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RESEARCH ARTICLE

Effects of organic and inorganic nitrate on aortic and carotid haemodynamics in heart failure with preserved ejection fraction

Julio A. Chirinos[1]*†, Francisco Londono-Hoyos[1,2†], Payman Zamani[1†], Melissa Beraun[1], Philip Haines[3], Izzah Vasim[1,4], Swapna Varakantam[1,4], Timothy S. Phan[1], Thomas P. Cappola[1], Kenneth B. Margulies[1], Raymond R. Townsend[1], and Patrick Segers[2]

[1]University of Pennsylvania Perelman School of Medicine, Hospital of the University of Pennsylvania, Philadelphia, PA, USA; [2]Institute of Biomedical Technology, Ghent University, Ghent, Belgium; [3]Warren Alpert Medical School of Brown University, Providence, RI, USA; and [4]Philadelphia VA Medical Center, Philadelphia, PA, USA

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Aims To assess the haemodynamic effects of organic vs. inorganic nitrate administration among patients with heart failure with preserved ejection fraction (HFpEF).

Methods and results We assessed carotid and aortic pressure–flow relations non-invasively before and after the administration of 0.4 mg of sublingual nitroglycerin ($n = 26$), and in a separate sub-study, in response to 12.9 mmoL of inorganic nitrate ($n = 16$). Nitroglycerin did not consistently reduce wave reflections arriving at the proximal aorta (change in real part of reflection coefficient, 1st harmonic: −0.09; $P = 0.01$; 2nd harmonic: −0.045, $P = 0.16$; 3rd harmonic: +0.087; $P = 0.05$), but produced profound vasodilatation in the carotid territory, with a significant reduction in systolic blood pressure (133.6 vs. 120.5 mmHg; $P = 0.011$) and a marked reduction in carotid bed vascular resistance (19 580 vs. 13 078 dynes · s/cm^5; $P = 0.001$) and carotid characteristic impedance (3440 vs. 1923 dynes · s/cm^5; $P = 0.002$). Inorganic nitrate, in contrast, consistently reduced wave reflections across the first three harmonics (change in real part of reflection coefficient, 1st harmonic: −0.12; $P = 0.03$; 2nd harmonic: −0.11, $P = 0.01$; 3rd harmonic: −0.087; $P = 0.09$) and did not reduce blood pressure, carotid bed vascular resistance, or carotid characteristic impedance (P = NS).

Conclusions Nitroglycerin produces marked vasodilatation in the carotid circulation, with a pronounced reduction in blood pressure and inconsistent effects on central wave reflections. Inorganic nitrate, in contrast, produces consistent reductions in wave reflections, and unlike nitroglycerin, it does so without significant hypotension or cerebrovascular dilatation. These haemodynamic differences may underlie the different effects on exercise capacity and side effect profile of inorganic vs. organic nitrate in HFpEF.

Keywords Arterial load • Cerebrovascular input impedance • Heart failure with preserved ejection fraction • Inorganic nitrate • Organic nitrate

Introduction

Heart failure (HF) affects ~2% of the western population and is the most common cause of hospitalization in adults >65 years of age. Approximately half of patients with HF have a preserved left ventricular (LV) ejection fraction (HFpEF). Multiple effective pharmacological therapies that result in substantial clinical benefit in HF with reduced ejection fraction are available. In contrast, there are currently no proven effective pharmacological therapies to improve outcomes in HFpEF.

*Corresponding author. South Tower, Rm. 11–138. Perelman Center for Advanced Medicine, 3400 Civic Center Blvd, Philadelphia, PA 19104, USA. Tel: +1 215 5736606, Fax: +1 215 7467415, Email: Julio.chirinos@uphs.upenn.edu
†These authors contributed equally to this work.

Pulsatile arterial load exerts important effects on LV function and remodelling. In particular, wave reflections originating at the periphery and conducted back to the heart, have been shown to cause LV diastolic dysfunction, hypertrophy and fibrosis in experimental models, a concept supported by an increasing body of human studies.[1–6] Acute administration of organic nitrates has been shown to reduce wave reflections arriving at the central aorta in hypertensive or healthy subjects in some, but not all studies.[7–11] The effects of organic nitrates on pulsatile arterial haemodynamics have not been well characterized in HFpEF. In recent trials in patients with HFpEF,[12,13] organic nitrates have been poorly tolerated; important side effects of organic nitrate therapy included hypotension and headaches, suggesting adverse effects on the cerebrovascular territory.

In contrast to the unfavourable effects of organic nitrates in HFpEF, recent randomized controlled trials have demonstrated that inorganic nitrate, administered as a single dose[14] or after one week of sustained administration,[15] improves exercise capacity in patients with HFpEF. Inorganic nitrate undergoes a two-step reduction to nitric oxide (NO) via the nitrate–nitrite–NO pathway.[16,17] The conversion of nitrite to NO occurs in conditions of hypoxia and acidosis, but a recent report indicates that it also occurs via paradoxical normoxic activation in conduit arteries,[18] indicating potential effects on pulsatile arterial haemodynamic function and arterial wave reflections. Furthermore, in contrast to the frequent occurrence of headache with organic nitrate, the administration of inorganic nitrate has not been associated with headaches or other side effects in two recent trials in this population.[14,15]

We aimed to assess the effect of organic and inorganic nitrate in HFpEF on (i) wave reflections arriving at the central aorta, and (ii) carotid arterial haemodynamics (i.e. to characterize cerebrovascular effects).

