

Heart Failure, Left Ventricular Remodeling, and Circulating Nitric Oxide Metabolites

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Background—Stable plasma nitric oxide (NO) metabolites (NO_M), composed predominantly of nitrate and nitrite, are attractive biomarkers of NO bioavailability. NO_M levels integrate the influence of NO-synthase-derived NO production/metabolism, dietary intake of inorganic nitrate/nitrite, and clearance of NO_M. Furthermore, nitrate and nitrite, the most abundant NO_M, can be reduced to NO via the nitrate-nitrite-NO pathway.

Methods and Results—We compared serum NO_M among subjects without heart failure (n=126), subjects with heart failure and preserved ejection fraction (HFpEF; n=43), and subjects with heart failure and reduced ejection fraction (HFrEF; n=32). LV mass and extracellular volume fraction were measured with cardiac MRI. Plasma NO_M levels were measured after reduction to NO via reaction with vanadium (III)/hydrochloric acid. Subjects with HFpEF demonstrated significantly lower unadjusted levels of NO_M (8.0 μmol/L; 95% CI 6.2–10.4 μmol/L; ANOVA *P*=0.013) than subjects without HF (12.0 μmol/L; 95% CI 10.4–13.9 μmol/L) or those with HFrEF (13.5 μmol/L; 95% CI 9.7–18.9 μmol/L). There were no significant differences in NO_M between subjects with HFrEF and subjects without HF. In a multivariable model that adjusted for age, sex, race, diabetes mellitus, body mass index, current smoking, systolic blood pressure, and glomerular filtration rate, HFpEF remained a predictor of lower NO_M (β=−0.43; *P*=0.013). NO_M did not correlate with LV mass, or LV diffuse fibrosis.

Conclusions—HFpEF, but not HFrEF, is associated with reduced plasma NO_M, suggesting greater endothelial dysfunction, enhanced clearance, or deficient dietary ingestion of inorganic nitrate. Our findings may underlie the salutary effects of inorganic nitrate supplementation demonstrated in recent clinical trials in HFpEF. (*J Am Heart Assoc.* 2016;5:e004133 doi: 10.1161/JAHA.116.004133)

Key Words: diastolic heart failure • heart failure • hypertrophy/remodeling • myocardial fibrosis • myocardial structure

Nitric oxide (NO) is a key bioactive molecule for cardiovascular function. NO is synthesized by various NO synthase (NOS) isoforms, including neuronal, inducible, and endothelial isoforms. In addition, NO can also be

produced via the nitrate-nitrite-NO pathway, which is increasingly recognized as an important source of NO in vivo.^{1,2}

The plasma concentration of stable NO metabolites (NO_M) represents an attractive biomarker of NO bioavailability. Nitrate (NO₃[−]) and nitrite (NO₂[−]), the most abundant circulating NO_M species, are generated as byproducts of NOS-derived NO³⁻⁵ as well from the ingestion of dietary inorganic nitrate and nitrite.^{1,2,6} Therefore, NO_M levels integrate the influence of endogenous nitric oxide (NO) production, clearance, and dietary intake of NO precursors. Regardless of their source, plasma nitrate and nitrite constitute a circulating storage pool of NO precursors that can be readily converted to NO via the nitrate-nitrite-NO pathway.^{1,2,6-8} In this pathway nitrate undergoes a 2-step reduction (to nitrite and then to NO) via the combined action of bacterial oxidoreductases and various nitrite reductases (predominantly deoxyhemoglobin and deoxymyoglobin).^{1,2,6-8}

Recently, plasma NO_M levels have been shown to independently correlate with electrocardiographic LV hypertrophy (LVH) in a Japanese population of normotensive men.⁹

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However, whether NO_M are related to the presence of heart failure (HF) is unknown. In particular, it is unknown whether low NO_M levels are present in patients with HF with preserved ejection fraction (HFpEF), an epidemic condition in which abnormal phenotypes consistent with reduced NO bioavailability have been demonstrated.^{1,10-15} Furthermore, the relationship between NO_M and LVH/remodeling in patients with and without HF requires further study. The previous report of a relationship between NO_M and electrocardiographic LVH⁹ included normotensive men from Japan, a country in which dietary nitrate intake is high. Whether a relationship between LVH and NO_M is present in Western populations with and without HF is unknown. Moreover, LVH is a complex process that may be driven by cellular hypertrophy, extracellular volume expansion due to diffuse interstitial fibrosis, or both. Cardiac MRI with T1 mapping before and after the injection of gadolinium contrast has emerged as a novel, highly precise method for the quantification of diffuse myocardial fibrosis.¹⁶

