopsy where they will be asked to complete 2 muscle biopsies. One biopsy will be completed prior to or as close as possible to the beginning of cardiac rehabilitation, the other as close to the end of ET . The muscle biopsy sample will be collected from the vastus lateralis muscle (outer thigh). Muscle biopsy will be performed with a percutaneous biopsy needle (modified Bergstrom needle). Local subcutaneous injection of 2% lidocaine buffered with 8.4% sodium bicarbonate will be used for analgesia. All biopsies will be done under sterile conditions with all necessary aseptic precautions. A small incision will be made through the skin and fascia. After the biopsy, pressure will be held over the incision until hemostasis is achieved. The incision is closed with sterile strips, gauze and an Ace wrap. Risks of this procedure include hematoma, infection, and pain. Hematoma likelihood is minimized by holding pressure after the biopsy to ensure hemostasis, followed by a pressure dressing. The risk of infection is minimized by using sterile surgical techniques. Pain is managed by local analgesia during the procedure and Tylenol following the procedure. Subjects will receive $50/biopsy for participating.

At the conclusion of the main trial, subjects will be offered participation in an optional ancillary study where they will be randomized to standard care or behavioral/motivation intervention (once weekly visits with co-investigator +2 phone calls per week) to evaluate durability of ET effects. For subjects participating in this component of the study, there will be one final visit 8-10 weeks following the final study visit where QOL assessment, Borg symptom severity (effort and dyspnea scores) will be recorded during standardized exercise training at a common objective workload, 2 mph on treadmill at 2° grade for 5 minutes and accelerometry data will be obtained (post trial intervention).

3.2 Primary Study Endpoints

Exercise capacity assessed by peak oxygen consumption (VO_2) achieved during exercise testing will be the primary endpoint for Aim 1. Chronic activity levels assessed by accelerometry will be the primary endpoint for Aim 2. Assessments were performed after 12 weeks of intervention.

3.3 Secondary Study Endpoints

Cardiac output reserve, peak exercise workload, rest and exercise hemodynamics assessed by echocardiography, Borg dyspnea and fatigue scores recorded during ET, endothelial function assessed by tonometry, QOL assessed by the Kansas City Cardiomyopathy Questionnaire and body composition assessed by dual energy X-ray absorptiometry (DEXA). Cardiac structure and function will be assessed by cardiac MRI and adipose content will be assessed by MRI in subjects participating in the optional MRI substudy. The muscle biopsy will tell us about what improvements we see in the leg muscles, and specifically in the mitochondria of leg muscles.

There will be ancillary trial endpoints to assess QOL, Borg symptom scores, DEXA, AXM data, and exercise capacity following completion of the main trial, comparing subjects randomized to usual care or behavioral/motivation intervention.

3.4 Primary Safety Endpoints

Safety endpoints will include blood pressure, methemoglobin levels, symptoms of dizziness, lightheadedness, headaches, and cough.

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

1. Age ≥ 40 years
2. Symptoms of dyspnea (II-IV) without evidence of a non-cardiac or ischemic explanation for dyspnea
3. EF of > or = 50% determined on most recent imaging study within the preceding 5 years, with no change in clinical status suggesting potential for deterioration in systolic function
4. One of the following:

- Previous hospitalization for HF with radiographic evidence (pulmonary venous hypertension, vascular congestion, interstitial edema, pleural effusion) of pulmonary congestion **or**

- Catheterization documented elevated filling pressures at rest (PCWP ≥15 or LVEDP ≥18) or with exercise (PCWP ≥25) **or**
- Elevated NT-proBNP (>400 pg/ml) or BNP(>200 pg/ml) **or**
- Echo evidence of diastolic dysfunction/elevated filling pressures manifest by medial E/e' ratio≥15 and/or left atrial enlargement **and** chronic treatment with a diuretic for signs or symptoms of heart failure

5. Heart failure is primary factor limiting activity as indicated by answering # 2 to the following question:

   My ability to be active is most limited by:

   1. Joint, foot, leg, hip or back pain
   2. Shortness of breath and/or fatigue and/or chest pain
   3. Unsteadiness or dizziness
   4. Lifestyle, weather, or I just don't like to be active

6. No chronic nitrate therapy or not using intermittent sublingual nitroglycerin (requirement for >1 SL nitroglycerin per week).
7. No daily use of phosphodiesterase 5 inhibitors or soluble guanylyl cyclase activators and willing to withhold prn use of phosphodiesterase 5 inhibitors for duration of study
8. Ambulatory (not wheelchair / scooter dependent)
9. Body size allows wearing of the accelerometer belt as confirmed by ability to comfortably fasten the test belt provided for the screening process
10. Willingness to wear the accelerometer belt for the duration of the trial

4.2 Exclusion Criteria

1. Recent (< 1 month) hospitalization for heart failure
2. Ongoing requirement for PDE5 inhibitor, organic nitrate or soluble guanylyl cyclase activators
3. Hemoglobin (Hgb) < 8.0 g/dl within 30 days prior to randomization
4. GFR < 20 ml/min/1.73 m^2 within 30 days prior to randomization
5. Systolic blood pressure < 115 mmHg seated or < 90 mmHg standing
6. Resting HR > 110
7. Previous adverse reaction to the study drug which necessitated withdrawal of therapy
8. Significant chronic obstructive pulmonary disease that is a primary contributor to symptoms in the opinion of the investigator
9. Ischemia thought to contribute to dyspnea in the opinion of the investigator
10. Documentation of previous EF < 40%
11. Acute coronary syndrome within 3 months defined by electrocardiographic (ECG) changes and biomarkers of myocardial necrosis (e.g., troponin) in an appropriate clinical setting (chest discomfort or anginal equivalent)
12. PCI, coronary artery bypass grafting, or new biventricular pacing within past 3 months
13. Obstructive hypertrophic cardiomyopathy
14. Known infiltrative cardiomyopathy (amyloid)

15. Constrictive pericarditis or tamponade
16. Active myocarditis
17. Complex congenital heart disease
18. Active collagen vascular disease
19. More than mild aortic or mitral stenosis
20. Intrinsic (prolapse, rheumatic) valve disease with more than moderate mitral, tricuspid or aortic regurgitation
21. Acute or chronic severe liver disease as evidenced by any of the following: encephalopathy, variceal bleeding, INR > 1.7 in the absence of anticoagulation treatment
22. Terminal illness (other than HF) with expected survival of less than 1 year
23. Enrollment or planned enrollment in another therapeutic clinical trial in next 3 months.
24. Inability to comply with planned study procedures
25. Pregnancy or breastfeeding mothers

4.3 Subject Recruitment, Enrollment and Screening

Eligible patients will be identified from screening of the inpatient and outpatient heart failure services of the Mayo Clinic in Rochester, Minnesota. As well as the cardiovascular medicine and general medical services of the Mayo Clinic Health Systems. Patients that participated in the first stage (with inhaled nitrite) will also be eligible to participate in the second stage with oral nitrite, provided that at least 6 months have passed since the first exercise training intervention. Data from the same patients in different phases will not be analyzed together, but rather separately.

Eligible patients will be paid up to $500 if the entire study is completed. If they are not able to complete the entire study, they will be paid as follows for the study visits they do complete: $100 each for completion of Visits 1 & 4, $50 each for Visits 2 & 3 and $5 for each cardiac rehab session completed.