Methods

We performed two sub-studies. In the first sub-study, we assessed aortic and carotid haemodynamics at baseline and after the administration of 0.4 mg of sublingual nitroglycerin (NTG). In the second sub-study, we analysed aortic and carotid pressure–flow data from our previous randomized controlled trial of inorganic nitrate administration in HFpEF.[14] Protocols were approved by the University of Pennsylvania and Philadelphia VA Medical Center Institutional Review Boards, as appropriate. All subjects provided written informed consent.

Study population

For sub-study 1, we included subjects with HFpEF who met the following criteria: (i) symptomatic HF with a LV ejection fraction >50%; (ii) at least one of the following within one year prior to consent: hospitalization for decompensated HF, acute treatment for HF with intravenous diuretics or haemofiltration, chronic treatment with a loop diuretic for control of HF symptoms, or chronic diastolic dysfunction evidenced by left atrial enlargement (left atrial volume index >34 mL/m^2), or Doppler signs of increased left atrial pressure, as defined by the European Society of Cardiovascular Imaging/American Society of Echocardiography;[19] (iii) stable medical therapy. Key exclusion criteria were: (i) clinically significant valve disease (more than mild aortic/mitral stenosis or more than moderate aortic/mitral regurgitation); (ii) atrial fibrillation/flutter; (iii) current nitrate therapy; (iv) significant ischaemia on stress testing within the past year that was not revascularized; (v) other clinically important causes of dyspnoea; (vi) hypertrophic, infiltrative, or inflammatory cardiomyopathy; (vii) pericardial disease; (viii) primary pulmonary arteriopathy; (ix) blood pressure <110/40 mmHg or >180/100 mmHg; (x) resting heart rate >100 b.p.m.; (xi) LV ejection fraction <50% in the past; (xii) adverse reactions to organic nitrates or phosphodiesterase inhibitor use; (xiii) severe renal dysfunction (glomerular filtration rate <30 mL/min/1.73 m^2), or liver disease. In sub-study 2, we utilized very similar inclusion/exclusion criteria, as previously described.[14]

Study protocol

Sub-study 1

Twenty-six subjects participated in this sub-study. After >10 min of rest in the supine position, blood pressure was taken in the right arm with a validated oscillometric device (Omron HEM-705CP, Omron Corporation, Kyoto, Japan). Carotid pressure (*Figure 1*) was recorded via applanation tonometry, using a SphygmoCor-CPV System (AtCor Medical, Itasca, IL, USA) equipped with a high-fidelity Millar tonometer (Millar Instruments, Houston, TX, USA).

Pulsed-wave Doppler measurements of flow velocities in the LV outflow tract (*Figure 1*) were performed using a GE-e9 ultrasound machine (GE Healthcare, Fairfield, CT, USA), with the Doppler sample immediately proximal to the aortic valve leaflets within the centerline of the LV outflow tract. We computed LV outflow tract cross-sectional area from its radius measured in the parasternal long-axis view (area = πr^2). Carotid diameters and blood velocities were also acquired, using a vascular linear probe. Carotid flow was computed as flow velocity multiplied by lumen cross-sectional area (area = πr^2). After baseline measurements were obtained, a single dose (0.4 mg) of NTG was administered sublingually, and measurements were repeated starting 2 min after administration. Comparisons were made between the measurements obtained pre- vs. post-NTG administration.

Sub-study 2

Seventeen subjects participated in a randomized, double-blind, cross-over study of a single dose of inorganic nitrate given as concentrated nitrate-rich beetroot juice (NO_3^-, BEET-IT Sport, James White Drinks Ltd, Ipswich, UK) containing 12.9 mmoL NO_3^- in 140 mL, vs. an otherwise identical nitrate-depleted placebo juice (James White Drinks, Ltd). The interventions were separated by a wash-out period of at least 5 days. We measured aortic and carotid heamodynamics 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 vs. nitrate-depleted beetroot juice. One subject was excluded from these analyses due to lack of carotid flow data during one of the study visits.

Pressure–flow analyses

Pressure and Doppler flow velocity files were processed off-line using custom-designed software written in Matlab (The Mathworks, Natick, MA, USA) as previously described.[20] A representative example of pressure–flow data processing is shown in *Figures 1* and *2*. Time alignment of pressure and LV outflow curves was performed to maximize: (i) the rapid systolic upstroke of pressure and flow; (ii) concordance