In this study, we aimed to (1) compare NO_M levels among subjects with HFpEF, HF with reduced ejection fraction (HFrEF), and subjects without HF and (2) assess the relationship between NO_M and LV remodeling (both macroscopic hypertrophy and diffuse interstitial myocardial fibrosis) in these populations.

Methods

We prospectively enrolled a convenience sample of patients at the Corporal Michael J. Crescenz VA Medical Center. The protocol was approved by the Philadelphia VA Medical Center Institutional Review Board, and all subjects provided written informed consent.

HFrEF was defined as a symptomatic HF in the presence of a left ventricular ejection fraction (LVEF) <50%. HFpEF was defined as (1) NYHA Class II-IV symptoms consistent with HF in the absence of significant aortic stenosis; (2) LV ejection fraction >50%; (3) a mitral E wave to annular e' ratio >14¹⁷; or at least 2 of the following: (a) a mitral E wave to annular e' ratio >8; (b) treatment with a loop diuretic for control of HF symptoms; (c) left atrial volume index >34 mL/m² of body surface area (BSA); (d) NT-pro B-type natriuretic peptide level >200 pg/mL; and (e) LV mass index >149 g/m² in men and 122 g/m² in women (measured by cardiac MRI). Subjects without HF had an LVEF >50%, no significant valvular disease, and no symptoms and signs consistent with HF.

Key exclusion criteria were as follows: (1) claustrophobia; (2) presence of metallic objects or implanted medical devices in body; (3) more than mild aortic stenosis; (4) atrial fibrillation; (5) conditions that would make the study measurements less accurate or unreliable (ie, arrhythmia affecting cardiac gating, inability to perform an adequate breath hold

for cardiac MRI acquisitions); (6) known infiltrative or hypertrophic cardiomyopathy or extracardiac amyloidosis or sarcoidosis.

Plasma NO_M Measurements

Venous blood samples were obtained at the time of enrollment and stored at -80°C for batch analysis. NO_M were measured as previously described.¹⁸ Briefly, samples were passed through a filter (AmiconUltra-0.5 Centrifugal Filter Unit, EMD Millipore, Billerica, MA) to remove proteins with molecular weight >30 kDa. Samples were then injected into a custom-made ice-water-cooled reaction chamber containing vanadium (III)/hydrochloric acid solution heated to 95°C . NO generated from the reduction of NO_M was quantified by its gas-phase chemiluminescence reaction with ozone (Nitric Oxide Analyzer, Sievers Instruments, Boulder, CO). Signal peaks (mV) were manually integrated, and the corresponding areas were used for the quantification of NO_M concentrations. Authentic nitrate in the range of 0 to 50 $\mu\text{mol/L}$ was injected into the system, and a 10-point standard curve was constructed by plotting area against nitrate concentration. The detection limit of this assay was 1.6 $\mu\text{mol/L}$. The intraclass coefficient of variation of this method in our laboratory has been shown to be 0.96 (95% CI 0.92–0.98; $P<0.001$).

Measurement of LV Mass

Participants underwent a cardiac MRI examination to assess LV structure and function, using a 1.5-Tesla (T) whole-body MRI scanner (Avanto or Espree, Siemens, Malvern, PA) equipped with a phase-array cardiac coil. LV volumes and ejection fraction (EF) were determined using balanced steady-state free-precession (SSFP) cine imaging. Typical parameters were as follows: TR=30.6 milliseconds; TE=1.3 milliseconds; phases=30; slice thickness=8 mm; matrix size=192×192; parallel image (IPAT) factor=2. LV short-axis stack cine images were manually traced at end-diastole and end-systole using CMR42 software (Circle CVI, Calgary, AB, Canada). LV mass (LVM) was computed as the difference between epicardial and endocardial volumes, multiplied by myocardial density. LVM was normalized for body height in meters raised to the allometric power of 1.7.¹⁹

