The inorganic NItrate and eXercise performance in Heart Failure (iNIX-HF) phase II clinical trial: Rationale and study design

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ABSTRACT

Background: Heart failure (HF) is a debilitating and often fatal disease that affects millions of people worldwide. Diminished nitric oxide synthesis, signaling, and bioavailability are believed to contribute to poor skeletal muscle function and aerobic capacity. The aim of this clinical trial (iNIX-HF) is to determine the acute and longer-term effectiveness of inorganic nitrate supplementation on exercise performance in patients with HF with reduced ejection fraction (HFrEF).
Methods: This clinical trial is a double-blind, placebo-controlled, randomized, parallel-arm design study in which patients with HFrEF (n = 75) are randomized to receive 10 mmol potassium nitrate (KNO_3) or a placebo capsule daily for 6 wk. Primary outcome measures are muscle power determined by isokinetic dynamometry and peak aerobic capacity (VO_2peak) determined during an incremental treadmill exercise test. Endpoints include the acute effects of a single dose of KNO_3 and longer-term effects of 6 wk of KNO_3. The study is adequately powered to detect expected increases in these outcomes at $P < 0.05$ with $1-\beta > 0.80$.
Discussion: The iNIX-HF phase II clinical trial will evaluate the effectiveness of inorganic nitrate supplements as a new treatment to ameliorate poor exercise capacity in HFrEF. This study also will provide critical preliminary data for a future 'pivotal', phase III, multi-center trial of the effectiveness of nitrate supplements not only for improving exercise performance, but also for improving symptoms and decreasing other major cardiovascular endpoints. The potential public health impact of identifying a new, relatively inexpensive, safe, and effective treatment that improves overall exercise performance in patients with HFrEF is significant.

1. Background

There are over six million patients with heart failure (HF) in the United States and millions more worldwide [1]. The hallmark symptom of this debilitating and often ultimately fatal disease is exercise intolerance. This is due in part to a diminished peak O_2 consumption (VO_2peak) [2] and especially an exaggerated ventilatory response to exercise, resulting from elevated afferent signaling from the exercising limbs [3]. The muscles of patients with HF are also weaker, slower, and less powerful than those of healthy individuals [4–6], even when participants are carefully matched for physical activity level, muscle mass,

and statin use [6]. Together, these maladaptations lead to functional deficits, loss of independence, and reduced quality of life, and are powerful predictors of mortality in patients with HF [7–10]. Unfortunately, despite treatment advances HF remains not only a mortal disease but also a leading cause of disability. New treatments that operate via novel pathways to improve exercise capacity are therefore needed and have the potential to enhance clinical care dramatically.

Numerous factors underlie the impairments in exercise performance described above. These include a reduction in cardiac output due to ventricular dysfunction [2], alterations in blood flow regulation/distribution [11], abnormalities in muscle energetics [12], and molecular

derangements in the contractile apparatus of muscle itself [13]. In addition, a reduction in nitric oxide (NO) signaling may also play a role. NO regulates many physiological processes vital to exercise performance, including cardiac function [14] and skeletal muscle blood flow [15,16], energy metabolism [17], and contractile function [18]. Importantly, NO bioavailability is reduced in patients with HF, due to a decreased rate of NO synthesis [19] and/or an increased rate of NO destruction resulting from increased oxidative stress [20]. Thus, a relative deficiency in NO likely contributes to the diminished exercise capacity in patients with HF.

Based on the above mechanisms, we [21,22] and others [23–28] have investigated the effects of nitrate (NO_3^-) -rich beetroot juice (BRJ), a source of NO via the $NO_3^- \rightarrow$ nitrite (NO_2^-) $\rightarrow$ NO enterosalivary pathway [29,30], on exercise capacity in patients with HF. Most [21–25], albeit not all [26–28], of these studies have found that BRJ supplementation can ameliorate the functional deficits described above. In particular, we have demonstrated that ingestion of a single dose of NO_3^--rich BRJ can improve muscle speed and power [21] and VO_2peak [22] in patients with non-ischemic HF with reduced ejection fraction (HFrEF) (Fig. 1). The magnitudes of these changes are likely to be clinically relevant. For example, NO_3^- ingestion increased maximal muscle power by 13%, which would be sufficient to erase 1/3rd of the deficit normally observed in HFrEF [6]. Similarly, VO_2peak increased by 8% following NO_3^- intake, which theoretically would reduce the annual risk of cardiac transplantation or death by almost 10% [31]. Notably, these improvements were observed in patients *already receiving* stable, optimal pharmacotherapy, including use of beta blockers, angiotensin converting enzyme inhibitors/receptor blockers, and/or aldosterone antagonists. Indeed, quantitatively the effect of *acute* dietary NO_3^- intake on VO_2peak compares favorably to *chronic* treatment with the first two medications [32–38] (aldosterone antagonists do not alter VO_2peak in patients with HFrEF [39]), and unlike these conventional therapies, dietary NO_3^- also improves muscle power. Finally, this approach provides potential advantages over other methods for increasing NO production and/or signaling in HFrEF, as shown in Table 1.