Patients will also be given a 6 hour parking pass for the screening visit, final visit and visits at week 4, 8 and 12.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Development of any condition that requires study withdrawal related to safety, disease progression, subject decision or failure to adhere to protocol requirements could qualify as reason to withdraw for the study.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Subjects withdrawing from the study will be contacted 2 weeks following last dose of study drug to ensure that there have been no adverse events. Participants that withdraw may be replaced to allow for achievement of target enrollment.

5 Study Drug

5.1 Description

Inhaled nitrite (Stage I): Inhaled, nebulized placebo or inhaled nebulized sodium nitrite (80 mg 3 times daily) was given at a minimum of 4 hours apart, with the first dose starting at the beginning of the active part of the day (for example, 8:00, 12:00, and 16:00). The Participant dispersed the doses by at least 4 hours and delivers them over their normal active day time. The doses do not need to be given at the same time each day if the Participant's active period varies by day, for example if the Participant arises later on the weekends.

Inhaled, nebulized sodium nitrite or placebo was administered utilizing the Phillips I-neb AAD nebulizer over 10-15 minutes for each dose. The nebulizer device has a Participant logging system that automatically records adherence and compliance with protocol-specified treatments. The device log was reviewed at the time of ET sessions by the research coordinator with study participants to optimize adherence.

Oral Nitrite (Stage II): Because the inhaled form of nitrite is no longer available, the oral formulation of sodium nitrite will be used in Stage II of the study to test the study hypotheses. Sodium nitrite capsules and matching placebo capsules will be compounded by The University of Iowa Pharmaceuticals, in Iowa City, IA, then shipped to the Research pharmacy at the University of Pittsburgh according to specifications in the IND of Dr. Mark Gladwin for ongoing stability testing, and then study drug will be shipped to Mayo Clinic, where they will be stored and dispensed by the Mayo Clinic Research Pharmacy.

Oral sodium nitrite will be administered at a dose of 40 mg capsule, 3 times daily, using the same dosing frequency as in Stage I of the study with inhaled nitrite. The Participant should disperse the doses by at least 4 hours and deliver them over their normal active day time. The doses do not need to be given at the same time each day if the Participant's active period varies by day, for example if the Participant arises later on the weekends.

Permitted dose adjustment: If significant headache occurs, participants will be encouraged to treat headaches with acetaminophen. If participants cannot tolerate the 80 mg dose of inhaled nitrite, because of headaches, despite acetaminophen, they will be able to down-titrate to the 46 mg dose. If a dose is missed, the 4 hour minimum regimen should be resumed as prescribed with the next scheduled dose. An extra dose or increase in dose should not be taken to make up for the missed dose.

For the oral dose, headaches will be treated with acetaminophen as above. If participants cannot tolerate the study medication 40 mg three times daily even with acetaminophen, the dosage can be reduced to 20 mg three times daily. If 20 mg three times daily is not tolerated, the frequency can be reduced to twice daily or once daily, in consultation with study staff. It will be critically

important that the single day dose be administered 30 minutes prior to exercise training visits and patients will be instructed of this.

If a participant has a temporary discontinuation for reasons other than intolerance, (e.g., such as hospitalization, lost device, etc), the investigator can restart at the last tolerated dose.

Drug interactions: Potential excess nitric oxide mediated effects (e.g., hypotension, headache) might be observed with co-administration of organic nitrates or phosphodiesterase-5 inhibitors or soluble guanylyl cyclase activators. Therefore, co-administration of nitrates, any phosphodiesterase-5 inhibitor formulation or soluble guanylyl cyclase activators is strictly contraindicated due to the risk of hypotension. There are no other known drug interactions and study drug absorption is not affected by food or administration of other inhalers (inhaled steroids or bronchodilators).

5.2 Treatment Regimen

Inhaled nitrite (Stage I): The dose used for this part of the study was 80 mg three times per day.

Oral nitrite (Stage II): In Stage II of the study, participants will receive an oral dose of 40 mg sodium nitrite or matching placebo three times daily. If there is intolerance, patients may (under the supervision of study staff) down-titrate to 20 mg tid dosing, or 20 mg at lower frequency (as noted above).

5.3 Method for Assigning Subjects to Treatment Groups

Medidata, Rave, and *Balance* will be used for clinical data management and randomization. These tools are institutionally supported and allow for rapid database development. The implementation of the software within a project includes training on the system's use, robust error checking, security considerations, and full audit trail. Balance, which is integrated into *Rave*, allows for a variety of randomization and minimization algorithms.

5.4 Preparation and Administration of Study Drug

Drug dispensing will be managed by the research pharmacy who will obtain and store supplies from the contracted drug supply vendors for both the inhaled and oral nitrite/placebo formulations. At baseline, week 4, and week 8 study visits, participants will receive a sufficient supply of inorganic nitrite or placebo to permit three doses a day for 4 weeks (Stage I). At baseline, and week 4 participants will receive a sufficient supply of inorganic nitrite or placebo to permit three doses a day until final visit (Stage II). To account for potential damage or loss of drug, additional study drug doses will be supplied.

Participants will be instructed to take the medication as required by the protocol, and compliance will be discussed weekly with the participants and assessed every other week (at the time of QOL assessment). Adherence with the inhaled nitrite formulation will also be assessed by download of the compliance data from the nebulizer device, with oral nitrite adherence will be assessed by pill

counts. Participants will be instructed to bring the nebulizer (Stage I only) and unused drug supplies (inhaled or pills) to study visits where drug is dispensed.

5.5 Subject Compliance Monitoring

Inhaled nitrite (Stage I): The I-neb device has a Participant logging system that automatically records adherence and compliance with protocol-specified treatments. The device log was reviewed every other week by the research coordinator with study participants to optimize adherence in Stage I.

Oral nitrite (Stage II): Administration of the oral capsule form of nitrite is expected to be much easier for participants compared with the inhaled delivery system in Stage I. Adherence will be encouraged through phone calls with participants and monitored by pill counts at weeks 4, 8, and 12.

5.6 Prior and Concomitant Therapy

Participants should be treated with standard HFpEF strategies (diuretics for congestion, blood pressure control and heart rate control if Participant is in atrial fibrillation) as per recommended guidelines. Participants should be on stable medications and with adequate blood pressure control prior to entry as outlined in the entry criteria. Further adjustment of diuretics or blood pressure medications during the study period is discouraged and should only be performed according to new and clinically compelling worsening of clinical status. As above, therapy with organic nitrates, phosphodiesterase-5 inhibitors or soluble guanylyl cyclase activators is contra- indicated during the study period.

Data for baseline medication usage will be obtained for descriptive purposes at Study Visit 1.

5.7 Packaging

The inhaled nitrite drug or matching placebo was supplied in one ml squeezable plastic ampules. The ampules are produced in snap-apart groups of 5 vials and are placed in a nitrogen filled pouch. Participants are instructed to open and utilize one pouch at a time. The medication is stable at room temperature.

The oral nitrite drug or matching placebo using in Stage II will be supplied in capsules compounded by The University of Iowa Pharmaceuticals, Iowa City, IA, then shipped to the Research pharmacy at the University of Pittsburgh according to specifications in the IND of Dr. Mark Gladwin then stored and dispensed by the Research pharmacy at the Mayo Clinic.

5.8 Blinding of Study

Only research pharmacy staff will be aware of randomization scheme and all study personnel and subjects will remain blinded to the identity of study drug. Placebo and nitrite ampules (Stage I) and capsules (Stage II) have an identical appearance assuring maintenance of the blind.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Drug dispensing will be managed by the research pharmacy who will obtain and store supplies from the contracted drug supply vendor. At the first study visit, participants will receive a sufficient supply of inorganic nitrite or placebo ampules (Stage I) or capsules (Stage II) to permit three doses a day for 4 weeks. To account for potential damage or loss of study drug, additional study drug doses will be supplied.