Assessment of Diffuse Myocardial Fibrosis

In a subset of participants (n=107), we assessed myocardial fibrosis with cardiac MRI. We used a modified Look-Locker inversion recovery (MOLLI)²⁰ sequence to assess T1 times prior to and following the intravenous administration of gadolinium contrast (gadopentetate dimeglumine,

0.15 mmol/kg or equivalent) in a midventricular short-axis slice (Figure 1A). Scan parameters for the MOLLI protocol included field of view (FOV) ~340 mm; matrix size=144×192; slice thickness=6 mm; repetition time=24.9 milliseconds; echo time=1.18 milliseconds; flip angle=30. Myocardial T1 measurements were performed before and at several time points (~5, 10, 15, and 20-40 minutes) post-gadolinium administration. All available blood and myocardial T1 measurements were used to compute λ (myocardium-blood partition coefficient) as the slope of the blood 1/T1 over the myocardial 1/T1 change, via linear regression²¹ (Figure 1B through 1D). The λ was used to compute the ECV as follows: $ECV = \lambda \times (1 - \text{hematocrit})$.

Statistical Methods

Continuous variables are presented as mean±SD unless otherwise stated. Categorical variables are presented as frequencies and percentages. Comparisons in NO_M among subjects without HF, subjects with HFrEF, and subjects with HFpEF were performed with 1-way analysis of variance (ANOVA), with post-hoc pairwise comparisons performed with Bonferroni correction, after testing for homogeneity of variance with the Levine test. Linear regression was performed to determine the relationship between the presence of HFpEF or HFrEF and NO_M, adjusting for multiple comorbidities. We also used linear regression to assess the association between NO_M and measures of LV geometry (LV mass, end-diastolic volume, myocardial ECV). NO_M levels were log-transformed because of their positively skewed distribution. All probability values are 2-tailed. Statistical significance was defined $\alpha < 0.05$. Statistical analyses were performed using SPSS for Windows v22 (SPSS Inc, Chicago, IL).

Results

We included 201 subjects in this study, among whom 126 had no evidence of HF, 43 had HFpEF, and 32 had HFrEF. Baseline clinical characteristics for these groups are presented in Table 1. Subjects with HFrEF had lower body mass indexes, lower blood pressure, and significantly more coronary artery disease than control subjects and subjects with HFpEF. The prevalence of hypertension was high in all 3 groups, without significant differences among the groups.

NO_M Levels in Subjects With HFpEF, HFrEF, and no HF

NO_M levels in subjects without HF versus those with HFrEF and HFpEF are shown in Figure 2. There was a significant difference between the groups (ANOVA $P = 0.013$). Post-hoc

pairwise comparisons revealed that subjects with HFpEF exhibited significantly lower levels of NO_M (8.0 $\mu\text{mol/L}$; 95% CI 6.2-10.4 $\mu\text{mol/L}$) compared to (12.0 $\mu\text{mol/L}$; 95% CI 10.4-13.9 $\mu\text{mol/L}$; $P = 0.025$) or to those with HFrEF (13.5 $\mu\text{mol/L}$; 95% CI 9.7-18.9 $\mu\text{mol/L}$; $P = 0.03$). There were no significant differences in NO_M between subjects with HFpEF and subjects without HF ($P = 1.00$).

Multivariable Predictors of NO_M Levels

Results of multivariable linear models in which HF status and various confounders were included as predictors of NO_M levels are shown in Table 2.

In a model that adjusted for age, sex, and race, HFpEF was a significant predictor of lower log-NO_M levels ($\beta = -0.42$; $P = 0.009$). Similarly, in a model that further adjusted for the presence of diabetes mellitus, body mass index, current smoking, systolic blood pressure, and glomerular filtration rate, HFpEF remained a significant predictor of lower log-NO_M ($\beta = -0.43$; $P = 0.013$). With further adjustment for ACE inhibitor use, angiotensin receptor blocker use, β -blocker use, spironolactone use, statin use, and long-acting nitrate use, HFpEF remained a significant predictor of lower log-NO_M ($\beta = -0.41$; $P = 0.028$). The presence of HFrEF was not a significant predictor of NO_M levels in any of these multivariable models ($P > 0.05$, Table 2).