Inorganic NO_3^- supplementation is therefore a promising new treatment for improving muscle and cardiovascular dysfunction in patients with HFrEF. Before this promise can be realized, however, the above-described findings need to be corroborated in a larger study of such individuals. It also needs to be determined whether the beneficial effects of dietary NO_3^- on VO_2peak and muscle power are maintained, or perhaps even enhanced, with repeated ingestion, or if tolerance or endothelial dysfunction develops as with inorganic nitrates, e.g.,
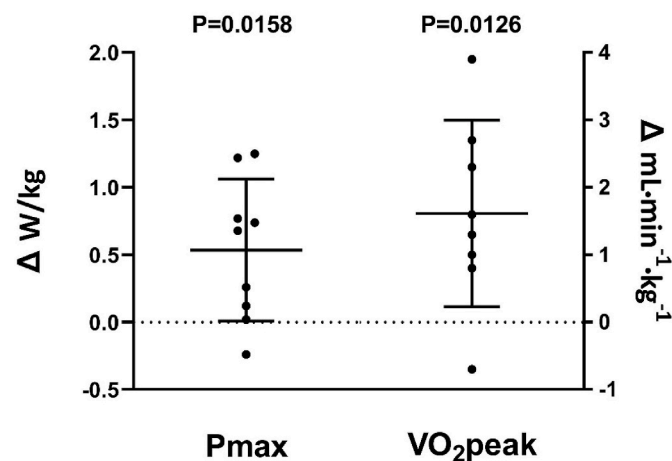

Fig. 1. Acute (i.e., single dose) dietary NO_3^--induced changes in maximal knee extensor power (Pmax) and peak O_2 consumption (VO_2peak) in patients with heart failure with reduced ejection fraction (HFrEF). Data are from Refs. 21 and 22, respectively.

Table 1
KNO$_3$ versus other possible approaches for increasing NO signaling in patients with HFrEF.

Alternative approach	Advantages of KNO$_3$ Versus Alternative Approach
Pharmacological nitrates (e.g., nitroglycerin)	• Does not cause tolerance ● Does not cause endothelial dysfunction ● Does not increase reactive oxygen species (ROS) in mitochondria ● May be less likely to cause hypotension[a] ● May be less likely to cause headaches[a]
Phosphodiesterase 5 inhibitors (e.g., sildenafil)	● Does not cause changes in retinal function/vision ● May be less likely to cause hypotension[a] ● May be less likely to cause headaches[a]
Soluble guanyl cyclase stimulators/ activators (e.g., riociguat)	● Does not cause fetal toxicity (can be used by pregnant persons) ● Does not cause severe bleeding ● May be less likely to cause hypotension[a] ● May be less likely to cause headaches[a]
ʟ-arginine	● Not dependent upon functioning of NO synthase (NOS) ● Does not cause airway inflammation ● Functions well in acidic tissues ● Functions well in ischemic tissues
Beetroot juice	● Easier to control exact NO_3^- dose ● Does not contain oxalate (reduced risk of kidney stones) ● More portable ● More palatable ● No allergic reactions ● No beeturia to be confused with/mask urinary/renal tract disease ● No red stool to be confused with/mask gastrointestinal disease

[a] No such symptoms were observed in our prior studies of patients with HFrEF treated with dietary NO_3^-(21,22).

glyceryl trinitrate [40]. These negative consequences are thought to be due to increased production of highly-reactive oxygen species during drug activation, which does not occur with inorganic nitrate supplementation [41]. Finally, in addition to efficacy the longer-term safety and tolerability of NO_3^- supplementation needs to be established. Supported by a grant from the National Heart, Lung, and Blood Institute (R61 HL155858), the **i**norganic **NI**trate and e**X**ercise performance in **H**eart **F**ailure (iNIX-HF) phase II registered clinical trial (NCT05562167) has therefore been designed to achieve the following specific aims:

Specific Aim 1 (R61 phase). To complete all the regulatory, procedural, and safety steps required to launch the iNIX-HF clinical trial and enroll the first participants;

Specific Aim 2 (R33 phase). To determine the short-term (acute) effectiveness of dietary NO_3^- on exercise performance in patients with HFrEF; and.