Used and unused investigational product can be destroyed at the site according to accepted pharmacy practice, local and national guidelines, using the site's destruction procedure. A copy of the current investigational product (IP) destruction SOP should be maintained in the pharmacy section of the Regulatory Binder and available for review in case of an audit.

Study IP must not be destroyed until IP accountability has been documented and verified. Study IP destruction should be documented within the Participant Specific IP Accountability Log (or equivalent tracking system).

5.9.2 Storage

Trial products (both unused and in-use) should not be exposed to moisture but can be stored at room temperature. For inhaled nitrite, the plastic ampules come in a nitrogen filled foil pouch in snap-apart groups of 5. Participants are instructed to open one pouch at a time and utilize each ampule before opening another pouch. Oral nitrite is stored at room temperature in accordance with the manufacturer's instructions.

5.9.3 Dispensing of Study Drug

Study drug will be provided by research pharmacy throughout the trial. Study drug may be mailed to participants if circumstances prevent them from collecting the drug in person, cause them to miss scheduled visits or any other unforeseen circumstances arise.

For the inhaled nitrite formulation, the device has a Participant logging system that automatically records adherence and compliance with protocol-specified treatments. The device log will be reviewed every other week at ET sessions by the research coordinator with study participants to optimize adherence. For the oral formulation, adherence will be monitored by study staff using pill counts at follow up visits (see schedule of assessments).

Regular study drug reconciliation will be performed to document drug assigned, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team.

5.9.4 Return or Destruction of Study Drug

Participants are instructed to return all used, partly used and unused trial product at the 12 week visit (4, 8 and 12 week visit for Stage I, which has been completed). Returned trial product(s) (used, partly used or unused including empty packaging material) must be stored separately from the non-allocated trial product(s) until drug accountability has been reconciled. The investigators will keep track of all received, used, partly used and unused trial products.

6 Study Procedures

6.1 Screening Visit 1

At Visit 1, patients will provide written informed consent followed by history and physical examination performed by a cardiologist. Medications will be documented. Participants will complete a blood draw in the CRTU where (NT-proBNP levels, blood methemoglobin, blood sulfhemoglobin, fasting blood glucose and insulin levels will be tested. An optional blood collection drawing an additional 20mL's of whole blood will be collected, separated into serum and plasma by the CRTU and stored by BAP. This will be done at both the screening and final visits and stored for additional testing. We are storing the blood for future analysis and will be examining circulating inflammatory biomarkers, adipokines and how they relate to adipose tissue and HFpEF symptom severity. Assays to be run at a later date will include (subject to change): C-reactive protein (C-RP), Interleukin-**3** (IL-1β), Interleukin 6 (IL-6), Leptin, and Adiponectin.
Assessment of endothelial function, baseline echocardiography, dual energy X-ray absorptiometry (DEXA) and a QOL assessment consisting of the KCCQ and SF 36 will be performed. Maximal effort cardiopulmonary exercise testing with expired gas analysis will then be performed with simultaneous echocardiography. Borg symptom severity (effort and dyspnea scores) will be recorded during standardized exercise training at a common objective workload, 2 mph on treadmill at 2° grade for 5 minutes.

Following visit 1 assessments, subjects will be instructed on the use of accelerometer (AXM) devices and devices will be dispensed. Participants are to wear AXM at all times starting at Visit 1. Participants will be randomized in a double-blind fashion at the time but will not start active treatment, and study drug will not be dispensed until the first ET visit (below).

In contrast, Mayo health system participants (who undergo ET visits outside of Mayo Rochester) will receive study drug and supplies during the screening visit, as part of the nebulizer education the participant will be instructed on how to use the nebulizer and will self-administer a saline treatment (for Stage I). Mayo health system participants will be instructed to start assigned study drug the day prior to their initial cardiac rehab visit (ET1).

6.2 Exercise Training (ET) Visits

Following the screening visit 1, subjects will enroll in standard cardiopulmonary exercise training (cardiac rehab) in the Mayo Cardiovascular Health Clinic (CVHC). This will involve ~1 hour sessions 3 times weekly wherein subjects participate in a combination of endurance and strength training as per current practice in the Mayo CVHC. As a result of COVID-19, Mayo Clinic

(Rochester and Health System sites) cardiac rehabilitation centers remain closed or are open with significantly reduced capacity. Thus, we feel it prudent to offer our participants (who are all considered high-risk for contracting COVID-19) a home-based cardiac rehabilitation program (HBCR). This program will model the center-based cardiac rehab program implemented by the Mayo Clinic CVHC. Participants who agree to complete the HBCR program will be seen in clinic for their baseline assessments, 6 minute walk, and given a home exercise protocol. A member from the study team will follow up with patient twice weekly to assess program compliance, discuss successes/barriers with the exercise program, provide modifications to the exercise program (if necessary) and offer support and encouragement.

Once weekly, adverse events, safety and tolerability will be assessed., along with promotion of adherence to training regimen (for example on the third ET visit of each week). For Mayo health system participants this assessment will be done during a weekly telephone call. Every other week, study drug compliance information will be downloaded from the I-Neb device (for Stage I), reviewed, and discussed with the participant. For Mayo health system participants the I Neb device will be downloaded and reviewed during the week 4 visit, week 8 visit and final visit (for Stage I). For Stage II, adherence will be assessed by pill counts at these visits and review of diary.

ET Visit 1:
At the first ET visit (ET1), subjects received training on the use of I-neb device to administer study drug (for Stage I only). For Stage II, subjects will take their first dose of oral study drug 30 minutes prior to the initiation of the ET session (at ET1 and all training visits). One to two days after ET Visit 1, a study team member will call the participant at home to ask about compliance and tolerability of study drug.

Prior to start of exercise training at ET1 and each subsequent ET visit, subjects will administer one dose of study drug (if the Visit is in the morning this will be the first dose of the day, if the Visit is in the afternoon, this will be the second dose of the day but must be separated from the first dose by ≥4 hours). For inhaled study drug (Stage I), this was administered immediately prior to ET visit. For the oral study drug (Stage II), the capsule is to be taken 30 minutes prior to the start of the ET visit.

At one of the ET sessions every other week, measurement of QOL will be performed using the Kansas City Cardiomyopathy Questionnaire (KCCQ) and Borg symptom severity (effort and dyspnea scores) will be recorded during standardized exercise training at a common objective workload, 2 mph on treadmill at 2° grade for 5 minutes. Mayo health system participants will complete the KCCQ via biweekly phone visits with the study coordinator.

6.3 ET Visits 12 (week 4) and 24 (week 8)

At one of the ET visits during week 4 and week 8, the following additional assessments will be performed: New medications over the last 4 weeks will be documented. Blood tests (NT- proBNP levels, blood methemoglobin, blood sulfhemoglobin, fasting blood glucose and insulin levels will be drawn in the CRTU. Data from the accelerometer will be downloaded from the device and the device will be exchanged with a fully charged device. Study drug will be administered prior to

start of training, as at all other ET visits. (Participants will receive additional study drug supplies from research pharmacy for Stage I). Mayo health system participants will be required to do the week 4 and week 8 visits at Mayo Clinic Rochester; they cannot be completed during an ET visit at their participating cardiac rehab site.

