

Does cardiac resynchronization therapy restore peripheral circulatory homeostasis?

Chukwudiebube N. Ajaero^{1,2,3}, Cher-Rin Chong^{4,5}, Nathan E.K. Procter⁶, Saifei Liu⁷, Yuliy Y. Chirkov^{2,7}, Tamila Heresztyn⁷, Wai Ping Alicia Chan^{1,2,8}, Margaret A. Arstall^{2,9}, Andrew D. McGavigan^{3,10}, Michael P. Frenneaux⁶ and John D. Horowitz^{1,2*}

¹The Queen Elizabeth Hospital, Adelaide, Australia; ²The University of Adelaide, Adelaide, Australia; ³Flinders Medical Centre, Adelaide, Australia; ⁴Cardiac Metabolism Research Group, Department of Physiology, Anatomy and Genetics, University of Oxford, Oxford, UK; ⁵School of Medicine, The University of Adelaide, Adelaide, Australia; ⁶Bob Champion Research and Education Building, University of East Anglia, Norwich, UK; ⁷Basil Hetzel Institute, Adelaide, Australia; ⁸The Lyell McEwin Hospital, Adelaide, Australia; ⁹Northern Adelaide Local Health Network, Adelaide, Australia; ¹⁰Flinders University, Adelaide, Australia

Abstract

Aims To evaluate whether peripheral circulatory ‘remodelling’ as measured by changes in vascular compliance and in markers of nitric oxide signalling contributes to patient response to cardiac resynchronization therapy (CRT).

Methods and results Effects of CRT were evaluated in 33 patients pre-procedure and 6 months post-procedure. Peak oxygen consumption, 6 min walk distance, New York Heart Association class, and quality of life score were evaluated. Augmentation index and its interactions with nitric oxide (NO) were evaluated by applanation tonometry. Platelet NO responsiveness and content of thioredoxin-interacting protein were assessed. Plasma concentrations of N-terminal proBNP, asymmetric and symmetric dimethylarginine (SDMA), high sensitivity C-reactive protein, catecholamines, and matrix metalloproteinases-2 and -9 were assessed. Despite significant improvement in 6 min walk distance ($P = 0.005$), New York Heart Association class ($P < 0.001$), quality of life ($P = 0.001$), and all echocardiographic parameters post-CRT, there were no significant changes in augmentation index measurements, thioredoxin-interacting protein content, and platelet NO response. Significant falls in N-terminal proBNP ($P = 0.008$) and SDMA ($P = 0.013$; independent of renal function) occurred. Falls in SDMA predicted reduction in high-sensitivity C-reactive protein ($P = 0.04$) and increases in peak oxygen consumption ($P = 0.04$). There were no correlations between changes in echocardiographic parameters and those in vascular function.

Conclusions These data suggest that the beneficial effects of CRT over 6 months are independent of any change in peripheral NO-related signalling. However, there is evidence that suppression of inflammation occurs, and its magnitude predicts extent of clinical improvement.

Keywords Augmentation index (AI_x); Symmetric dimethylarginine (SDMA); Thioredoxin-interacting protein (TXNIP); Cardiac resynchronisation therapy (CRT); Nitric oxide (NO) signalling; Left ventricular dyssynchrony

Received: 5 June 2017; Revised: 24 July 2017; Accepted: 9 August 2017

*Correspondence to: John D. Horowitz, The Queen Elizabeth Hospital, 28 Woodville Road, Woodville South, Adelaide 5011, Australia. Tel: +61 8 8222 7432; Fax: +61 8 8222 6422. Email: john.horowitz@adelaide.edu.au

Institutions where work was performed: (A) The Queen Elizabeth Hospital, 28 Woodville Road, Woodville South, South Australia. (B) Basil Hetzel Institute, Woodville South, South Australia (C) The Lyell McEwin Hospital, Haydown Road, Elizabeth Vale, South Australia (D) The Flinders Medical Centre, Flinders Drive, Bedford Park, South Australia

Introduction

Over the last two decades, as a result of several landmark trials demonstrating mortality and morbidity benefits with cardiac resynchronization therapy (CRT) with or without defibrillation insertion,^{1–3} CRT has become a standard of care in select patients with systolic heart failure and concomitant

left ventricular dyssynchrony. Substantial limitations of CRT include not only the 30–40% of recipients classified as ‘non-responders’² but also significant heterogeneity in the commonly used criteria of response and poor agreement between these different clinical and echocardiographic parameters of response.⁴ There has been considerable interest in understanding the mechanisms underlying benefits associated with