Specific Aim 3 (R33 phase). To determine the longer-term effectiveness of dietary NO_3^- on exercise performance in patients with HFrEF.

These aims will be addressed using a double-blind, placebo-controlled, randomized parallel-arm study design to determine the effects of short- (i.e., an acute dose) and longer-term (i.e., once daily for 6 wk) supplementation with 10 mmol KNO$_3$ on muscle power and VO_2peak (co-primary outcomes) in patients with HFrEF. We hypothesize that an acute dose of KNO$_3$ will increase both muscle power and VO_2peak, as we observed in our preliminary studies [21,22], and that this positive effect will be maintained, but not enhanced, after 6 wk of treatment.

2. Methods

2.1. Study participants

Patients with known HFrEF will be recruited from various sources,

including the Washington University School of Medicine (WUSM) HF clinic, the WUSM Cardiology clinic, the WUSM Cardiac Diagnostic Laboratory's database of patients with outpatient transthoracic echocardiograms, the WUSM Volunteer for Health Research Participant Registry, WUSM electronic medical records, and from three other hospitals in the St. Louis metropolitan area. Inclusion and exclusion criteria for the trial are shown in Table 2. All potential participants will provide written, informed consent before participating in the study, which is approved by the WUSM Institutional Review Board and the Indiana University Human Subjects Office.

2.2. Experimental protocol

As shown in Fig. 2, participants will complete three study visits, each involving identical assessments of exercise capacity. Visit 1 will be conducted without ingestion of any study drug. Participants then will be randomized to KNO_3 or placebo therapy, stratifying by sex and ischemic/nonischemic status. The effect of an acute dose of 10 mmol KNO_3 or placebo on VO_2peak and muscle power will then be assessed during Visit 2. Afterwards, participants will continue on the same daily dose of 10 mmol KNO_3 or placebo for 6 wk before returning for Visit 3, during which the longer-term effects of KNO_3 (or placebo) will be determined.

2.2.1. Screening/phenotyping/baseline assessment (visit 1)

Participants will report to the WUSM Clinical Translational Research Unit (CTRU) in the morning after a 12 h fast and complete a medical history form, the Kansas City Cardiomyopathy Questionnaire (KCCQ), and the Minnesota Living with HF Questionnaire (MLHFQ). They will then undergo a physical examination, including measurement of heart rate (HR) and blood pressure (BP), and phlebotomy for screening/phenotyping laboratories: NT-proBNP, chemistries (including K^+), glucose,

Table 2
Inclusion and exclusion criteria for the iNIX-HF phase II clinical trial.

Inclusion criteria	Exclusion criteria
•Age ≥ 18 and ≤ 80 y	•Age <18 or >80 y
•LVEF ≥ 15 and $\leq 45\%$	•LVEF <15 or $>45\%$
•NYHA class II-III HF	•NYHA class I or IV HF
•Systolic blood pressure ≥ 90 and ≤ 180 mmHg	•Systolic blood pressure <90 or >180 mmHg
•Diastolic blood pressure ≥ 40 and ≤ 100 mmHg	•Diastolic blood pressure <40 or >100 mmHg
•eGFR ≥ 45 mL min^{-1}•1.73 m^{-2}	•eGFR <45 mL min^{-1}•1.73 m^{-2}
•On stable, optimal medical therapy including beta-blockers, aldosterone antagonists, and angiotensin converting enzyme inhibitors, angiotensin receptor blockers, or angiotensin receptor/neprilysin inhibitors, as appropriate	•Unstable or suboptimal medical therapy
•Occasional use of proton pump inhibitors or antacids as long as not within 5 half-lives of any study procedure	•Daily use of proton pump inhibitors or antacids
	•Use of pharmacological (organic) nitrates during last 3 mo
	•Use of phosphodiesterase or xanthine oxidase inhibitors
	•Angina/ischemia requiring supplemental O_2 at rest or during exercise
	•Primary hypertrophic cardiomyopathy, infiltrative cardiomyopathy (e.g., amyloid), active myocarditis, complex congenital heart disease, active collagen vascular disease, severe valvular heart disease, or percutaneous coronary intervention, new bi-ventricular pacing, or coronary bypass grafting in the last 3 mo
	•Acute or chronic severe liver disease
	•Major orthopedic, psychiatric, neurological, or other condition that would interfere with exercise testing
	•Vulnerable population including prisoners
	•Pregnancy

