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Effect of organic and inorganic nitrates on cerebrovascular pulsatile power transmission in patients with heart failure and preserved ejection fraction

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Abstract

Objective: Increased penetration of pulsatile power to the brain has been implicated in the pathogenesis of age-related cognitive dysfunction and dementia, a common comorbidity in patients with heart failure and preserved ejection fraction (HFpEF). However, there is a lack of knowledge on the effects of organic and inorganic nitrates administration in this population on the power carried by pressure and flow waves traveling through the proximal aorta and penetrating the carotid artery into the brain microvasculature.

Approach: We assessed aortic and carotid hemodynamics non-invasively in two sub-studies: (1) at baseline and after administration of 0.4 mg of sublingual nitroglycerine (an organic nitrate; $n = 26$); and (2) in a randomized controlled trial of placebo (PB) versus inorganic nitrate administration (beetroot-juice (BR), 12.9 mmol NO₃; $n = 16$).

Main results: Wave and hydraulic power analysis demonstrated that NTG increased total hydraulic power (from 5.68% at baseline to 8.62%, $P = 0.001$) and energy penetration (from 8.69% to 11.63%; $P = 0.01$) from the aorta to the carotid, while inorganic nitrate administration did not induce significant changes in aortic and carotid wave power (power: 5.49%PB versus 6.25%BR, $P = 0.49$; energy: 8.89%PB versus 10.65%BR, $P = 0.27$).

Significance: Organic nitrates, but not inorganic nitrates, increase the amount of hydraulic energy transmitted into the carotid artery in subjects with HFpEF. These findings may have implications for the adverse effect profiles of these agents (such as the differential incidence of headaches) and for the pulsatile hemodynamic stress of the brain microvasculature in this patient population.

Keywords

heart failure with preserved ejection fraction; pulsatile arterial hemodynamics; organic nitrates; inorganic nitrates; brain circulation

Introduction

Heart failure (HF) affects approximately 6.5 million adults in the United States, with its prevalence rising to $\geq 10\%$ among persons 70 years of age or older (Benjamin *et al* 2017). Approximately half of patients with HF have a preserved left ventricular (LV) ejection fraction (HFpEF) (Udelson 2011). Patients with HFpEF demonstrate increased arterial stiffness and pulsatile arterial load, including increased late systolic pressure augmentation from arterial wave reflections arriving at the LV during systole (Weber *et al* 2008).

Pharmacological treatment of HFpEF aims at lowering ventricular load, with nitrates, both in organic and inorganic form, being part of the therapeutic arsenal. Organic nitrates have been shown to reduce arterial load to the LV after acute administration (Heart Failure Society of America 2006, Hunt *et al* 2009), but they are also associated with a high incidence of side effects such as headache, dizziness and hypotension in patients with HFpEF, and recent trials failed to demonstrate a clinical benefit in this population (Zamani *et al* 2016, 2017). In contrast to available data with organic nitrates, several recent studies have demonstrated that inorganic nitrate improves hemodynamics in HFpEF and has not been associated with a high incidence of headache or important reductions in blood pressure (Zamani *et al* 2015, Eggebeen *et al* 2016).

In a recent study (Chirinos *et al* 2017), we found that the acute administration of organic nitrates has a profound effect on carotid hemodynamics, with a marked reduction in carotid bed vascular resistance and dilation of the carotid artery, which may account for the high incidence of headaches and dizziness, respectively. The effects of vasoactive drugs on cerebrovascular hemodynamics, however, may be relevant beyond acute side effects such as headache. Accumulating evidence links increased penetration of pulsatile energy (power) to the brain, with the development and/or progression of cognitive dysfunction and dementia (Mitchell *et al* 2011, Chirinos *et al* 2013), which are common comorbidities in the HFpEF population. The brain is a high-flow, low-resistance organ, in which the microvasculature is more directly exposed to the pulsatility that occurs in central arteries. Although organic nitrates reduce central pulse pressure in HFpEF (Chirinos *et al* 2017), the marked associated cerebrovascular vasodilatory effect, with reduction of carotid characteristic impedance and carotid bed vascular resistance, may actually facilitate pulsatile power penetration into the brain microvasculature, potentially undermining the beneficial effects of these drugs on central pulse pressure. The effects of organic nitrates on power penetration to the brain have not been previously assessed, nor have these effects been compared with the effects of inorganic nitrates.

