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Pharmacokinetics and Pharmacodynamics of Inorganic Nitrate in Heart Failure With Preserved Ejection Fraction

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Abstract

Rationale—Nitrate-rich beetroot juice has been shown to improve exercise capacity in Heart Failure with Preserved Ejection Fraction (HFpEF), but studies using pharmacologic preparations of inorganic nitrate are lacking.

Objectives—To determine: (1) the dose-response effect of potassium nitrate (KNO_3) on exercise capacity; (2) the population-specific pharmacokinetic and safety profile of KNO_3 in HFpEF.

Methods and Results—We randomized 12 subjects with HFpEF to oral KNO_3 (n=9) or potassium chloride (KCl, n=3). Subjects received 6mmol twice-daily during Week-1, followed by 6mmol thrice-daily during Week-2. Supine cycle ergometry was performed at baseline (Visit 1) and after each week (Visits 2&3). Quality of life (QOL) was assessed with the Kansas City Cardiomyopathy Questionnaire (KCCQ). The primary efficacy outcome, peak O_2-uptake, did not significantly improve (P=0.13). Exploratory outcomes included exercise duration and quality of life. Exercise duration increased significantly with KNO_3 (Visit 1: 9.87 [95%CI=9.31–10.43];

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DISCLOSURES

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Visit 2: 10.73 [95%CI=10.13–11.33]; Visit 3: 11.61 [95%CI=11.05–12.17] minutes, *P*=0.002). Improvements in the KCCQ total symptom (Visit 1: 58.0 [95%CI=52.5–63.5], Visit 2: 66.8 [95%CI=61.3–72.3]; Visit 3: 70.8 [95%CI=65.3–76.3], *P*=0.016) and functional status scores (Visit 1: 62.2 [95%CI=58.5–66.0], Visit 2: 68.6 [95%CI=64.9–72.3], Visit 3: 71.1 [95%CI=67.3–74.8]; *P*=0.01) were seen after KNO_3. Pronounced elevations in trough levels of nitric oxide metabolites (NO_m) occurred with KNO_3 (Visit 2: 199.5 [95%CI=98.7–300.2]; Visit 3: 471.8 [95%CI=377.8–565.8]) versus baseline (Visit 1: 38.0 [95%CI=0.00–132.0] μM; *P*<0.001). KNO_3 did not lead to clinically-significant hypotension or methemoglobinemia. Following 6 mmol of KNO_3, systolic blood pressure was reduced by a maximum of 17.9 (95%CI −28.3-[−7.6]) mmHg 3.75 hours later. Peak NO_m concentrations were 259.3 (95%CI 176.2–342.4) μM 3.5 hours after ingestion, and the median half-life was 73.0 (IQR 33.4–232.0) minutes.

Conclusions—KNO_3 is potentially well-tolerated and improves exercise duration and QOL in HFpEF. This study reinforces the efficacy of KNO_3 and suggests that larger randomized trials are warranted.

ClinicalTrials.gov—NCT02256345; https://www.clinicaltrials.gov/ct2/show/NCT02256345

Subject Terms

Endothelium; Vascular Type; Nitric Oxide; Heart Failure; Exercise

Keywords

Heart failure; exercise capacity; nitric oxide

INTRODUCTION

The prevalence of heart failure with preserved ejection fraction (HFpEF) is rising.[1, 2] Recent tissue data demonstrate decreased cyclic GMP (cGMP) concentration, and increased oxidative stress in subjects with HFpEF,[3, 4] suggesting impaired nitric oxide (NO) signaling. However, efforts at increasing NO bioavailability, either through phosphodiesterase-5 inhibition or the administration of organic nitrate, have not led to improvements in exercise capacity in HFpEF.[5, 6]

Traditionally, NO synthesis has been known to occur via the enzymatic oxidation of L-arginine by nitric oxide synthases (NOS), in the presence of molecular oxygen, to yield citrulline and NO.[7] Given its dependence on oxygen, NO synthesis from NOS may be inefficient in the setting of hypoxia. Inorganic nitrate, ingested in the diet or via supplementation, is rapidly absorbed in the gastrointestinal tract, concentrated in the salivary glands, and then released into the oral cavity where bacteria facilitate its reduction to nitrite. Nitrite is then reabsorbed into the circulation and can be reduced to NO by deoxyhemoglobin.[8] Conversion of nitrite to NO is potentiated by hypoxia and acidosis,[9, 10] as would be seen in exercising muscle,[11] leading to vasodilation and increased perfusion at sites of greater metabolic need.[12] Thus, circulating nitrate/nitrite may serve as an alternative pool for bioactive NO, which can sustain/enhance NO signaling when the traditional pathway for NO generation is less functional.[9, 13] Indeed, nitrate/nitrite supplementation has

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been shown to increase blood flow to exercising muscle in rats with[14, 15] and without[16, 17] heart failure. NO-induced vasodilation during exercise may facilitate the matching of perfusion to metabolic activity.[18]

Recently, our group demonstrated that acute supplementation with beetroot juice, which is rich in inorganic nitrate, improves vasodilatory reserve and peak oxygen uptake in HFpEF subjects.[19] A more recent study demonstrated improvements in endurance during submaximal exercise following one-week of supplementation with beetroot juice.[20] However, studies using pharmacologic preparations of inorganic nitrate are lacking. The current study was an in-depth pharmacodynamic and pharmacokinetic study of oral KNO_3 in HFpEF with the specific goals of: (1) Determining the safety profile of short-term (2-week) inorganic nitrate supplementation, administered as potassium nitrate (KNO_3) capsules; and (2) Determine the physiologic effects and the dose-response associated with KNO_3 administration over 2-weeks on exercise capacity and key physiologic adaptations to exercise; and (3) Assess the pharmacokinetic profile of KNO_3 in this population.