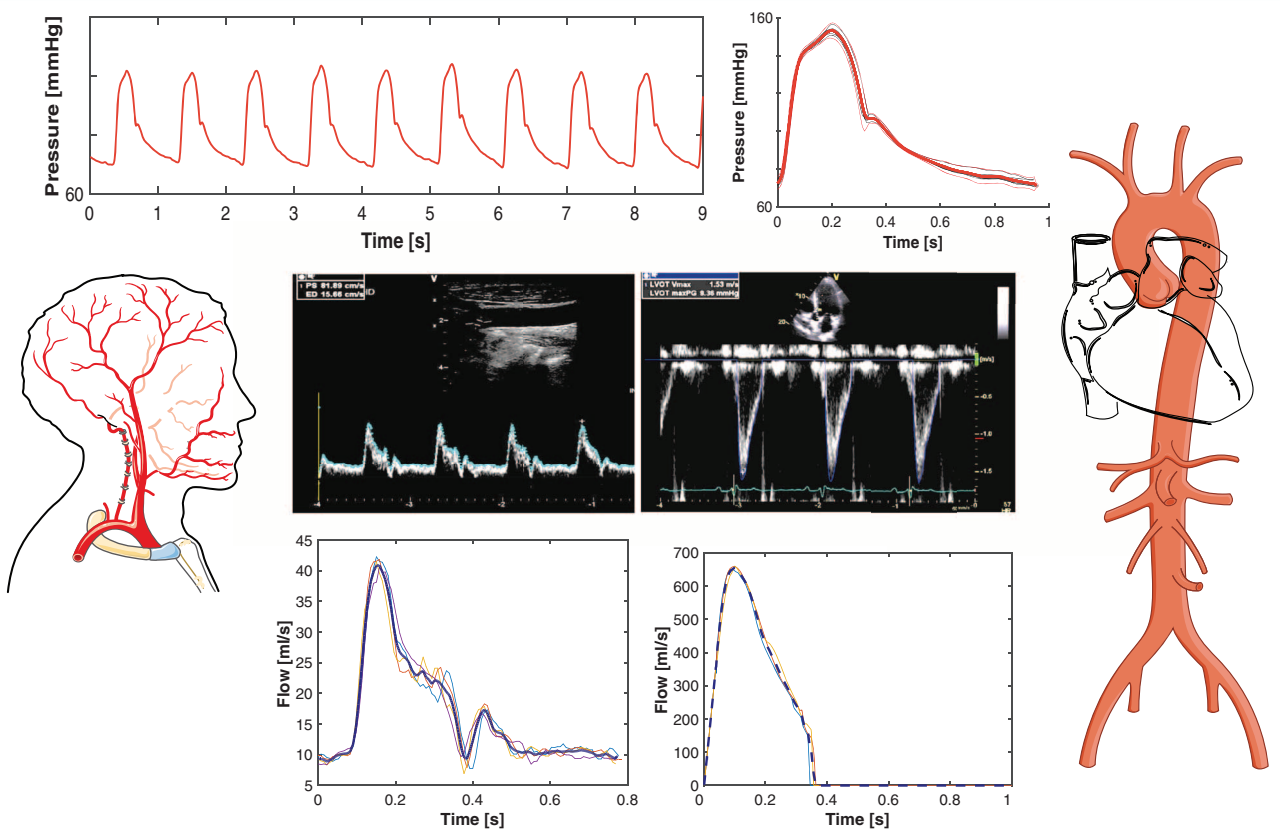

Figure 1 Example of pressure–flow analyses. Tonometric pressure data are shown at the top (*left*), along with the signal-averaged pressure waveform (top *right*, red). Doppler flow interrogations were performed, as shown for the carotid (*left*) and left ventricular outflow (*right*) locations. Tracing and signal averaging of flow waveforms (bottom blue curves) was subsequently performed. The carotid pressure waveform is used for both carotid pressure–flow analyses and aortic pressure–flow analyses, since the carotid pressure is a good surrogate of the aortic pressure waveform.

of the pressure dicrotic notch and cessation of flow in the LV outflow tract, or the flow dicrotic notch in the carotid; (iii) linearity of the early systolic pressure–flow relationship.

We computed aortic input impedance (*Figure 2*), which characterizes the ratio of pulsatile pressure over flow in each harmonic of heart rate. In this analysis, the fundamental frequency, or 1st harmonic, is the heart rate, and higher harmonics are multiples of that frequency. Proximal aortic characteristic impedance (Zc) was computed in the frequency domain. Each pressure and flow harmonic was separated into forward and backward components using wave separation analysis.[21,22] We assessed the reflection coefficient in the first three harmonics. These are the relevant harmonics for assessing wave reflections because: (i) they contain the vast majority of the pulsatile energy in pressure and flow signals; (ii) at higher frequencies, reflections cancel out at random, and the input impedance spectrum hovers around aortic root characteristic impedance, mimicking a reflection-free system.

As the reflection coefficient is derived from the ratio of two sine waves, it is a complex number with an amplitude and phase-angle, which can correspond to different degrees of destructive or constructive interference between forward and backward waves. Therefore, the net effect of reflections was expressed as the real part of the reflection coefficient, which becomes increasingly positive as pressure from wave reflections increases (constructive interference), and negative when destructive interference leads to a net decrease in pressure by wave reflections at a given harmonic.[23]

We also reconstructed the forward and backward pressure waves in the time domain. The sum of forward and backward pressure harmonics yields the forward and backward waves, respectively (*Figure 2*). The time of onset of the reflected wave was defined as the time at which the reflected wave starts adding to mean pressure. We computed the Buckberg index (also known as the sub-endocardial viability ratio), as the ratio of diastolic/systolic pressure–time integrals (i.e. areas under the pressure curve). This index provides an assessment of the effect of pulsatile haemodynamics on myocardial oxygen demand (systolic load) vs. supply (perfusion pressure).[24]

We also performed analyses of carotid pressure–flow relations. Haemodynamic 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.[25]

In our laboratory, repeated measurements of all indices from aortic and carotid pressure–flow analyses yielded coefficients of variation of 17% or less.