6.4 Final Visit

The Final Visit of the drug interventional part of the study for Stage I occurred at week 12

This visit will include all of the assessments performed in Screening Visit 1 (see above). Participants will be asked not to take their study medications the day of this visit; they will be asked to administer a treatment directly prior to the final visit CPET under direct investigator observation. For Stage I, inhaled study drug was administered immediately prior to CPET. For Stage II, oral study drug is administered (under direct observation by study staff) 30 minutes prior to CPET. AXM data will be downloaded and devices returned or changed out. Participants will return all other study materials at the final visit and all unused study drug.

At the Final ET Visit (week 12), participants will be offered enrollment in an ancillary study to evaluate the effects of behavioral/motivational intervention on chronic activity levels and QOL. Subjects agreeing to this part of the trial will then be randomized to behavioral/motivational intervention (motivational phone calls twice weekly with one investigator visit per week) or standard care (no additional contact. Participants will continue to wear AXM devices following this visit to assess how well increases in chronic activity levels are maintained following ET intervention. Involvement in this part of the trial is optional.

Study subjects not participating in the ancillary study will be contacted by phone 2 weeks following the final ET visit (week 12) to verify that there have been no adverse events.

6.5 Ancillary Study Visits

6.5.1 Weekly Ancillary Study Visits

Subjects who agree to participate in the ancillary study will be randomized to standard care or behavioral/motivation intervention (once weekly visits with investigator +2 phone calls per week) to evaluate durability of ET effects.

6.5.2 Final Ancillary Study Visit

The final ancillary study visit will occur 8-10 weeks after ET Visit 36. This visit will include a safety assessment, DEXA, cardiopulmonary exercise test, AXM return and final data download, repeat QOL assessment, Borg symptom severity (effort and dyspnea scores) will be recorded during standardized exercise training at a common objective workload, 2 mph on treadmill at 2° grade for 5 minutes.

6.5.3 Optional MRI and Muscle biopsy Study

Subjects who agree to participate in the MRI ancillary study will undergo contrast MRI at the beginning of the trial (after consent but prior or as close to the start of exercise training) and again

at the conclusion of the drug intervention portion of the trial (within 2 weeks). Subjects who agree to participate in the optional muscle biopsy study will undergo a muscle biopsy at the beginning of the trial (after consent but prior or as close to the start of exercise training) and again as close to the end of ET as possible.

Study Assessments:

Accelerometry (AXM): daily activity will be assessed using externally-worn hip accelerometers (GT3XP-BTLE, Actigraph; Pensacola, FL), which participants will receive along with a belt at Visit 1. The belt will be tailored by the site study coordinators to provide a comfortable fit over or under clothing. Participants will wear the belt continuously throughout the study, including while sleeping. The device will be removed only for bathing or swimming.

Baseline (pre-intervention) daily activity will be assessed from AXM data obtained following Visit 1 but prior to ET1 (1-2 weeks). AXM data will be downloaded from the devices and units exchanged (to ensure adequately charged batteries) at least every 4 weeks. Mayo health system participants will be sent home with an accelerometer charger and instructed to charge the device daily while it is off for showering or bathing. At the final visit on-trial accelerometer data will be downloaded.

Subjects will be encouraged to walk for at least 30-60 minutes on non ET-days and will record outpatient activity levels in a log book to compare with accelerometer recorded activity levels. Subjects with low reported activity in log books will be encouraged to increase activity levels.

Assessment of Exercise tolerance will be performed by measuring Borg perceived effort (6-20 scale) and dyspnea (0-10 scale) scores during matched, low-level submaximal effort (2 mph on treadmill at 2° grade for 5 minutes). This assessment will be performed at Visit 1, on the third ET visit of each week (i.e. ET3, ET6, ET9…up to ET36), and at Visit 2. Submaximal exercise assessment of symptom severity at Visits 1 and 2 will occur *after* the primary endpoint assessment of peak exercise capacity. Patient-reported activity levels will be assessed by the IPAQ questionnaire, which will be completed at the screening visit and final endpoint visit (week 12).

Measurement of QOL will be performed every other week at ET sessions using the Kansas City Cardiomyopathy Questionnaire (KCCQ).[56, 57] The KCCQ score reflects clinical status in HF with high fidelity;[56, 57] the score changes rapidly with clinical improvement or deterioration,[58, 59] is strongly associated with outcome,[60] and performs equally well in HFpEF and HFrEF.[61]The SF36 will be completed at the screening visit and final endpoint visit (week 12).

Endothelial function will be assessed at Visit 1 and Visit 2 using digital arterial tonometry (reactive hyperemia peripheral arterial tonometry, RH-PAT, EndoPAT, Itamar Medical).[9] These noninvasive assessments are routinely performed for both research and clinical care purposes. Changes in activity level, QOL and symptoms will then be correlated with changes in microvascular endothelial function (RH-PAT).

Assessment of Exercise capacity will be performed at Visit 1 (pre drug) and Visit 2 (week 10 for Stage II of study) using expired gas analysis in the laboratory of Dr. Bruce Johnson (Joseph 4).

Subjects participating in the additional ancillary study will also undergo an additional cardiopulmonary exercise test at the final ancillary study visit. Participants will undergo standard, maximal effort cardiopulmonary testing on a semi-recumbent cycle ergometer to maximal subjective effort level with assessments of gas exchange as described below:

- Measurement of Gas Exchange and Ventilation will be performed during exercise testing to measure the volumes of VO2, carbon dioxide produced (VCO2), breathing frequency (fb), tidal volume (VT), minute ventilation (VE), partial pressure of end-tidal oxygen and carbon dioxide (PETO2 and PETCO2), and derived variables (e.g., respiratory exchange ratio, VE/VCO2, etc.) using a low resistance, open circuit, automated metabolic system (Medical Graphics Co., St. Paul, MN) integrated to a mass spectrometer (Perkin Elmer, model 1100) as we have previously described.[4,9,13,35,36]

- Cardiac Output (CO) will be measured at rest and during exercise by the acetylene (C2H2) wash-in technique first described by Stout et al., refined by Gan et al. and Nielson et al. and validated against direct Fick in our laboratory.[45-48]

Echocardiography will be performed at rest and during exercise at Visit 1 and Final Visit to estimate central cardiac hemodynamics and ventricular function, including EF, Doppler-estimated pulmonary artery systolic pressure, and LV filling pressures (E/e' ratio).

Dual Energy X-ray Absorptiometry (DEXA) will be performed at the first and final visit in the primary study, and for subjects participating in the ancillary trial, at the final visit of that component, to measure changes in body composition during the trial.

Magnetic Resonance Imaging (MRI) will be performed in subjects participating in the optional MRI substudy to measure cardiac structure and function as well as stiffness along with quantification of central and peripheral adipose tissue. As part of the MRI examination we will measure intramyocardial fat content using multiecho Dixon water and fat separated managing with variable projection (VARPRO). We will also measure interstitial myocardial fibrosis by extracellular volume (ECV) using the MOLLI TI mapping sequence. These additional cardiac MRI measurements will be analyzed offline by our external collaborators, from completely de-identified MRI scan data. No personal health information will be included in the scans that are transmitted for external analysis.

Muscle Biopsy
Will be performed in subjects participating in the optional muscle biopsy substudy to tell us about what improvements we see in the leg muscles, and specifically in the mitochondria of leg muscles

Biological Samples
Venous blood samples will be collected at baseline, week 4, 8, and 12 to assess clinical stability (NT-proBNP) and safety (blood methemoglobin levels). Methemoglobin levels will be used as safety markers during the study and will be shared with the DSMB. All female subjects of childbearing potential will undergo a urine pregnancy test at study enrollment and end of study.