The aim of this study is therefore to quantify and characterize the patterns of hydraulic power carried by waves traveling through the proximal aorta and the carotid artery, and the effects of organic and inorganic nitrates on these parameters among patients with HFpEF.

Methods

The present work is based on previous studies of organic (Chirinos *et al* 2017) and inorganic nitrates (Zamani *et al* 2015) in HFpEF, here referred to as sub-studies 1 and 2. In sub-study 1, aortic and carotid hemodynamics were assessed non-invasively at baseline and after the administration of 0.4 mg of sublingual Nitroglycerine (NTG), an organic nitrate. In sub-study 2, aortic and carotid pressure-flow data was analyzed from a PB-controlled randomized trial of inorganic nitrate administration (nitrate-rich beetroot juice) in HFpEF (Zamani *et al* 2015). Protocols were approved by the University of Pennsylvania and Philadelphia Veterans Affairs (VA) Medical Center Institutional Review Boards, as appropriate. All subjects provided written informed consent before enrollment. Inclusion and exclusion criteria are described in detail elsewhere (Zamani *et al* 2015, Chirinos *et al* 2017).

Study protocol

Sub-study 1—Twenty-six participants were included in this protocol. After at least 10 min of rest in the supine position, BP was taken on the right arm with a validated oscillometric device (Omron HEM-705CP, Omron Corporation, Kyoto, Japan). For echocardiographic and Doppler ultrasound assessments, a GE-e9 ultrasound machine (GE Healthcare; Fairfield, CT) equipped with a cardiac and vascular probe was used. Pulsed wave Doppler measurements of flow velocities in the LV outflow tract were performed and recorded placing the Doppler sample immediately proximal to the aortic valve leaflets within the centerline of the LV outflow tract, and its cross-sectional area was computed from the radius (r) measured in the parasternal long axis view (area = πr^2). Carotid diameter and blood velocity were acquired using a vascular linear probe.

Carotid artery applanation tonometry was performed using a SphygmoCor CPV System (AtCor Medical Inc, Itasca, IL) that operates with a high-fidelity applanation tonometer (Millar Instruments; Houston, TX). Aortic pressure waveforms are only obtainable through invasive measurements, which is not applicable in clinical practice. Therefore, derived measurements should be used instead and, previously, it has been demonstrated that carotid pressure waveforms represent reasonable surrogates of the aortic pressure waveform (Chen *et al* 1996, Segers *et al* 2005). After baseline measurements were obtained, a single dose (0.4 mg) of NTG was administered sublingually, and measurements were repeated starting at least 2 min after administration. Comparisons were made between measurements obtained pre-versus post-NTG administration.

Sub-study 2—Seventeen subjects participated in a randomized, double-blind, crossover study of a single dose of inorganic nitrate given as concentrated nitrate-rich beetroot juice (BR) (NO_3^- , BEET IT Sport, James-White Drinks Ltd, Ipswich, UK) containing 12.9 mmol NO_3^- in 140 ml versus an otherwise identical nitrate-depleted PB juice (James White Drinks, Ltd). There was a washout period of at least 5 d separating the interventions. Measurements of aortic and carotid hemodynamics were performed using identical methods as in sub-study 1, ~2.5 h after juice ingestion. Comparisons were made between measurements obtained after administration of nitrate-rich (BR) versus nitrate-depleted (PB) beetroot juice. One

subject was excluded from these analyses due to the lack of carotid flow data during one of the study visits.

Hemodynamic analyses

A custom-designed software was built in Matlab (The Mathworks, Natick, MA) (Segers *et al* 2007, Chirinos *et al* 2009) to process off-line central arterial tonometry recordings and Doppler flow velocity files. Signal averaging was performed first, followed by the time alignment of both signals as previously described (Segers *et al* 2007, Nichols *et al* 2011). Hemodynamic analysis commonly assumes a parabolic or flat flow velocity profile to convert velocity measured in a sample volume into a volumetric flow. However, this simplification may be inadequate for the carotid artery, as the flow velocity profile is neither of both. We therefore implemented a conversion accounting for the Womersley number (a well-established dimensionless fluid-dynamics parameter for oscillatory flow), as previously described (Ponzini *et al* 2010).