Relationship Between NO_M Levels and LV Mass

Results of multivariable linear models examining the predictors of LV mass index in the study population are shown in Table 3. In a model that included age, sex, race, log-NO_M, and the presence of HFpEF and HFrEF, both HFpEF ($\beta = 15.08 \text{ g/m}^{1.7}$; $P < 0.0001$) and HFrEF ($\beta = 15.06 \text{ g/m}^{1.7}$; $P < 0.0001$) were significantly associated with a greater LV mass index. However, NO_M levels were not associated with LV mass index ($\beta = 0.16 \text{ g/m}^{1.7}$; $P = 0.92$). With incremental adjustment for the presence of diabetes mellitus, body mass index, current smoking, systolic blood pressure, and glomerular filtration rate (Table 3), HFpEF and HFrEF remained significant predictors of LV mass index, whereas NO_M was not (Table 3).

Relationship Between NO_M Levels and Myocardial ECV

Results of multivariable linear models examining the predictors of LV mass index in the study population are shown in Table 3. In unadjusted analyses ECV was significantly different among the groups (ANOVA $P = 0.004$). This was due to a significantly greater ECV in HFrEF compared to participants without HF ($P = 0.004$). In analyses that adjusted for age, sex,

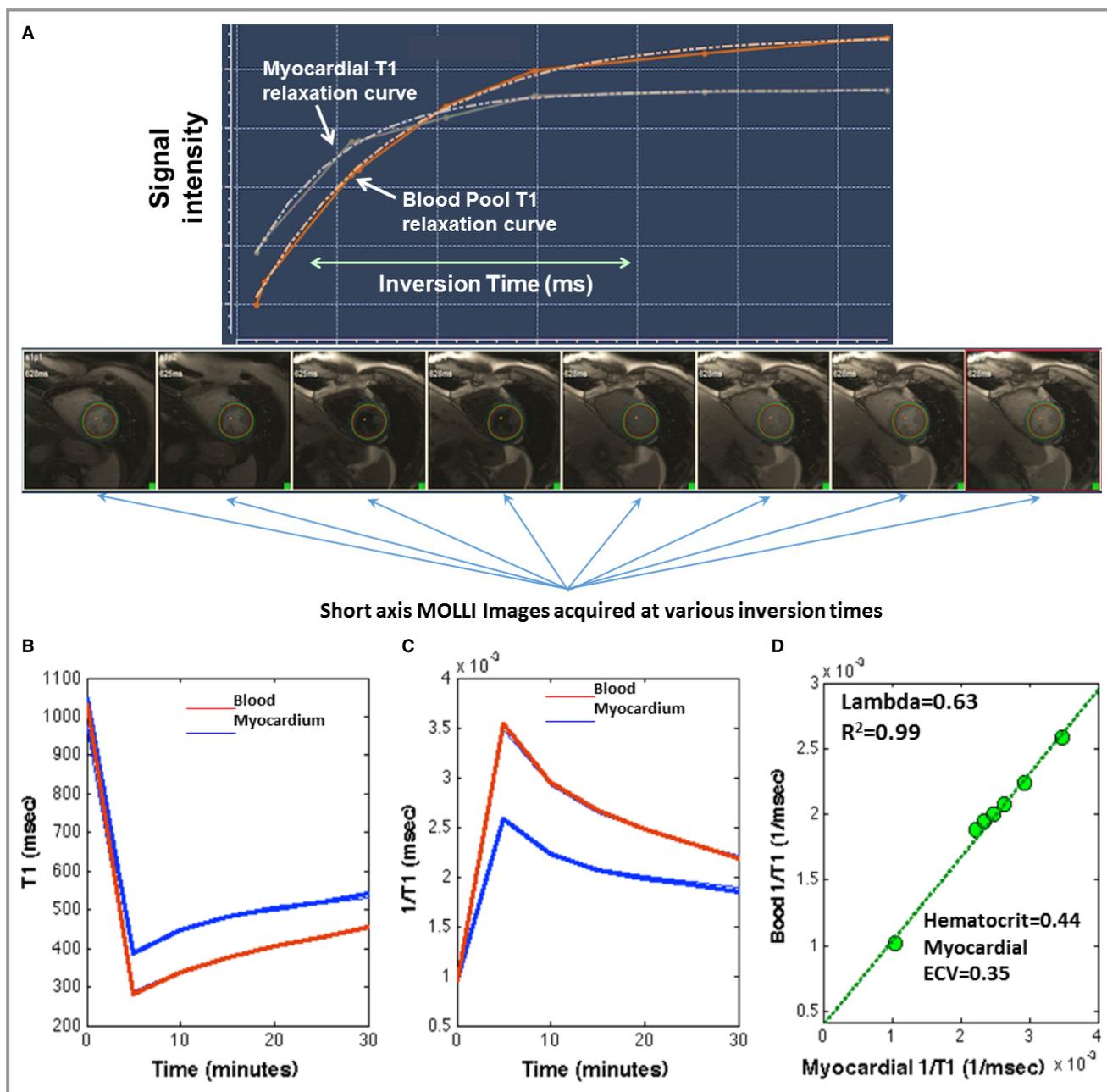


Figure 1. A, Myocardial T1 mapping using the modified Look-Locker inversion recovery sequence (MOLLl). Example of MOLLl single-slice T1 determinations performed at 8 different inversion times in a single breath hold. A region of interest (ROI) is defined manually in the images for the myocardium and for the blood pool. Then, the signal intensity in the images acquired at various inversion times is used to compute the time course of longitudinal relaxation (T1) for myocardium and blood. The myocardial T1 relaxation (white) and blood (orange) relaxation curves are shown. T1 for blood and myocardium is computed as the exponential time constant of the respective curves. B, MOLLl images are acquired before the administration of gadolinium-based contrast (time “zero”) and at several time points after the administration of gadolinium (~5, 10, 15, 20, and 30 minutes postinjection) in