7 Statistical Plan

7.1 Statistical Overview

7.2 The full statistical details for this protocol are documented in the supplementary Statistical Analysis Plan (SAP). This protocol provides the key information, namely the justification for the sample size and the details on the primary analysis. The details regarding secondary endpoints, subgroup analyses, missing data and analysis sets are provided in the SAP.Sample Size Determination

In the RELAX trial, the mean±SD change in peak VO_2 with placebo in subjects with HFpEF treated for 6 months was 0±2.0 ml/kg/min,[36] and in a previous trial of ET in HFpEF, the increase in peak VO in the ET arm was +2.3±2.2 ml/kg/min.[22] An increase of 75% (additional 1.5 ml/kg/min) in this change would be considered clinically relevant. Thus, we estimate that a sample size of N=34 participants per group will be required to provide 80% statistical power (two-tailed, alpha=0.05) to detect the difference of 1.5 ml/kg/min between subjects randomized to NO_2^- with ET versus placebo with ET. To account for attrition, we will increase our number of randomized subjects to 40/group (enroll 80 subjects total to accrue 68 total or 34 in each group).

Stage 1 was discontinued early because of loss of inhaled nitrite availability, therefore we will accrue a total of 68 patients with complete data combining the patients with complete data receiving inhaled placebo in Stage I (n=17) with another 51 patients in Stage II (17 receiving placebo and 34 receiving nitrite) to ensure that the study is adequately powered to test the study hypotheses.

7.3 Primary Outcome Analysis

Primary Statistical Hypotheses:

H0: Change from baseline to 12 weeks in Peak VO2 in participants randomized to NO_2^- will be equal to the change in Peak VO2 in participants randomized to placebo.

Ha: The change from baseline to 12 weeks in Peak VO2 will be different between the two randomized groups

Analysis Summary:
The primary statistical hypothesis will be tested using a regression framework. The dependent variable will be the change (baseline to 12 week) in Peak VO2. An indicator variable will be used to indicate the randomized treatment assignment (NO_2^- vs. placebo as the referent). This analysis is equivalent to a two-sample t-test. While the study design is randomized, the number of participants is relatively small, so imbalance between putative confounding variables may be observed. To address this, we will conduct an a priori sensitivity analysis that will adjust for baseline Peak VO2, BMI, Sex, and Age group strata through an extension of the regression model used for the primary analysis. The type III estimate of the treatment effect will be compared to the unadjusted estimate to assess the robustness of the randomization process.

Statistical testing for the primary analysis will be two-sided at the alpha=0.05 level of significance.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event

An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event

Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

For this study, the study treatment follow-up period is defined as 2 weeks following the last administration of study treatment. The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up.

Preexisting Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event

All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Hospitalization, Prolonged Hospitalization or Surgery

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to

be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.2.1 Assessment of Causal Relationship

A medically-qualified investigator must assess the relationship of any AE to the use of study drug, based on available information, using the following guidelines:

- **Not related:** There is not a reasonable causal relationship to the investigational product and the adverse event.
- **Unlikely related:** No temporal association or the cause of the event has been identified, or the drug or biologic cannot be implicated.
- **Possibly related:** There is reasonable evidence to suggest a causal relationship between the drug and adverse event.
- **Related:** There is evidence to suggest a causal relationship, and the influence of other factors is unlikely.

8.2.2 Assessment of Adverse Event Severity

The determination of adverse event severity rests on medical judgment of a medically-qualified Investigator. The severity of the AEs will be graded using the following definitions:

- **Mild:** Awareness of sign, symptom, or event, but easily tolerated;
- **Moderate:** Discomfort enough to cause interference with usual activity and may warrant intervention;
- **Severe:** Incapacitating with inability to do usual activities or significantly affects clinical status, and warrants intervention.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When a serious adverse event has been identified, the study team will take appropriate action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

Information collected on the adverse event worksheet

- Subject's name:
- Medical record number:
- Disease (if applicable):

- The date the adverse event occurred:
- Description of the adverse event:
- Relationship of the adverse event to the research (drug, procedure, or intervention):
- If the adverse event was expected:
- The severity of the adverse event:
- If any intervention was necessary:
- Resolution: (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution:

The investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs will be reported to the IRB.

8.3.2 Sponsor-Investigator reporting: Notifying the FDA

The investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Findings from other studies in human or animals that suggest a significant risk in humans exposed to the drug will be reported. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigators initial receipt of the information about the event.

8.4 Unblinding Procedures

The investigators will be given access to the treatment code for their Participants for emergency un-blinding. Due to the safety profile of inorganic nitrite it is anticipated that there should be no

need to un-blind the study drug for any reason. Any suspected study drug-related events should be treated as though the Participant received active therapy.

Randomization data are kept strictly confidential, accessible only to authorized persons, until the time of un-blinding.

8.5 Stopping Rules

The study would be stopped only if advised because of unanticipated adverse events as determined by the Data and Safety Monitoring Board (DSMB).

8.6 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the conduct of the study and safety of subjects. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.6.1 Internal Data and Safety Monitoring Board

An internal Data and Safety Monitoring Board (DSMB) has been formed, chaired by Dr. Bernard Gersh. The DSMB Charter is included as an Attachment.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated

instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF will be recorded. All missing data will be explained.

Medidata Rave and *Balance* will be used for clinical data management and randomization (see *Facilities and Resources* for expanded description). These tools are institutionally supported and allow for rapid database development. The implementation of the software within a project includes training on the system's use, robust error checking, security considerations, and full audit trail. Balance, which is integrated into *Rave*, allows for a variety of randomization and minimization algorithms.

9.4 Records Retention

The investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents.

The investigator will retain the specified records and reports for;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Access to and Retention of Research Data Policy" http://mayocontent.mayo.edu/research-policy/MSS_669717

Whichever is longer.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored on a routine basis during the conduct of the trial. The Mayo Clinic Office of Research Regulatory Support will provide periodic study monitoring as a service for the sponsor-investigator, to verify the validity and integrity of the data and protection of human research subjects. Written monitoring reports will be provided to the Sponsor-Investigator and should be provided to the IRB at the time of continuing review. This will assist with compliance with Food and Drug Administration regulations and GCP Guidelines.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or the subject's legally authorized representative, and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

This study is financed through a grant from the US National Institute of Health.

Study drug for Stage 1 was provided by Aires Pharmaceuticals.

Study drug for Stage 2 of the trial (oral nitrite/placebo) is being compounded at The University of Iowa Pharmaceuticals, in Iowa City, IA, shipped to the Research Pharmacy at the University of Pittsburgh, and then, shipped to Mayo Clinic where it will be stored and dispensed by the Mayo Research Pharmacy.

12.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must

have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor-investigator prior to participation in this study.

13 Publication Plan

The primary responsibility for publication of the results lies with the principal investigator. The trial will be registered on ClinicalTrials.gov.

14 References

1. Heidenreich PA, Albert NM, Allen LA, Bluemke DA, Butler J, Fonarow GC, Ikonomidis JS, Khavjou O, Konstam MA, Maddox TM, Nichol G, Pham M, Pina IL, Trogdon JG. Forecasting the impact of heart failure in the United States: A policy statement from the American heart association. Circ Heart Fail. 2013;6:606-19. PMCID: PMC3908895.

2. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. N Engl J Med. 2006; 355:251-59.

3. Borlaug BA, Paulus WJ. Heart failure with preserved ejection fraction: Pathophysiology, diagnosis, and treatment. Eur Heart J. 2011;32: 670-79. PMCID: PMC3056204.

4. Borlaug BA, Melenovsky V, Russell SD, Kessler K, Pacak K, Becker LC, Kass DA. Impaired chronotropic and vasodilator reserves limit exercise capacity in patients with heart failure and a preserved ejection fraction. Circulation. 2006; 114: 2138-47.

5. Borlaug BA, Lam CS, Roger VL, Rodeheffer RJ, Redfield MM. Contractility and ventricular systolic stiffening in hypertensive heart disease insights into the pathogenesis of heart failure with preserved ejection fraction. J Am Coll Cardiol. 2009;54:410-18. PMCID: PMC2753478.

6. Tan YT, Wenzelburger F, Lee E, Heatlie G, Leyva F, Patel K, Frenneaux M, Sanderson JE. The pathophysiology of heart failure with normal ejection fraction: Exercise echocardiography reveals complex abnormalities of both systolic and diastolic ventricular function involving torsion, untwist, and longitudinal motion. J Am Coll Cardiol. 2009; 54:36- 46.

7. Phan TT, Abozguia K, Nallur Shivu G, Mahadevan G, Ahmed I, Williams L, Dwivedi G, Patel K, Steendijk P, Ashrafian H, Henning A, Frenneaux M. Heart failure with preserved ejection fraction is characterized by dynamic impairment of active relaxation and contraction of the left ventricle on exercise and associated with myocardial energy deficiency. J Am Coll Cardiol. 2009; 54: 402-09.

8. Maeder MT, Thompson BR, Brunner-La Rocca HP, Kaye DM. Hemodynamic basis of exercise limitation in patients with heart failure and normal ejection fraction. J Am Coll Cardiol. 2010;56: 855-63.

9. Borlaug BA, Olson TP, Lam CS, Flood KS, Lerman A, Johnson BD, Redfield MM. Global cardiovascular reserve dysfunction in heart failure with preserved ejection fraction. J Am Coll Cardiol. 2010;56:845-54. PMCID: PMC2950645.

10. Borlaug BA, Nishimura RA, Sorajja P, Lam CS, Redfield MM. Exercise hemodynamics enhance diagnosis of early heart failure with preserved ejection fraction. Circ Heart Fail. 2010;3: 588-95. PMCID: PMC3048586.

11. Borlaug BA, Jaber WA, Ommen SR, Lam CS, Redfield MM, Nishimura RA. Diastolic relaxation and compliance reserve during dynamic exercise in heart failure with preserved ejection fraction. Heart. 2011;97:964-69. PMCID: PMC3767403.

12. Tartiere-Kesri L, Tartiere JM, Logeart D, Beauvais F, Cohen Solal A. Increased proximal arterial stiffness and cardiac response with moderate exercise in patients with heart failure and preserved ejection fraction. J Am Coll Cardiol. 2012;59:455-61.

13. Abudiab MM, Redfield MM, Melenovsky V, Olson TP, Kass DA, Johnson BD, Borlaug BA. Cardiac output response to exercise in relation to metabolic demand in heart failure with preserved ejection fraction. Eur J Heart Fail. 2013;15:776-785. PMCID: PMC3857919.

14. Haykowsky MJ, Brubaker PH, John JM, Stewart KP, Morgan TM, Kitzman DW. Determinants of exercise intolerance in elderly heart failure patients with preserved ejection fraction. J Am Coll Cardiol. 2011;58:265-74. PMCID: PMC3272542.

15. Bhella PS, Prasad A, Heinicke K, Hastings JL, Arbab-Zadeh A, Adams-Huet B, Pacini EL, Shibata S, Palmer MD, Newcomer BR, Levine BD. Abnormal hemodynamic response to exercise in heart failure with preserved ejection fraction. Eur J Heart Fail. 2011; 13:1296- 1304. PMCID: PMC3220394.

16. Kitzman DW, Nicklas B, Kraus WE, Lyles MF, Eggebeen J, Morgan TM, Haykowsky M. Skeletal muscle abnormalities and exercise intolerance in older patients with heart failure and preserved ejection fraction. Am J Physiol Heart Circ Physiol. 2014;306:H1364-70. PMCID: PMC4010668.

17. Haykowsky MJ, Kouba EJ, Brubaker PH, Nicklas BJ, Eggebeen J, Kitzman DW. Skeletal muscle composition and its relation to exercise intolerance in older patients with heart failure and preserved ejection fraction. Am J Cardiol. 2014;113:1211-16. PMCID: PMC4282135.

18. Schwartzenberg S, Redfield MM, From AM, Sorajja P, Nishimura RA, Borlaug BA. Effects of vasodilation in heart failure with preserved or reduced ejection fraction implications of distinct pathophysiologies on response to therapy. J Am Coll Cardiol. 2012;59:442-51. PMCID: PMC3740089.

19. van Heerebeek L, Hamdani N, Falcao-Pires I, Leite-Moreira AF, Begieneman MP, Bronzwaer JG, van der Velden J, Stienen GJ, Laarman GJ, Somsen A, Verheugt FW, Niessen HW, Paulus WJ. Low myocardial protein kinase g activity in heart failure with preserved ejection fraction. Circulation. 2012; 126:830-39.

20. Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: Comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. J Am Coll Cardiol. 2013;62:263-71.

21. Smart N, Haluska B, Jeffriess L, Marwick TH. Exercise training in systolic and diastolic dysfunction: Effects on cardiac function, functional capacity, and quality of life. Am Heart J. 2007;153: 530-36.

22. Kitzman DW, Brubaker PH, Morgan TM, Stewart KP, Little WC. Exercise training in older patients with heart failure and preserved ejection fraction: A randomized, controlled, single-blind trial. Circ Heart Fail. 2010;3:659-67. PMCID: PMC3740089.

23. Edelmann F, Gelbrich G, Dungen HD, Frohling S, Wachter R, Stahrenberg R, Binder L, Topper A, Lashki DJ, Schwarz S, Herrmann-Lingen C, Loffler M, Hasenfuss G, Halle M, Pieske B. Exercise training improves exercise capacity and diastolic function in patients with heart failure with preserved ejection fraction: Results of the ex-dhf (exercise training in diastolic heart failure) pilot study. J Am Coll Cardiol. 2011;58:1780-91.

24. O'Connor CM, Whellan DJ, Lee KL, Keteyian SJ, Cooper LS, Ellis SJ, Leifer ES, Kraus WE, Kitzman DW, Blumenthal JA, Rendall DS, Miller NH, Fleg JL, Schulman KA, McKelvie RS, Zannad F, Pina IL. Efficacy and safety of exercise training in patients with chronic heart failure: Hf-action randomized controlled trial. JAMA. 2009;301:1439-50. PMCID: PMC2916661.

25. Flynn KE, Pina IL, Whellan DJ, Lin L, Blumenthal JA, Ellis SJ, Fine LJ, Howlett JG, Keteyian SJ, Kitzman DW, Kraus WE, Miller NH, Schulman KA, Spertus JA, O'Connor CM, Weinfurt KP. Effects of exercise training on health status in patients with chronic heart failure: Hf-action randomized controlled trial. JAMA. 2009;301:1451-59. PMCID: PMC2690699.