Wave power analysis

Recently, Mynard *et al* (Mynard and Smolich 2016) introduced the concept of wave power as a means to analyse the energy carried by the incident and reflected pressure and flow waves. This technique finds its origin in wave intensity analysis but has the major advantage of allowing for a quantitative analysis of how the energy transmitted by the heart into the aorta is distributed over the arterial tree.

Figure 1 shows a graphical depiction of wave power analysis. After digitally re-sampling signals to 500 Hz for a more accurate time-alignment of pressure and flow, wave power ($d\pi$) was defined as the product of the instantaneous changes in pressure and flow ($dP * dQ$). Wave power can be separated into the power carried by forward-traveling ($d\pi_+$) and backward-traveling ($d\pi_-$) waves:

$$d\pi_{\pm} = \pm \frac{1}{4Z_c} (dP \pm Z_c dQ)^2 \quad (1)$$

where Z_c = characteristic impedance (calculated in the frequency domain), and total power ($d\pi$) equals the sum of power carried by forward ($d\pi_+$) and backward ($d\pi_-$) waves.

Wave power is a function of time and demonstrates two major positive peaks: a forward compression wave (FCW), generated by the contraction of the LV in early systole, and a forward expansion wave (FEW), generated as the rate of myocardial shortening is reduced in late systole (Penny *et al* 2008, Mynard and Smolich 2016). We report the area under the curve (AUC) of FCW (grey area) and FEW (light grey area) at the aorta and the carotid arteries, while the black area is the backward wave (figure 1(B)).

We also computed total hydraulic power which is related to the rate of energy production and expenditure in the cardiovascular system, as previously described (Mynard and Smolich 2016). We calculated the AUC in total hydraulic power as the total energy transmitted from

the heart to the aorta and to the carotid artery. We also computed the peak values of the total hydraulic power curve at both locations (figure 1(C)).

We computed the power penetration from the aorta into the carotid vascular circulation, as an index based on total hydraulic power parameters (peak power or energy as the AUC) as follows:

$$\%P_{\text{etration}} = \frac{\text{Carotid Power Parameter}}{\text{Aortic Power Parameter}} * 100. \quad (2)$$

Statistical analysis

Descriptive data are presented as mean $\pm$ standard deviation (SD) for continuous variables or counts (%) for categorical variables. Comparisons between pre- and post-NTG values for sub-study 1, and between values corresponding to nitrate-rich (BR) versus nitrate-depleted (PB) beetroot juice administration for sub-study 2, were performed using paired t-tests. Physiologic indices were expressed as absolute values at each time point, as well as absolute differences between measurements (with 95% confidence intervals, CIs). Positive differences represent an increase in the values associated with organic/inorganic nitrate administration, whereas negative differences represent a decrease in such values. A two-tailed P value < 0.05 was considered statistically significant. Statistical analyses were performed using SPSS v-21 for Windows (SPSS Inc., Chicago, IL).

Results

General characteristic parameters obtained from both sub-study populations are shown in table 1. The mean age of participants was 60 and 65 years in sub-studies 1 and 2, respectively. Consistent with the HFpEF phenotype, subjects in both studies were obese, had a high prevalence of hypertension and diabetes, as well as left atrial enlargement. Both sample populations were composed predominantly of males, with a high proportion of African-Americans. All subjects in both sub-studies had NYHA class II–III symptoms.

