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Cardiac effects of 6 months' dietary nitrate and spironolactone in patients with hypertension and with/at risk of type 2 diabetes, in the factorial design, double-blind, randomised-controlled, VASERA TRIAL

Short running title: Chronic cardiac effects of dietary nitrate

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Abstract

Aims: To explore whether long-term intervention with dietary nitrate ((NO_3^-)), a potential tolerance-free source of beneficial vasoactive nitric oxide) and spironolactone (to oppose aldosterone's potential deleterious cardiovascular effects) improve cardiac structure/function, independent of blood pressure (BP), in patients with/at risk of type 2 diabetes (a population at risk of heart failure).

Methods: A sub-sample of participants in our double-blind, randomised, factorial-design intervention (VaSera) trial of active beetroot juice as a nitrate source (≤11.2 mmol) or placebo (nitrate-depleted) beetroot juice, and either ≤50 mg spironolactone or ≤16 mg doxazosin (control), had trans-thoracic cardiac ultrasounds at baseline (n=105), 3 and 6 months (n=87) of intervention. Analysis was by modified intention-to-treat.

Results: Nitrate-containing juice (n=40) decreased left ventricular (LV) end diastolic volume: -6.3 mL (95% confidence intervals (CI) -11.1,-1.6), and end systolic volume: -3.2 mL (-5.9,-0.5), and increased end diastolic mass/volume ratio: +0.04 (0.00,0.07), relative to placebo juice (n=47). Spironolactone (n=44) reduced relative wall thickness compared to doxazosin (n=43): -0.01 (-0.02,-0.00). Whilst spironolactone reduced LV mass index relative to baseline: -1.48 $g/m^{2.7}$ (-2.08,-0.88), there was no difference versus doxazosin: -0.85 $g/m^{2.7}$ (-1.76,0.05). Spironolactone also decreased the E/A ratio: -0.12 (-0.19,-0.04) and increased S' (a tissue-Doppler systolic function index) by 0.52 (0.05,1.0 cm/s). BP did not differ between the juices, or between the drugs.

Conclusions: 6 months' dietary nitrate decreased LV volumes ~5%, representing new, sustained, BP-independent benefits on cardiac structure, extending mechanisms characterised in pre-clinical models of heart failure. Spironolactone's effects on cardiac remodeling and systo-diastolic function whilst confirmatory, were independent of BP.

Key words: dietary nitrate, beetroot juice, echocardiography, cardiac remodelling, nitrate-nitrite-NO pathway, type 2 diabetes,

What is already known about this subject:

- Type 2 Diabetes (T2DM) is a major risk factor for heart failure (HF), especially with preserved ejection fraction (HFpEF), for which there are no established cures
- Acutely, inorganic nitrite improves central haemodynamics and left heart filling pressures in patients with HFpEF
- Chronic administration of nitrite (4 and 9 weeks') in murine models of heart failure reduces left ventricular (LV) volumes

What this study adds:

- In the longest study yet completed with dietary nitrate, 6 months' beetroot juice decreased LV volumes ~5%
- This was independent of blood pressure and represents a sustained beneficial effect on cardiac structure
- Dietary nitrate has potential to prevent diabetic cardiomyopathy/heart failure

Introduction

Type 2 Diabetes (T2DM) is a major risk factor for heart failure (HF) [1], with either reduced (HFrEF) or preserved ejection fraction (HFpEF) [2]. Patients with T2DM are particularly susceptible to increased LV volumes with drugs which cause fluid retention/increase pre-load, such as pioglitazone [3]. Conversely, simply lowering BP with losartan or atenolol in the LIFE study did not alter LV volumes in patients with diabetes [4].

Decreased production of nitric oxide (NO), a key regulator of vascular homeostasis, by NO synthases and/or decreased bioavailability of NO, (eg: due to excess reactive oxygen species, ROS), is implicated in vascular dysfunction in cardiovascular disease and T2DM [5], LV diastolic dysfunction [6], HF [7], and HFpEF [8]. However, standard approaches to supplement NO using organic nitrates, such as isosorbide mononitrate, lack benefit [9]. This loss of effect with chronic ingestion may be due to nitrate tolerance via decreased bioactivation, increased ROS production and endothelial dysfunction [10]. An alternative therapeutic approach may be via dietary inorganic nitrate (NO_3^-), as found in green leafy vegetables and beetroot [11]. Nitrate is reduced to nitrite (NO_2^-) via the entero-salivary circulation, and further reduced to NO in a hypoxia-dependent process. This "nitrate-nitrite-NO pathway" appears to lack these tolerance issues [12], suppress ROS [13] and reverse endothelial dysfunction [14], and has been extensively investigated clinically in studies up to 4-6 weeks, particularly for blood pressure (BP)-lowering [12][14-16][17]. By contrast, patients with T2DM appear to lack any effect of dietary nitrate on BP [17][18][19].

However, we recently reported that dietary nitrate lowered central aortic systolic BP (–2.6 mm Hg [–4.5 to –0.75 mm Hg], (mean [95% CIs]) p=0.007), despite no effect on brachial

BP, with the main haemodynamic findings of the current study [20]. This is consistent with our findings whereby inorganic nitrite acutely and selectively lowers central aortic pressure through a normoxia-dependent dilatory effect on conduit arteries (radial) in healthy volunteers [21, 22], and selectively dilates epicardial coronary arteries in patients undergoing coronary angiography [23].

Another important cause of heart failure in patients with T2DM is myocardial infarction due to coronary artery disease, with nitrite displaying a potential role in coronary ischaemia-reperfusion injury (IRI) [24], acute ST-elevation myocardial infarction (STEMI) [25, 26], and remote ischaemic preconditioning (RIPC) [27, 28]. Moreover, Lefer and colleagues showed that chronic, 4-9 weeks' oral sodium nitrite supplementation prevented the increases in end-diastolic volume (EDV) and end-systolic volume (ESV)) in murine models of IRI following left coronary artery occlusion [29], and pressure-overload induced LVH with trans-aortic constriction [7].

In contrast to NO-supplementation, mineralocorticoid antagonists are established treatments in HF and hypertension, combatting aldosterone-mediated deleterious cardiovascular effects [30], with 40 weeks' spironolactone improving LV mass, arterial stiffness measured as pulse wave velocity (PWV), augmentation index, and aortic distensibility, in parallel with the reduction in BP, over in patients with stage 2-3 chronic kidney disease [31].

Given the potential for long-term dietary nitrate, and spironolactone, to improve cardiac structure or function, alongside, or independently of, any changes in arterial haemodynamics, we prospectively performed echocardiograms in a sub-sample of patients participating in our VaSera factorial RCT [20, 32], with the *a priori* intention of exploring these specific

mechanisms independently of BP, following a chronic, 6 months' treatment with dietary nitrate ('Beet-it®' or 'Beet-it Sport®' beetroot juice), and/or spironolactone.

