HHS Public Access

Author manuscript

Am J Cardiol. Author manuscript; available in PMC 2017 December 15.

Published in final edited form as:

Am J Cardiol. 2016 December 15; 118(12): 1855–1860. doi:10.1016/j.amjcard.2016.08.077.

Effect of Heart Failure with Preserved Ejection Fraction on Nitric Oxide Metabolites

Payman Zamani, MD, MTR[a], **Benjamin French, PhD**[b], **Jeffrey A. Brandimarto, MS**[a], **Paschalis-Thomas Doulias, PhD**[c], **Ali Javaheri, MD, PhD**[d], **Julio A. Chirinos, MD, PhD**[a], **Kenneth B. Margulies, MD**[a], **Raymond R. Townsend, MD**[e], **Nancy K. Sweitzer, MD, PhD**[f], **James C. Fang, MD**[g], **Harry Ischiropoulos, PhD**[c], and **Thomas P. Cappola, MD, ScM**[a]

[(a)]Division of Cardiovascular Medicine; Hospital of the University of Pennsylvania; Philadelphia, PA

[(b)]Department of Biostatistics and Epidemiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

[(c)]Children's Hospital of Philadelphia Research Institute, Philadelphia, PA

[(d)]Cardiovascular Division; Washington University School of Medicine, St. Louis, MO

[(e)]Division of Nephrology/Hypertension, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA

[(f)]Division of Cardiovascular Medicine, University of Arizona, Tucson, AZ

[(g)]Division of Cardiovascular Medicine, University of Utah, Salt Lake City, UT

Abstract

Endothelial function may be deranged in Heart Failure with Preserved Ejection Fraction (HFpEF). Serum NO-derived metabolites (NO_m) might provide a biochemical surrogate of endothelial function in heart failure (HF) patients. We measured serum NO_m in 415 participants in the Penn HF Study. Participants with HFpEF (n=82) and participants whose EF had recovered (Recovered-HF [n=125]) were matched 1:1 to Heart Failure with Reduced Ejection Fraction (HFrEF) participants based on age, sex, race, tobacco use, and eGFR. Serum NO_m levels were quantified after chemical reduction coupled with gas-phase chemiluminescence detection. After adjustment

Corresponding Author: Payman Zamani, MD, MTR, Hospital of the University of Pennsylvania, 3400 Spruce Street, 9026 Gates, Philadelphia, PA 19104, pzamani@upenn.edu, pH: (215) 662-6192, fax: (215) 349-8017.

Disclosures

PZ: none. TPC: Funding from BG medicine, Abbott Diagnostics. JAC: Consultant to Bristol Myers Squibb, OPKO Healthcare, Fukuda Denshi, Microsoft and Merck; received research grants from National Institutes of Health, American College of Radiology Network, Fukuda Denshi, Bristol Myers Squibb, Microsoft and CVRx Inc, and a device loan from Atcor Medical. JAC named as inventor in a University of Pennsylvania patent application for the use of inorganic nitrates/nitrites for the treatment of Heart Failure and Preserved Ejection Fraction. KBM: Research Grant, Modest: Juventis Therapeutics, Celladon Corporation, Thoratec Corporation, Innolign Biomedical, LLC. Research Grant, Significant: Merck, Inc. Consultant/Advisory Board, Modest: NovoNordisk (unpaid), Janssen, Merck, Pfizer, Ridgetop Research, AstraZeneca. RRT: consultant for Medtronic, Fukuda Denshii, Relypsa. HI received grants from National Institutes of Health and Sanofi.

for matching covariates and BMI, HFpEF (34.5 [IQR: 25.0, 51.5] μM) participants had lower NO_m levels than HFrEF (41.0 [IQR: 28.3, 58.0] μM; ratio of HFpEF:HFrEF 0.82 [95% CI: 0.67-0.99]; *P*=0.04), which further decreased when adjusted for covariates that impact endothelial function (ratio 0.79 [95% CI: 0.65-0.98]; *P*=0.03). There were no differences between HFrEF (34.0 [IQR: 25.3, 49.0]) and matched Recovered-HF (36.0 [IQR: 25.0, 55.0] μM) or HFpEF and Recovered-HF. Age (+21%/10-year increase, *P*<0.001) and black race (−28%, *P*=0.03) associated with NO_m in HFpEF; whereas, age (+11%/10-year increase, *P*=0.03), current tobacco use (+67%, *P*=0.01) and eGFR (*P*=0.01) associated with NO_m in Recovered-HF. In conclusion, HFpEF participants have reduced NO_m as compared to HFrEF in this matched cohort. This might suggest either compromised endothelial function or poor dietary intake. Black race was associated with lower NO_m in HFpEF.