CRT and specifically whether these arise purely from mechanical correction of dyssynchrony. Of particular interest is the putative effect of CRT on peripheral vascular function. Two small studies have shown consistent improvement in microvascular cutaneous reactive hyperaemia post-CRT insertion,^{5,6} while studies of the effects of CRT on large conduit vessels utilizing flow-mediated [i.e. nitric oxide (NO)-dependent] dilatation (FMD) have shown conflicting results.^{7–9} In this present study, we sought to evaluate the effect of CRT on peripheral vascular function and associated markers of inflammation, as well as vascular and platelet NO signalling, we also examined the effects of CRT on parameters of inflammatory and neurohumoral activation.

Objectives of the study were as follows:

- (1) To evaluate whether the optimal outcome post-CRT is engendered not only by reversal of left ventricular systolic dysfunction but also by amelioration of peripheral circulatory dysfunction.
- (2) To evaluate the effects of CRT on neurohumoral activation.

Methods

Thirty-three patients with conventional indications for CRT were prospectively evaluated before and 6 months after CRT insertion. Clinical characteristics were noted, including New York Heart Association functional class status, and quality of life was evaluated using the standard (and well-validated) Minnesota Living with Heart Failure Questionnaire.

Exercise capacity was evaluated both by 6 min walk distance and by cardiopulmonary exercise testing.

Six minute walk distance

The protocol for the 6 min walk distance has been described.¹⁰ This test was performed indoors in a dedicated and marked hallway that is 30 m long with a hard floor. Cones were used to delineate the turn-around points. The procedure was duly explained, and patients were asked to walk as far as possible and as fast as they could for a timed period of 6 min. They were allowed to slow down, to rest or to lean against the wall if necessary, at their own discretion, and to resume walking again at their possible fastest pace. Running or jogging was not allowed. The test was terminated if a patient developed chest pain, became unduly dyspnoeic, or looked ashen with significant diaphoresis. If any patient ordinarily mobilized with a walking aid, he or she was allowed to use the same during the test. The maximum distance walked at 6 min was recorded for each patient.

Cardiopulmonary exercise testing

Cardiopulmonary exercise testing was performed according to established guidelines.¹¹ Briefly, a bicycle ergometer (Model: ergoline/100/200 GmbH, Germany) and linked to ExpAir Medisoft S.A Belgique 1.31.02 software were utilized in all cases. Before performing each test, the equipment was calibrated both for airflow and volume, including low and high flows (with calculated volumes within $\pm 3\%$) using a 3 L syringe and also for gases with carbon dioxide set at 4% and oxygen at 20%. A semi-automated progressive incremental (ramp) protocol in which the pre-test was set at 0 W while the workload started from 10 W and increased by 10 W every minute was used. Patients pedalled for a minute on the pre-test setting of 0 W before loading commenced. They were encouraged to exercise as long as possible, ideally for up to 8 to 12 min, and especially aiming to achieve a respiratory exchange ratio of 1 and above, with cycling rate kept at 60–70 revolutions per minute. Volitional exhaustion was the usual endpoint although exercise was terminated if a patient developed chest pain, acute ischaemic changes on the electrocardiogram, or hypotension. After unloading, the patient pedalled for a further 1 min. Measurements of oxygen consumption (VO_2), carbon dioxide output, ventilator equivalent, and respiratory exchange ratio were automatically acquired and finally averaged and displayed at 10 s interval. The highest reading of the three last averages at peak exercise was chosen as the peak oxygen consumption (VO_2 max).

Echocardiographic measurements

All echocardiographic measurements were performed according to the American Society of Echocardiography and the European Association of Cardiovascular Imaging guidelines.¹² A Phillips echocardiogram machine model iE33, 2009, Bothell WA, 98041 USA was used for image acquisition, and analyses were performed using Echopac Software Only BT 11 Version 113, 2013 General Electric Co. M-mode echocardiographic analysis was used to assess left ventricular interventricular dyssynchrony. Septal to posterior wall delay was calculated as the time difference between the onset of the QRS to that of the peak of deformation of the interventricular septum and the left ventricular posterior. Although extent of dyssynchrony was analysed as a continuum, septal to posterior wall delay of 130 ms or more was considered diagnostic of clinically significant of intraventricular mechanical dyssynchrony.¹³ Left ventricular volumes including end-diastolic, end-systolic, and stroke volumes were measured in two dimensions and ejection fraction calculated by the modified Simpson's method in biplane using 2D images.