Sub-study 1: effects of organic nitrate

Parameters obtained from wave and hydraulic power analysis before and after NTG intake are summarized in table 2. Aortic and carotid FCW and FEWs were not significantly different after NTG administration. There was a reduction in the total aortic hydraulic power energy (1.53 J/beat to 1.35 J/beat, change: 11.8% decrease, $P = 0.01$) and a trend towards a decrease in peak hydraulic power (7.26 W to 6.59 W; change: 9.2% decrease, $P = 0.06$). In contrast, there was a significant increase in peak carotid hydraulic power (0.39 W to 0.54 W; change: +38.5% increase, $P = 0.008$) after NTG administration. Figure 2(A) demonstrates the post-NTG change in total hydraulic power parameters. The observed increase in carotid hydraulic power despite a reduced aortic hydraulic power implies an increase in the percentage of power transmission from the aorta into the carotid (peak total hydraulic power penetration: 5.68% versus 8.62%; $P = 0.001$; total hydraulic energy penetration: 8.69% versus 11.63%; $P = 0.01$, figure 3(A)).

Sub-study 2: effects of inorganic nitrate

Table 2 displays the calculated wave and hydraulic power parameters for sub-study 2. No significant changes were found for aortic or carotid values between PB and beetroot intake. Figure 2(B) shows the percent difference in total hydraulic power parameters at the aorta and the carotid arteries in the BR intervention compared to the PB intervention. Figure 3(B) displays a comparison of the percent changes in power penetration parameters between these two conditions, demonstrating non-significant differences between these hemodynamic parameters after PB versus BR intake.

Discussion

In this study, we quantified the effect of organic and inorganic nitrates on pulsatile power penetration to the brain in patients with HFpEF. Administration of NTG, an organic nitrate, led to a significant reduction of aortic hydraulic power, an increase in power penetration to the carotid artery (due to cerebrovascular vasodilation), and an absolute increase in carotid hydraulic power. In contrast, inorganic nitrate administration did not induce significant changes in aortic and carotid wave power or percentage of power penetration. Our findings have important implications for our understanding of the cerebrovascular hemodynamic effects of organic and inorganic nitrates in this population, which in turn may have implications for the adverse effect profile of these drugs and cerebrovascular microvascular health.

In contrast to inorganic nitrate (Chirinos and Zamani 2016), organic nitrates result in undesirable side effects, such as pulsatile headache, flushing, hypotension or even syncope (Yaginuma *et al* 1986, Chirinos *et al* 2017). In particular, recent trials of organic nitrates in HFpEF have demonstrated poor tolerability, with a high incidence of side effects (Redfield *et al* 2015, Zamani *et al* 2016) and frequent discontinuation of long-term use (Zamani *et al* 2017). The important incidence of headaches is possibly related to the increase on pulsatile power penetration to the cerebrovascular territory demonstrated in our study. However, the importance of this hemodynamic effect may extend beyond its role in headaches. Increased penetration of pulsatile power to the brain is progressively recognized as an important factor in the development of cognitive dysfunction and dementia (Mitchell *et al* 2011, Chirinos *et al* 2013), which are common comorbidities in the HFpEF population (Cannon *et al* 2015, Cermakova *et al* 2015). Due to its high blood flow needs, the brain vasculature is a low-resistance bed, in which the microvasculature is more directly exposed to the pulsatility that occurs in conduit vessels. Therefore, the pulsatile power transmission from the aorta to the conduit branches that feed the cerebrovascular territory is a key determinant of pulsatile hemodynamics in the cerebrovascular microcirculation. We demonstrate, for the first time, that despite the reduction in blood pressure induced by NTG in HFpEF, the marked associated cerebrovascular vasodilatory effect actually leads to increased (rather than decreased) hydraulic power transmission into the carotid territory. In contrast, we demonstrate that inorganic nitrates do not increase pulsatile power penetration to the brain.

Nitrates/nitrites-related adverse events can limit compliance with these medications and have an undesired impact on various outcomes (such as outpatient physical activity) in HFpEF. Detailed assessments of their hemodynamic effects can contribute to elucidate the

mechanisms of these side effects. Given that the incidence of headaches has been reported to be greater with organic versus inorganic nitrates, our study focused on the differential effects of these agents on brain hemodynamics. However, we acknowledge that the relationship between our hemodynamic findings and the incidence of adverse events should be further investigated in future studies.