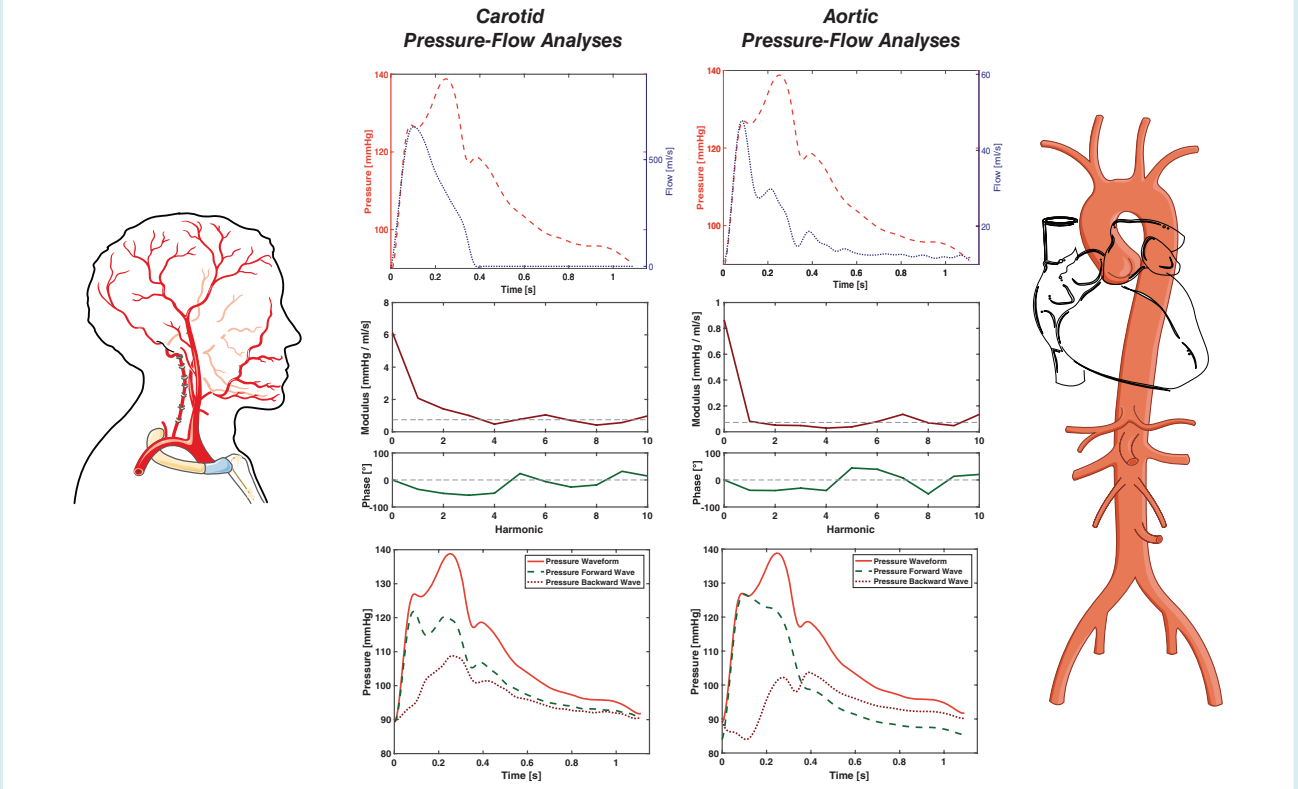

Figure 2 Carotid (*left panels*) and aortic (*right panels*) pressure–flow pairs are processed via mathematical analyses in the frequency domain. The input impedance spectrum, which consists of modulus (red) and phase (blue) is obtained. Characteristic impedance (Zc, dashed line in modulus plot) is computed based on the average modulus of higher harmonics of input impedance modulus. Zc is a local vascular property of the aortic root or carotid artery, which governs the local pressure–flow relation in the absence of wave reflections. Once Zc is known, the modulus and phase of the reflection coefficient in the frequency domain (at each harmonic) can be computed (not shown), along with the net contribution of reflections to pressure at each harmonic (i.e. the real part of the reflection coefficient). Reflection coefficients in the frequency domain are derived purely from the input impedance spectrum and thus depend purely on arterial load. The first three harmonics contain the vast majority of the pulsatile energy and are the relevant harmonics. We also performed wave separation in the time domain, as shown in the *bottom panels*. This approach yields forward (green dashed line) and backward (red dotted line) waves reconstructed in the time domain. At the aorta, forward and backward wave amplitude and morphology in the time domain are not purely dependent on arterial load, but depend also on re-reflections in the heart and the left ventricular contraction pattern.

Statistical analysis

Descriptive data are presented as mean ± standard deviation 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 vs. nitrate-depleted beetroot juice administration for sub-study 2, were performed using paired Student's *t*-test. Physiologic indices were expressed as absolute values at each time point, as well as absolute differences between measurements (with 95% confidence intervals). A two-tailed *P* value of <0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 15 for Mac-OS (SPSS Inc., Chicago, IL, USA).

Results

General characteristics of the study populations for sub-studies 1 and 2 are shown in *Table 1*. The characteristics of study subjects in both sub-studies were very similar. In both sub-studies, subjects were obese, with a high prevalence of hypertension and diabetes, as well as left atrial enlargement and a high mitral E/e' ratio. Both study samples were composed predominantly of males, with a high proportion of African-Americans. All subjects in both sub-studies had New York Heart Association class II–III symptoms.