Vascular endothelial function

Vascular endothelial function was assessed by changes in augmentation index (AI_x) using radial artery applanation tonometry as previously described.¹⁴ Briefly, patients were first rested in a supine position for 30 min. Using a commercially available pulse waveform analyser, the SphygmoCor system (AtCor Medical, Sydney, Australia, model CvMS V9), baseline AI_x was computed as the average of three readings. Sublingual glycerine trinitrate (GTN 300 μ g) was administered and AI_x remeasured every 5 min for 20 min. The difference between the lowest value of AI_x with GTN and the baseline AI_x (that is, the maximum fall in AI_x with GTN) was utilized as a measure of endothelium-independent NO signalling. Subsequently, 400 μ g of inhaled salbutamol was administered, and measurements were repeated every 5 min for 20 min. The difference between the lowest AI_x with salbutamol and the baseline (the maximum fall in AI_x with salbutamol) represents a measure of endothelium-dependent NO signalling.¹⁵ Using the acquired radial artery waveform, a validated, generalized transfer function was used to generate the corresponding central aortic pressure waveform from which AI_x values were calculated. All measurements were indexed to a heart rate of 75 b.p.m., and only high fidelity tracings were used.

Platelet expression of thioredoxin-interacting protein

Platelet expression of thioredoxin-interacting protein (TXNIP) was quantified by immunofluorescence as previously described.¹⁶ Briefly, blood was collected in ethylenediaminetetraacetic acid (EDTA) tubes and spun at 250 g for 10 min at room temperature. Slides were smeared, air-dried, and fixed with 4% paraformaldehyde and stored in -70°C freezer, and immunofluorescence staining performed within 6 months.

For immunofluorescence staining, the slides were allowed to warm to room temperature and then washed three times in phosphate-buffered saline (PBS) for 5 min per wash. A 100 μ L of diluted goat's serum 1:5 with PBS (as a blocking solution) was added and incubated at room temperature for 30 min. Primary antibody was prepared by diluting rabbit anti-Vitamin D3 Up-regulated Protein (VDUP) TXNIP, (Invitrogen Corporation, Carlsbad, CA, USA) with 1% (w/v) bovine serum albumin in PBS in 1:50 ratio. The blocking solution was discarded without washing the slides, and then, 100 μ L of the primary antibody was added and incubated overnight at 2–4°C. The primary antibody was discarded the next day, and the slides were washed three times in PBS for 5 min per wash. Two conjugated 2° antibodies: dilute phycoerythrin-labelled CD41 antibody (Becton, Dickinson and Company, USA), in 1:50 with PBS, serving as a platelet

marker, and dilute fluorescein isothiocyanate-labelled anti-rabbit polyclonal antibody (Becton, Dickinson and Company, USA), in 1:100 with PBS were prepared while shielded from light. A 100 μ L of each of the 2° antibodies was added to the slide and incubated at room temperature for 60 min. The slides were again washed three times in PBS for 5 min per wash, dried with lens tissue, and one drop of Dako fluorescent mounting medium was added, covered with a slip, and allowed to sit at room temperature for 10 min. Image acquisition was performed with Carl Zeiss Microscope, Germany, using $\times 400$ magnification, multichannel for rhodamine and green fluorescent protein and exposure time set at 5000 ms. The intensities of TXNIP staining for each platelet were obtained utilizing image analysis software (AxioVision 40 version 4.8.2, Carl Zeiss Microscopy, Germany)

Subsequent analysis or counting was performed by randomly identifying 100 platelets per slide and average TXNIP obtained.

Asymmetric dimethylarginine and symmetric dimethylarginine

For asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) concentration estimation in plasma, 10 mL of blood was collected in heparinized tubes and immediately put in ice, before centrifugation at 4°C at 1800 g for 15 min. Plasma was collected in Eppendorf tubes and stored at -70°C until analysed. Plasma ADMA and SDMA concentrations were determined by high performance liquid chromatography, as described previously.¹⁷ Briefly, this involved extraction, derivatization with fluorescent derivatizing reagent (AccQ-Fluor™), and chromatography.