order to compute blood and myocardial T1 values at various time points for each subject (data shown correspond to 1 subject). Gadolinium administration reduces both myocardial and blood T1 (B) and thus increases T1 relaxivity ($1/T1$, C). Equilibrium is reached between blood and myocardium, such that when blood and myocardial T1 values are plotted against each other (D), a linear slope of the myocardial $1/T1$ over the blood $1/T1$ change (D) can be obtained with linear regression. This slope represents the gadolinium partition coefficient (lambda). Extracellular volume (ECV) is then computed as $\lambda \times (1 - \text{hematocrit})$.

race, presence of diabetes mellitus, body mass index, current smoking, systolic blood pressure, and glomerular filtration rate, both HFpEF ($\beta=3.31$; $P=0.039$) and HFrEF ($\beta=4.44$;

$P=0.009$) were significantly associated with a greater ECV. However, NO_M levels were not associated with ECV ($\beta=0.43$; $P=0.55$).

Table 1. Demographic, Clinical, and Laboratory Characteristics of the Study Population

	No HF	HFrEF	HFpEF	P Value*
	n=126	n=32	n=43	
Age, y	60.9, 12.7	65.2, 7.5	65.2, 10	0.038
Male	116 (92.1)	31 (96.9)	38 (88.4)	0.40
Race				0.43
White	58 (46)	13 (40.6)	12 (27.9)	
Black	61 (48.4)	19 (59.4)	30 (69.8)	
Other	7 (5.5)	0 (0)	1 (2.3)	
Pro B-type natriuretic peptide, pg/mL	184.1, 200.6	3809.2, 4932.8	911.2, 1508	<0.0001
Hypertension	100 (80)	27 (84.4)	39 (90.7)	0.26
Diabetes mellitus	65 (52)	15 (46.9)	29 (67.4)	0.14
Current smoking	27 (21.6)	11 (34.4)	9 (20.9)	0.28
Body mass index, kg/m ²	31.7, 7.2	28.2, 6.3	35.1, 6.7	<0.0001
Systolic BP, mm Hg	142.7, 17.4	139.5, 21.3	152.4, 18.8	0.004
Diastolic BP, mm Hg	83, 11.5	79.5, 10.4	85.4, 12.5	0.10
LDL cholesterol, mg/dL	97.9, 33.4	89.1, 41.1	97.9, 33.8	0.44
HDL cholesterol, mg/dL	42.2, 11.9	45.9, 12.2	42.9, 11.1	0.30
eGFR, mL/min per 1.73 m ²	89.1, 26.9	76.7, 22.8	76.7, 37.7	0.016
ACE inhibitor use	61 (48.8)	26 (81.3)	22 (51.2)	0.004
Angiotensin receptor blocker use	5 (4)	2 (6.3)	10 (23.3)	<0.0001
β-Blocker use	48 (38.4)	29 (90.6)	33 (76.7)	<0.0001
Spironolactone	3 (2.4)	3 (9.4)	1 (2.3)	0.14
Statin use	76 (60.8)	29 (90.6)	31 (72.1)	0.004