Keywords

Heart failure; endothelial function; nitric oxide; race

Introduction

Heart Failure with preserved ejection fraction (HFpEF) is associated with reduced quality of life similar in magnitude to patients with heart failure with reduced ejection fraction (HFrEF).[1] While abnormalities within the myocardium have been demonstrated, increasing evidence suggests that peripheral mechanisms, such as endothelial dysfunction, can be important in the pathogenesis of HFpEF.[2,3] Yet despite this notion, studies of endothelial function in HFpEF have yielded mixed results.[4-10] Nitric oxide (NO), a ubiquitous signaling molecule with important cardiovascular effects, is generated by the nitric oxide synthases (NOS) as well as from the reduction of inorganic nitrate and nitrite.[11] Once produced, NO activates soluble guanylate cyclase (sGC) to increase the levels of cyclic guanosine monophosphate (cGMP). NO also engages in numerous secondary reactions that generate bioactive nitrosated and nitrated metabolites as well as nitrate and nitrite.[11] These NO-derived metabolites (NO_m) complement and expand vascular responses under physiological conditions and provide an additional pool of NO for when NOS-derived NO signaling is compromised. Increased NO_m have been quantified after acute exercise, indicating increased NO production.[12,13] Additionally, dietary supplementation with inorganic nitrate has been shown to increase NO_m.[14] Thus, the circulating levels of NO-derived metabolites could be used to track both endogenous and exogenous NO bioavailability and monitor vascular responses. We hypothesized that circulating NO_m, a biochemical reflection of diet and endothelial activity, would be reduced in HFpEF participants as compared to HFrEF.

Methods

Details of the Penn Heart Failure Study (PHFS) have been reported previously.[15] In brief, the PHFS recruited participants from three outpatient heart failure centers (University of Pennsylvania, Philadelphia, PA; Case Western Reserve University, Cleveland, OH; and the University of Wisconsin, Madison, WI) between 2003-2012. Clinical data were obtained via a standardized questionnaire administered to the patient and treating physician, with

verification from the medical record. Venous blood samples were obtained at the time of enrollment, regardless of dietary state, and stored at −80 °C for later analysis. An institutional review board from each of the participating centers approved the protocol; participants gave written informed consent.

Participants were classified into 3 heart failure phenotypes: HFrEF (ejection fraction < 50% on the entry echocardiogram), HFpEF (all echocardiograms demonstrating an LVEF>50%), and recovered heart failure (prior demonstration of an LVEF<50% with an LVEF>50% on the entry echocardiogram, Recovered-HF).[15]

For the present analysis, participants without a prior echocardiogram, without serum available, or with known infiltrative or hypertrophic cardiomyopathy were excluded. Participants who were on chronic NO-donating medications, such as organic nitrates or hydralazine, were excluded to avoid pharmacologic NO contributions. Because inorganic nitrate is renally-cleared, participants on dialysis or who had undergone a renal transplant were excluded.

Due to limited resources, propensity score matching was used to select participants for NO_m measurement. All PHFS participants with either HFpEF or Recovered-HF who met inclusion criteria were eligible for matching. Separately, participants with HFpEF and participants with Recovered-HF were matched 1-to-1 to participants with HFrEF. Nearest-neighbor matching was performed based on logit differences in propensity scores, which were obtained from logistic regression models that included age, sex, race (white, black, other or unknown), tobacco use (current, former, never, unknown), and estimated glomerular filtration rate (<45, 45-60, 60-90, >90 mL/min/1.73 m^2, or unknown). 'Unknown' categories were included such that the propensity score balanced the distribution of missing data between groups. Matching was performed using the MatchIt extension package to the R programming environment.[16]