The primary hypothesis for the main study was that spironolactone, dietary nitrate, or both could reduce arterial stiffness, measured by PWV, as a treatment target formally independent of BP. We have recently reported that the primary outcome, change in arterial stiffness as cardio-ankle vascular index (CAVI), a nominally BP-independent measure, was not different between spironolactone and doxazosin, P=0.08 [20]. Also, and against the hypothesis, the secondary outcome, aortic PWV by arteriography adjusted for peripheral BP differences at baseline and BP change between trial arms from the trial's start to end, was lower with doxazosin than spironolactone (P=0.045). Dietary nitrate had no effect on PWV.

Methods

Study Population

A sub-sample of patients (with, or at risk of, T2DM) who were consented and randomised in our VaSera factorial RCT had serial trans thoracic cardiac ultrasound performed during the course of the study. The study design and methods have previously been described in detail [32]. Briefly, participants with or at risk of T2DM were recruited from Guy's and St Thomas' Hospitals, London, UK and surrounding areas between 2013-2015. Inclusion criteria were age 18-80 years, clinically diagnosed T2DM or at risk of T2DM (as body mass index (BMI) ≥27 kg/m2, positive family history or glucose intolerance after 75g challenge), ability to understand and comply with the protocol. Exclusion criteria: interfering chronic illness, adverse reaction to either drug, known allergy to beetroot, eGFR <45 mL min-1, HbA1c >11% (97mM/M), pregnant, breast feeding or atrial fibrillation.

The results for the primary outcome – (arterial stiffness) are described above and have been published separately [20]. The study was reviewed and approved by Central London National Research Ethics Service (NRES) and took place in the Clinical Research Facility (CRF) of St Thomas's Hospital. (Clinical trial registration: ISRCTN25003627/ DOI 10.1186/ISRCTN25003627). After initial consent and screening/familiarisation, visit 1 (V1), and having met inclusion criteria, patients were invited to return for double randomisation (in blocks of 6) at visit 2 (V2), with simultaneous allocation to both types of intervention for each patient, therefore into 1 of 4 groups [32]; see **Figure 1** for Study Flow Diagram. After cardiac and vascular measurements, treatments were: either spironolactone 12.5mg once daily for one week titrated to twice daily, OR doxazosin 2mg once daily for one week titrated to twice daily, AND either nitrate -containing beetroot juice (BEET-IT®, nitrate 4.5mmol/day) or placebo beetroot juice. The juices were identical in appearance, smell and taste, with the

nitrate having been removed from placebo juice by ion exchange (nitrate ~0mmol/day). Following two check-up visits (V3 and V4; 2 and 8 weeks, respectively), cardiac and vascular measures were repeated at 3 months (V5). Then, provided there were no contraindications, medication doses were increased (to spironolactone 25mg twice daily or doxazosin 8mg twice daily) and to more concentrated nitrate -containing beetroot juice (BEET-IT® Elite Sports Shot, ~11.2mmol nitrate/day, or matching placebo juice, ~0 mmol nitrate/day). The final visit (V6) was at 6 months' post-randomisation, when V2 and V5 cardiovascular assessments were repeated.

Thus, in this factorial design, approximately half the patients were randomised to active, nitrate-containing beetroot juice, and the other half to the placebo nitrate-depleted juice (with no difference in BP expected, based on other studies of dietary nitrate in patients with T2DM described above). Also, half the patients were randomised to spironolactone, and the other half to doxazosin as control (expected to produce similar changes in BP from baseline, but no difference between the treatments). The factorial design is intended to permit determination of the independent effects of nitrate v placebo, and spironolactone v doxazosin, following testing for drug-dietary nitrate interactions for BP and for echocardiographic parameters.

Echocardiography: Echocardiography was added to the protocol and offered to as many of the patients as possible, to explore mechanisms related to standard cardiac structure and function assessments, in parallel with the key haemodynamic outcome measures of the main study.

Trans-thoracic cardiac ultrasound was performed using a GE Vivid 7 Ultrasound system. All measurements were performed by two expert operators and all images analysed by a single operator blinded to the intervention. Acquisitions were individually optimized for depth, gain,

and frame rate to maximize image quality and minimize inconsistency in acoustic windows prior to analysis. Standard M-mode and 2D imaging was undertaken at rest. Images were saved in raw data format for offline analysis. Left atrial volume (LAV) was calculated by the ellipsoid method and subsequently normalized to body surface area to obtain left atrium volume index (LAVI). Recommendations of the American Society of Echocardiography and the European Association of Cardiovascular Imaging [33] were used to estimate left ventricular mass (LVM) which was indexed to height$^{2.7}$, for LVM index (LVMI) to avoid systematic misclassification of cardiovascular risk in overweight and obesity - likely in these patients. Left ventricular ESV and EDV were measured using Simpson's method and to estimate ejection fraction (EF). The ratio between LVM and EDV (mass/volume, M/V ratio) was calculated. Left ventricular (LV) systolic function was evaluated by peak systolic tissue Doppler imaging (TDI) of S' wave (averaged between septal and later mitral annulus) and global longitudinal strain (GLS) assessed by 2-dimensional speckle tracking echocardiography. Diastolic function of the left ventricle was estimated by conventional Doppler mitral inflow (ratio of transmitral Doppler early (E) to late (A) filling velocity (E/A)) and tissue Doppler imaging (TDI) of mitral annulus (ratio of transmitral Doppler early filling velocity (E) to tissue Doppler early diastolic mitral annular velocity (E′) – (E/E')), as per recommendations [34] as was the ratio E/E' for evaluating LV filling pressure.

Statistical considerations

Analyses were conducted by our independent biostatistician (SM) modified intent-to-treat, consisting of all randomised patients except those with no outcome data at any follow up visit. Patients with missing data at some visits were included, and we assumed that data were missing at random (i.e. unrelated to the unobserved value). We used mixed effect models to estimate the effect of the interventions, and included gender, age, ethnicity (European,

223 African-Caribbean, West African and other), a diagnosis of diabetes and the baseline value of
224 the outcome as covariates. This was a pre-specified/prospectively-conducted, hypothesis-
225 generating, exploratory mechanistic part of the main study. Thus, we present least squares
226 mean changes from baseline and differences between drugs and between juices averaged over
227 both follow-up visits for each outcome, with 95% confidence intervals (95% CI), rather than
228 as hypothesis-testing *P*-values, in accordance with the recent editorial, "Statistical reporting
229 of clinical pharmacology research" [35].
230
231

Results

One hundred and five participants had echocardiograms at baseline (V2), of whom 87 (83%) also had follow-up data at V5 (3 months) and V6 (6 months); see **Figure 2**, CONSORT diagram. Participant details and baseline echo parameters in each treatment arm are shown in **Table 1**. Baseline LVMI (mean±SD) was 53±13.5g/$m^{2.7}$; 52% met criteria for LV hypertrophy (LVH) [33], whilst 95% had normal LV filling pressure (average E/A 1±0.4, E/E' 7.8±2.2).