26. Haykowsky MJ, Brubaker PH, Stewart KP, Morgan TM, Eggebeen J, Kitzman DW. Effect of endurance training on the determinants of peak exercise oxygen consumption in elderly

patients with stable compensated heart failure and preserved ejection fraction. J Am Coll Cardiol. 2012;60:120-28. PMCID: PMC3429944.

27. Kitzman DW, Brubaker PH, Herrington DM, Morgan TM, Stewart KP, Hundley WG, Abdelhamed A, Haykowsky MJ. Effect of endurance exercise training on endothelial function and arterial stiffness in older patients with heart failure and preserved ejection fraction: A randomized, controlled, single-blind trial. J Am Coll Cardiol. 2013;62:584-92. PMCID: PMC3740089.

28. Sullivan MJ, Higginbotham MB, Cobb FR. Exercise training in patients with severe left ventricular dysfunction. Hemodynamic and metabolic effects. Circulation. 1988;78:506-15.

29. Esposito F, Reese V, Shabetai R, Wagner PD, Richardson RS. Isolated quadriceps training increases maximal exercise capacity in chronic heart failure: The role of skeletal muscle convective and diffusive oxygen transport. J Am Coll Cardiol. 2011;58:1353-62. PMCID: PMC3180857.

30. Borlaug BA. The pathophysiology of heart failure with preserved ejection fraction. Nat Rev Cardiol. 2014;11:507-15

31. Keteyian SJ, Squires RW, Ades PA, Thomas RJ. Incorporating patients with chronic heart failure into outpatient cardiac rehabilitation: Practical recommendations for exercise and self-care counseling-a clinical review. J Cardiopulm Rehabil Prev. 2014;34:223-32.

32. Paulus WJ, Tschope C, Sanderson JE, Rusconi C, Flachskampf FA, Rademakers FE, Marino P, Smiseth OA, De Keulenaer G, Leite-Moreira AF, Borbely A, Edes I, Handoko ML, Heymans S, Pezzali N, Pieske B, Dickstein K, Fraser AG, Brutsaert DL. How to diagnose diastolic heart failure: A consensus statement on the diagnosis of heart failure with normal left ventricular ejection fraction by the heart failure and echocardiography associations of the european society of cardiology. Eur Heart J. 2007;28:2539-50.

33. Borbely A, Falcao-Pires I, van Heerebeek L, Hamdani N, Edes I, Gavina C, Leite-Moreira AF, Bronzwaer JG, Papp Z, van der Velden J, Stienen GJ, Paulus WJ. Hypophosphorylation of the stiff n2b titin isoform raises cardiomyocyte resting tension in failing human myocardium. Circ Res. 2009;104:780-86.

34. van Heerebeek L, Borbely A, Niessen HW, Bronzwaer JG, van der Velden J, Stienen GJ, Linke WA, Laarman GJ, Paulus WJ. Myocardial structure and function differ in systolic and diastolic heart failure. Circulation. 2006;113:1966-73.

35. Redfield MM, Borlaug BA, Lewis GD, Mohammed SF, Semigran MJ, Lewinter MM, Deswal A, Hernandez AF, Lee KL, Braunwald E. Phosphdiesterase-5 inhibition to improve

clinical status and exercise capacity in diastolic heart failure (relax) trial: Rationale and design. Circ Heart Fail. 2012;5:653-59.

36. Redfield MM, Chen HH, Borlaug BA, Semigran MJ, Lee KL, Lewis G, Lewinter MM, Rouleau JL, Bull DA, Mann DL, Deswal A, Stevenson LW, Givertz MM, Ofili EO, O'Connor CM, Felker GM, Goldsmith SR, Bart BA, McNulty SE, Ibarra JC, Lin G, Oh JK, Patel MR, Kim RJ, Tracy RP, Velazquez EJ, Anstrom KJ, Hernandez AF, Mascette AM, Braunwald E. Effect of phosphodiesterase-5 inhibition on exercise capacity and clinical status in heart failure with preserved ejection fraction: A randomized clinical trial. JAMA. 2013;309:1268-77. PMCID: PMC3530955.

37. Munzel T, Daiber A, Mulsch A. Explaining the phenomenon of nitrate tolerance. Circ Res. 2005;97:618-28.

38. Cosby K, Partovi KS, Crawford JH, Patel RP, Reiter CD, Martyr S, Yang BK, Waclawiw MA, Zalos G, Xu X, Huang KT, Shields H, Kim-Shapiro DB, Schechter AN, Cannon RO, 3rd, Gladwin MT. Nitrite reduction to nitric oxide by deoxyhemoglobin vasodilates the human circulation. Nat Med. 2003;9: 1498-05.

39. Dejam A, Hunter CJ, Tremonti C, Pluta RM, Hon YY, Grimes G, Partovi K, Pelletier MM, Oldfield EH, Cannon RO, 3rd, Schechter AN, Gladwin MT. Nitrite infusion in humans and nonhuman primates: Endocrine effects, pharmacokinetics, and tolerance formation. Circulation. 2007;116:1821-31.

40. Maher AR, Milsom AB, Gunaruwan P, Abozguia K, Ahmed I, Weaver RA, Thomas P, Ashrafian H, Born GV, James PE, Frenneaux MP. Hypoxic modulation of exogenous nitrite-induced vasodilation in humans. Circulation. 2008;117:670-77.

41. Huang Z, Shiva S, Kim-Shapiro DB, Patel RP, Ringwood LA, Irby CE, Huang KT, Ho C, Hogg N, Schechter AN, Gladwin MT. Enzymatic function of hemoglobin as a nitrite reductase that produces no under allosteric control. J Clin Invest. 2005;115:2099-07. PMCID: PMC1177999.

42. Larsen FJ, Weitzberg E, Lundberg JO, Ekblom B. Effects of dietary nitrate on oxygen cost during exercise. Acta Physiol (Oxf). 2007;191:59-66.

43. Bailey SJ, Winyard P, Vanhatalo A, Blackwell JR, Dimenna FJ, Wilkerson DP, Tarr J, Benjamin N, Jones AM. Dietary nitrate supplementation reduces the o2 cost of low-intensity exercise and enhances tolerance to high-intensity exercise in humans. J Appl Physiol (1985). 2009;107:1144-55.

44. van Empel VP, Mariani J, Borlaug BA, Kaye DM. Impaired myocardial oxygen availability contributes to abnormal exercise hemodynamics in heart failure with preserved ejection fraction. J Am Heart Assoc. 2014; Dec 2;3(6). PMCID: PMC4338724.

45. Stout RL, Wessel HU, Paul MH. Pulmonary blood flow determined by continuous analysis of pulmonary n2o exchange. J Appl Physiol. 1975;38:913-18.

46. Gan K, Nishi I, Chin I, Slutsky AS. On-line determination of pulmonary blood flow using respiratory inert gas analysis. IEEE Trans Biomed Eng. 1993;40:1250-59.

47. Nielsen OW, Hansen S, Gronlund J. Precision and accuracy of a noninvasive inert gas washin method for determination of cardiac output in men. J Appl Physiol. 1994;76:1560-65.

48. Johnson BD, Beck KC, Proctor DN, Miller J, Dietz NM, Joyner MJ. Cardiac output during exercise by the open circuit acetylene washin method: Comparison with direct fick. J Appl Physiol. 2000;88:1650-58.

49. Franciosa JA, Goldsmith SR, Cohn JN. Contrasting immediate and long-term effects of isosorbide dinitrate on exercise capacity in congestive heart failure. Am J Med. 1980;69:559-66.