Matrix metalloproteinase-2 and matrix metalloproteinase-9

Matrix metalloproteinase-2 was estimated from blood samples collected in EDTA tubes, while matrix metalloproteinase-9 was estimated from blood samples collected in heparinized tubes. For both, collected blood was immediately put in ice and was centrifuged for 15 min at 1800 g at 4°C within 30 min of collection. Platelet-poor plasma was collected and stored at -70°C until analysed. Assays were performed with R&D Quantikine quantitative sandwich enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems Ltd, Minneapolis USA) according to the manufacturer's instructions. Plasma concentrations of matrix metalloproteinase-2 and matrix metalloproteinase-9 were determined by ELISA (R&D Systems Ltd) according to manufacturer's instructions.

Platelet aggregometry test; responsiveness to nitric oxide

Platelet response to NO was assessed *in vitro* in whole blood using a 4-channel impedance aggregometer (Chrono-Log Corporation, model 700) according to a previously described protocol, which required temporary interruption of therapy with P2Y₁₂ receptor antagonists.¹⁸ Briefly, 9 mL of blood was collected into plastic tubes containing 1 mL of acid citrate anticoagulant. The blood was allowed to stand at room temperature for 20 min before testing. A 500 µL of normal saline was pre-warmed to 37°C in a 4-channel impedance aggregometer after which 450 µL of whole blood was added to the saline and both pre-warmed for 5 min. All tests were run at 37°C and a stirring speed of 900 rpm. Platelet aggregation was induced with adenosine 5'-diphosphate (ADP) (2.5 µM), and responses were measured as an increase in impedance (in Ohms), at 7 min. When NO donor sodium nitroprusside (10 µM) was added to samples 1 min before adenosine 5'-diphosphate, the resultant inhibition of aggregation was evaluated as a percentage relative to control.

N-terminal proBNP levels

These were analysed by the South Australia Pathology laboratory at the Queen Elizabeth Hospital. Samples were collected in heparinized tubes and analysed for N-Terminal proBNP with the Elecsys proBNP system (Roche diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim). The Sandwich principle was used: this involves a first incubation with a biotinylated monoclonal NT-proBNP-specific antibody and a monoclonal NT-proBNP-specific antibody labelled with a ruthenium complex. A second incubation was performed with the addition of streptavidin-coated microparticles, and the microparticles were subsequently captured on the surface of the electrode magnetically. A photomultiplier finally measured the chemiluminescent emission induced by application of voltage to the electrode.

Plasma metanephrine and normetanephrine

Analyses for catecholamine metabolites were performed with fresh samples collected in K₃EDTA bottles and analysed utilizing liquid chromatography-tandem spectrometry. The upper limits of the normal ranges for plasma concentrations were <500 pmol/L for free metanephrine and <900 pmol/L for free normetanephrine.

High sensitivity C-reactive protein

Samples were collected in K₃EDTA bottles and analysed by the immuno-turbidimetric quantification method on Beckman

Coulter AU analysers (Beckman Coulter, Inc., Brea, CA, USA) according to the manufacturer's recommendations.

Ethics approval

The study complies with the Declaration of Helsinki, and approval for the study was granted by the Ethics and Human Research Committee of The Queen Elizabeth Hospital. All participants provided prior written informed consent.

Statistical analyses

The power of the study was calculated on the basis of variability in responsiveness of AI_x to salbutamol and was approximately 80% to detect a 5% variation in response at $P < 0.05$.

All data are expressed as mean \pm standard deviation unless stated otherwise. The effects of cardiac resynchronization therapy on clinical, biochemical, endothelial, and echocardiographic parameters were assessed using paired *t*-test for normally distributed variables and Wilcoxon matched-pairs signed-rank test for non-parametric data. Each patient served as his/her control. Interactions between changes in left ventricular contractility and endothelial functions as well as the interactions between changes in function and neurohumoral parameters were correlated using Pearson correlation coefficients for normally distributed data and Spearman correlation for non-parametric data. Relationship between categorical changes in SDMA and changes in high-sensitivity C-reactive protein concentrations and in VO₂ max were evaluated by non-paired *t*-tests. A two-tailed *P* value < 0.05 was considered statistically significant.

All data were analysed with Prism 6 for Mac OS X version 6.0h October 2015.