Haemodynamic Parameters

Spironolactone and doxazosin both reduced systolic BP (SBP) by about 6 mmHg compared to baseline by with no difference between treatments **(Table 2, Figure 3 (A-B))**. Changes in diastolic BP (DBP) were also similar on each drug (~5 mmHg); see **Figure 3 (C-D)** with no change in heart rate (HR). There were no differences in brachial SBP, DBP or HR between nitrate-containing versus nitrate-depleted juice (**Figure 4**). No drug-dietary nitrate interactions were detected for BP or for echocardiographic parameters; therefore, the effects of the drugs and dietary nitrate were estimated from models with no interaction term.

Echocardiographic Morphological Parameters

Compared to placebo juice (*n*=47), nitrate-containing beetroot juice (*n*=40) decreased EDV: -6.33 mL (-11.1,-1.57) and ESV: -3.2 mL (-5.9,-0.5); see **Table 2 and Figure 5**. Also, EDV and ESV decreased relative to baseline on nitrate-containing beetroot juice: -4.77 mL (-8.10,-1.44) and -2.77 mL (-4.66,-0.89), respectively, but not on placebo juice (n=47): -1.56 mL (-1.67,4.80) and -0.40 mL (-1.43,2.22). The reduction in LVMI from baseline was similar between nitrate-containing and placebo juices, with no difference between interventions. Therefore, the ratio between LV mass and volume – the M/V ratio, increased by 0.04 (0.00,0.07) between active and nitrate-containing beetroot juices. Relative to baseline, LAVI

fell on active juice: -1.59 ml/m^2 (-2.64,-0.54), but not placebo juice: -0.26 ml/m^2 (-1.29,0.78); however, there was no difference between the interventions: -1.33(-2.83,0.17).

In contrast to nitrate, the only between-group difference in morphological parameters with spironolactone (n=44) was a marginal reduction in relative wall thickness (RWT): -0.01(-0.02,0.00) vs doxazosin (n=43); see **Table 2**. Whilst spironolactone reduced LVMI relative to baseline: -1.48 g/$m^{2.7}$ (-2.08, -0.88), there was no difference versus doxazosin: -0.85 g/$m^{2.7}$ (-1.76,0.05). Similarly, LAVI appeared to be reduced by doxazosin relative to baseline: -1.16 ml/m^2 (-2.24,-0.07), but not versus spironolactone.

Echocardiographic Systo-Diastolic Function

Among parameters of systo-diastolic function, the E/A ratio fell on spironolactone from baseline: -0.07 (-0.12,-0.02), and versus doxazosin: -0.12 (-0.19,-0.04)); see **Figure 6 (A-B)**. The tissue Doppler systolic function index, S', increased on spironolactone versus doxazosin, by 0.52 cm/s (0.05, 1.00); see **Figure 6 (C-D)**.

The only change in systo-diastolic function parameters observed with nitrate-containing beetroot juice was a prolongation of the deceleration time (DT) by 19.50 ms (8.40, 30.60) and 19.74 ms (4.47,36.01) relative to baseline and nitrate-depleted juice, respectively.

Discussion

We found that 6 months' intervention with dietary nitrate as beetroot juice may reduce LV volumes (EDV and ESV) compared to placebo nitrate-depleted juice. The lack of any change in LV mass by active juice suggests a favourable effect of nitrate on LV structure and possibly myocardial wall stress (since LV volumes were reduced, whilst BP was unaffected). In addition, spironolactone decreased RWT, suggesting a beneficial effect on myocardial remodelling, and improved parameters of systo-diastolic function (S', E/A) compared to doxazosin. These effects were also independent of BP (which was not different between spironolactone and doxazosin).

Nitrate's chronic effect on reducing LV volumes have important implications for HFpEF, and builds on the beneficial acute actions of nitrate/nitrite recently demonstrated on exercise performance and left heart filling pressures (PCWP), in patients with HFpEF [36-39]. Indeed, across two randomised double-blind placebo-controlled studies by Borlaug and colleagues, one in 28 patients [37], the other in a subset of 52 of 98 patients with HFpEF [39], who were undergoing invasive haemodynamic exercise testing, sodium nitrite (either intravenous or nebulised-inhaled) acutely decreased aortic wave reflections (at rest), improved arterial compliance, elastance and central hemodynamics (during exercise), and left heart filling pressures (pulmonary capillary wedge pressure [PCWP]), compared to placebo [37]. However, no clinical data are available on the long-term effects of dietary nitrate on cardiac structure and function. This is a key question, given the problems of tolerance associated with organic nitrates. Therefore, the current study provides the most extensive evidence to date of long-term cardiac effects of dietary nitrate and has biological plausibility, building on the translational study of 9 weeks' supplementation with oral nitrite showing decreased EDV and ESV versus placebo in the mouse model of pressure-overload induced LVH with trans-aortic constriction [7]. Regarding the specific changes in ventricular volumes in our study, it can be

speculated that dietary nitrate-derived nitrite likely acts on ventricular pre-load by influencing venous dilatation [22, 40], and pressure; though since nitrate salts are also known to have diuretic activity, this could play a role. No direct measures of pre-load were collected here; however, changes in indirect parameters, such as ventricular volumes, as demonstrated with intravenous organic nitrate therapy [41]) support this. Indeed, previous invasive studies have used EDV to define LV pre-load [42, 43]. Moreover, the reduction in LV volumes, but not LVMI, resulted in an increased M/V ratio. This suggests a positive action on cardiac remodelling [44] and myocardial wall stress [45], with potential favourable prognostic implications [46].

In contrast to nitrate, ventricular volumes were not altered by spironolactone, which instead improved other structural and functional cardiac parameters. Spironolactone has previously been demonstrated to improve cardiac hypertrophy and remodelling in hypertensive patients [47], although this was not shown in patients with T2DM [48]. In our population, there was some evidence that spironolactone decreased RWT and LVMI, suggesting a direct action of spironolactone on cardiac remodelling (that was independent of BP). If confirmed, this finding would be relevant because cardiac remodelling is a prognostic factor for CV events - even in the absence of LVH [49].

Our results also suggest important differential actions of the two drugs on cardiac performance (S' and E/A). S' is considered a sensitive TDI index of systolic function [50] which was increased by spironolactone compared with doxazosin.

Spironolactone has previously been found to have beneficial effects on diastolic function [51, 52]. In subclinical diabetic cardiomyopathy, spironolactone decreased conventional Doppler parameters (E/A), without affecting E/E' [47], despite evidence of elevated LV diastolic filling pressure (E/E' 14.3±7). We also observed a reduction in E/A ratio with spironolactone,

with no change in E/E', which was within the normal range at baseline. Therefore, the reduction in E/A may reflect an improvement in diastolic function that is not limited to alterations in pre-load, since other parameters sensitive to pre-load, such as EDV, ESV and LAVI were not affected by spironolactone, **Table 2**, (and neither were blood pressure or heart rate). However, diastolic function is a complex phenomenon and a "single parameter" approach does not provide a comprehensive overview [34].