METHODS

Participants

Inclusion criteria included symptomatic heart failure in the context of a preserved (≥50%) ejection fraction. Subjects were required to have evidence of elevated filling pressures, demonstrated by at least one of the following: (1) Elevated invasively-measured filling pressures; (2) Septal E/e'>15; (3) Septal E/e'>8 with either elevated natriuretic peptide levels within the past year, left atrial enlargement, or chronic loop diuretic use for control of symptoms. Exclusion criteria included any rhythm other than sinus with native conduction, inability to exercise, ≥ moderate valvular disease, known hypertrophic/infiltrative/inflammatory cardiomyopathy, pericardial disease, current angina, acute coronary syndrome or coronary intervention within the past 2 months, primary pulmonary arteriopathy, clinically-significant lung disease, current ischemia, chronic treatment with organic nitrates or allopurinol, significant liver disease, eGFR<30 mL/min/1.73 m^2 or creatinine > 2.5 mg/dL, current smoking, alcohol dependency, Barrett's esophagus, G6PD deficiency, and a baseline methemoglobin >3%. Background medications were not altered. The study was approved by the Institutional Review Board of the University of Pennsylvania and conducted under an FDA IND (#123,785). Written informed consent was obtained from all subjects. This study was registered on ClinicalTrials.gov (NCT02256345).

Study medication and dosing regimen

This was a randomized trial of KNO_3 given in two dosing regimens: 6 mmol twice daily for one week followed by dose-escalation to 6 mmol thrice daily for one week. While a primary goal of the study was to assess the safety of KNO_3 and within-group changes in various endpoints in KNO_3-treated subjects, a small number of placebo-treated (PB, n=3) subjects were included, only to assess for any potential training-effect on repeated exercise and KCCQ measurements.[21] Potassium chloride (KCl), given in equivalent doses, was used as the PB to account for differences in blood pressure or flow that could be attributed to potassium.[22]

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Design

The study was initially designed to be single-blinded, to allow the Principal Investigator to be aware of arm allocation due to potential concerns for methemoglobinemia with drug administration. As described below; however, no clinically-significant elevations in methemoglobin occurred. One investigator (PZ), who was the Primary Investigator responsible for supervising all visits and measurements during the study, remained blinded to treatment allocation throughout the entirety of the study. All physiologic and imaging data were analyzed in a double-blind manner.

Study protocol

Subjects presented to the Clinical and Translational Research Center at the University of Pennsylvania in the fasted state. Resting blood pressure was obtained using an automated oscillometric device (Accutorr Plus, Datascope Corp., Paramus, NJ). Blood was obtained for serum nitric oxide metabolites (NO_m) and nitrite determination, basic chemistries, NT-pro-BNP determination (Cobas e411 Analyzer, Roche Diagnostics, Indianapolis, IN), and measurement of hemoglobin and methemoglobin (ABL800 Flex Analyzer, Radiometer America, Brea, CA). Subjects refrained from using mouthwash and were provided with a list of nitrate-rich foods to avoid during the study. A registered dietician met with subjects at each study visit to reinforce dietary restrictions. During the baseline visit (Visit 1), subjects proceeded directly to the exercise testing protocol after verification of inclusion/exclusion criteria, a physical examination, echocardiography with arterial tonometry, and laboratory testing. During Visit 2 and Visit 3, study drug was administered with a standardized low-nitrate meal. Two hours later, orthostatic blood pressure measurements were made, followed by echocardiography, arterial tonometry, and the exercise protocol, as described below. The Kansas City Cardiomyopathy Questionnaire was administered at each study visit.[23]

Echocardiography and arterial tonometry

Subjects underwent resting echocardiography using a GE Vivid I machine (GE Healthcare, Fairfield, CT). Metrics were quantified in triplicate, according to the American Society of Echocardiography guidelines.[24] Left atrial volumes were quantified using the Area-Length method and indexed to body-surface area (BSA). Left ventricular outflow tract (LVOT) Doppler velocity-time integrals (VTI) were obtained from the 5-chamber view to obtain stroke volume (LVOT cross-sectional area * VTI) at rest and at peak exercise.

Resting arterial tonometry (Millar Instruments, Houston, TX) was performed at the left radial artery and calibrated using brachial oscillometric blood pressures (Scholar III507EL, Criticare Systems, Inc., Waukesha, WI). Tonometric signals were processed using Sphygmocor software (AtCor Medical, Australia) to derive central pressures. Augmentation index (AIx) was calculated as the ratio of the amplitude of the second peak to the first peak ($P_2/P_1 \times 100\%$).

Exercise studies

As described previously,[19] subjects underwent peak effort exercise testing using supine cycle ergometry with gas exchange measurements (Parvo Medics, Sandy, UT). Peak oxygen uptake ($VO_{2,peak}$) was defined as the average value obtained during the last 30 seconds of

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exercise. Exercise economy was estimated as the ratio of total work performed (kilojoules) to total oxygen consumed (liters) above baseline during the entirety of the exercise study. Prior work demonstrates an increased benefit for inorganic nitrate supplementation at higher exercise intensities, when Type II muscle fibers are utilized to a greater degree.[16, 25–28] In *post hoc* analysis, we quantified total oxygen uptake and work performed during the last 3- and 6-minutes of exercise, when subjects were exercising at higher intensities, and obtained estimates of exercise economy over those time periods.

After at least 15 minutes of rest, subjects exercised at 25W for 6 minutes with steady-state VO_2 measured during the final minute of exercise. Radial tonometry and LVOT VTIs were obtained at rest and during steady-state to determine input impedance, as described previously.[29] VO_2 recovery kinetics (τ) were modeled according to a mono-exponential decay.

Skeletal muscle mitochondrial oxidative function

We used near infrared spectroscopy (NIRS) to determine skeletal muscle mitochondrial oxidative function after a standardized exercise protocol, as described by Ryan et al.,[30] and reported previously by our group.[19]

Safety and pharmacokinetic determination

On Visit 1 and Visit 2, subjects were given study medications (either 6 mmol KNO_3 or 6 mmol KCl) with a standardized nitrate-free meal following exercise testing. For the next 5 hours, blood pressure was measured every 15 minutes, and serum was drawn every 30 minutes for determination of NO_m and methemoglobin. The elimination rate (K_e) was the negative of the slope from the linear regression of log concentration versus time over the last 3 time points and used to calculate the half-life ($t_{1/2} = \ln 2/K_e$).