LVEF, left ventricular ejection fraction. NYHA, New York Heart Association. eGFR, estimated glomerular filtration rate.

and creatinine (for estimation of glomerular filtration rate). Women of child-bearing age will be administered a urine pregnancy test. Participants will also undergo a standard 2D Doppler, tissue Doppler, and strain imaging echocardiographic study to quantify left ventricular structure and function in accordance with ASE guidelines. If there are clinically significant abnormalities that are new relative to the patient's medical history (e.g., low renal function), they will be withdrawn from the study and the patient's cardiologist will be informed of the results. Eligible participants will proceed with measurement of body composition via DXA, pulse wave velocity (PWV) at time 0 and 1 h, and HR, BP, plasma NO_3^- and NO_2^-, and breath NO at time 0, 1, 2, 3, and 4 h. During this period, participants will be familiarized with all procedures and will also perform practice exercise tests as described below.

2.2.2. Randomization

Participants will be randomized to the KNO_3 or placebo arm using the REDCap randomization module, with stratification for sex and non-ischemic/ischemic cardiomyopathy status. The assignment order will be generated using SAS and then fed into REDCap.

2.2.3. Acute dose visit (visit 2)

As with Visit 1, participants will report to the CTRU fasted (and not having used mouthwash) and have blood drawn for measurement of K^+ and creatinine. If hyperkalemia is present or if eGFR is <45 mL min^{-1}•1.73 m^{-2}, the patient will be withdrawn from the study and their cardiologist contacted; otherwise, the study visit will proceed as follows. HR, BP, plasma NO_3^- and NO_2^-, and breath NO will be measured at time 0 and hourly for 3 h and PWV will be measured at time 0 and 1 h after the participant ingests a single gelatin capsule containing either 10 mmol KNO_3 or placebo (microcrystalline cellulose). This dose was chosen after preliminary experiments demonstrated that a higher dose (i.e., 20 mmol) did not result in greater improvements in muscle power or VO_2peak, but was accompanied by a 3-fold higher frequency of nausea (i.e., 27% vs. 9%) [42]. Based on our novel pharmacokinetic model of the NO_3^-/NO_2^- system [43], a dose of 10 mmol is expected to increase average 24 h plasma NO_3^- and NO_2^- concentrations by ~1200% and ~50%, respectively (Table 3). The capsules will be produced under subcontract by the University of Iowa Pharmacy using USP-grade KNO_3 (Spectrum Chemical, New Brunswick, NJ) and tested for appearance, disintegration, weight variation, capsule closure, microbial enumeration (USP 61), and absence of *e. Coli* (USP 62) before use. After allowing 2 h for attainment of peak plasma NO_3^-, plasma NO_2^-, and breath NO levels, the power of the knee extensor muscles will be determined using isokinetic dynamometry. After 10 min of recovery, participants will perform an incremental treadmill exercise test to determine their VO_2peak and ventilatory responses to exercise. Details of the exercise protocols are provided in sections 2.4 and 2.5.

2.2.4. Intervention

After completion of Visit 2, participants will be given a 6 wk supply of capsules (i.e., n = 42) containing either 10 mmol KNO_3 or placebo, based on the randomization scheme. Participants will be instructed to not use mouthwash before ingesting the study capsules and to not change their diet or level of physical activity during the study (in particular, to not begin or cease an exercise program while enrolled in the study).

2.2.4.1. 6 wk dose visit (visit 3).
The procedures for this visit will be identical to those described for Visit 2.