Sub-study 1: effects of sublingual nitroglycerin on aortic and carotid haemodynamics

Key haemodynamic parameters measured before and after the administration of NTG are shown in *Table 2* and *Figure 3*. Nitroglycerin reduced central systolic and mean pressure. Nitroglycerin did not have any significant effect on the amplitude of forward or backward waves, the ratio of backward/forwards wave amplitudes, or the time to reflected wave onset. Nitroglycerin tended to delay the peak of the reflected wave, but did not improve the Buckberg

Table 1 General characteristics of study subjects

Variable	NTG sub-study (sub-study 1) ($n = 26$)	Beetroot study (sub-study 2) ($n = 16$)	*P*-value
Age, years [median (IQR)]	60 (56–65)	65 (62.5–70.5)	0.10
Male sex	20 (76.9)	14 (87.5)	0.68
Race			0.15
African-American	16 (61.5)	14 (87.5)	
Caucasian	9 (34.6)	2 (12.5)	
BMI, kg/m^2	36.5 (6.5)	34.4 (3.5)	0.24
Obesity (BMI >30 kg/m^2)	22 (84.6)	15 (93.8)	0.63
Current smoker	4 (15.4)	1 (6.3)	0.63
Hypertension	24 (92.3)	16 (100)	0.52
Diabetes	17 (65.4)	11 (68.8)	0.82
Coronary artery disease	8 (30.8)	3 (18.8)	0.49
Chronic kidney disease (eGFR <60 mL/min/1.73 m^2)	9 (34.6)	5 (31.3)	0.82
Drug therapy			
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 diuretic	13 (50.0)	6 (37.5)	0.43
Laboratory data			
eGFR*, mL/min/1.73 m^2 [median (IQR)]	74.1 (53.5–95.4)	65.5 (52.4–89.5)	0.95
Echocardiography			
LV ejection fraction, % [median (IQR)]	57.4 (55.0–65.5)	62.4 (57.5–69.8)	0.30
Left atrial volume index, mL/m^2	30.3 (10.9)	35.7 (10.9)	0.13
Mitral E-wave velocity, cm/s	81.8 (24.9)	71.7 (16.4)	0.16
Mitral A-wave velocity, cm/s	79.2 (24.6)	73.3 (24.2)	0.45
Mitral septal tissue Doppler velocity, cm/s	6.6 (2.2)	6.5 (1.7)	0.88
Mitral E/e' ratio [median (IQR)]	12.8 (11.0–14.4)	11.4 (9.2–13.3)	0.17
TAPSE, cm [median (IQR)]	2.3 (2.1–2.6)	2.6 (2.1–2.8)	0.44
TAPSE <1.6 cm	0 (0)	0 (0)	
Tricuspid regurgitant jet peak gradient†, mmHg	31.8 (3.7)	27.9 (8.9)	0.054
LV end-diastolic diameter, cm	4.8 (0.6)	4.5 (0.6)	0.12
LV mass, g [median (IQR)]	243.7 (214.1–318.3)	236.6 (183.2–275.2)	0.62
LV mass indexed to height, g/m$^{1.7}$ [median (IQR)]	96.9 (71.7–114.1)	91.6 (70.7–102.8)	0.39
LV mass indexed to BSA, g/m^2	109 (23.5)	106.3 (37.6)	0.77
Relative wall thickness, cm [median (IQR)]	0.52 (0.47–0.58)	0.59 (0.54–0.66)	0.041
NT-proBNP, pg/mL [median (IQR)]	250.5 (90.5–510.6)	148.0 (61.7–272.5)	0.16
Ejection duration, ms	318 (28)	330 (35)	0.22

Values are expressed and mean ± standard deviation, or number (%), unless otherwise specified.
ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; BMI, body mass index; BSA, body surface area; eGFR, estimated glomerular filtration rate; IQR, interquartile range; LV, left ventricular; NT-proBNP, *N*-terminal pro-brain natriuretic peptide; TAPSE, tricuspid annular plane systolic excursion.
*eGFR was calculated using the Modification of Diet in Renal Disease equation.
†Could be assessed reliably from the tricuspid regurgitation envelope in only nine subjects in sub-study 1 and in seven subjects in sub-study 2.

index. Nitroglycerin did not significantly reduce aortic root characteristic impedance.

Table 2 and *Figure 3* show the effect of NTG on the real part of the reflection coefficient in the first three harmonics. Nitroglycerin reduced the reflection coefficient of the 1st harmonic, but did not reduce it in the 2nd harmonic and actually tended to increase the reflection coefficient of the 3rd harmonic.

Despite these inconsistent effects on systemic wave reflections, NTG markedly reduced carotid artery Zc, increased carotid cross-sectional area and reduced carotid bed vascular resistance.

Effects of inorganic nitrate on aortic and carotid haemodynamics

Key comparisons of haemodynamic parameters obtained after administration of nitrate-rich beetroot juice vs. placebo juice are shown in *Table 3* and *Figure 3*. Inorganic nitrate did not significantly reduce central systolic or mean pressure. Similar to NTG, inorganic nitrate did not have any significant effect on the amplitude of forward or backward waves or the ratio of backward/forwards wave amplitudes, and did not reduce aortic

Table 2 Aortic and carotid pulsatile haemodynamics before (pre) and after (post) administration of sublingual nitroglycerin (NTG)