Serum NO_m quantification

Serum levels of NO_m were measured as reported previously using vanadium (III)/ hydrochloric acid reduction, heated at 95°C, and coupled with chemiluminescence detection using the Sievers-Nitric Oxide Analyzer (Sievers-NOA 280, Nitric Oxide Analyzer, Sievers Instruments, Boulder, CO). This method quantifies nitrate, as well as nitrite, NO-metal and heme complexes, low molecular weight thiol-NO adducts and protein cysteine-NO adducts.[19] NO_m samples for 3 individuals were repeated in each of the 9 batches performed. The intra-class correlation coefficient for NO_m was 0.98 (95%CI 0.93–1.00; $P<0.001$), demonstrating excellent reproducibility. Serum nitrite levels were quantified using reduction with potassium iodide/crystalline iodide and the same Sievers instrument. Nitrite samples were measured in each of the 6 batches performed. The intra-class correlation coefficient for nitrite was 0.94 (95%CI 0.77–1.00; $P<0.001$).

Activity monitoring and 24-hour blood pressure measurements

At the conclusion of Visit 1 and Visit 2, subjects were given an ambulatory blood pressure monitor (Mobil-O-Graph PWA, I.E.M. GmbH, Stolberg, Germany) and an activity monitor (wActiSleep-BT Monitor, Actigraph, LLC., Pensacola, FL). Blood pressure measurements were obtained every 30 minutes over a 24-hour period. Actigraphy was used to obtain daily

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steps taken and estimated kilocalorie expenditure. Because follow-up visits were scheduled during the last 1–2 days of each week, data was abstracted for days 1–5 in all subjects. Values for days 3–5 were averaged to obtain estimates corresponding to steady-state drug effects (SS).

Study outcomes

The primary efficacy endpoint was the change in $VO_{2,peak}$. Secondary endpoints included changes in vasodilatory reserve (percent reduction in systemic vascular resistance during exercise, compared to the resting measurement), changes in mitochondrial oxidative function using NIRS, and changes in AIx. Exploratory endpoints included other metrics of exercise capacity, and changes in the KCCQ. Pharmacokinetics were assessed via measurement of NO_m after single-dose administration of 6 mmol and with trough levels after repeated twice- and thrice-daily administration.

Statistical methods

Demographic data are presented as n(%) or means(SD). Mixed-effects models were generated in STATA (Stata/SE 13.1, StataCorp, College Station, TX), incorporating the correlation between repeated measurements in the same individual. No assumption of linearity was made. As pre-specified in the protocol, only within group comparisons were performed. Marginal means with 95% confidence intervals (CI) were determined from the mixed models and presented in the tables and figures. An overall P-value<0.05 was taken to be significant for each model, with post-hoc comparisons between visits performed subsequently. No adjustment for multiple comparisons was made. The elimination half-life was determined using the *PK* package in STATA. Linear regression was performed to assess the correlation between changes in serum NO_m and nitrite levels and the change in exercise metrics. Regression coefficients are presented as standardized units describing the standard deviation change in the endpoint for each standard deviation change in the predictor variable. In a previous trial, we demonstrated a 1.0±1.2 mL/min/kg improvement in peak VO_2 following a single-dose (12.9 mmol) of inorganic nitrate, given in the form of beetroot juice.[19] Using a correlation between studies of 0.80, beta=0.80, and alpha=0.05, we estimated that 7 subjects would be needed to demonstrate a similar change. We thus planned for 9 subjects in the active arm to allow for drop-out. Randomization was performed by the University of Pennsylvania Investigational Drug Pharmacy. Randomization tables were constructed using a 3:1 KNO_3:PB ratio in blocks of 4 (www.randomization.com).

RESULTS

From 703 subjects screened, a total of 15 subjects were enrolled (Figure 1). An alternative explanation for dyspnea was identified during baseline testing in 3 individuals, who were subsequently excluded. Twelve individuals met all inclusion/exclusion criteria and completed the study (Table 1). Of these 12, 7 (58%) previously had elevated invasively-determined intra-cardiac pressures. The remaining individuals had an elevated E/e' ratio>8 along with at least one of the following: (a) an elevated NT-pro-BNP previously (n=1); (b) left atrial enlargement (n=1); (c) or the need for chronic loop diuretics for control of symptoms (n=3). Mean (SD) age of participants was 62.5 (5.8) years, 4 (33%) were males,

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and 3 (25%) were African-American. Most participants had comorbidities typical for HFpEF: hypertension (100%), obesity (67%), hyperlipidemia (83%), diabetes (58%), obstructive sleep apnea (50%), coronary disease (33%), and chronic renal insufficiency (33%).

Maximal effort exercise test

$VO_{2,peak}$, the primary efficacy endpoint, tended to increase, without reaching statistical significance (P=0.13). Vasodilatory reserve did not change with supplementation (P=0.72). The exploratory endpoint of exercise duration was significantly increased in the KNO_3 group (P=0.002; Table 2, Figure 2). Both total work performed (P=0.02) and total oxygen uptake (P=0.019) increased. Additional hemodynamic data from the maximal effort study are presented in Supplemental Table I. Interestingly, the change in $VO_{2,peak}$ from the baseline to final visit correlated with the change in serum NO_m levels: Standardized β=0.74; P=0.006. The change in nitrite levels did not correlate with any metrics of exercise capacity.

In *post hoc* analysis, we evaluated oxygen uptake and work performed during the final 3 and final 6 minutes of exercise (i.e. heavy and severe intensity). Work performed in the final 3 minutes increased significantly (P=0.002), as did total O_2 uptake in the final 6 minutes (P=0.041) and total work performed in the final 6 minutes (P=0.002; Figure 2). Overall exercise economy in the final 3-minute (P=0.007) and 6-minute (P=0.004) periods increased (Supplemental Table II). The increase in oxygen uptake during the final 3 minutes of exercise correlated with the increase in serum NO_m levels (Standardized β=0.64; P=0.024).

Pharmacodynamics following administration of 6 mmol of KNO_3

Blood pressure was measured every 15 minutes, and serum collected every 30 minutes, following the administration of 6 mmol of KNO_3. The maximal reduction in SBP occurred at 3 hours and 45 minutes (−17.9 [95%CI −28.3-[−7.6]] mmHg; Figure 3). We observed a statistically-significant, though clinically-irrelevant, increase in methemoglobin concurrent with the reduction in SBP (Figure 3). Peak NO_m concentrations were 259.3 (95%CI 176.2–342.4) μM occurring 3.5 hours after ingestion. The median half-life was 73.0 (IQR 33.4–232.0) minutes.