2.3. Measurement of plasma NO_3^-, plasma NO_2^- and breath NO

Blood samples will be rapidly centrifuged to obtain plasma, which will be frozen at -80 °C until being shipped on dry ice to Indiana University Purdue University Indianapolis (IUPUI) for analysis. There,
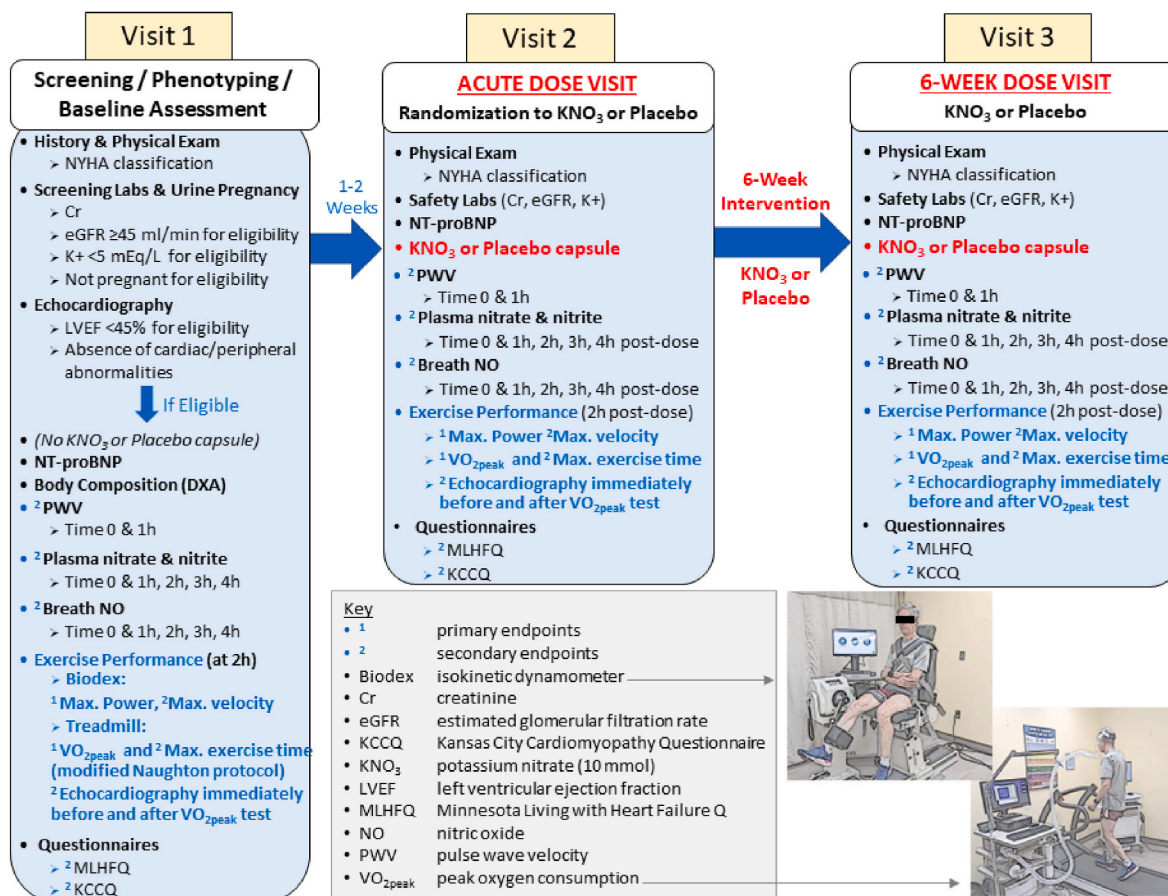
Fig. 2. Overall experimental design and primary and secondary outcome measurements of the inorganic NItrate and eXercise performance In Heart Failure (iNIX-HF) phase II clinical trial.

Table 3
Pharmacokinetic model-predicted changes in steady-state plasma NO_3^- and NO_2^- in response to various dietary NO_3^- dosing regimens.

	10 mmol NO_3^- QD		5 mmol NO_3^-/kg BD		20 mmol NO_3^-/kg QD	
	Plasma NO_3^-	Plasma NO_2^-	Plasma NO_3^-	Plasma NO_2^-	Plasma NO_3^-	Plasma NO_2^-
Δ C_{min} (µmol/L)	209	0.090	293	0.140	418	0.180
Δ C_{max} (µmol/L)	568	0.245	451	0.215	1135	0.490
Δ C_{ave} 0–24 h (µmol/L)	380	0.164	381	0.182	761	0.329
Δ C_{ave} 0–12 h (µmol/L)	476	0.205	381	0.182	951	0.411