Variable	Pre-NTG	Post-NTG	*P*-value
Central systolic blood pressure, mmHg	133.6 (27.6)	120.5 (29.8)	0.011
Central pulse pressure, mmHg	59.5 (23.6)	50 (19.4)	0.06
Central mean arterial pressure, mmHg	96.8 (17)	90.5 (20.4)	0.019
Central diastolic blood pressure, mmHg	74 (15.4)	70.5 (15.3)	0.18
Aortic pressure–flow relations			
Aortic characteristic impedance, dynes · s/cm^5	172 (69)	174 (119)	0.93
Reflection coefficient, 1st harmonic	0.093 (26)	0.002 (26)	0.017
Reflection coefficient, 2nd harmonic	−0.071 (26)	−0.117 (26)	0.16
Reflection coefficient, 3rd harmonic	−0.012 (26)	0.058 (26)	0.054
Forward wave amplitude, mmHg*	52.6 (20.2)	47.6 (16.6)	0.26
Backward wave amplitude, mmHg*	19.1 (9.1)	16.9 (7.5)	0.21
Backward/forward wave amplitude*	0.36 (0.07)	0.35 (0.08)	0.33
Time to reflected wave onset, ms	78 (30)	89 (44)	0.22
Buckberg index, %	130 (33)	129 (33)	0.81
Carotid pressure–flow relations			
Carotid characteristic impedance, dynes · s/cm^5	3440 (2757)	1923 (1277)	0.002
Carotid cross-sectional area, cm^2	0.38 (0.09)	0.43 (0.1)	<0.0001
Carotid bed vascular resistance, dynes · s/cm^5	19 580 (13 402)	13 078 (8974)	0.001

*Computed in the time domain.

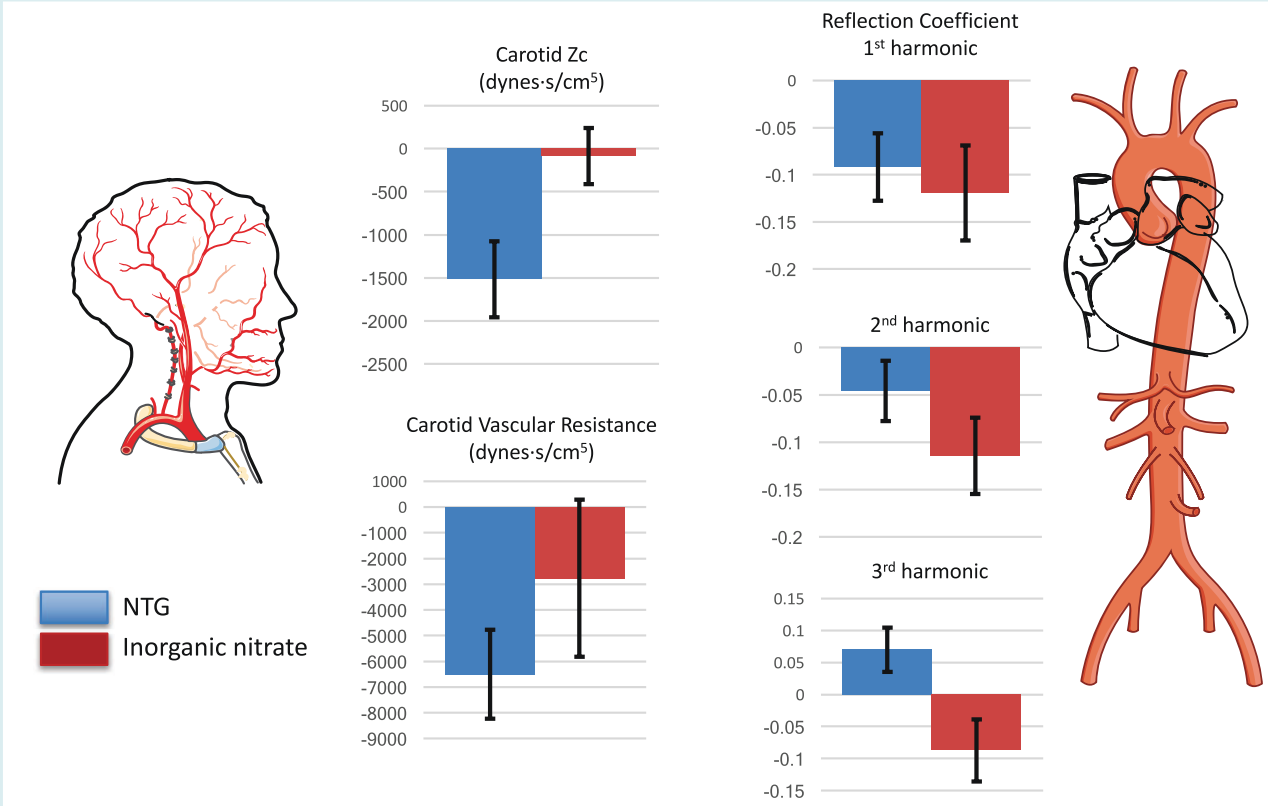

Figure 3 Changes in key pulsatile haemodynamic indices in the aorta (*right*) and the carotid artery (*left*), in sub-study 1 [sublingual nitroglycerin (NTG), solid blue bars] and sub-study 2 (inorganic nitrate, solid red bars). Mean changes ± standard errors are shown.