Tolerability of KNO_3

KNO_3 was well-tolerated, with all subjects completing the dosing and study protocols. Five individuals experienced side-effects during the study (Supplemental Table III): 4 (44%) in the KNO_3 group and 1 (33%) in PB. Gastrointestinal symptoms and headache were the most common side-effect. There were numerically fewer side-effects in the KNO_3 group during Week 2 (18 mmol/d of KNO_3).

Serum NO_m, blood pressure and laboratory testing at each visit

KNO_3 significantly increased fasting serum NO_m concentration in a dose-dependent manner (Table 3). This primarily reflects an increase in nitrate levels, as nitrite levels did not change (data not shown). We observed a reduction in systolic blood pressure (SBP) with KNO_3 (Table 3). Importantly, percent methemoglobin was no different between visits (Table 3).

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Likely due to the serial blood sampling during the safety/pharmacokinetics portions of Visit 1 and Visit 2, hemoglobin was significantly reduced in the KNO_3 group (Table 3).

Orthostatic blood pressure measurements

Orthostatic measurements were performed 2 hours following dose administration. Inorganic nitrate caused an asymptomatic reduction in SBP following 2 minutes of standing from a supine position, particularly at the highest doses (Visit 1: +1.2 [95%CI −6.1–8.4]; Visit 2: −1.1 [95%CI −7.8–5.6]; Visit 3: −11.6 [95%CI −18.3-[−4.9]] mmHg, P=0.051).

Kansas City cardiomyopathy questionnaire

The KNO_3 group experienced an improvement in HF symptoms over the 2-week study, as evidenced by higher scores on the KCCQ in several domains: symptom stability (P=0.03), symptom burden (P=0.049), total symptom score (P=0.016), and the functional status score (P=0.01; Figure 4). The overall summary score tended to improve with KNO_3 (P=0.067).

Resting echocardiography

Diastolic function and left-ventricular end-diastolic volumes were quantified at rest. Diastolic function was not impacted by KNO_3. Additionally, EDV did not change (P=0.99, Supplemental Table IV).

Oxygen uptake and input impedance during submaximal exercise

Using radial tonometry and LVOT flow, input impedance was determined at rest and while at steady-state during submaximal (25W) exercise. No changes in input impedance were observed (Supplemental Table V). Neither steady-state VO_2 (P=0.85) nor economy (P=0.98) were impacted by KNO_3 (Supplemental Table VI).

Ambulatory BP

During the 24-hours following Visit 1 (first day of 6 mmol twice daily) and Visit 2 (first day of increase to 6 mmol thrice daily), no differences in 24-hour ambulatory blood pressure were seen (data not shown).

Actigraphy

KNO_3 did not alter activity. Specifically, neither average daily steps taken during each week (Week 1: 9244.1 [95%CI 8435.8–10052.4]; Week-2: 9469.8 [95%CI 8661.5–10278.1] steps; P=0.71) nor estimated daily kilocalories expended (Week-1: 1683.3 [95%CI 1555.5–1811.1]; Week-2: 1678.7 [95%CI 1550.9–1806.4]; P=0.96) were altered with KNO_3.

Augmentation index and mitochondrial oxidative function

There was no difference in AIx or mitochondrial oxidative function between visits in either the KNO_3 or the PB group (P=NS for all).

Lack of training effect in PB-Treated subjects

There were no significant changes in any metric of exercise with PB. Furthermore, KCCQ scores did not change in these subjects during the course of the study, suggesting lack of a

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training effect on repeated testing. Data on study-endpoints for the PB-treated subjects can be found in the online supplement (Supplemental Table VII).

DISCUSSION

In subjects with HFpEF, the principal findings of this investigation are that KNO_3 (1) appears to be potentially safe at a dose of up to 18 mmol/day; (2) did not significantly improve the primary efficacy endpoint of peak VO_2, although a non-significant trend was observed; (3) significantly increased the exploratory endpoint of exercise duration; (3) significantly improved several domains of the Kansas City Cardiomyopathy Questionnaire; (4) did not worsen outpatient activity; and (5) this preparation demonstrates favorable pharmacokinetics, with high trough levels of NO metabolites achieved with doses of 6 mmol twice daily and substantially higher levels with thrice daily dosing, but without significant methemoglobinemia for either dosing interval.

KNO_3 appeared to be potentially safe up to a dose of 18 mmol/day, and no subject prematurely discontinued study medications for any reasons. The side-effects experienced were mild and were not more frequent at the higher dose. Similar to other studies, we demonstrate that KNO_3 mildly reduced resting blood pressure.[20, 31] We did, however, note a greater decrease in orthostatic SBP at the higher dose of 18 mmol/day, suggesting that this approaches the maximum tolerated dose. The reduction of nitrite to NO leads to the generation of methemoglobin, raising the theoretical concern for methemoglobinemia with KNO_3 administration. Consistent with this biology, we demonstrate a clinically-insignificant rise in methemoglobin immediately after dose administration. However, there was no elevation in steady-state methemoglobin levels at any study visit, suggesting that the endogenous mechanisms of hemoglobin reduction operate fast enough to prevent any sustained rise in methemoglobin.