Overall, these results indicate that dietary nitrate may have BP-independent beneficial actions on myocardial remodelling over and above established effects of spironolactone. This could be explained by different mechanisms of action – mainly cardiac pre-load for dietary nitrate versus a direct anti-remodelling effect for spironolactone; although a direct action of nitrate/nitrite on the myocardium cannot be excluded and should be further investigated.

We acknowledge several limitations of our study: cardiac analysis was not the primary outcome of the Vasera trial and our analyses are therefore exploratory; the overall sample size was relatively small (87 patients with follow-up data); confidence intervals are therefore wide; follow up data was incomplete, and the mixed effects model may not adequately account for any bias this could have introduced.

Conclusion

Six months' dietary nitrate decreased LV volumes ~5%, representing sustained, BP-independent effects on cardiac structure, suggesting a beneficial action on cardiac remodelling, with potential consequences on CV prevention/treatment. Spironolactone independently decreased LV wall thickness and modified parameters of systo-diastolic function.

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Table 1: Baseline Clinical and Cardiac Parameters of the Study Population

		Doxazosin		*Spironolactone*	
		Placebo Juice	***Active Juice***	***Placebo Juice***	***Active Juice***
Patients	*n*	27	16	20	24
Sex -female	*n (%)*	6 (22)	5 (31)	8 (40)	8 (33)
Diabetes -at risk	*n (%)*	11 (41)	6 (38)	6 (30)	12 (50)
Previous CV event	*n (%)*	1 (3.7)	2 (12.5)	2 (10.0)	2 (8.3)
Smoker	*n (%)*	3 (12.0)	2 (15.4)	2 (13.3)	1 (5.6)
Age (years)	*mean (SD)*	54.9 (13.8)	58.4 (14.7)	58.2 (9.9)	57.1 (13.2)
BMI- Kg/m^2)	*Mean (SD)*	30.2 (5.1)	32.7 (6.5)	33.0 (4.2)	33.7 (5.2)
SBP (mmHg)	*Mean (SD)*	135.1 (16.8)	134.3 (16.6)	139.2(17.6)	138.3 (21.6)
DBP (mmHg)	*Mean (SD)*	79.4 (11.1)	80.7 (8.2)	79.8 (11.8)	83.1 (15.6)
HR (beat/min)	*Mean (SD)*	73.3 (14.4)	70.2 (11)	73 (13.1)	66.7 (10.4)
LAVI (ml/m^2)	*Mean(SD)*	23.0 (8.4)	25.3 (8.6)	25.6 (9.8)	24.9 (6.9)
LVMI (g/m$^{2.7}$)	*Mean(SD)*	52.7 (12.6)	50.9 (11.9)	54.1 (15.2)	51.8 (10.5)
RWT	*Mean(SD)*	0.401 (0.064)	0.389 (0.068)	0.403 (0.057)	0.373 (0.052)
EDV (ml)	*Mean(SD)*	127.4 (35.7)	138.4 (45.6)	127.6 (19.3)	134.7 (34.1)
ESV (ml)	*Mean(SD)*	53.6 (16.9)	63.9 (29.7)	55.3 (10.7)	57.5 (15.2)
Mass/Volume (g/ml)	*Mean(SD)*	0.96 (0.33)	0.81 (0.23)	0.88 (0.24)	0.81 (0.23)
E/A	*Mean(SD)*	1.00 (0.30)	0.98 (0.31)	1.09 (0.66)	0.94 (0.26)
DT (msec)	*Mean(SD)*	233.0 (51.6)	232.4 (59.9)	212.3 (59.0)	220.3(53.6)
E/E'	*Mean(SD)*	7.55 (2.16)	8.38 (2.70)	7.63 (2.29)	7.50 (2.23)
EF (%)	*Mean(SD)*	58.20 (3.1)	54.90 (5.3)	56.80 (4.2)	57.4 (3.7)
S' (cm/s)	*Mean(SD)*	9.0 (1.6)	9.0 (1.7)	8.8 (2.1)	8.8 (1.2)

Table 1: Baseline clinical and cardiac parameters of the study population. Abbreviations: systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), left atrium volume index to body surface area (LAVI), left ventricular mass index (LVMI), relative wall thickness (RWT), end-diastolic volume (EDV), end-systolic volume (ESV), ratio of transmitral Doppler peak early (E) to late (A) filling velocity (E/A), ratio of transmitral Doppler peak early filling velocity (E) to pulsed-wave tissue-Doppler imaging (TDI)-derived early mitral annular diastolic velocity (E′) – (E/E'), early transmitral deceleration time (DT), ejection fraction (EF), pulsed-wave TDI-derived mitral annular systolic velocity – a systolic function index (S'), global longitudinal strain (GLS).