Results

The baseline characteristics of patients are listed in *Tables 1* and *2*. The mean QRS duration was 158.7 \pm 23.6 ms. Approximately half of the patients had an ischaemic aetiology of heart failure, and the majority were in New York Heart Association functional class III–IV pre-CRT implantation. All patients were also extensively treated for heart failure, and the majority were receiving β -adrenoceptor antagonists. One patient died 2 months after CRT insertion, while three patients declined the 6 month follow-up visit. Among the whole cohort, there was significant improvement post-CRT in all echocardiographic parameters and in all clinical parameters, with the exception of VO₂ max, as shown in *Figures 1* and *2* and *Table 3*. There was no significant change overall in vascular and platelet functions except for a statistically significant

Table 1 Baseline characteristics of patients

Age (years)	71.2 ± 9.7
Female, <i>n</i> (%)	10 (30.3)
Weight (kg)	87.1 ± 18.6
Height (cm)	172.5 ± 8.1
BMI (kg/m ²)	29.3 ± 6.1
Ischaemic aetiology of CHF, <i>n</i> (%)	17 (52)
History of current/past smoking, <i>n</i> (%)	19 (57.6)
NYHA class	
• I, <i>n</i> (%)	3 (9)
• II, <i>n</i> (%)	7 (21)
• III, <i>n</i> (%)	19 (58)
• IV, <i>n</i> (%)	4 (12)
Comorbidities	
• Hypertension, <i>n</i> (%)	21 (64)
• Diabetes, <i>n</i> (%)	14 (42)
• COPD, <i>n</i> (%)	4 (12)
• Atrial fibrillation, <i>n</i> (%)	5 (15)
Medications	
• ACE inhibitor	22 (67)
• Angiotensin receptor blocker	8 (24)
• β-adrenoceptor blocker	25 (76)
• Aldosterone Antagonist	18 (55)
• Digoxin	9 (27)
• Frusemide	24 (73)
• Statin	18 (55)
• Aspirin	17 (52)
• Clopidogrel	7 (21)
• Perhexiline	5 (15)
Clinical assessment	
Systolic BP (mmHg)	126.4 ± 17.1
Diastolic BP (mmHg)	71.2 ± 9.2
Heart rate (b.p.m.)	69 ± 13.6
6MWD (m)	321.74 ± 104.62
VO ₂ max (mL/min/kg)	13.8 ± 4.7
QOL score	41.9 ± 25.6
Biochemistry	
NT-proBNP (ng/L)	1814.0 (1091–3073)
eGFR (mL/min/1.73 m ²)	56.4 ± 22.2
Plasma metanephrine (pmol/L)	252.1 ± 158.3
Plasma normetanephrine (pmol/L)	996.5 ± 396.2
Plasma MMP-2 (ng/mL)	217.5 ± 51.2
Plasma MMP-9 (ng/mL)	27.7 ± 9.1
high-sensitivity C-reactive protein (mg/L)	2.4 (1.4–6.1)
Ventricular function	
LVESV (mL)	137.4 ± 55.3
LVEDV (mL)	192.8 ± 67.4
EF (%)	29.8 ± 6.1
SPWD (ms)	120.0 ± 195.0
IVMD (ms)	43.9 ± 44.0

6MWD, 6 min walk distance; ACE, angiotensin-converting enzyme; BMI, body mass index; BP, blood pressure; CHF, chronic heart failure; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; EF, ejection fraction; eGFR, estimated glomerular filtration rate; IVMD, interventricular mechanical delay; LVEDV, left ventricular end-diastolic volume; LVESV, left ventricular end-systolic volume; MMP, matrix metalloproteinase; NT-proBNP, N terminal proBNP; NYHA, New York Heart Association; QOL, quality of life; SPWD, septal to posterior wall delay; VO₂ max, peak oxygen consumption during exercise.

Normally distributed data are mean ± SD; skewed data are expressed as median values and interquartile values.

reduction in plasma concentrations of SDMA (paired *t*-test, *P* = 0.013).

As shown in *Figure 3A–C*, although a statistically significant inverse correlation existed at baseline between estimated glomerular filtration rate and SDMA concentration, at 6 months,

Table 2 Endothelial function/no signalling pre-CRT

Baseline Al _x (%)	19.9 ± 7.8
GTN Al _x change (%)	–13.9 ± 10.0
Salbutamol Al _x change (%)	–11.3 ± 10.2
ADMA (μM)	0.6 ± 0.07
SDMA (μM)	0.73 (0.60–1.0)
Platelet SNP response (%)	33.9 ± 26.5
Platelet TXNIP (AU)	136.9 ± 111.2

ADMA, asymmetric dimethyl arginine; Al_x, augmentation index; CRT, cardiac resynchronization therapy; GTN, glycerine trinitrate; SDMA, symmetric dimethyl arginine; SNP, sodium nitroprusside; TXNIP, thioredoxin-interacting protein.