QD, once per day. BD, twice per day. Δ C_{min}, minimum increase in concentration above baseline. C_{max}, maximum increase in concentration above baseline. C_{ave} 0–24 h, average increase in concentration above baseline over 24 h Δ C_{ave}, average increase in concentration during first 12 h after ingestion. Calculations are based on data from Refs. 21,22,42, and unpublished observations. For context, in our hands average baseline (fasting) plasma NO_3^- and NO_2^- concentrations in patients with HFrEF are 32 $\pm$ 17 and 0.366 $\pm$ 0.415 µmol/L, respectively.

plasma NO_3^- and NO_2^- concentrations will be determined using a dedicated high-performance liquid chromatography system (ENO-30, Eicom USA, San Diego, CA) as previously described [22]. To reduce variability, all samples from a single participant will be analyzed together.

The level of NO in each participant's breath, a biomarker of whole-body NO production, will be measured using a portable electro-chemical analyzer (NIOX VERO, Aerocrine Inc., Morrisville, NC) following American Thoracic Society/European Respiratory Society guidelines [44].

2.4. Measurement of muscle contractile function

A Biodex System4 isokinetic dynamometer (Biodex Medical Systems, Shirley, NY) will be used to determine the maximal power (Pmax) and velocity (Vmax) of the quadriceps muscles of the participant's dominant leg as previously described [21]. Briefly, peak torque and hence peak power (= torque x angular velocity) will be measured during 3–4 knee extensions performed at angular velocities of 0, 1.57, 3.14, 4.71, and 6.28 rad/s (0, 90, 180, 270, and 360°/s). The peak power-velocity data will then be fit with a parabolic function to determine Pmax and Vmax. Strong verbal encouragement will be provided throughout the test.

2.5. Measurement of VO2peak and ventilatory responses

These measures will be determined during an incremental treadmill exercise test (modified Naughton protocol [45]) performed to volitional fatigue. Respiratory gas exchange will be measured at 15 s intervals using a ParvoMedics TrueOne 2400 metabolic cart. VO2peak (mL min^{-1}•kg^{-1}) will be defined as the highest average of four consecutive VO2 values. The ventilatory threshold (VT; mL min^{-1}•kg^{-1}) will be

determined using the V-slope method [46]. The ratios of ventilation (V_e) to VO_2 (i.e., V_e/VO_2) and VCO_2 (i.e., V_e/VCO_2) will be determined by regressing V_e on VO_2 and VCO_2, respectively, using all data obtained during sub-VT exercise. The oxygen uptake efficiency slope (OUES; L O_2/L V_e) will be calculated in a similar fashion by regressing VO_2 (in L/min) on the log of V_e (also in L/min) throughout exercise [47]. Total exercise duration will also be recorded.

2.6. Other measures

Aortic stiffness will be determined by measuring the carotid-to-femoral PWV in duplicate with the cuff-based SphygmoCor XCEL (AtCor Medical, Itasca, IL) [48]. This device enables simultaneous acquisition of carotid (via tonometer) and femoral (via cuff) pulse waves. Transit time between carotid and femoral pressure waves will be calculated with the "foot-to-foot method". Wave "feet" will be identified with intersecting tangent algorithms. PWV (m/s) will be calculated as distance traveled by the pulse wave divided by pulse transit time. In addition, these data will be subjected to pulse wave analysis to derive novel measures of arterial hemodynamics and wave reflections that have been recently shown to be associated with clinical outcomes in patients with HFrEF [49].

Before and immediately after the treadmill test, 2D and Doppler echocardiography recordings will be obtained via the suprasternal notch while the participant is seated upright in a chair next to the treadmill. These data will be used to determine pulsatile arterial hemodynamics [50] and stroke volume (SV; mL/beat), cardiac output (Q; L/min), and whole-body arteriovenous O_2 difference (a-$\bar{v}$ O_2diff; mL/L) [51]. Stroke volume (SV) will be calculated by multiplying the peak flow rate in the left ventricular outflow tract (LVOT) by the cross-sectional area of the LVOT. Q will be calculated by multiplying SV by heart rate. A-$\bar{v}$ O_2diff will be calculated from Q and VO_2peak (in L/min) via rearrangement of the cardiovascular Fick equation, i.e., a-$\bar{v}$ O_2diff = VO_2peak/Q x 1000.