Table 2: Haemodynamic and Echocardiographic Parameters

	Active juice (n=40)	Placebo juice (n=47)	Active vs placebo juice	Spironolactone (n=44)	Doxazosin (n=43)	Spironolactone vs Doxazosin
Haemodynamic parameters						
SBP (mmHg)	**-6.34 (-9.09,-3.59)**	**-6.57 (-9.41,-3.73)**	0.23 (-3.77, 4.22)	**-6.49 (-9.31,-3.67)**	**-6.42 (-9.20,-3.64)**	-0.07 (-4.07, 3.93)
DBP (mmHg)	**-5.06 (-6.80,-3.33)**	**-4.96 (-6.76,-3.17)**	-0.10 (-2.63, 2.43)	**-5.19 (-6.96,-3.42)**	**-4.84 (-6.59,-3.09)**	-0.35 (-2.86, 2.16)
HR (bpm)	0.14 (-1.51, 1.79)	-0.94 (-2.65, 0.76)	1.08 (-1.33, 3.49)	-0.05 (-1.73, 1.63)	-0.76 (-2.41, 0.89)	0.71 (-1.67, 3.09)
Morphological parameters						
LAVI (ml/m^2)	**-1.59 (-2.64, -0.54)**	-0.26 (-1.29, 0.78)	-1.33 (-2.83, 0.17)	-0.69 (-1.69, 0.32)	**-1.16 (-2.24, -0.07)**	0.47 (-1.05, 1.99)
LVMI (g/m$^{2.7}$)	**-0.96 (-1.60, -0.32)**	**-1.16 (-1.75, -0.57)**	0.20 (-0.68, 1.09)	**-1.48 (-2.08, -0.88)**	-0.63 (-1.28, 0.01)	-0.85 (-1.76, 0.05)
RWT	**-0.00 (-0.01, 0.00)**	**-0.00 (-0.01, 0.00)**	**-0.00 (-0.01, 0.01)**	**-0.001 (-0.01, -0.00)**	0.00 (-0.00, 0.001)	**-0.01 (-0.02, -0.00)**
EDV (ml)	**-4.77 (-8.10, -1.44)**	1.56 (-1.67, 4.80)	**-6.33 (-11.1, -1.57)**	-2.36 (-5.52, 0.79)	-0.85 (-4.26, 2.59)	-1.51 (-6.28, 3.25)
ESV (ml)	**-2.77 (-4.66, -0.89)**	0.40 (-1.43, 2.22)	**-3.17 (-5.86, -0.48)**	-1.52 (-3.31, 0.27)	-0.86 (-2.78, 1.07)	-0.67 (-3.37, 2.03)
Mass/Volume (g/ml)	0.01 (-0.01, 0.03)	**-0.03 (-0.05, -0.00)**	**0.04 (0.00, 0.07)**	-0.01 (-0.03, 0.02)	-0.01 (-0.03, 0.02)	0.00 (-0.03, 0.04)
Systo-diastolic function parameters						
E/A	-0.00 (-0.05, 0.10)	-0.03 (-0.07, 0.02)	0.02 (-0.05, 0.10)	**-0.07(-0. 12, -0.02)**	0.05 (-0.01, 0.10)	**-0.12 (-0.19, -0.04)**
DT (ms)	**19.50 (8.40, 30.60)**	-0.24 (-10.6, 10.08)	**19.74 (4.47, 36.01)**	**11.32 (0.82, 21.82)**	7.93 (-3.42, 19.26)	3.39 (-12.5, 19.25)
E/E'	0.26 (-0.15, 0.68)	-0.19 (-0.57, 0.19)	0.45 (-0.12, 1.02)	-0.13 (-0.53, 0.26)	0.21 (-0.21, 0.62)	-0.34 (-0.93, 0.24)
EF (%)	0.46 (-0.29, 1.21)	0.09 (-0.64, 0.81)	0.38 (-0.69, 1.45)	0.38 (-0.34, 1.10)	0.17 (-0.60, 0.94)	0.21 (-0.88, 1.29)
S' (cm/s)	-0.19 (-0.53, 0.15)	0.02 (-0.29, 0.34)	-0.21 (-0.68, 0.26)	0.18 (-0.14, 0.50)	**-0.35 (-0.68, -**	**0.52 (0.05, 1.00)**

					0.01)	
GLS (%)	0.72 (-0.43, 1.87)	-0.58 (-1.88, 0.72)	1.30 (-0.48, 3.08)	-0.44 (-1.68, 0.80)	0.58 (-0.61, 1.78)	-1.02 (-2.77, 0.73)

Table 2: Change from baseline, active nitrate-containing beetroot juice versus the placebo nitrate-depleted juice, and spironolactone versus doxazosin. Least square means (LSM) estimated from a model using data from all follow up visits, adjusted for baseline value, gender age, ethnicity and diagnosis of diabetes. Data shown as LSM and 95% confidence intervals (CIs). Sets of data where the 95% CIs do not cross zero are highlighted in bold. Abbreviations: systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), left atrium volume index to body surface area (LAVI), left ventricular mass index (LVMI), relative wall thickness (RWT), end-diastolic volume (EDV), end-systolic volume (ESV), ratio of transmitral Doppler peak early (E) to late (A) filling velocity (E/A), ratio of transmitral Doppler peak early filling velocity (E) to pulsed-wave tissue-Doppler imaging (TDI)-derived early mitral annular diastolic velocity (E′) – (E/E’), early transmitral deceleration time (DT), ejection fraction (EF), pulsed-wave TDI-derived mitral annular systolic velocity – a systolic function index (S'), global longitudinal strain (GLS).

Figure Legends

Figure 1. Study Flow Diagram. At Visit 2, spironolactone dosage regimen was 12.5mg once daily for one week titrated to twice daily (indicated in the Diagram as (1-2x/d)); doxazosin was 2mg once daily for one week titrated to twice daily (indicated in the Diagram as (1-2x/d)). At Visit 5 the doses of each were doubled, but frequencies maintained at twice daily (2x/d).

Figure 2. CONSORT flow diagram for VaSera trial and subsample of participants who had an echo at baseline and follow up visits.

Figure 3. Blood pressure (BP) responses to spironolactone and doxazosin: (A) change from baseline in systolic BP (SBP) for spironolactone and doxazosin; (B) overall effect of spironolactone versus doxazosin on SBP; (C) change from baseline in diastolic BP (DBP) for spironolactone and doxazosin; (B) overall effect of spironolactone versus doxazosin on DBP. Data shown as least square means (LSM) with 95% Confidence Intervals.

Figure 4. Blood pressure (BP) responses to dietary nitrate (beetroot juice): (A) change from baseline in systolic BP (SBP) for active nitrate-containing beetroot juice and placebo nitrate-depleted juice; (B) overall effect of active nitrate-containing beetroot juice versus placebo nitrate-depleted juice on SBP; (C) change from baseline in diastolic BP (DBP) for active nitrate-containing beetroot juice and placebo nitrate-depleted juice; (B) overall effect of active nitrate-containing beetroot juice versus placebo nitrate-depleted juice on DBP. Data shown as least square means (LSM) with 95% Confidence Intervals.

Figure 5. Left ventricular (LV) volume responses, measured by echocardiography, to dietary nitrate (beetroot juice): (A) change from baseline in LV end-diastolic volume

(LVEDV) for active nitrate-containing beetroot juice and placebo nitrate-depleted juice; (B) overall effect of active nitrate-containing beetroot juice versus placebo nitrate-depleted juice on LVEDV; (C) change from baseline in LV end-systolic volume (LVESV) for active nitrate-containing beetroot juice and placebo nitrate-depleted juice; (B) overall effect of active nitrate-containing beetroot juice versus placebo nitrate-depleted juice on LVESV. Data shown as least square means (LSM) with 95% Confidence Intervals.

Figure 6. Echocardiographic systo-diastolic responses to spironolactone and doxazosin: (A) change from baseline in E/A ratio for spironolactone and doxazosin; (B) overall effect of spironolactone versus doxazosin on E/A ratio; (C) change from baseline in tissue Doppler systolic function index (S') for spironolactone and doxazosin; (B) overall effect of spironolactone versus doxazosin on S'. Data shown as least square means (LSM) with 95% Confidence Intervals.

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555 **Figure 1**: Study Flow Diagram.
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Figure 1: Study Flow Diagram.