Normally distributed data are mean ± SD; skewed data are expressed as median values and interquartile values.

significant decreases in plasma levels of SDMA had occurred without any change in estimated glomerular filtration rate. It is therefore unlikely that changes in renal function had contributed to the observed fall in SDMA levels. A categorical reduction in SDMA concentrations was predictive of both a reduction in high-sensitivity C-reactive protein concentrations (*P* = 0.04; *Figure 3D*) and an increase in VO₂ max (*P* = 0.04; *Figure 3E*). With regard to neurohumoral and inflammatory activation, there was a statistically significant reduction in plasma levels of NT-proBNP (paired *t*-test, *P* = 0.008) but no significant change in any other marker of NO signalling or of inflammatory activation. It must be noted, however, that in the absence of significant changes, effects of both salbutamol and GTN on Al_x tended to increase (by about 20%). On univariate analysis, there was no significant correlation between change at 6 months in left ventricular end-systolic volume and changes at 6 months in baseline Al_x, GTN-mediated fall in Al_x, salbutamol-mediated fall in Al_x, or with platelet NO response.

Discussion

In this study, the first point to be noted is perhaps the fact that the cohort on the whole ‘responded’ to CRT, in the sense that there was significant improvement in parameters of left ventricular ejection fraction, as well as significant improvement in symptomatic status as shown in *Table 3*. Therefore, we have a cohort in whom there was effective mechanical cardiac resynchronization; otherwise, it would have been futile for us to proceed with the pursuit of understanding of any further mechanistic effect of CRT. The lack of statistically significant improvement in VO₂ max was similar to the results of some previously reported CRT studies^{19,20} although CRT has also been shown to improve VO₂ max.² In addition, there was a significant reduction in the plasma concentrations of N-terminal proBNP, a similar finding to the CARE-HF trial.² In this regard, a *post hoc* analysis of the MADIT-CRT trial²¹ found that reduction in BNP following CRT was associated

Figure 1 Effects of cardiac resynchronization therapy (CRT) on clinical parameters in individual patient. (A) New York Heart Association (NYHA) functional class ($n = 29$; this is partially obscured graphically by identical changes in 20 patients). (B) 6 min walk distance (6MWD). (C) Peak oxygen consumption (VO_2 max). (D) Quality of life (QOL) score. All analyses were performed via paired t -tests.