Serum levels of NO metabolites (NO_m, primarily composed of nitrate, nitrite, NO-metal complexes, and low molecular weight protein-NO adducts) were measured as performed by our group previously.[14] In brief, samples were passed through a filter (AmiconUltra-0.5 Centrifugal Filter Unit, EMD Millipore) to remove proteins with molecular weight >30 kilodalton. Samples were then injected into a custom-made ice-water cooled reaction chamber containing vanadium(III)/hydrochloric acid solution heated to 95°C. The 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 concentration. Authentic nitrate in the range of 0 to 50 μmol/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 μmol/L. Samples were run in a total of 6 batches. Forty-three participants had replicate measurements performed in two different batches with an intraclass coefficient of variation of 0.96 (95% CI 0.92-0.98; $P<0.001$). Additionally, four participants had serum NO_m measurements performed from the same sample in all 6 batches with an intraclass coefficient of variation of 0.96 (95% CI 0.86-1.00; $P<0.001$), demonstrating excellent reproducibility.

NO_m levels were log transformed due to their positively skewed distribution. Linear regression models compared average NO_m levels between groups; exponentiated regression coefficients were converted to ratio differences. Adjusted models included variables in the propensity score in addition to body mass index (Model 1). Further adjustment was also performed for covariates known to impact endothelial function or NO_m levels: ischemic etiology, history of hypertension, history of hypercholesterolemia or statin use, history of diabetes or use of diabetes medications, and systolic blood pressure (Model 2). The variables used for adjustment were pre-specified. We considered the minimally adjusted model (Model 1) to be the primary analysis, for which $P<0.05$ indicated statistical significance.

In secondary analyses, we explored the relationship between our *a priori* identified covariates and NO_m levels in the preserved and recovered groups. Parsimonious models were created in which variables that did not improve model fit (based on the Akaike information criterion) were removed. Because the HFrEF group was comprised of individuals who were matched to baseline characteristics of either the HFpEF or Recovered-HF subjects, the distribution of covariates in the HFrEF group was felt to be artificial; therefore, correlates of NO_m were not explored in this group. Finally, in the pooled group of all participants, we compared NO_m levels between NYHA classes and determined the correlation between NO_m and BNP levels.

All analyses were performed in the R programming environment (version 3.2.0; R Foundation for Statistical Computing, Vienna, Austria).

Results

A total of 415 participants were included in this analysis of the PHFS. Eighty-two participants with HFpEF were matched to 82 participants with HFrEF; 125 participants with Recovered-HF were matched to 126 participants with HFrEF. Baseline demographic characteristics are presented in Table 1.

Median NO_m level in the HFpEF group was 34.5 (IQR 25.0, 51.5) μM versus 41.0 (IQR 28.3, 58.0) μM in the matched HFrEF group, corresponding to a ratio of 0.87 (95% CI 0.72-1.06; $P=0.16$). After adjustment for variables in the propensity score (age, sex, race, tobacco use, eGFR) and BMI, HFpEF participants had lower NO_m levels than HFrEF ($P=0.041$; Table 2). Further adjustment for covariates known to impact endothelial function increased the difference in NO_m levels between HFpEF and HFrEF participants (Table 2).

Median NO_m levels were 34.0 (IQR 25.3, 49.0) μM for the HFrEF group as compared to 36.0 (IQR 25.0, 55.0) μM for the matched Recovered-HF participants. There was no difference in the ratio of NO_m levels between Recovered-HF and HFrEF ($P>0.20$ for all analyses) or in the ratio between HFpEF versus Recovered-HF ($P>0.10$ for all analyses; Table 2).

Within HFpEF, age ($P<0.001$) and race (black versus non-black; −28.0%; $P=0.034$) were significantly correlated to NO_m levels (Table 3). Within the Recovered-HF group, age ($P=0.031$), current tobacco use ($P=0.014$), and eGFR ($P=0.010$) were associated with NO_m levels (Table 4).

In the pooled group of all participants, NO_m levels increased with increasing NYHA Class (NYHA Class I: median 31.0 [IQR 21.0, 44.0]; NYHA Class II: median 36.5 [IQR 25.3, 51.8]; NYHA Class III: median 39.5 [IQR 29.0, 62.0]; NYHA Class IV: median 47.0 [IQR 30.0, 82.0]; P=0.001). There was a modest correlation between NO_m and BNP levels (r=0.25, P<0.001).