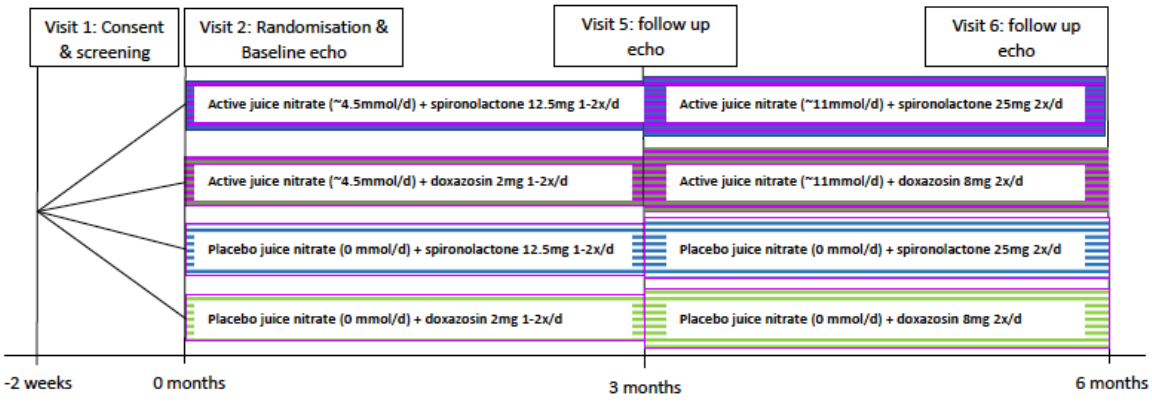
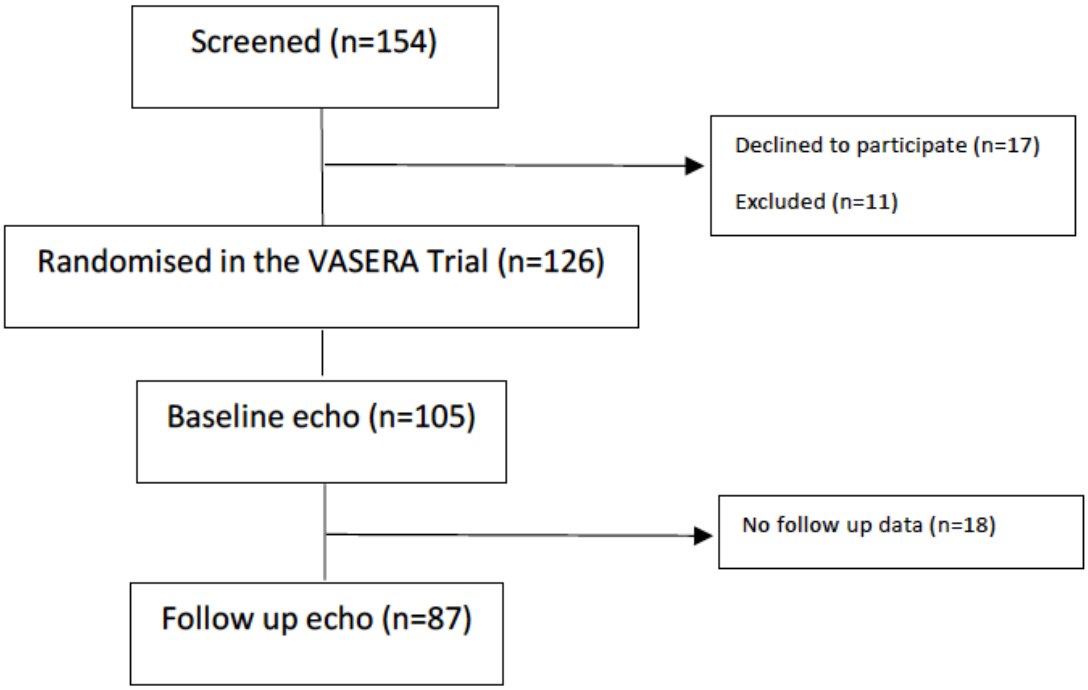
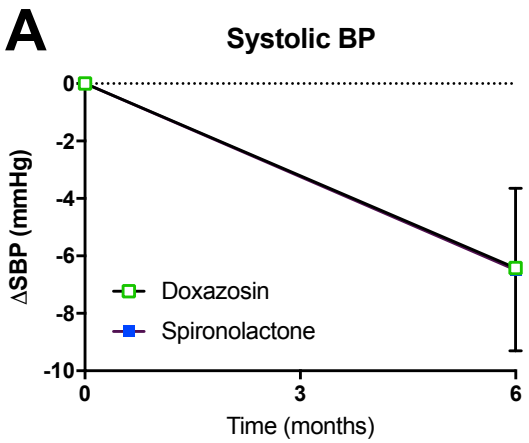
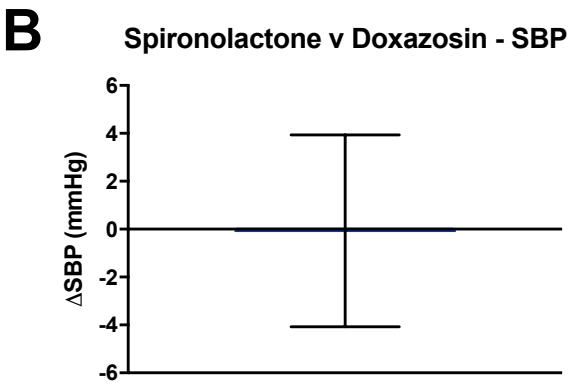
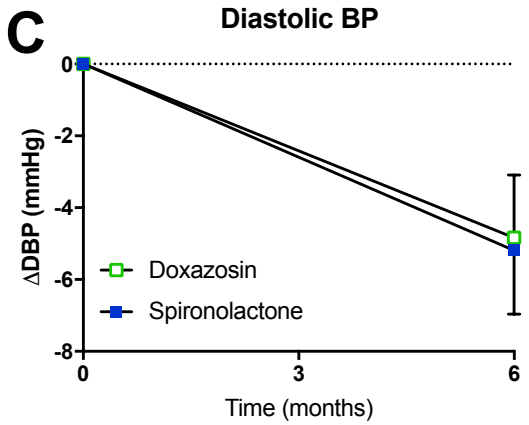


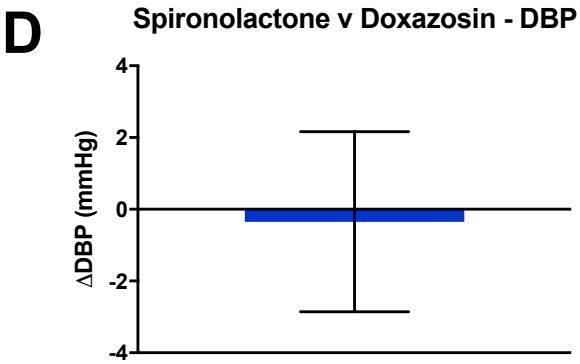
Figure 2 CONSORT flow diagram for VaSera trial and subsample of participants who had an echo at baseline and follow up visits.