In contrast to NO production via NOS, which requires molecular oxygen, KNO_3 may provide an alternative source of NO that causes vasodilation and increased perfusion within hypoxic exercising muscle, leading to improved exercise duration. During our progressive ramp protocol, exercise duration increased via improved tolerance to heavy/severe exercise. As the intensity of exercise increases, a greater proportion of Type II fibers is activated.[25] Type II fibers, with their lower capillary volume density and increased dependence on glycolytic pathways for ATP production, reside in a relatively more hypoxic and acidotic milieu, particularly during exercise thus providing an ideal environment for the reduction of nitrite to NO.[25] Our findings are in line with recent evidence for the preferential activation of nitrite by Type II fibers[16] and at higher exercise intensities.[26–28]

Participants randomized to KNO_3 experienced an improvement in HF-related symptoms, as evidenced by higher total symptom, symptom burden, and functional status scores. The total symptom score has previously been demonstrated to be the most responsive metric for the evaluation of HF symptoms, and lower functional status scores have been associated with an increased incidence of death or HF hospitalization.[23] Importantly, the magnitude of the changes seen in our study were generally >5 points, suggesting the potential for clinical relevance.[32] Given the substantial morbidity associated with HFpEF,[33] strategies that

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improve quality of life and exercise represent important progress for this patient population that has few therapeutic options. Beyond the short-term effects demonstrated in this trial, inorganic nitrate might enable HFpEF patients to better tolerate the initiation/maintenance of an exercise prescription, improving the adherence to, and efficacy of, an exercise regimen.

Recently, a randomized placebo controlled crossover study of an organic nitrate, isosorbide mononitrate (ISMN), was performed in HFpEF patients.[5] This study demonstrated a reduction in physical activity with ISMN. In our study, no decrease in activity was identified, suggesting that KNO_3 may not have the same harmful impact.

Limitations

There are several limitations to our study. First, the sample size was small because a larger population could not be justified prior to assessing safety. Given the small number of patients exposed to active agent and the very small number of placebo patients, the study had low power to detect safety signals. Gathering more robust safety data is a goal of the ongoing registered larger clinical trial that was supported by our present findings (KNO_3CK OUT HFpEF Trial; ClinicalTrials.gov: NCT02840799). Second, the pharmacokinetics portions of our study required serial blood draws, which caused a significant decrease in hemoglobin concentration from 13.6 g/dL to 12.8 g/dL (−5.9%). Because $VO_{2,peak}$ is limited by oxygen delivery, particularly in heart failure subjects,[18] the decrease in hemoglobin may have constrained the increase in $VO_{2,peak}$ and reduced the magnitude of the improvement in exercise capacity and quality of life. Indeed, in our current study, we found a 3.4% improvement in $VO_{2,peak}$. Had the hemoglobin remained constant, mathematically $VO_{2,peak}$ would be expected to improve by ~6%, leading to a similar magnitude of change as demonstrated in our prior study where a single acute dose of 12.9 mmol of inorganic nitrate improved $VO_{2,peak}$ by approximately 8%.[19] Differences in patient characteristics may also have contributed to the non-significant increase in $VO_{2,peak}$. Our prior study included a greater number of African-American males. It is conceivable that hypertensive African-Americans represent a unique population with particularly deranged NOS function,[34] and stand to benefit more from interventions that increase NO bioavailability, as demonstrated in HFrEF.[35] Furthermore, there may be differences in KNO_3 responsiveness between genders, as females demonstrate less reduction in blood pressure following inorganic nitrate supplementation despite greater increases in serum nitrate concentration.[36] Given the forced up-titration of study medications, we cannot exclude the possibility that the improvement in exercise duration was due to longer exposure to study medications versus the higher dose administered. That the change in $VO_{2,peak}$ and oxygen uptake in the last 3 minutes of exercise correlated with the change in serum NO_m levels suggests that the supplementation dose is also important. Additionally, we did not obtain serial urine and saliva samples during the pharmacokinetics segment of the study, precluding more detailed assessments. Finally, it is possible that more PB-treated subjects were needed to demonstrate a training effect; however, we were limited in the number of subjects to be performed in this initial pilot study. Our group is currently conducting a follow-up cross-over study of KNO_3 versus PB in HFpEF subjects, which will be adequately powered to detect most relevant treatment-related differences and will also specifically address possible gender and race differences in the response to KNO_3 (KNO_3CK OUT HFpEF Trial; ClinicalTrials.gov: NCT02840799).

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Conclusions

KNO_3 improves exercise duration and QOL in subjects with HFpEF. KNO_3, at a dose of 18 mmol/day, appears to be potentially safe with only mild reduction in blood pressure, and no elevation in methemoglobin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Nonstandard Abbreviations and Acronyms

KNO_3,	potassium nitrate
KCl	potassium chloride
VTI	velocity time integral
LVOT	left ventricular outflow tract
AIx	augmentation index
VO_2	peak, peak oxygen uptake
NIRS	near infrared spectroscopy
SS	steady state
KCCQ	Kansas City Cardiomyopathy Questionnaire
SBP	systolic blood pressure
EDV	end-diastolic volume

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NOVELTY AND SIGNIFICANCE

What Is Known?

- Heart Failure with Preserved Ejection Fraction (HFpEF) is associated with marked reductions in quality of life and exercise tolerance.
- Although nitric oxide (NO) bioavailability is reduced in HFpEF, prior efforts to increase NO using organic nitrate or phosphodiesterase-5 inhibitors have not been beneficial.
- Inorganic nitrate, which is reduced to nitrite by oral anaerobic bacteria, can increase NO signaling via selective reduction of nitrite to NO at the site of exercising muscle, leading to increased muscle blood flow.

What New Information Does This Article Contribute?

- Inorganic nitrate supplementation appears to be potentially safe and well-tolerated in HFpEF subjects.
- Inorganic nitrate supplementation increases exercise duration and quality of life in HFpEF subjects in a dose-dependent fashion.
- The benefits of inorganic nitrate in HFpEF subjects are most manifest at high exercise intensities.

We studied the population-specific safety and efficacy data for inorganic nitrate in HFpEF. We performed detailed phenotypic and pharmacokinetic assessments of inorganic nitrate in HFpEF. We found that a dose of up to 18 mmol/day of inorganic nitrate potentially appears to be safe, with dose-dependent improvements in exercise duration and quality of life. In contrast to organic nitrate therapy, inorganic nitrate did not reduce physical activity over the course of our 2-week study. These findings strengthen the rationale for performing a larger trial of inorganic nitrate in HFpEF patients.