2.7. Statistical analysis

2.7.1. Sample size

The null hypothesis of the iNIX-HF trial is that acute changes in peak power (W/kg) and VO_2peak (mL.min^{-1}•kg^{-1}) in the KNO_3 group are equal to the corresponding changes in the placebo group. Sample size requirements for testing this hypothesis were estimated based on two-sided tests using p values of 0.05 and 0.25 and power values of 0.8 and 0.9. Results for peak power were based on our prior data which showed an acute intervention-induced change of 0.46 ± 0.44 [21]. To be conservative, we assumed a standard deviation of 0.50 instead of the previously observed value of 0.44. We also assumed a change of 0.0 ± 0.50 in the control group. Results for VO_2peak were based on our prior data which showed an acute intervention-induced change of 1.6 ± 1.4 [22]. To be conservative, we assumed a standard deviation of 1.5 instead of the previously observed value of 1.4. We also assumed a change of 0.0 ± 1.5 in the control group. Based on these calculations, and assuming an estimated 33% screen failure rate and a 20% dropout/unusable data rate, we plan to enroll 75 participants, in order to have a total of 40 patients (20 per arm) finish the study. As shown in Table 4, with this sample size the statistical power for testing both primary outcomes is excellent. Assuming no attenuation of treatment benefits, we will also have sufficient power to test our secondary aim, which is to determine the longer-term effects of KNO_3 supplementation. Furthermore, even if there is attenuation that reduces power values below what would be ideal, the third aim of providing effect size estimates for a future phase 3, multi-center trial will still be realized.

2.7.2. Data analysis

Initial analyses will involve *t*-test and chi-square tests to compare baseline values across groups. If the assumptions of a *t*-test are violated, we will explore the use of data transformations and, if appropriate transformations cannot be found, we will use Wilcoxon's test as an alternative to the *t*-test. For Specific Aim 2, where data are available on each participant at two time points, we will use analysis of covariance with the post intervention value of the outcome variable as the dependent variable and with the predictor variable being the pre-treatment value and the group assignments. The fit of the model will be evaluated using regression residuals. For the third aim, mixed model repeated measures analysis of variance will be used to evaluate the three data points available for each participant. The comparison of primary interest will be evaluated using the statistical contrast that compares the change from baseline to 6 wk in the control group with the corresponding change in the intervention group. The covariance structure to be used in these analyses will be determined using information criteria. If the information criteria do not suggest a preference for any particular correlation structure, we will employ the least complex of the equivalent criteria, where least complex is defined as the criterion with the smallest number of parameters that must be estimated. Significance testing will be at the $p < 0.05$ level.

To better understand whether sex as a biological variable has an impact on our results, we will include sex in our analytic models to determine whether any observed between-group differences can be explained by the sex of the participant. We will also evaluate the sex by group interaction to assess whether the efficacy of the intervention is different in male as compared to female participants. It should be noted that the study is not powered to evaluate this interaction term and it is possible that genuine efficacy differences by sex will be missed because of inadequate power. However, the project will still provide effect size estimates to adequately power a future phase 3, multicenter trial.

3. Summary

HF is a mortal and morbid disease, impairing the ability of millions of people to exercise and even to perform routine activities of daily living. Improving aerobic exercise capacity, decreasing ventilatory effort, and bolstering muscle speed and power should decrease the morbidity of HF. NO is a key mediator of muscle function at the molecular level. Bioavailability of NO is decreased in HF, particularly HFrEF. Taken orally, inorganic NO_3^- increases NO bioavailability, and small studies have shown that dietary NO_3^- increases NO production and improves exercise performance in patients with HFrEF. The inorganic NItrate and eXercise performance In Heart Failure (iNIX-HF) phase II clinical trial will further evaluate the effectiveness of dietary NO_3^- as a new treatment to ameliorate poor exercise capacity in HFrEF. This study will also provide critical preliminary data for a future 'pivotal', phase III, multicenter trial of the effectiveness of dietary NO_3^-, not only for improving exercise performance, but also for improving symptoms and decreasing other major cardiovascular endpoints. The potential public health impact of identifying a new, relatively inexpensive, safe, and effective treatment that improves overall exercise performance in patients with HFrEF is significant.

Table 4
Sample size estimates.

	Power (1-β) = 0.8		Power (1-β) = 0.9	
Outcome measure	α = 0.050	α = 0.025	α = 0.050	α = 0.025
Muscle power (W/kg)	20	24	26	31
VO_2peak (mL min^{-1}•kg^{-1})	15	18	20	23

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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