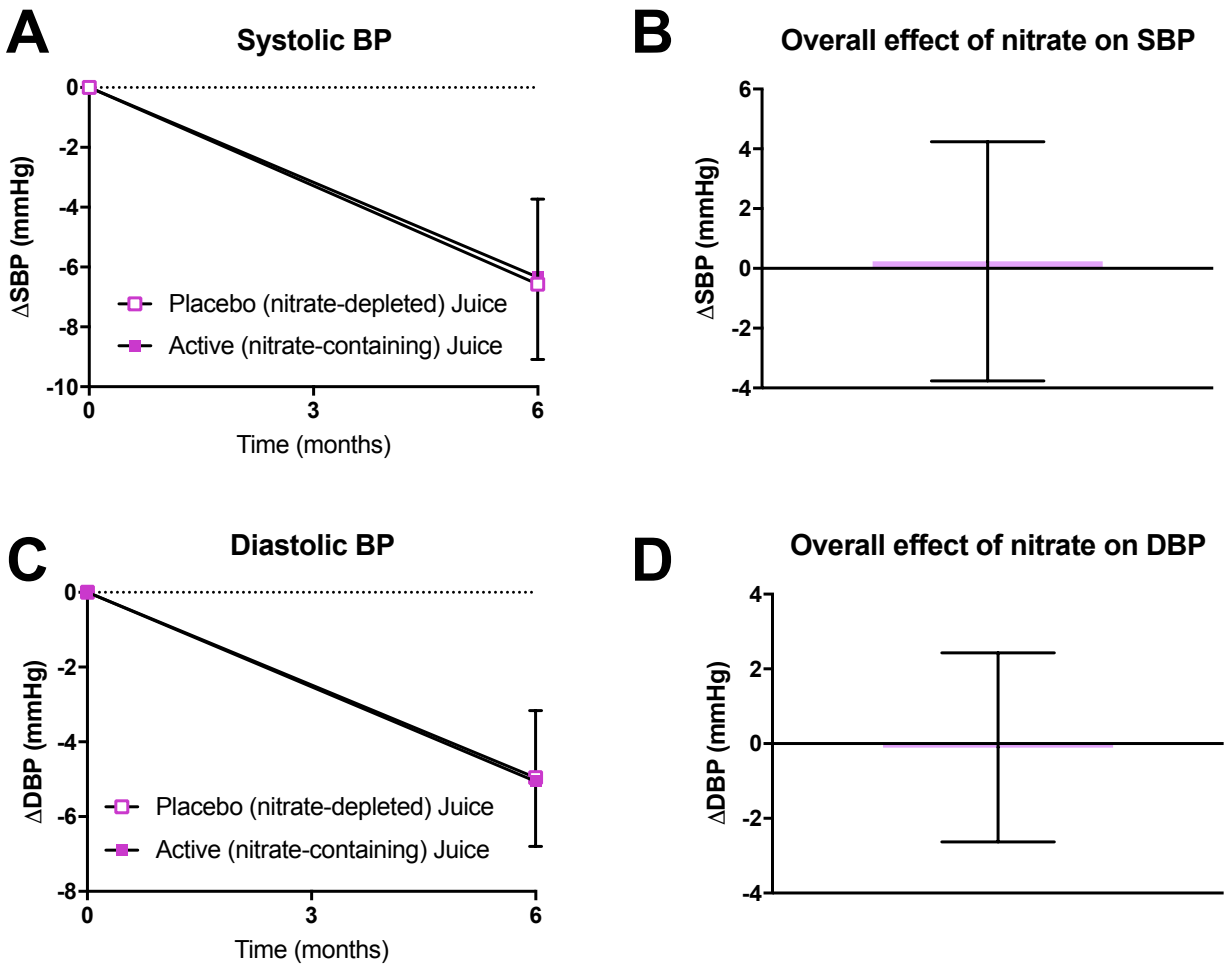


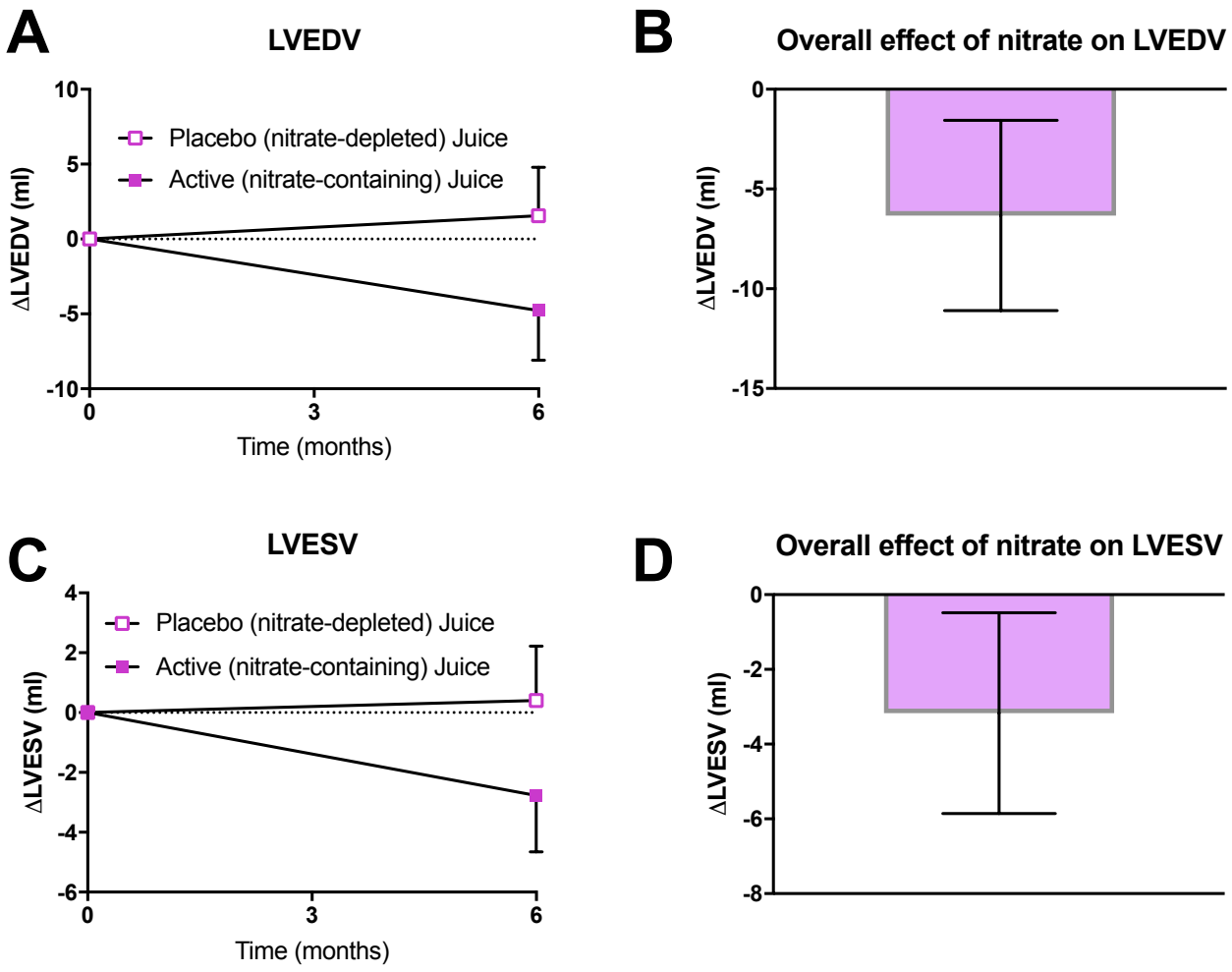

A
Systolic BP
0
-2
-4
-6
-8
-10
ΔSBP (mmHg)
Doxazosin
Spironolactone
0
3
6
Time (months)