Table 3 Aortic and carotid pulsatile haemodynamics after administration of 12.9 mmoL of inorganic nitrate (nitrate-rich beetroot juice, NO_3) vs. placebo juice (nitrate-depleted beetroot juice)

Variable	Placebo	NO_3	*P*-value
Central systolic blood pressure, mmHg	130 (20.8)	126.6 (24.2)	0.50
Central pulse pressure, mmHg	55.3 (16.7)	51 (18)	0.34
Central diastolic blood pressure, mmHg	74.7 (12.6)	75.6 (9.8)	0.70
Central mean arterial pressure, mmHg	97.6 (14.8)	96.4 (15.2)	0.70
Aortic pressure–flow relations			
Aortic characteristic impedance, dynes · s/cm^5	163 (74)	185 (64)	0.20
Reflection coefficient, 1st harmonic	0.119 (0.15)	−0.001 (0.21)	0.032
Reflection coefficient, 2nd harmonic	−0.03 (0.13)	−0.144 (0.15)	0.012
Reflection coefficient, 3rd harmonic	0.046 (0.16)	−0.042 (0.16)	0.091
Forward wave amplitude, mmHg*	47.2 (13.8)	47.1 (14.1)	0.98
Backward wave amplitude, mmHg*	16.8 (6)	18.8 (7.9)	0.30
Backward/forward wave amplitude*	0.35 (0.05)	0.39 (0.08)	0.08
Time to reflected wave onset, ms	64 (30)	93 (30)	0.016
Buckberg index, %	128 (29)	141 (25)	0.053
Carotid pressure–flow relations			
Carotid characteristic impedance, dynes · s/cm^5	3013 (1867)	2928 (1279)	0.80
Carotid cross-sectional area, cm^2	0.37 (0.08)	0.40 (0.12)	0.15
Carotid bed vascular resistance, dynes · s/cm^5	19 391 (13 557)	16 624 (6800)	0.38

*Computed in the time domain.

root characteristic impedance. However, it significantly delayed the systolic onset of the reflected wave, moved its peak well into diastole, and tended to improve the Buckberg index.

Table 3 and *Figure 3* show the effect of inorganic nitrate on the real part of the reflection coefficient in harmonics 1–3. Inorganic nitrate significantly reduced the reflection coefficient of the 1st and 2nd harmonics, and tended to reduce the reflection coefficient of the 3rd harmonic. In contrast to NTG, inorganic nitrate did not reduce reduced carotid artery Zc, carotid cross-sectional area, or carotid bed vascular resistance.

Discussion

We assessed the effects of organic and inorganic nitrate on aortic and carotid pulsatile haemodynamics in HFpEF. We demonstrate that organic nitrate substantially reduced blood pressure, but reduced arterial wave reflections inconsistently across the first three harmonics of the pressure–flow relation (in which most of the pulsatile energy is contained). Nitroglycerin did not significantly improve the Buckberg index and produced profound vasodilatation in the carotid territory, with a reduction in cerebrovascular resistance and carotid characteristic impedance. In contrast, inorganic nitrate produced consistent reductions in wave reflections across the first three harmonics, with a delay in the reflected wave and a trend for improvement in the Buckberg index, without significant cerebrovascular dilatation. These haemodynamic differences likely underlie the differential clinical effects of organic vs. inorganic nitrate observed in recent clinical trials.

While both organic and inorganic nitrate/nitrite ultimately act by increasing NO bioavailability, biochemical differences exist between the two classes of drugs that lead to important differences in their action. Inorganic nitrate is reduced to nitrite and NO via the nitrate–nitrite–NO pathway,[16,17] which involves the reduction of nitrate to nitrite upon ingestion and when nitrate is subsequently excreted by the salivary glands (enterosalivary circulation).[26] Subsequent reduction of nitrite to NO occurs via: (i) a hypoxia/acidosis-dependent mechanism (which enhances reductions in microvascular resistance during exercise),[14] and (ii) a 'paradoxic' normoxia-dependent mechanism operating in muscular conduit arteries,[18] which explains the effect on arterial wave reflections.[14,18] There also appears to be non-enzymatic nitrite reduction to NO in the acid gastric medium.[27] In contrast, organic nitrates require activation in the cytochrome-P450 system, leading to tonic NO release.[28] Alternative activation via mitochondrial aldehyde dehydrogenase for NTG and other organic nitrates also occurs.[28]

Headache is a common side effect of organic nitrates, and can limit compliance with these medications. Hypotension can be seen, and may result in syncope.[12,16] In contrast, inorganic nitrate has been well tolerated in HFpEF, with no limiting side effects, as reported in two recent trials.[14,15] No significant hypotension and, in particular, no vasoactive symptoms (such as headache) were reported. These side effect differences are consistent with the observed haemodynamic effects observed in our study. The profound carotid bed vasodilatation seen in response to NTG, but not inorganic nitrate, is likely due to differences in the activation of these compounds. The high mitochondrial content of neurons may facilitate the activation of NTG by mitochondrial aldehyde dehydrogenase in the brain, thus reducing microvascular resistance in the cerebrovascular bed. In contrast, inorganic nitrite (produced via reduction in inorganic nitrate) is reduced to NO in the microvasculature, but this conversion occurs preferentially in

conditions of hypoxia/acidosis.[16,17] Such conditions are not present in the brain, a highly aerobic organ which demonstrates relatively preserved microvascular oxygenation[29] due to its low resistance and high arterial flow. Therefore, conditions of hypoxia and acidosis are not present in the cerebral microvasculature, explaining the lack of significant effects of inorganic nitrate in our study.