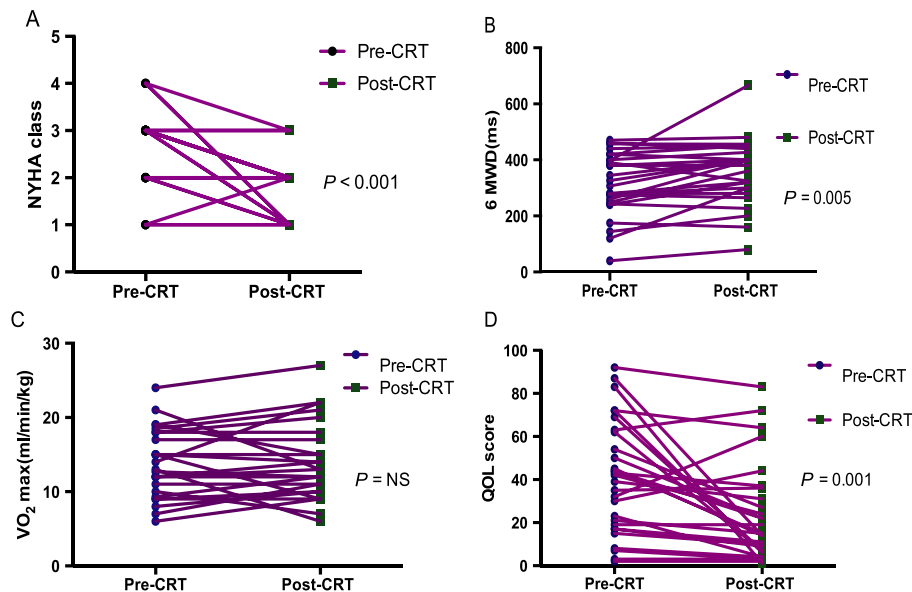
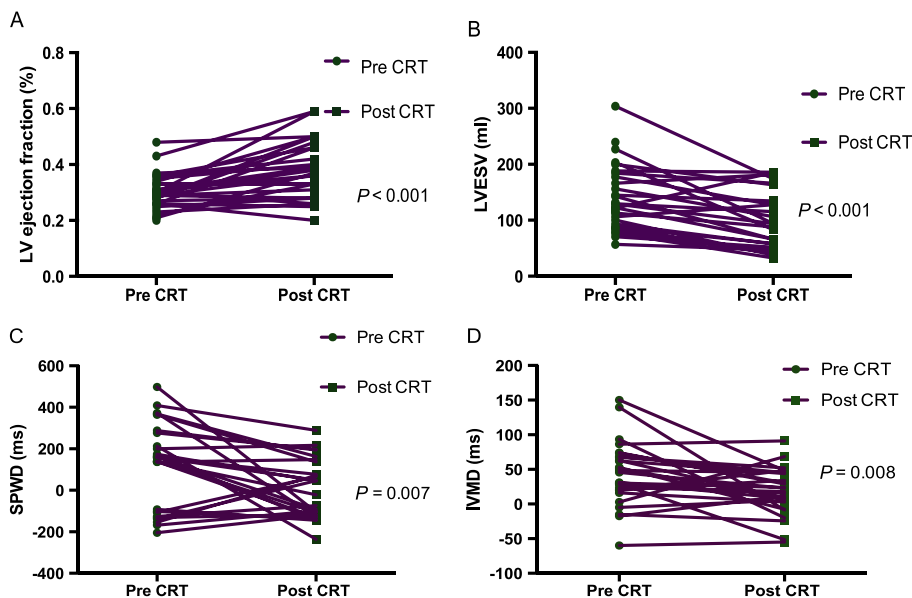


Figure 2 Effects of cardiac resynchronization therapy (CRT) on echocardiographic parameters in individual patients. (A) Left ventricular (LV) ejection fraction. (B) Left ventricular end-systolic volume (LVESV). (C) Septal to posterior wall delay (SPWD). (D) Interventricular mechanical delay (IVMD). All analyses were performed via paired t -test.



with significant decrease in hospitalized heart failure and mortality.

In spite of these salutary effects of CRT in our cohort, there was no significant change in conventional markers of peripheral vascular endothelial function as assessed by AI_x with

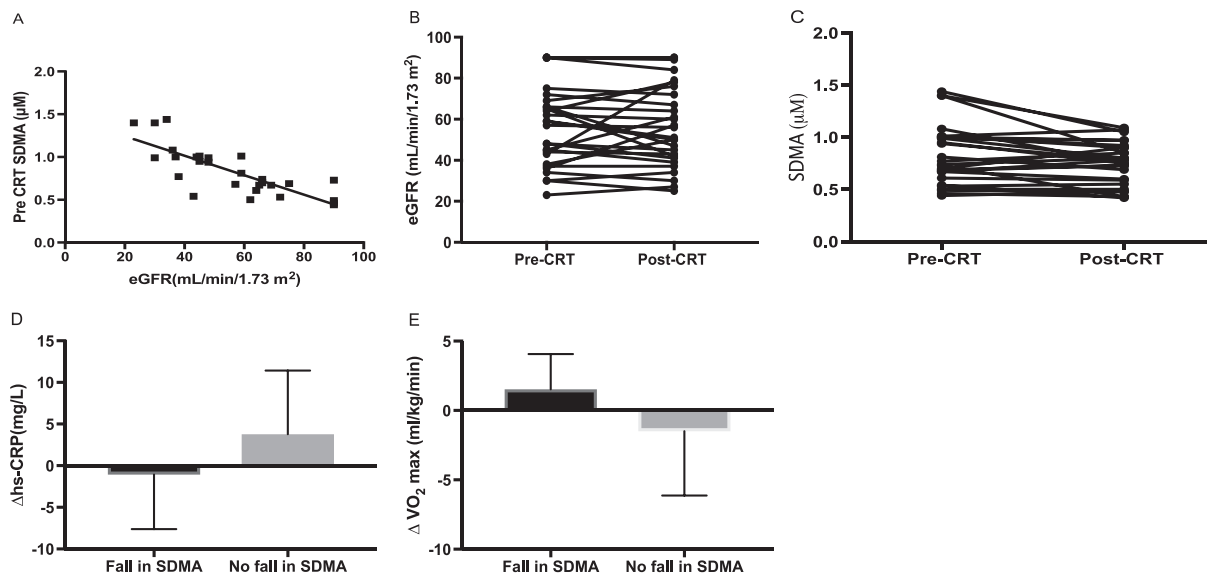
radial artery applanation tonometry. Specifically, there were no significant changes either in baseline AI_x or in endothelium-independent and endothelial-dependent AI_x , as assessed with GTN and salbutamol, respectively. A potential confounder to this apparent lack of effect on vascular