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Reason	Exclusions
Any rhythm other than sinus	75
Inability to Exercise	31
Significant Valvular Disease	54
Hypertrophic or Infiltrative Cardiomyopathy	22
Pericardial Disease	3
Pulmonary Arterial Hypertension	19
Recent Ischemia	1
Non-Cardiac Dyspnea	64
Uncontrolled HTN	1
Hemoglobin < 10 g/dL	20
Cirrhosis	6
Current Ischemia or angina	19
Therapy with PDE5-I	2
Use of Nitrates	23
eGFR<30/Hemodialysis	42
Reduced LVEF	52
Prior reduced LVEF, now recovered	29
No Symptoms of HF	67
Deceased	54
No recent records available to review	32
Eligible but Not Interested	72
Total	688

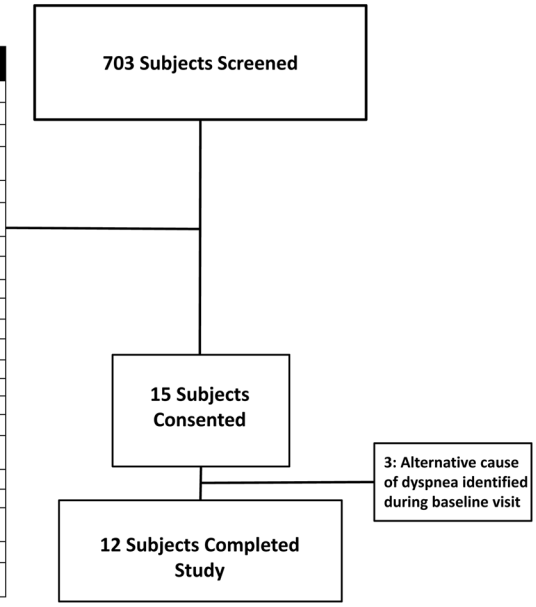

Figure 1. CONSORT diagram

Summary of the screened subjects, including reasons for exclusions.

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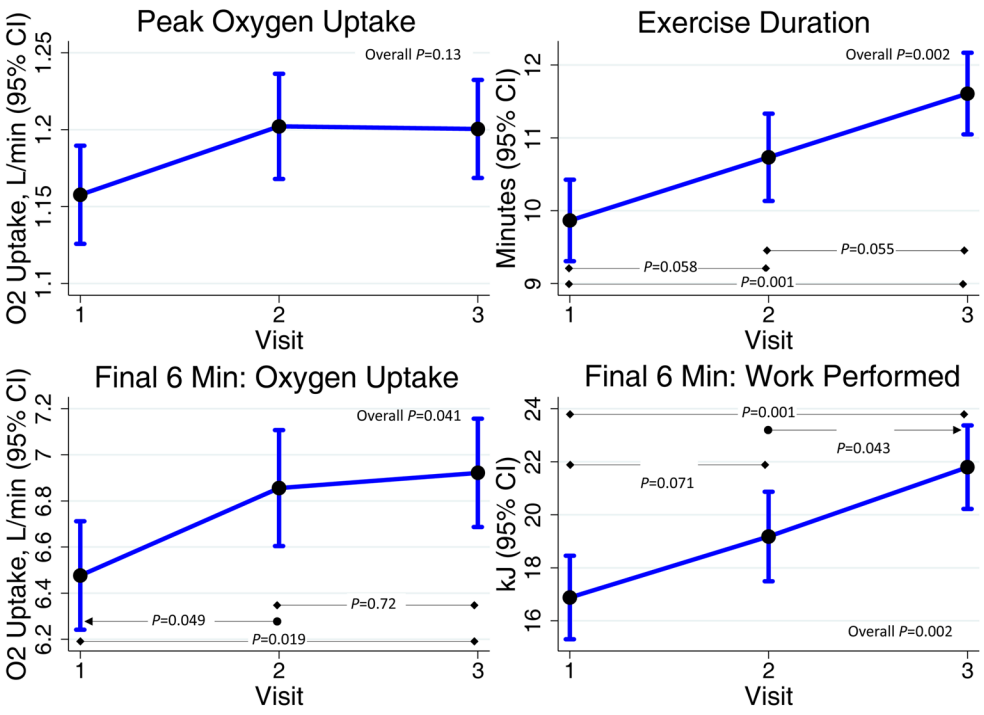

Figure 2.
Maximal Effort Exercise Study Results.

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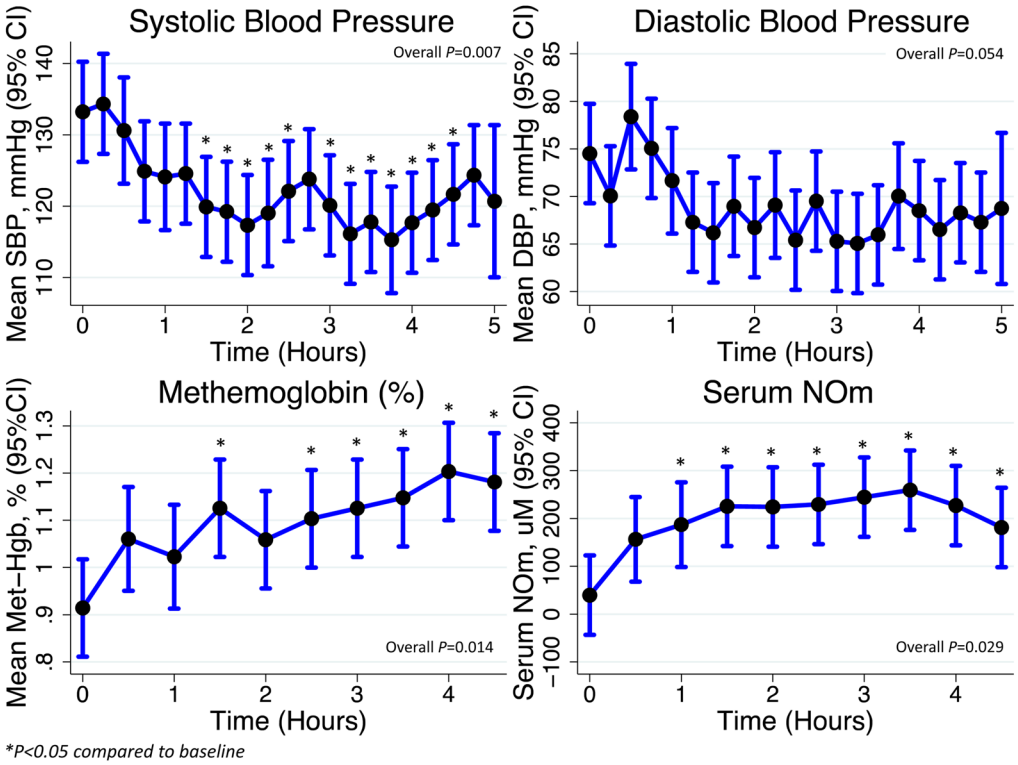

Figure 3.