Numbers represent mean, SD or counts (%). ACE indicates angiotensin-converting enzyme; BP, blood pressure; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; LDL, low-density lipoprotein; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

*ANOVA *P* value for differences among the groups.

Effect Modification

We found no significant effect modification by either HFpEF or HFrEF status on the relationship between NO_M and either LV mass index or ECV (all *P*>0.05).

Discussion

In this study we demonstrate that HFpEF, but not HFrEF, is associated with reduced plasma NO_M, suggesting greater endothelial dysfunction, enhanced clearance, or deficient ingestion or metabolism of dietary inorganic nitrate. These findings support the concept of reduced NO bioavailability in HFpEF and may underlie the recently demonstrated salutary effects of inorganic nitrate supplementation observed in recent randomized trials in HFpEF.^{1,2,18,22} We also assessed the relationship between NO_M and LV remodeling. In contrast to a previous report that reported a relationship between electrocardiographic LVH and NO_M levels in a Japanese

normotensive population,⁹ we did not observe such a relationship with LV mass measured with cardiac MRI in the current study, which included a Western population. Furthermore, we did not find a relationship between NO_M and diffuse myocardial fibrosis.

Circulating NO_M represents an attractive integrated biomarker of NO bioavailability. Several studies have demonstrated that plasma NO_M levels are influenced by endogenous NO production by NOS.^{3,4} However, plasma NO_M levels can also be substantially increased via the ingestion of dietary nitrate.^{1,2,18} Nitrate and nitrite, the main NO_M, undergo enterosalivary cycles and are ultimately excreted by the kidneys.¹ Therefore, circulating NO_M integrate the influence of endogenous NOS-derived NO production, dietary intake of NO precursors, and clearance of NO metabolites. Interestingly, HFpEF remained associated with reduced NO_M levels after adjustment for estimated glomerular filtration rate, suggesting that renal clearance is unlikely to account for the observed difference.