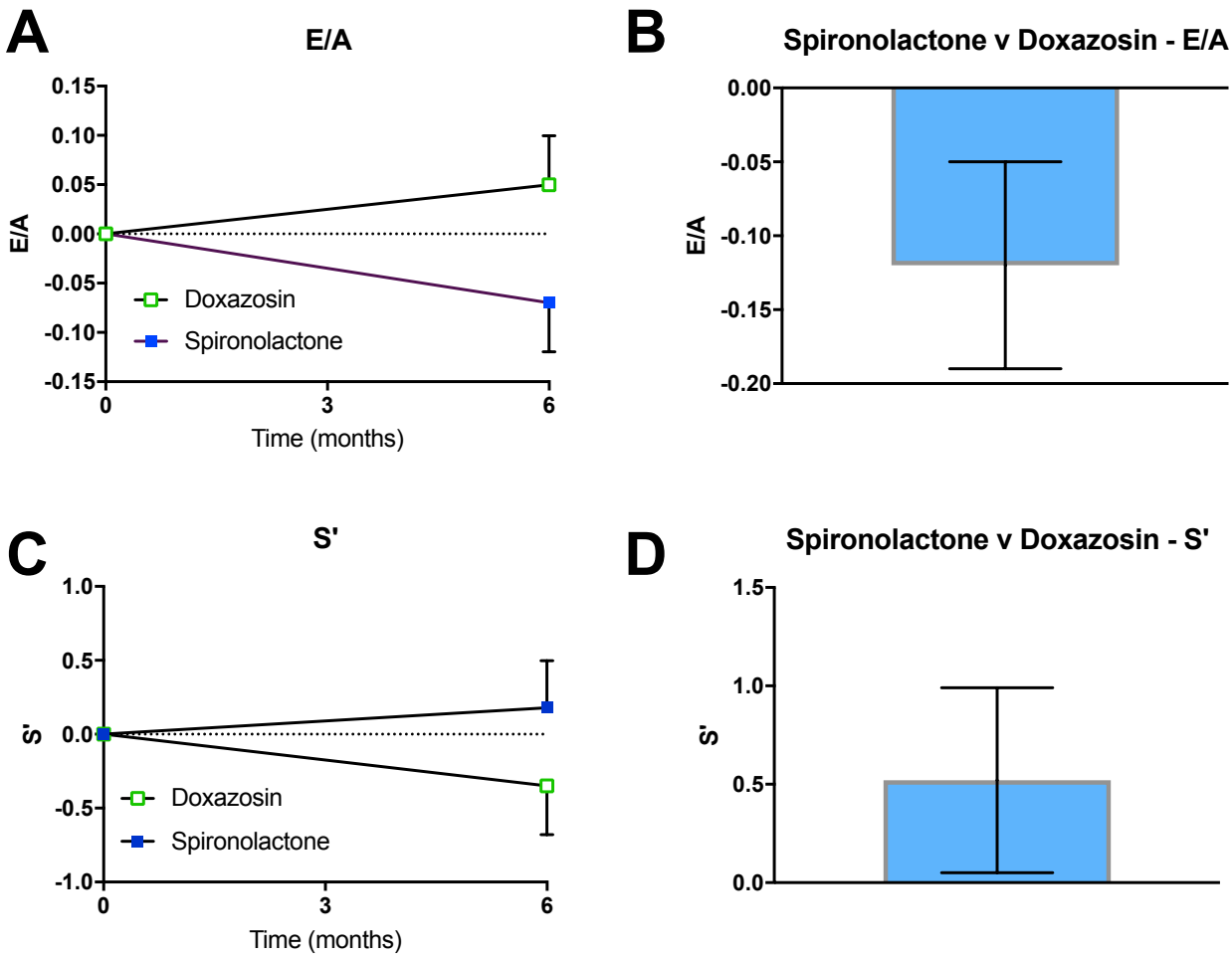

B
Spironolactone v Doxazosin - SBP
6
4
2
0
-2
-4
-6
ΔSBP (mmHg)


C
Diastolic BP
0
-2
-4
-6
-8
ΔDBP (mmHg)
Doxazosin
Spironolactone
0
3
6
Time (months)


D
Spironolactone v Doxazosin - DBP
4
2
0
-2
-4
ΔDBP (mmHg)


A
Systolic BP
0
-2
-4
-6
-8
-10
ΔSBP (mmHg)
Placebo (nitrate-depleted) Juice
Active (nitrate-containing) Juice
0
3
6
Time (months)
B
Overall effect of nitrate on SBP
6
4
2
0
-2
-4
ΔSBP (mmHg)
C
Diastolic BP
0
-2
-4
-6
-8
ΔDBP (mmHg)
Placebo (nitrate-depleted) Juice
Active (nitrate-containing) Juice
0
3
6
Time (months)
D
Overall effect of nitrate on DBP
4
2
0
-2
-4
ΔDBP (mmHg)


A
LVEDV
10
5
0
-5
-10
ΔLVEDV (ml)
Placebo (nitrate-depleted) Juice
Active (nitrate-containing) Juice
0
3
6
Time (months)
B
Overall effect of nitrate on LVEDV
0
-5
-10
-15
ΔLVEDV (ml)
C
LVESV
4
2
0
-2
-4
-6
ΔLVESV (ml)
Placebo (nitrate-depleted) Juice
Active (nitrate-containing) Juice
0
3
6
Time (months)
D
Overall effect of nitrate on LVESV
0
-2
-4
-6
-8
ΔLVESV (ml)


A
E/A
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
E/A
Doxazosin
Spironolactone
0
3
6
Time (months)
B
Spironolactone v Doxazosin - E/A
0.00
-0.05
-0.10
-0.15
-0.20
E/A
C
S'
1.0
0.5
0.0
-0.5
-1.0
S'
Doxazosin
Spironolactone
0
3
6
Time (months)
D
Spironolactone v Doxazosin - S'
1.5
1.0
0.5
0.0
S'