Wave reflections originate at sites of impedance mismatch throughout the arterial tree and return to the heart during ejection, increasing pulsatile load and affecting the LV loading sequence (early vs. late systolic load).[22,30] Wave reflections and late systolic load have been shown to cause diastolic dysfunction and myocardial remodelling in animal models.[1,2] Human studies demonstrated a relationship between increased wave reflections/late systolic load and worse longitudinal LV function,[3] LV hypertrophy,[4] and a higher risk of incident new-onset HF[5] and readmission after an episode of established acute decompensated HF.[6]

In our study, both NTG and inorganic nitrate reduced wave reflections. However, inorganic nitrate produced numerically greater and more consistent reductions in the real part of the reflection coefficient across the first three harmonics, which contain most of the pulsatile energy. In contrast, NTG reduced the real component of the reflection coefficient only in the 1st harmonic, without an effect in the 2nd harmonic, and an increase in the 3rd harmonic. The real part of the reflection coefficient characterizes the net effect of wave reflections on the pressure–flow relation in the aorta at a given harmonic.[23] Inorganic nitrate, but not NTG, delayed the reflected wave and tended to improve the Buckberg index (which characterizes the effect of pulsatile haemodynamics on LV systolic load vs. diastolic perfusion pressure). The reduction in wave reflections with inorganic nitrate occurred without reductions in systemic vascular resistance or blood pressure. This clearly indicates that the effects of inorganic nitrate on microvascular resistance/blood pressure and those on wave reflections are not necessarily linked. Reductions in wave reflections can thus be achieved in the absence of significant hypotension. Although these haemodynamic effects are unexpected from the well-known hypoxia-mediated reduction of nitrite in the microvasculature, they are consistent with the recently described paradoxic normoxia-dependent activation of inorganic nitrite in the wall of muscular conduit arteries,[18] because muscular arteries are known to modify the magnitude and phase of wave reflections returning to the proximal aorta.[22,24]

The effects of inorganic nitrate on pulsatile load from wave reflections demonstrated in our study, along with the absence of cerebrovascular effects and side effects, is helpful not only to interpret its demonstrated short-term clinical effects (i.e. improvements in exercise tolerance and the absence of side effects such as headache), but may also have implications for its long-term clinical effects. By virtue of reducing wave reflections, which are deleterious for the left ventricle, inorganic nitrate may exert long-term 'disease-modifying' effects in HFpEF, potentially reducing LV diastolic dysfunction and remodelling. We are currently assessing the efficacy of sustained administration of inorganic nitrate (oral potassium nitrate) in a randomized cross-over phase IIb trial funded by the National Heart, Lung and Blood Institute (KNO_3CK OUT HFPEF trial; clinicaltrials.gov NCT02840799). A single-dose study with inorganic nitrite yielded promising results in HFpEF,[31] and a larger study with inhaled sodium nitrite (INDIE HFPEF; clinicaltrials.gov NCT02742129) is ongoing.

An interesting observation is the absence of significant effects of either NTG or inorganic nitrate on the ratio of amplitudes of backward/forward waves, which is a commonly used index of wave reflections. It should be noted that this ratio does not account for the time-resolved shape of the waveforms (which can be different despite identical amplitudes). Similarly, this ratio does not account for the time at which the reflected wave exerts its effects on central pressure (late systole vs. diastole). Furthermore, the amplitudes of both forward and backward waves are not purely a function of arterial properties, but are heavily dependent on ventricular contraction dynamics. In addition, the ratio of backward/forward waves is confounded by rectified reflections (i.e. re-reflections in the heart), which substantially contribute to forward wave amplitude.[32] Despite the lack of change in reflection magnitude, detailed analyses in the time domain demonstrated favourable changes (delay in the onset of the reflected wave after ejection, and a trend towards improvement in the Buckberg index) with inorganic nitrate, but not with NTG.

Our study should be interpreted in the context of its strengths and limitations. Strengths include the careful assessment of pulsatile carotid and aortic haemodynamics using state-of-the-art non-invasive pressure–flow analyses, rather than pressure-only approaches. Input impedance assessments distinctly characterize arterial properties distal to the point of measurement, whereas pressure-only approaches (such as assessments of augmentation index) can be confounded by changes in ventricular contraction or preload. Additional strengths of our study include the use of identical methods to measure haemodynamics after organic and inorganic nitrate administration, facilitating the interpretation of differential haemodynamic effects. Our study also has limitations. Our study populations were relatively small; however, the paired nature of the analyses reduced measurement variability and enhanced detection of drug effects in each sub-study. Owing to the characteristics of the patient population at the VA Medical Center, where most subjects were enrolled, our study populations were composed primarily of men. Our population was predominantly African-American. Larger studies will be required to assess whether the haemodynamic effects of inorganic nitrate differ by ethnicity. Enrollment in the ongoing KNO_3CK OUT HFpEF trial is stratified based on gender and ethnicity; this study is also assessing detailed haemodynamic phenotypes and will clarify this issue. The exclusion of patients with atrial fibrillation reduces generalizability of the findings to the important subject of patients who have atrial fibrillation in the setting of HFpEF. We performed two separate sub-studies with different designs. We tested the effects of NTG compared to the values before drug administration, whereas the effects of inorganic nitrate were tested in a cross-over blinded design. This was an acute administration study, and the chronic effects of these drugs on the carotid and peripheral circulations could be different. The patient population had relatively mild HFpEF as evidenced by the relatively low use of loop diuretics; therefore, these results may not apply to patients who have more severe or advanced HFpEF.

Conclusions

We compared the effects of organic and inorganic nitrate on aortic and carotid pulsatile haemodynamics in patients with HFpEF. We demonstrate that organic nitrate administration reduced arterial wave reflections less consistently than observed with inorganic nitrate. Organic nitrate also produced profound vasodilatation in the carotid territory, with a reduction in cerebrovascular resistance and carotid characteristic impedance, whereas inorganic nitrate did not produce significant cerebrovascular dilatation. These important haemodynamic differences are likely related to the differential clinical effects of these agents documented in recent clinical trials.

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