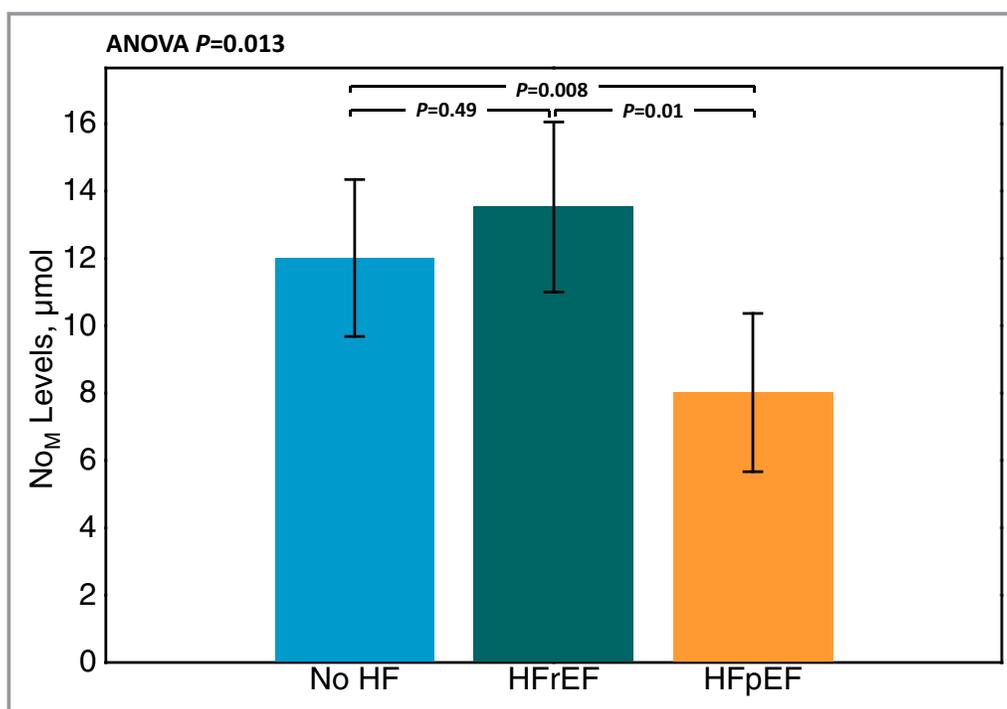


Figure 2. Comparison of metabolites (NO_M) levels between subjects without HF, subjects with heart failure and preserved ejection fraction (HFpEF) and subjects with heart failure and reduced ejection fraction (HFrEF).

Overall, our findings support the concept of reduced NO bioavailability in HFpEF, as suggested by recent studies.^{23,24} Reduced NO bioavailability has been demonstrated in myocardial tissue of patients with HFpEF compared to those with HFrEF,^{25,26} with reduced nitrate/nitrite content within myocardial homogenates of HFpEF patients.²⁶ Similarly, increased oxidative stress, which tends to reduce NO bioavailability, has been demonstrated in HFpEF participants as compared to those with HFrEF.^{25,26} In addition to reduced NOS-derived endogenous NO production, it is also possible that the reduced NO_M levels observed in HFpEF are the result of deficient ingestion or bioavailability of dietary inorganic nitrate and nitrite. It seems unlikely, however, that HFpEF patients have dietary intake patterns different from both HFrEF and control subjects, although this will need to be properly assessed in future studies.

It should be noted that, regardless of their source, plasma nitrate and nitrite (the most abundant circulating NO_M species) constitute a physiologic circulating storage pool of NO precursors, which can be readily converted to NO via the nitrate-nitrite-NO pathway.^{1,2,6-8} This conversion is particularly effective in the presence of hypoxia and acidosis, as occurs in skeletal muscle during exercise.^{1,2} Interestingly, a reduced vasodilatory reserve with exercise has been demonstrated in HFpEF, and inorganic nitrate supplementation led to an improvement in this vasodilatory reserve and increased oxygen consumption in this patient population.¹⁸ Our findings are therefore consistent and may underlie the positive effects of inorganic nitrate administration in recent randomized trials in patients with HFpEF. These trials have shown that inorganic nitrate supplementation with either a single dose of 12.9 mmol or sustained administration of 6 mmol/day for

Table 2. HFpEF and HFrEF as Predictors of log-NO_M Levels in Adjusted Linear Regression Models

	Model 1		Model 2		Model 3	
	β±SE	P Value	β±SE	P Value	β±SE	P Value
HFpEF	-0.42±0.16	0.009	-0.43±0.17	0.013	-0.41±0.19	0.028
HFrEF	0.1±0.18	0.573	0.03±0.19	0.887	0.04±0.20	0.83

Model 1: adjusted for age, sex, and race. Model 2: adjusted for variables in model 1 and further adjusted for the presence of diabetes mellitus, body mass index, current smoking, systolic blood pressure, and glomerular filtration rate. Model 3: adjusted for variables in model 2 and further adjusted for ACE inhibitor, angiotensin receptor blocker, β-blocker, spironolactone, statin, and long-acting nitrate use. ACE indicates angiotensin-converting enzyme; HFpEF, heart failure and preserved ejection fraction; HFrEF, heart failure and reduced ejection fraction; NO_M, metabolites;

Table 3. HFpEF, HFrEF, and log-NO_M as Predictors of LV Mass Index and Myocardial Extracellular Volume (ECV) in Linear Regression Models