Discussion

In this report, we demonstrate that participants with HFpEF have reduced NO_m levels as compared to matched HFrEF participants. We observed no difference in NO_m levels between HFpEF and Recovered-HF or between Recovered-HF and HFrEF participants. We demonstrate that black race was associated with reduced NO_m in HFpEF.

The reduction in the circulating levels of NO_m in HFpEF participants as compared to HFrEF suggests either: (1) impaired endothelial synthesis of NO, (2) increased tissue utilization of NO_m, (3) increased renal clearance of NO_m, (4) decreased inflammatory stimuli required for the activation of the inducible NOS (iNOS), or (5) decreased dietary intake of nitrate/nitrite. The participants were matched based on renal function, and group differences persisted despite adjustment for eGFR, making differences in renal clearance unlikely to account for the difference in NO_m levels. Moreover, increased oxidative stress, which would reduce NO production and bioavailability, has been demonstrated in myocardial tissue from HFpEF participants as compared to those with HFrEF.[17,18] To the degree that increased tissue oxidative stress and decreased NO bioavailability are reflective of systemic changes, our finding of decreased NO_m levels suggests differences in NO generation and/or bioavailability in HFpEF patients.

Numerous studies have demonstrated impaired endothelial function in HFrEF,[7,19-21] yet the data regarding endothelial function in HFpEF has been mixed.[4-10] While flow mediation dilation of the brachial artery is a standard metric of endothelial function, this test is based on increased flow generation by the microvasculature with a subsequent response by the brachial (conduit) artery. It is possible that these two processes are distinct, with some individuals demonstrating microvasculature impairments in augmenting flow to an ischemic stimulus, yet preserved conduit artery dilatory response to the flow generated.[10] Both of these processes are dependent, in part, on the endothelium. Our study provides complimentary data that might suggest systemic impairment in the generation of NO in HFpEF participants, as compared to those with HFrEF.

It is important to note that the relation between NO_m and endothelial function could be confounded in HFrEF, as additional sources of NO, aside from eNOS, can also be present. Activation of inducible nitric oxide synthase (iNOS), leading to higher NO and NO_m levels, has been reported in HFrEF.[22,23] We cannot exclude the possibility that our findings are due to elevated levels of NO_m in HFrEF, as opposed to reduced levels in HFpEF.

Decreased endothelial function has been demonstrated in African-Americans with chronic heart failure.[24] Increasing NO bioavailability in African-Americans with HFrEF has been shown to improve outcomes.[25] To the best of our knowledge, this is the first report

demonstrating decreased NO_m specifically in African-Americans with HFpEF. Our data suggest that African-Americans with HFpEF represent a unique group who might derive additional benefit from treatments that improve endothelial function.

Attempts at increasing nitric oxide signaling specifically in HFpEF have produced neutral findings in multi-center trials. Sildenafil, a phosphodiesterase-5 inhibitor that decreases the breakdown of cGMP, did not improve exercise capacity in HFpEF.[26] It is possible that mechanisms upstream of cGMP are deranged in HFpEF, leading to reduced cGMP generation,[17,18] as opposed to upregulated destruction. Alternatively it is plausible that non-cGMP signaling by NO, mediated by secondary nitrosated or nitrated molecules, is required for beneficial effects. More recently, isosorbide mononitrate did not improve activity, as assessed using an accelerometer, in a cross-over study of HFpEF participants,[27] with worse activity in participants who received the highest dose. Given that organic nitrate can worsen endothelial function in the setting of established disease,[28] it is possible that further decrements in NO bioavailability induced by organic nitrate contributed to the failure of isosorbide mononitrate to improve activity.

Conversely, supplementation with inorganic nitrate might provide an alternative pathway for increasing NO bioavailabilty in HFpEF. Inorganic nitrate is not subject to tolerance, improves exercise capacity,[14,29] and might improve skeletal muscle function,[30] presumably through increased NO signaling. Further study of inorganic nitrate in HFpEF is underway (ClinicalTrials.Gov: NCT02840799).