Pharmacokinetics following initial administration of 6 mmol of KNO_3.

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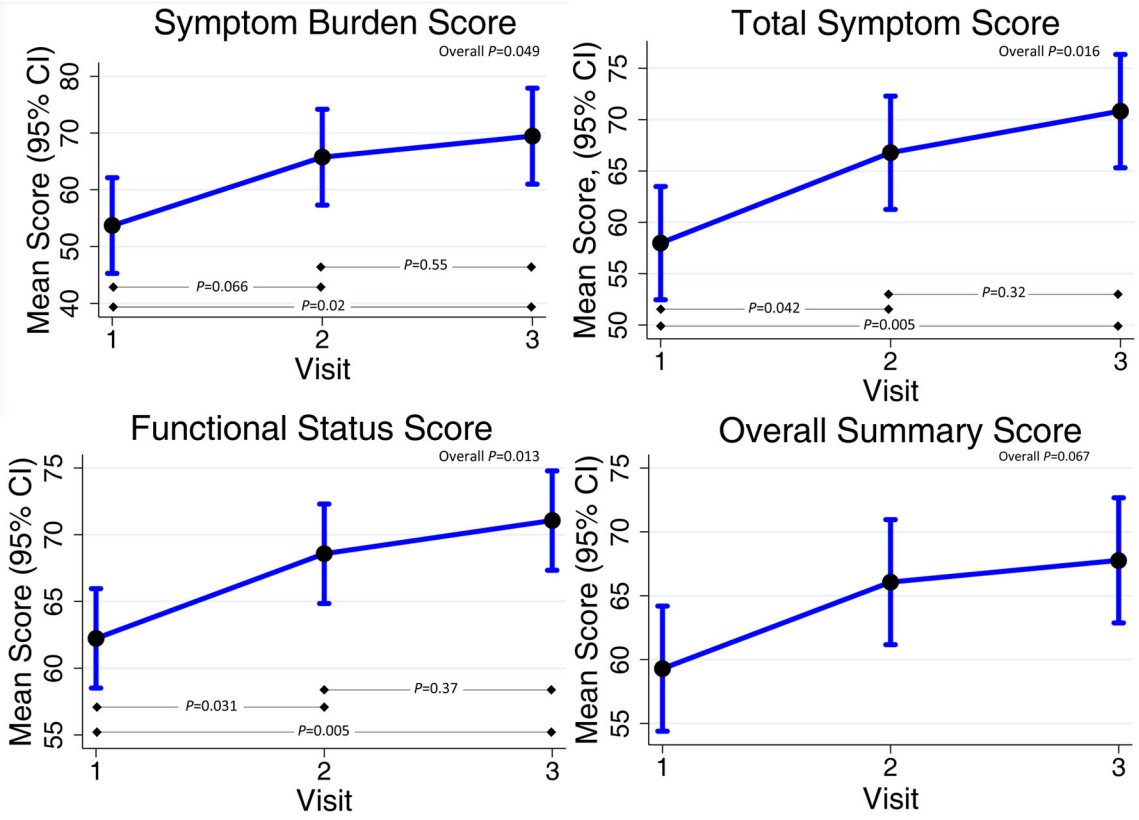

Figure 4.
Kansas City Cardiomyopathy Questionnaire Results.

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Table 1

Demographic, Laboratory, and Echocardiographic Characteristics of the Study Population at Baseline

Variable	Overall	KNO3	Placebo
	n=12	n=9	n=3
Age (years), mean (SD)	62.5 (5.8)	62.4 (5.5)	62.7 (8.0)
Male, n (%)	4 (33.3)	3 (33.3)	1 (33.3)
Race, n (%)			
White	8 (66.7)	5 (55.6)	3 (100)
African-American	3 (25.0)	3 (33.3)	0 (0)
Pacific Islander	1 (8.3)	1 (11.1)	0 (0)
NYHA Class, n (%)			
Class II	10 (83.3)	8 (88.9)	2 (66.7)
Class III	2 (16.7)	1 (11.1)	1 (33.3)
Body Mass Index (kg/m^2), mean (SD)	34.4 (7.0)	33.4 (6.3)	37.4 (9.9)
Obese, n (%)	8 (66.7)	5 (55.6)	3 (100)
Hypertension, n (%)	12 (100)	9 (100)	3 (100)
Hyperlipidemia, n (%)	10 (83.3)	7 (77.8)	3 (100)
Coronary Artery Disease, n (%)	4 (33.3)	3 (33.3)	1 (33.3)
History of Atrial Fibrillation, n (%)	1 (8.3)	0 (0)	1 (33.3)
Diabetes, n (%)	7 (58.3)	5 (55.6)	2 (66.7)
Obstructive Sleep Apnea, n (%)	6 (50.0)	3 (33.3)	3 (100)
Current CPAP use, n (%)	5 (41.7)	2 (22.2)	3 (100)
Obstructive Lung Disease, n (%)	3 (25.0)	2 (22.2)	1 (33.3)
Medical Therapy			
Beta-Blockers, n (%)	9 (75.0)	7 (77.8)	2 (66.7)
Ace-I/ARB, n (%)	9 (75.0)	7 (77.8)	2 (66.7)
Mineralocorticoid Receptor Antagonists, n (%)	3 (25.0)	2 (22.2)	1 (33.3)
Calcium-Channel Blockers, n (%)	4 (33.3)	3 (33.3)	1 (33.3)
Loop Diuretics, n (%)	9 (75.0)	7 (77.8)	2 (66.7)
Thiazide Diuretics, n (%)	4 (33.3)	3 (33.3)	1 (33.3)
Statin, n (%)	7 (58.3)	5 (55.6)	2 (66.7)
Baseline Laboratories			
eGFR (mL/min/1.73m^2), mean (SD)	69.4 (12.6)	71.0 (14.1)	64.7 (5.9)
eGFR<60 mL/min/1.73m^2, n(%)	4 (33.3)	3 (33.3)	1 (33.3)
NT-pro-BNP (pg/mL), mean (SD)	111.0 (89.0)	108.3 (94.7)	119 (87.0)
Elevated NT-pro-BNP, n (%)	5 (41.7)	3 (33.3)	2 (66.7)
Hemoglobin (g/dL), mean (SD)	13.6 (0.8)	13.6 (0.8)	13.5 (1.0)
Methemoglobin (%), mean (SD)	1.0 (0.3)	1.0 (0.2)	1.0 (0.5)
Baseline Echocardiographic Data (mean, SD)			