Our study should be interpreted in the context of its strengths and limitations. Strengths include the careful assessment of pulsatile carotid and aortic hemodynamics in an experimental design, using state-of-the-art non-invasive pressure-flow analyses. In particular, we implemented an ad hoc method to assess pulsatile power in the aorta and carotid arteries, as recently proposed by Mynard *et al* (Mynard and Smolich 2016). This method quantifies the distribution of wave power at junctions, is relatively insensitive to diameter variations, and is quantitatively linked to transient changes in hydraulic pressure power, a concept related to the rate of energy production and expenditure in the cardiovascular system. Additional strengths of our study include the use of identical methods to measure hemodynamics after organic and inorganic nitrate administration, facilitating the interpretation of differential hemodynamic effects. Our study also has several limitations. Our study samples were relatively small; however, the paired design of the studies greatly reduces measurement variability and allows for a robust assessment of the drug effects in each sub-study. However, our sub-studies represent post hoc analyses of two separate paired experiments, which prevents a direct comparison of the effects of organic versus inorganic nitrates. Both studies were performed as acute administration studies, and the chronic effects of these drugs on the patterns of power carried by waves traveling the aorta and the carotid artery could be different. Due to the characteristics of the patient population at the VA Medical Center, where subjects were primarily recruited, our study population was composed primarily of men.

We note that the hemodynamic effects reported herein for inorganic nitrate likely do not apply to shortacting inorganic nitrite preparations. Inhaled sodium nitrite, which is currently being studied in HFpEF, demonstrates markedly different pharmacokinetic properties compared to orally administered inorganic nitrate (Reddy *et al* 2017). The half-life of inhaled sodium nitrite is only ~35 min (Rix *et al* 2015), in contrast to several hours for inorganic nitrate (Kapil *et al* 2010), and intermittent inhaled administration is associated with pronounced circulating level fluctuations, which may cause hypotensive episodes in elderly patients with HFpEF. To our knowledge, no studies have been done assessing the cerebrovascular hemodynamic effects of sodium nitrite in HFpEF. Ongoing trials (the KNO₃CK OUT HFpEF trial with orally administered potassium nitrate and the INDIE HFpEF trial with inhaled sodium nitrite (Reddy *et al* 2017)) will help clarify the side effect profiles and hemodynamic differences between inorganic nitrite and nitrate in this patient population.

In conclusion, to the extent of our knowledge, this study is the first to assess the effects of organic and inorganic nitrate on aortic and carotid wave and hydraulic power and energy in patients with HFpEF. Our study demonstrates that organic nitrates increase power penetration from the aorta into the carotid, which leads to a significant increase in carotid pulsatile hydraulic power, despite a reduction in aortic power. In contrast, inorganic nitrate

administration did not induce significant changes in aortic and carotid wave and hydraulic power or percentage of power penetration. These hemodynamic effects likely have implications for the pulsatile hemodynamic stress of the microvasculature of the brain. In the short term, these hemodynamic differences may contribute to the differential incidence of side effects (such as headaches) with organic versus inorganic nitrate therapy. In the long term, they may have implications for cognitive decline. However, the causal link between carotid pulsatile hemodynamics and cognitive decline, particularly in the setting of vasodilator therapy, requires further study.