Strengths of our study include detailed matching of HFpEF and Recovered-HF participants to HFrEF participants in order to discern differences associated with the disease processes, independent of comorbid conditions. Limitations include the lack of a healthy control group as a reference for normative values of NO_m. An important limitation to this study is that dietary intake was not standardized at the time of our serum acquisition. Moreover, we did not measure nitrite and nitrate individually. However, that NO_m levels correlated with NYHA Class and BNP suggests that our NO_m measurements reflect the subjects' heart failure, as opposed to dietary changes alone. Finally, the PHFS was conducted in tertiary referral centers; hence, our results might not be generalizable to heart failure patients in the general population.

Acknowledgments

Sources of Funding:

PZ: Funding from the Institute for Translational Medicine and Therapeutics of the University of Pennsylvania (grant number: 5UL1TR000003-09 from the National Center for Research Resources), 5-T32-HL007843-17, and 1-K23-HL-130551-01. TPC: Funding from NIH (R01HL08577). JAC: Supported by NIH grants R56HL-124073-01A1, R01 HL 121510-01A1, and 5-R21-AG-043802-02. HI: funding from NIH HL54926, innovation Award from Sanofi and the Gisela and Dennis Alter Research Professor of Pediatrics. PTD: none. JAB: none. AJ is supported by 5T32HL007081-40. RRT: funding from NIH. BF: none. JCF: none. NKS: none.

References

1. Kitzman DW, Little WC, Brubaker PH, Anderson RT, Hundley WG, Marburger CT, Brosnihan B, Morgan TM, Stewart KP. Pathophysiological characterization of isolated diastolic heart failure in comparison to systolic heart failure. Jama. 2002; 288:2144–2150. [PubMed: 12413374]

2. Ferrari R, Bohm M, Cleland JG, Paulus WJ, Pieske B, Rapezzi C, Tavazzi L. Heart failure with preserved ejection fraction: uncertainties and dilemmas. Eur J Heart Fail. 2015; 17:665–671. [PubMed: 26079097]

3. Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. J Am Coll Cardiol. 2013; 62:263–271. [PubMed: 23684677]

4. Borlaug BA, Olson TP, Lam CS, Flood KS, Lerman A, Johnson BD, Redfield MM. Global cardiovascular reserve dysfunction in heart failure with preserved ejection fraction. J Am Coll Cardiol. 2010; 56:845–854. [PubMed: 20813282]

5. Akiyama E, Sugiyama S, Matsuzawa Y, Konishi M, Suzuki H, Nozaki T, Ohba K, Matsubara J, Maeda H, Horibata Y, Sakamoto K, Sugamura K, Yamamuro M, Sumida H, Kaikita K, Iwashita S, Matsui K, Kimura K, Umemura S, Ogawa H. Incremental prognostic significance of peripheral endothelial dysfunction in patients with heart failure with normal left ventricular ejection fraction. J Am Coll Cardiol. 2012; 60:1778–1786. [PubMed: 23040568]

6. Marechaux S, Samson R, van Belle E, Breyne J, de Monte J, Dedrie C, Chebai N, Menet A, Banfi C, Bouabdallaoui N, le Jemtel TH, Ennezat PV. Vascular and Microvascular Endothelial Function in Heart Failure With Preserved Ejection Fraction. J Card Fail. 2016; 22:3–11. [PubMed: 26386451]

7. Hundley WG, Bayram E, Hamilton CA, Hamilton EA, Morgan TM, Darty SN, Stewart KP, Link KM, Herrington DM, Kitzman DW. Leg flow-mediated arterial dilation in elderly patients with heart failure and normal left ventricular ejection fraction. Am J Physiol Heart Circ. 2007; 292:H1427–1434.

8. Haykowsky MJ, Herrington DM, Brubaker PH, Morgan TM, Hundley WG, Kitzman DW. Relationship of flow-mediated arterial dilation and exercise capacity in older patients with heart failure and preserved ejection fraction. J Gerontol A Biol Sci Med Sci. 2013; 68:161–167. [PubMed: 22522508]

9. Farrero M, Blanco I, Batlle M, Santiago E, Cardona M, Vidal B, Castel MA, Sitges M, Barbera JA, Perez-Villa F. Pulmonary hypertension is related to peripheral endothelial dysfunction in heart failure with preserved ejection fraction. Circ Heart Fail. 2014; 7:791–798. [PubMed: 25047042]

10. Lee JF, Barrett-O'Keefe Z, Garten RS, Nelson AD, Ryan JJ, Nativi JN, Richardson RS, Wray DW. Evidence of microvascular dysfunction in heart failure with preserved ejection fraction. Heart. 2015 10.1136/heartjnl-2015-308403.