	Model 1		Model 2	
	β±SE	P Value	β±SE	P Value
LV mass index				
HFpEF	15.08±3.61	<0.0001	12.58±3.59	0.001
HFrEF	15.06±3.7	<0.0001	17.77±3.67	<0.0001
Log-NO _M	0.16±1.6	0.92	0.88±1.57	0.58
Myocardial ECV				
HFpEF	1.87±1.52	0.223	3.31±1.58	0.039
HFrEF	5.01±1.55	0.002	4.44±1.67	0.009
Log-NO _M	0.3±0.71	0.676	0.43±0.72	0.553

Model 1: adjusted for age, sex, and race. Model 2: model 1, with further adjustment for the presence of diabetes mellitus, body mass index, current smoking, systolic blood pressure, and glomerular filtration rate. HFpEF indicates heart failure and preserved ejection fraction; HFrEF, heart failure and reduced ejection fraction; LV, left ventricular; NO_M, metabolites;

1 week increases NO_M levels and improves exercise capacity in HFpEF.^{1,2,18,22} Of note, these findings are in contrast to attempts at increasing endogenous NO signaling in this patient population using a phosphodiesterase inhibitor to prevent the breakdown of cyclic GMP, the second messenger of NO.²⁷ The latter findings have been attributed to low endogenous NO bioavailability. The low levels of NO_M observed in our study, which suggest reduced NO bioavailability, are consistent with this hypothesis.

Interestingly, NO_M were not reduced in HFrEF compared to subjects without HF. The reasons behind these observations are unclear and need to be assessed in future studies. Activation of inducible nitric oxide synthase (iNOS), leading to higher NO and NO_M levels, which has been reported in HFrEF, may partially explain this finding.^{28,29}

An additional novel contribution of our study is the assessment of the relationship between plasma NO_M and LV remodeling. We examined both macroscopic LVH (myocardial mass) and interstitial myocardial fibrosis using state-of-the-art MRI methods. We found that patients with HFpEF and, particularly, HFrEF exhibited greater myocardial fibrosis, which is consistent with other recent studies. However, in contrast to a previous study that demonstrated a relationship between ECG LVH and NO_M levels in a Japanese population of normotensive men,⁹ we did not find any relationship between NO_M levels and either LV mass or ECV (a measure of interstitial myocardial fibrosis). It is likely that multiple pathways are involved in the development of LVH and fibrosis, which may confound the association between NO_M and LV remodeling. It is also possible that high NO_M levels are “protective” against LV remodeling only in the presence of high dietary nitrate ingestion, as seen in Japanese diets. The

previous study from Japan reported a median NO_M level of 34.1 (IQR 22.6, 52.9) μmol/L, which is higher than the levels found in the present study; however, this comparison should be taken with caution, given potential differences in absolute NO_M levels due to different methods of measurement.

Our study should be interpreted in the context of its strengths and limitations. Strengths of our study include the inclusion of patients with and without HF, appropriate adjudication of HFpEF versus HFrEF, and the use of gold-standard noninvasive assessments of LV mass and diffuse myocardial fibrosis. Our study also has limitations. The sample size was limited, and some tests may have failed to reach significance due to limited statistical power. Dietary intake was not standardized at the time of our serum acquisition, and therefore, we cannot account for differences in inorganic nitrate/nitrite ingestion among the groups. Our population was a convenience clinical sample, which may not fully represent population-based trends. This is particularly true for the smaller subsample of subjects who underwent ECV measurements; our findings about ECV should be interpreted with caution and confirmed in future studies. Our study does provide inference about causality or reasons behind the reduced levels of NO_M in HFpEF. Further studies to assess the mechanisms leading to reduced levels of NO_M in HFpEF are needed. Similarly, further studies of interventions to increase NO_M levels (such as nitrate supplementation) are under way. Finally, we acknowledge that a measure of endothelial function (such as flow-mediated dilation) would have strengthened the study.

In conclusion, we report, for the first time, a reduction in plasma levels of NO_M in HFpEF, which may suggest either reduced endogenous synthesis of NO, increased clearance of NO_M, or decreased dietary intake of inorganic nitrate/nitrite. We did not observe a relationship between NO_M and either LV or diffuse myocardial fibrosis measured with cardiac MRI. Our findings regarding reduced NO_M levels in HFpEF may underlie the therapeutic effects of inorganic nitrate supplementation in HFpEF demonstrated in recent trials.

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