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References

- Benjamin EJ et al. 2017 Heart disease and stroke statistics—2017 update: a report from the American Heart Association *Circulation* 135 e146–603 [PubMed: 28122885]
- Cannon JA, McMurray JJ and Quinn TJ 2015 ‘Hearts and minds’: association, causation and implication of cognitive impairment in heart failure *Alzheimers Res. Ther* 7 22 [PubMed: 25722749]
- Cermakova P, Eriksdotter M, Lund LH, Winblad B, Religa P and Religa D 2015 Heart failure and Alzheimer’s disease *J. Intern Med* 277 406–25 [PubMed: 25041352]
- Chen CH et al. 1996 Validation of carotid artery tonometry as a means of estimating augmentation index of ascending aortic pressure *Hypertension* 27 168–75 [PubMed: 8567037]
- Chirinos JA and Zamani P 2016 The nitrate-nitrite-NO pathway and its implications for heart failure and preserved ejection fraction *Curr. Heart Fail. Rep* 13 47–59 [PubMed: 26792295]
- Chirinos JA et al. 2009 Time-varying myocardial stress and systolic pressure-stress relationship: role in myocardial-arterial coupling in hypertension *Circulation* 119 2798–807 [PubMed: 19451350]
- Chirinos JA et al. 2013 Central pulse pressure and its hemodynamic determinants in middle-aged adults with impaired fasting glucose and diabetes: the Asklepios study *Diabetes Care* 36 2359–65 [PubMed: 23610081]
- Chirinos JA et al. 2017 Effects of organic and inorganic nitrate on aortic and carotid haemodynamics in heart failure with preserved ejection fraction *Eur. J. Heart Fail* (10.1002/ejhf.885)
- Engelbeen J et al. 2016 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 Failure* 4 428–37 [PubMed: 26874390]
- Heart Failure Society of America 2006 Evaluation and management of patients with heart failure and preserved left ventricular ejection fraction *J. Cardiac Failure* 12 e80–5
- Hunt SA et al. 2009 Focused update incorporated into the ACC/AHA 2005 Guidelines for the diagnosis and management of heart failure in adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines: developed

in collaboration with the International Society for Heart and Lung Transplantation *Circulation* 119 e391–479 [PubMed: 19324966]

Kapil V et al. 2010 Inorganic nitrate supplementation lowers blood pressure in humans: role for nitrite-derived NO *Hypertension* 56 274–81 [PubMed: 20585108]

Mitchell GF et al. 2011 Arterial stiffness, pressure and flow pulsatility and brain structure and function: the age, gene/environment susceptibility—Reykjavik study *Brain* 134 3398–07 [PubMed: 22075523]

Mynard JP and Smolich JJ 2016 Novel wave power analysis linking pressure-flow waves, wave potential, and the forward and backward components of hydraulic power *Am. J. Physiol. Heart Circ. Physiol* 310 H1026–38 [PubMed: 26873972]

Nichols W, O'Rourke M and Vlachopoulos C 2011 McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles (Boca Raton, FL: CRC Press)

Penny DJ, Mynard JP and Smolich JJ 2008 Aortic wave intensity analysis of ventricular-vascular interaction during incremental dobutamine infusion in adult sheep *Am. J. Physiol. Heart Circ. Physiol* 294 H481–9 [PubMed: 18024544]

Ponzini R et al. 2010 Womersley number-based estimates of blood flow rate in Doppler analysis: in vivo validation by means of phase-contrast MRI *IEEE Trans. Biomed. Eng* 57 1807–15 [PubMed: 20659826]

Reddy YNV. et al. 2017; INDIE-HFpEF (inorganic nitrite delivery to improve exercise capacity in heart failure with preserved ejection fraction): rationale and design. *Circ. Heart Fail.* 10:e003862. [PubMed: 28476756]

Redfield MM et al. 2015 Isosorbide mononitrate in heart failure with preserved ejection fraction *New Engl. J. Med* 373 2314–24 [PubMed: 26549714]

Rix PJ et al. 2015 Pharmacokinetics, pharmacodynamics, safety, and tolerability of nebulized sodium nitrite (AIR001) following repeat-dose inhalation in healthy subjects *Clin. Pharmacokinet* 54 261–72 [PubMed: 25421879]

Segers P et al. 2005 Carotid tonometry versus synthesized aorta pressure waves for the estimation of central systolic blood pressure and augmentation index *Am. J. Hypertens* 18 1168–73 [PubMed: 16245411]

Segers P et al. 2007 Noninvasive (input) impedance, pulse wave velocity, and wave reflection in healthy middle-aged men and women *Hypertension* 49 1248–55 [PubMed: 17404183]

Udelson JE 2011 Heart failure with preserved ejection fraction *Circulation* 124 e540–3 [PubMed: 22105201]

Weber T, O'Rourke MF, Ammer M, Kvas E, Punzengruber C and Eber B 2008 Arterial stiffness and arterial wave reflections are associated with systolic and diastolic function in patients with normal ejection fraction *Am. J. Hypertens* 21 1194–202 [PubMed: 18787521]