11. Lundberg JO, Weitzberg E, Gladwin MT. The nitrate-nitrite-nitric oxide pathway in physiology and therapeutics. Nat Rev Drug Discov. 2008; 7:156–167. [PubMed: 18167491]

12. Allen JD, Cobb FR, Gow AJ. Regional and whole-body markers of nitric oxide production following hyperemic stimuli. Free Radic Biol Med. 2005; 38:1164–1169. [PubMed: 15808413]

13. Brown MD, Srinivasan M, Hogikyan RV, Dengel DR, Glickman SG, Galecki A, Supiano MA. Nitric oxide biomarkers increase during exercise-induced vasodilation in the forearm. Int J Sports Med. 2000; 21:83–89. [PubMed: 10727066]

14. Zamani P, Rawat D, Shiva-Kumar P, Geraci S, Bhuva R, Konda P, Doulias PT, Ischiropoulos H, Townsend RR, Margulies KB, Cappola TP, Poole DC, Chirinos JA. Effect of inorganic nitrate on exercise capacity in heart failure with preserved ejection fraction. Circulation. 2015; 131:371–380. discussion 380. [PubMed: 25533966]

15. Basuray A, French B, Ky B, Vorovich E, Olt C, Sweitzer NK, Cappola TP, Fang JC. Heart failure with recovered ejection fraction: clinical description, biomarkers, and outcomes. Circulation. 2014; 129:2380–2387. [PubMed: 24799515]

16. Ho D, Imai K, King G. Matching as Nonparametric Preprocessing for Reducing Model Dependence in Parametric Causal Inference. Polit Anal. 2007; 15:199–236.

17. van Heerebeek L, Hamdani N, Falcao-Pires I, Leite-Moreira AF, Begieneman MP, Bronzwaer JG, van der Velden J, Stienen GJ, Laarman GJ, Somsen A, Verheugt FW, Niessen HW, Paulus WJ. Low myocardial protein kinase G activity in heart failure with preserved ejection fraction. Circulation. 2012; 126:830–839. [PubMed: 22806632]

18. Franssen C, Chen S, Unger A, Korkmaz HI, De Keulenaer GW, Tschope C, Leite-Moreira AF, Musters R, Niessen HW, Linke WA, Paulus WJ, Hamdani N. Myocardial Microvascular

Inflammatory Endothelial Activation in Heart Failure With Preserved Ejection Fraction. JACC Heart Fail. 2016; 4:312–324. [PubMed: 26682792]