50. Leier CV, Huss P, Magorien RD, Unverferth DV. Improved exercise capacity and differing arterial and venous tolerance during chronic isosorbide dinitrate therapy for congestive heart failure. Circulation. 1983;67:817-22.

51. Elkayam U, Johnson JV, Shotan A, Bokhari S, Solodky A, Canetti M, Wani OR, Karaalp IS. Double-blind, placebo-controlled study to evaluate the effect of organic nitrates in patients with chronic heart failure treated with angiotensin-converting enzyme inhibition. Circulation. 1999;99:2652-57.

52. Borlaug BA, Redfield MM. Diastolic and systolic heart failure are distinct phenotypes within the heart failure spectrum. Circulation. 2011;123:2006-2013. PMCID: PMC3420141.

53. Jehn M, Schmidt-Trucksaess A, Schuster T, Hanssen H, Weis M, Halle M, Koehler F. Accelerometer-based quantification of 6-minute walk test performance in patients with chronic heart failure: Applicability in telemedicine. J Card Fail. 2009;15:334-40.

54. Conraads VM, Spruit MA, Braunschweig F, Cowie MR, Tavazzi L, Borggrefe M, Hill MR, Jacobs S, Gerritse B, van Veldhuisen DJ. Physical activity measured with implanted devices predicts patient outcome in chronic heart failure. Circ Heart Fail. 2014;7:279-87.

55. Kawabata M, Fantoni C, Regoli F, Raffa S, Pastori F, Fratini S, Prentice J, Klein HU, Auricchio A. Activity monitoring in heart failure patients with cardiac resynchronization therapy. Circ J. 2007;71:1885-92.

56. Green CP, Porter CB, Bresnahan DR, Spertus JA. Development and evaluation of the kansas city cardiomyopathy questionnaire: A new health status measure for heart failure. Journal of the American College of Cardiology. 2000;35:1245-55.

57. Spertus JA, Jones PG, Kim J, Globe D. Validity, reliability, and responsiveness of the kansas city cardiomyopathy questionnaire in anemic heart failure patients. Qual Life Res. 2008;17:291-98. PMCID: PMC2238779.

58. Eurich DT, Johnson JA, Reid KJ, Spertus JA. Assessing responsiveness of generic and specific health related quality of life measures in heart failure. Health Qual Life Outcomes. 2006;4:89. PMCID: PMC1675990.

59. Sauser K, Spertus JA, Pierchala L, Davis E, Pang PS. Quality of life assessment for acute heart failure patients from emergency department presentation through 30 days after discharge: A pilot study with the kansas city cardiomyopathy questionnaire. J Card Fail. 2014;20:18-22.

60. Heidenreich PA, Spertus JA, Jones PG, Weintraub WS, Rumsfeld JS, Rathore SS, Peterson ED, Masoudi FA, Krumholz HM, Havranek EP, Conard MW, Williams RE. Health status identifies heart failure outpatients at risk for hospitalization or death. J Am Coll Cardiol. 2006;47:752-56.

61. Joseph SM, Novak E, Arnold SV, Jones PG, Khattak H, Platts AE, Davila-Roman VG, Mann DL, Spertus JA. Comparable performance of the kansas city cardiomyopathy questionnaire in patients with heart failure with preserved and reduced ejection fraction. Circ Heart Fail. 2013;6:1139-46.

62. Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, Deanfield J, Drexler H, Gerhard-Herman M, Herrington D, Vallance P, Vita J, Vogel R. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: A report of the international brachial artery reactivity task force. J Am Coll Cardiol. 2002;39:257-65.

63. Shi Q, Pavey ES, Carter RE. Bonferroni-based correction factor for multiple, correlated endpoints. Pharm Stat. 2012;11:300-09.

15 Attachments

15.1 Schedule of Assessments

							Primary Study:						Ancillary Study:	
Visit windows	Screening Visit/ Day -14 to -7	Baseline Visit/ ET 1/Day 0	Phone f/u Day 1 to 2	Weeks 1 - 3	Wk 4	Weeks 5 - 7	Wk 8	Week 9	Week 10	Final Visit/Week 12	2 WeekF/U Visit	Wks 37 to 46 [C]	Final Visit/ Wk 44 to 46
Informed Consent	X												
Medical Hx	X												
Medication Review	X				X		X			X			
Physical Exam	X									X			
NYHA class	X									X			
CPET	X									X			X
Hemodynamic Echo	X									X			
Lab evaluation [A]	X				X		X			X			
Pregnancy Test	X									X			
DEXA Scan	X									X			X
Endothelial Function (Endopat)	X									X			
QOL assessment (KCCQ)	X	X		Wk 2	X	Wk 6	X		X	X			X
IPAQ	X									X			
SF-36 Questionnaire	X									X			X
Randomization	X												
Study Drug Education	X	X											
Dispense Study Drug		X				Wk5							
Collect un-used study drug					X		X			1x/wk			
Study drug compliance assessment and education			X	1x/wk	1x/wk	1x/wk	1x/wk	1x/wk	1x/wk				
Study drug accountability/ pill counts										X			
Accelerometer Education	X												
Dispense / Exchange / Return accelerometer	X				X		X			WK 12		X[C]	X
Accelerometer Data download					X		X			WK 12		X[C]	X
Exercise Training		X		3x/wk	3x/wk	3x/wk	3x/wk	3x/wk	3x/wk	3x/wk			
Borg Symptom Severity & Exercise Tolerance	X			1x/wk	1x/wk	1x/wk	1x/wk	1x/wk	1x/wk	X			X
AE assessment		X	X	1x/wk	X	1x/wk	X	1x/wk	1x/wk	X	X		X
Optional MRI Study	X (1)									X			X
Optional whole blood, lab draw study	X									X			X

A NT pro BNP, sulfhemoglobin, methemoglobin fasting blood glucose and insulin levels. (1) Optional MRI will be performed prior to or as close to the start of exercise as scheduling allows.

[B] Phone call to take place if not participating in the ancillary study

[C] Participants randomized to behavioral/motivational intervention will have once weekly visits with investigator and 2 phone visits per week.

[D] Exchange/Dispense/ Return accelerometer and download data

IRB 16-001467 V11 Nov. 2019

15 Attachments
15.1 Schedule of Assessments

Inorganic Nitrite To Amplify the Benefits and Tolerability of Exercise Training In Heart Failure With Preserved Ejection Fraction

Revised 16 Jan 2019

SERIES 7	RV Inflow FIESTA (2ch)	Scan Plane	Oblique	Matrix/Nex	224/224/1 NEX
Coil	Coil to cover anatomy	Mode	2D	Phase FOV	1
	SCAN TIMING	Pulse seq.	Fiesta	Freq. Direct	Unswap
#echoes	1	Image Opts.	Gat, Seq, Fast	FC Direction	
TE	Minimum	PSD Name		Phase Correct	No
TR			**ADDITIONAL PARAMETERS**	Shim	Auto
TI		User CV's			**SCANNING RANGE**
Flip Angle	60	Sat		FOV	38 +/-
ETL		Intensity Correct	SCIC	Slice/Space	8/0
BW	125	Acceleration		Locs Per Slab	
Scan Time		Intensity Filter			

IMAGING PARAMETERS **ACQUISITION TIMING**

comments: **On SAX make a plane through the pulmonary valve and the tricuspid valve (image1). Then on the 4 Ch make sure the plane goes through the tip of the RV apex (image 2). You get a plane that shows the RV outflow and pulmonary valve (image 3).**