Yaginuma T et al. 1986 Effect of glyceryl trinitrate on peripheral arteries alters left ventricular hydraulic load in man *Cardiovasc. Res* 20 153–60 [PubMed: 3085950]

Zamani P et al. 2015 Effect of inorganic nitrate on exercise capacity in heart failure with preserved ejection fraction *Circulation* 131 371–80 [PubMed: 25533966]

Zamani P et al. 2016 Effect of heart failure with preserved ejection fraction on nitric oxide metabolites *Am. J. Cardiol* 118 1855–60 [PubMed: 27742422]

Zamani P. et al. 2017; Isosorbide dinitrate, with or without hydralazine, does not reduce wave reflections, left ventricular hypertrophy, or myocardial fibrosis in patients with heart failure with preserved ejection fraction. *J. Am. Heart Assoc.* 6:e004262. [PubMed: 28219917]

Figure 1.

Graphical depiction of wave and hydraulic power analysis to illustrate steps and parameters extracted, including (A) tonometry (grey solid line) and ultrasound (black dotted line) signals alignment, and (B) wave power analysis ($d\pi$), and (C) total hydraulic power analysis and selected parameters: area under the curve (Energy, grey area) and peak values (Power, black diamond). At left and right panels, aortic and carotid signals and curves are shown, respectively. Presented signals are pre-NTG. AUC = area under the curve; FCW = forward compression wave (grey area); FEW = forward expansion wave (light grey area); black represents the backward wave. * Indicates a statistically significant change ($P < 0.05$).

Figure 2. Forest plots describing the change in percentage of selected parameters: after the administration of sublingual NTG (A) and between nitrate-depleted (PB) and nitrate-rich (BR) beet root juice intake (B). * Indicates a statistically significant change ($P < 0.05$)

Figure 3. Percentage of carotid total hydraulic power penetration after the administration of sublingual NTG (A) and between nitrate-depleted (PB) and nitrate-rich (BR) beet root juice intake (B).
* Indicates a statistically significant change against pre-NTG (3A) or PB (3B).

Table 1.

General characteristics of participants (Chirinos *et al* 2017).

Variable	Sub-study 1 (<i>n</i> = 26)	Sub-study 2 (<i>n</i> = 16)	<i>P</i> value
Age, median (IQR)	60 (56,65)	65 (62.5, 70.5)	0.10
Male, <i>n</i> (%)	20 (76.9)	14 (87.5)	0.68
Race			0.15
African-American, <i>n</i> (%)	16 (61.5)	14 (87.5)	
Caucasian, <i>n</i> (%)	9 (34.6)	2 (12.5)	
BMI(kg m ⁻²), mean (SD)	36.5 (6.5)	34.4 (3.5)	0.24
Obesity (BMI > 30 kg m ⁻²), <i>n</i> (%)	22 (84.6)	15 (93.8)	0.63
Current smoker, <i>n</i> (%)	4 (15.4)	1 (6.3)	0.63
Hypertension, <i>n</i> (%)	24 (92.3)	16 (100)	0.52
Diabetes, <i>n</i> (%)	17 (65.4)	11 (68.8)	0.82
Coronary artery disease, <i>n</i> (%)	8 (30.8)	3 (18.8)	0.49
Chronic kidney disease (eGFR < 60 ml min ⁻¹ /1.73 m ²)	9 (34.6)	5 (31.3)	0.82
Drug therapy, <i>n</i> (%)			
Beta blocker	14 (53.9)	10 (62.5)	0.58
ACE-inhibitor/ARB	18 (69.2)	10 (62.5)	0.65
Calcium-channel blocker	14 (53.9)	7 (43.8)	0.53
Mineralocorticoid receptor antagonist	0 (0)	1 (6.3)	0.38
Statin	15 (57.7)	9 (56.3)	0.93
Aspirin	17 (65.4)	14 (87.5)	0.16
Thiazide	14 (53.9)	4 (25.0)	0.07
Loop diuretics	13 (50.0)	6 (37.5)	0.43
eGFR ^a (ml min ⁻¹ /1.73 m ²), median (IQR)	74.1 (53.5, 95.4)	65.5 (52.4, 89.5)	0.95
Left ventricular ejection fraction (%), median (IQR)	57.4 (55.0, 65.5)	62.4 (57.5, 69.8)	0.30

^a eGFR was calculated using the modification of diet in renal disease (MDRD) study equation.