19. Hornig B, Maier V, Drexler H. Physical training improves endothelial function in patients with chronic heart failure. Circulation. 1996; 93:210–214. [PubMed: 8548890]
20. Katz SD, Schwarz M, Yuen J, LeJemtel TH. Impaired acetylcholine-mediated vasodilation in patients with congestive heart failure. Role of endothelium-derived vasodilating and vasoconstricting factors. Circulation. 1993; 88:55–61. [PubMed: 8391403]
21. Katz SD, Krum H, Khan T, Knecht M. Exercise-induced vasodilation in forearm circulation of normal subjects and patients with congestive heart failure: role of endothelium-derived nitric oxide. J Am Coll Cardiol. 1996; 28:585–590. [PubMed: 8772743]
22. Ishibashi Y, Shimada T, Sakane T, Takahashi N, Sugamori T, Ohhata S, Inoue S, Katoh H, Sano K, Murakami Y, Hashimoto M. Contribution of endogenous nitric oxide to basal vasomotor tone of peripheral vessels and plasma B-type natriuretic peptide levels in patients with congestive heart failure. J Am Coll Cardiol. 2000; 36:1605–1611. [PubMed: 11079665]
23. Winlaw DS, Smythe GA, Keogh AM, Schyvens CG, Spratt PM, Macdonald PS. Increased nitric oxide production in heart failure. Lancet. 1994; 344:373–374. [PubMed: 7914309]
24. Androne AS, Hryniewicz K, Hudaihed A, Dimayuga C, Yasskiy A, Qureshi G, Katz SD. Comparison of metabolic vasodilation in response to exercise and ischemia and endothelium-dependent flow-mediated dilation in African-American versus non-African-American patients with chronic heart failure. Am J Cardiol. 2006; 97:685–689. [PubMed: 16490438]
25. Taylor AL, Ziesche S, Yancy C, Carson P, D'Agostino R Jr. Ferdinand K, Taylor M, Adams K, Sabolinski M, Worcel M, Cohn JN, African-American Heart Failure Trial I. Combination of isosorbide dinitrate and hydralazine in blacks with heart failure. N Engl J Med. 2004; 351:2049–2057. [PubMed: 15533851]
26. Redfield MM, Chen HH, Borlaug BA, Semigran MJ, Lee KL, Lewis G, LeWinter MM, Rouleau JL, Bull DA, Mann DL, Deswal A, Stevenson LW, Givertz MM, Ofili EO, O'Connor CM, Felker GM, Goldsmith SR, Bart BA, McNulty SE, Ibarra JC, Lin G, Oh JK, Patel MR, Kim RJ, Tracy RP, Velazquez EJ, Anstrom KJ, Hernandez AF, Mascette AM, Braunwald E, Trial R. Effect of phosphodiesterase-5 inhibition on exercise capacity and clinical status in heart failure with preserved ejection fraction: a randomized clinical trial. Jama. 2013; 309:1268–1277. [PubMed: 23478662]
27. Redfield MM, Anstrom KJ, Levine JA, Koepp GA, Borlaug BA, Chen HH, LeWinter MM, Joseph SM, Shah SJ, Semigran MJ, Felker GM, Cole RT, Reeves GR, Tedford RJ, Tang WH, McNulty SE, Velazquez EJ, Shah MR, Braunwald E, Network NHFCR. Isosorbide Mononitrate in Heart Failure with Preserved Ejection Fraction. N Engl J Med. 2015; 373:2314–2324. [PubMed: 26549714]
28. Munzel T, Daiber A, Mulsch A. Explaining the phenomenon of nitrate tolerance. Circ Res. 2005; 97:618–628. [PubMed: 16195486]
29. Eggebeen J, Kim-Shapiro DB, Haykowsky M, Morgan TM, Basu S, Brubaker P, Rejeski J, Kitzman DW. One Week of Daily Dosing With Beetroot Juice Improves Submaximal Endurance and Blood Pressure in Older Patients With Heart Failure and Preserved Ejection Fraction. JACC Heart Fail. 2016; 4:428–437. [PubMed: 26874390]
30. Coggan AR, Leibowitz JL, Spearie CA, Kadkhodayan A, Thomas DP, Ramamurthy S, Mahmood K, Park S, Waller S, Farmer M, Peterson LR. Acute Dietary Nitrate Intake Improves Muscle Contractile Function in Patients With Heart Failure: A Double-Blind, Placebo-Controlled, Randomized Trial. Circ Heart Fail. 2015; 8:914–920. [PubMed: 26179185]

Table 1

Demographic, Clinical, and Laboratory Characteristics of this Substudy of the Penn Heart Failure Study

Variable	Preserved LVEF* (n=82)	Reduced LVEF* (n=82)	Recovered LVEF† (n=125)	Reduced LVEF† (n=126)
Median age (years, IQR)	61 (48, 71)	61 (47, 70)	56 (47, 66)	56 (47, 64)
Men	43 (52%)	40 (49%)	65 (52%)	59 (47%)
White	59 (72%)	56 (68%)	103 (82%)	102 (81%)
Black	17 (21%)	18 (22%)	19 (15%)	20 (16%)
Other race or unknown	6 (7%)	8 (10%)	3 (2%)	4 (3%)
Ischemic etiology	11 (13%)	31 (38%)	16 (13%)	33 (26%)
NYHA functional class III or IV	20 (24%)	29 (35%)	25 (20%)	41 (33%)
Hypertension	55 (67%)	45 (55%)	69 (55%)	64 (51%)
Hypercholesterolemia or statin use	49 (60%)	55 (67%)	71 (57%)	72 (57%)
Diabetes or use of diabetes medications	17 (21%)	20 (24%)	27 (22%)	26 (21%)
Tobacco use				
Current	4 (5%)	5 (6%)	9 (7%)	10 (8%)
Former	41 (50%)	38 (46%)	55 (44%)	64 (51%)
Never	33 (40%)	33 (40%)	57 (46%)	48 (38%)
Unknown	4 (5%)	6 (7%)	4 (3%)	4 (3%)
Median body mass index (kg/m^2, IQR)	31.0 (26.2, 38.9)	27.7 (23.8, 31.5)	27.8 (24.2, 33.5)	27.3 (24.4, 31.4)
Median systolic BP (mmHg, IQR)	126 (112, 138)	110 (98, 124)	114 (104, 127)	108 (97, 118)
Median eGFR (mL/min/1.73 m^2, IQR)	65.1 (53.2, 84.1)	65.7 (49.4, 83.9)	74.6 (59.4, 86.3)	72.6 (62.1, 89.2)
eGFR category				
<45 mL/min/1.73 m^2	11 (13%)	15 (18%)	13 (10%)	9 (7%)
45-60 mL/min/1.73 m^2	17 (21%)	16 (20%)	19 (15%)	17 (13%)
60-90 mL/min/1.73 m^2	40 (49%)	33 (40%)	64 (51%)	65 (52%)
≥90 mL/min/1.73 m^2	12 (15%)	15 (18%)	25 (20%)	27 (21%)
Unknown	2 (2%)	3 (4%)	4 (3%)	8 (6%)