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Variable	Overall	KNO3	Placebo
	n=12	n=9	n=3
LV Mass (g)	172.32 (66.36)	174.43 (77.09)	165.98 (19.27)
LV Mass Index (g/m^2), mean (SD)	78.88 (22.46)	79.78 (26.01)	76.18 (7.33)
Relative Wall Thickness	0.47 (0.09)	0.47 (0.09)	0.46 (0.09)
Mitral Early Inflow Velocity (E; cm/s)	77.33 (21.91)	74.53 (24.75)	85.73 (7.04)
Mitral Atrial Inflow Velocity (cm/s)	75.21 (20.99)	70.74 (20.06)	88.61 (21.28)
Septal Tissue Doppler Early Velocity (e'; mm/s)	76.41 (19.26)	70.64 (12.45)	93.71 (28.65)
Septal E/e' Ratio	10.42 (3.40)	10.70 (3.76)	9.60 (2.39)
Left Atrial Volume Index (mL/m^2)	27.74 (7.15)	27.20 (7.87)	29.38 (5.33)
Left Ventricular Ejection Fraction (%)	64.23 (9.27)	65.84 (7.74)	59.41 (13.66)

NYHA = New York Heart Association; CPAP = continuous positive airway pressure; ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; elevated NT-pro-BNP defined as >125 pg/mL; eGFR calculated using the MDRD formula

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Table 2

Peak Effort Gas Exchange Data

Marginal Mean (95% CI)	**KNO_3 (n=9)**			
	Visit 1	**Visit 2**	**Visit 3**	***P*-value**
Ventilatory Threshold				
Ventilatory Threshold (L/min)	0.75 (0.69–0.81)	0.68 (0.61–0.74)	0.74 (0.68–0.80)	0.23
iVentilatory Threshold (mL/kg/min)	8.18 (7.65–8.72)	7.51 (6.94–8.08)	8.07 (7.53–8.60)	0.24
Peak Exercise				
Peak VO_2 (L/min)	1.16 (1.13–1.19)	1.20 (1.17–1.24)	1.20 (1.17–1.23)	0.13
Peak iVO_2 (mL/kg/min)	12.50 (12.18–12.83)	12.83 (12.49–13.18)	12.87 (12.55–13.20)	0.26
Exercise Duration (min)	9.87 (9.31–10.43)	10.73 (10.13–11.33)	11.61 † (11.05–12.17)	0.002
Total Work Performed (kJ)	23.22 (18.82–27.61)	26.92 (22.22–31.63)	33.02 † (28.63–37.41)	0.02
Total Oxygen Consumed (L)	8.58 (7.72–9.44)	10.00 * (9.08–10.92)	10.54 † (9.67–11.40)	0.019
Total Exercise Economy (kJ/L O_2 Consumed)	2.52 (2.31–2.72)	2.61 (2.39–2.83)	2.85 (2.65–3.06)	0.10
RER	1.08 (1.06–1.10)	1.09 (1.06–1.11)	1.09 (1.07–1.11)	0.83
V_E/V_{CO2} Slope	34.37 (32.75–35.98)	36.10 (34.38–37.83)	33.81 (32.20–35.42)	0.18

RER = respiratory exchange ratio; kJ = kilojoules

* $p<0.05$ V_1 vs. V_2;

† $p<0.05$ V_1 vs. V_3;

‡ $p<0.05$ V_2 vs. V_3

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Table 3

Vital Signs and Laboratory Data

Marginal Means (95% CI)	KNO_3 (n=9)			
Vital Signs	**Visit 1**	**Visit 2**	**Visit 3**	***P***
Weight (kg)	95.0 (94.6–95.4)	95.1 (94.7–95.5)	95.0 (94.6–95.4)	0.94
SBP (mmHg)	127.2 (121.1–133.3)	118.6 (112.5–124.6)	115.0 † (108.9–121.1)	0.037
DBP (mmHg)	72.6 (69.5–75.6)	68.3 (65.2–71.4)	69.9 (66.8–73.0)	0.19
Heart Rate (bpm)	65.1 (59.9–70.3)	65.4 (60.2–70.7)	67.3 (62.1–72.5)	0.82
Laboratory Data				
Serum Sodium (mEq/dL)	137.8 (137.1–138.5)	137.0 (136.3–137.7)	136.4 (135.7–137.2)	0.06
Serum Potassium (mEq/dL)	3.8 (3.6–4.0)	4.0 (3.8–4.2)	4.1 (3.9–4.3)	0.28
eGFR (mL/min/1.73m^2)	71.0 (66.9–75.1)	70.5 (66.4–74.6)	69.6 (65.5–73.7)	0.89
NT-pro-BNP (pg/mL)	108.3 (84.0–132.6)	117.8 (93.5–142.1)	93.2 (68.9–117.5)	0.39
Hemoglobin (g/dL)	13.6 (13.4–13.8)	12.9 * (12.7–13.1)	12.8 † (12.6–13.0)	<0.001
Methemoglobin (%)	1.04 (0.92–1.17)	1.19 (1.06–1.32)	1.18 (1.05–1.31)	0.25
Serum NOm (μM)	38.0 (0.00–132.0)	199.5 * (98.7–300.2)	471.8 †,‡ (377.8–565.8)	<0.001

* p<0.05 V_1 vs. V_2;

† p<0.05 V_1 vs. V_3;

‡ p<0.05 V_2 vs.