Table 2.

Aortic and carotid hemodynamics and power analysis before (Pre-) and after (Post-) administration of sublingual nitroglycerin (NTG) (sub-study 1) and after nitrate-depleted (PB) and nitrate-rich beet root juice administration (sub-study 2).

Variable	Sub-study 1 (organic nitrates)			Sub-study 2 (inorganic nitrates)		
	Pre-NTG	Post-NTG	<i>P</i> value	Placebo	Beet root juice	<i>P</i> value
Hemodynamics						
Central systolic pressure (mmHg)	133.57 (27.61)	120.50 (29.77)	0.01	130.03 (20.76)	126.56 (24.23)	0.50
Mean arterial pressure (mmHg)	96.82 (16.99)	90.54 (20.42)	0.02	97.56 (14.85)	96.44 (15.23)	0.70
Central pulse pressure (mmHg)	59.53 (23.60)	49.99 (19.38)	0.06	55.29 (16.75)	50.98 (17.96)	0.34
Central diastolic pressure (mmHg)	74.03 (15.41)	70.51 (15.25)	0.18	74.74 (12.55)	75.58 (9.80)	0.70
Cardiac output (l min ⁻¹)	5.64 (1.40)	5.81 (1.23)	0.32	5.40 (1.42)	4.89 (1.15)	0.04
Stroke volume (ml)	90.41 (21.91)	89.07 (21.64)	0.65	83.58 (16.02)	78.71 (15.05)	0.07
Heart rate (bpm)	63.23 (10.82)	66.58 (11.06)	0.004	65.19 (12.78)	62.28 (10.39)	0.09
Peak aortic blood flow (cm ³ s ⁻¹)	408.61 (100.90)	410.03 (103.16)	0.93	371.71 (64.88)	350.39 (65.71)	0.20
Mean aortic blood flow (cm ³ s ⁻¹)	94.01 (23.31)	96.86 (20.42)	0.32	90.10 (23.59)	81.45 (19.12)	0.04
Peak carotid blood flow (cm ³ s ⁻¹)	23.92 (15.35)	34.31 (21.51)	0.001	21.25 (11.62)	22.19 (13.51)	0.74
Mean carotid blood flow (cm ³ s ⁻¹)	9.80 (6.73)	13.08 (8.38)	0.008	9.35 (5.24)	10.03 (6.91)	0.60
Carotid cross sectional area (cm ²)	0.38 (0.09)	0.43 (0.1)	<0.001	0.37 (0.08)	0.40 (0.12)	0.15
Aortic wave power analysis						
FCW _{AUC} (mJ)	0.129 (0.053)	0.117 (0.050)	0.26	0.107 (0.046)	0.093 (0.043)	0.31
FEW _{AUC} (mJ)	0.041 (0.031)	0.032 (0.023)	0.17	0.036 (0.017)	0.033 (0.015)	0.53
Aortic hydraulic power analysis						
Power (mW)	7256 (2549)	6591 (2204)	0.06	6666 (2165)	5845 (1846)	0.20
Energy (mJ)	1531 (511)	1351 (428)	0.01	1429 (447)	1275 (418)	0.18
Carotid wave power analysis						
FCW _{AUC} (mJ)	0.007 (0.006)	0.010 (0.011)	0.12	0.0056 (0.0040)	0.0049 (0.0027)	0.40
FEW _{AUC} (mJ)	0.0014 (0.0014)	0.0018 (0.0020)	0.25	0.0014 (0.0009)	0.0011 (0.00045)	0.13
Carotid hydraulic power analysis						
Power (mW)	387 (240)	536 (383)	0.008	356 (207)	351 (195)	0.91
Energy (mJ)	127 (78)	150 (84)	0.12	126 (76)	129 (68)	0.90

AUC = area under the curve; FCW = forward compression wave; FEW = forward expansion wave.