BP, blood pressure; eGFR, estimated glomerular filtration rate; IQR, interquartile range; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association

[*] Penn Heart Failure Study participants with preserved LVEF were matched to participants with reduced LVEF based on a propensity score that included age, sex, race, tobacco use, and eGFR category.

[†] Penn Heart Failure Study participants with reduced LVEF were matched to participants with reduced LVEF based on a propensity score that included age, sex, race, tobacco use, and eGFR category.

Table 2

Comparison of NO_m levels between participants with Heart Failure with Reduced, Preserved, and Recovered Ejection Fraction

	HFpEF vs. HFrEF Estimate* (95% CI); P	Recovered-HF vs. HFrEF Estimate* (95% CI); P	HFpEF vs. Recovered-HF Estimate* (95% CI); P
Unadjusted	0.87 (0.72, 1.06); 0.16	1.08 (0.94, 1.24); 0.27	0.96 (0.81, 1.14); 0.64
Model 1	0.82 (0.67, 0.99); 0.041	1.07 (0.93, 1.24); 0.34	0.87 (0.74, 1.04); 0.14
Model 2	0.79 (0.65, 0.98); 0.030	1.10 (0.95, 1.28); 0.21	0.88 (0.74, 1.05); 0.15

CI, confidence interval.

*Estimate corresponds to the ratio of average NO_m levels between groups, obtained by exponentiating the regression coefficient from a linear regression model for log-transformed NO_m levels.

Model 1: Age, sex, race, tobacco use, body mass index, and eGFR category.

Model 2: Model 1, plus ischemic etiology, history of hypertension, history of hypercholesterolemia or statin use, history of diabetes or use of diabetes medications, and systolic blood pressure.

Table 3

Determinants of NO_m levels among Heart Failure with Preserved Ejection Fraction Participants

	Estimate* (95% CI); P
Age (10-year increase)	+21% (+12%, +31%); <0.001
Race (Black versus non-Black)	−28% (−47%, −3.0%); 0.034
Current tobacco use (yes vs. no)	−5.3% (−45%, 63%); 0.85

CI, confidence interval.

*Estimate corresponds to the ratio of average NO_m levels between groups, obtained by exponentiating the regression coefficient from a linear regression model for log-transformed NO_m levels and converting it to a percentage.

Table 4

Determinants of NO_m among Recovered-Heart Failure Participants

	Estimate* (95% CI); P
Age (10-year increase)	+11% (+1.1%, +22%); 0.031
Current tobacco use (yes vs. no)	+67% (+11%, +149%); 0.014
eGFR category	0.010
45-60 vs. <45 mL/min/1.73 m^2	−33% (−57%, +2.6%)
60-90 vs. <45 mL/min/1.73 m^2	−42% (−61%, −14%)
≥90 vs. <45 mL/min/1.73 m^2	−19% (−48%, +27%)

CI, confidence interval.

*Estimate corresponds to the ratio of average NO_m levels between groups, obtained by exponentiating the regression coefficient from a linear regression model for log-transformed NO_m levels and converting it to a percentage.