Table 3 Effects of cardiac resynchronization therapy: analyses are limited to patients in whom 6 months data post-CRT were available

Parameters	Pre-CRT	Post-CRT	P-value
Clinical			
NYHA	2.7 (0.8)	1.9 (0.7)	<0.001
VO ₂ max (mL/min/kg)	13.8 (4.67)	14.1 (5.3)	NS
6MWD (M)	314.5 (112.8)	357.0 (117.0)	0.005
QOL score	40.7 (25.4)	22.9 (22.3)	0.001
Vascular and Platelet			
Baseline Al _x (%)	20.3 (8.2)	20.3 (8.1)	NS
GTN response [Al _x change (%)]	-14.1 (10.0)	-16.6 (8.1)	NS
Salbutamol response [Al _x change (%)]	-9.9 (10.5)	-11.9 (8.3)	NS
SNP response (%)	30.5 (21.8)	25.2 (19.7)	NS
TXNIP (AU)	144.8 (122.6)	188.5 (126.6)	NS
ADMA (μM)	0.66 (0.08)	0.65 (0.09)	NS
SDMA (μM)	0.83 (0.28)	0.74 (0.20)	0.013
Biochemistry			
Plasma metanephrine (pmol/L)	257.8 (167.2)	239.2 (88.8)	NS
Plasma normetanephrine (pmol/L)	918.8 (356.6)	900.7 (295.6)	NS
NT-proBNP (ng/L)	1862 (1091–3185)	1469 (774–2841)	0.008
MMP-2 (ng/mL)	217.2 (52.5)	219.8 (49.9)	NS
MMP-9 (ng/mL)	27.5 (9.6)	26.5 (10.0)	NS
High-sensitivity C-reactive protein (mg/L)	2.4 (1.4–7.1)	3.5 (1.6–11.0)	NS
Echocardiographic			
LV ejection fraction (%)	31.0 (6)	38 (10)	<0.001
LVESV (mL)	136.6 (57.5)	98.9 (52.1)	<0.001
SPWD (ms)	119.1 (201.2)	1.74 (141.4)	0.007
IVMD (ms)	43.6 (44.6)	19.9 (33.9)	0.008

6MWD, 6 min walk distance; ADMA, asymmetric dimethyl arginine; Al_x, augmentation index; CRT, cardiac resynchronization therapy; GTN, glycerine trinitrate; IVMD, interventricular mechanical delay; LV, left ventricular; LVESV, left ventricular end-systolic volume; MMP, matrix metalloproteinase; NS, non-significant; NT-proBNP, N terminal proBNP; NYHA, New York Heart Association; QOL, quality of life; SDMA, symmetric dimethyl arginine; SNP, sodium nitroprusside; SPWD, septal to posterior wall delay; VO₂ max, peak oxygen consumption; TXNIP, thioredoxin-interacting protein.

Figure 3 Implications of changes in plasma symmetric dimethyl arginine (SDMA) concentrations regarding renal excretion of SDMA, and variations in high-sensitivity C-reactive protein concentrations and peak oxygen consumption (VO₂ max) post-cardiac resynchronization therapy (CRT). (A) Correlation between baseline estimated glomerular filtration rate (eGFR) and SDMA concentrations ($r = -0.80$, $P < 0.001$). (B) Changes in eGFR post-CRT in individual patients ($P = NS$). (C) Changes in SDMA concentrations post-CRT in individual patients ($P = 0.013$). (D) Changes in high-sensitivity C-reactive protein concentrations ($P = 0.04$). (E) Changes in VO₂ max values ($P = 0.04$).



endothelial function is the possibility that CRT may indeed have opposing effects on AI_x : by increasing stroke volume into non-compliant vessels with already depressed Windkessel effect, there would be an increase in the velocity of both the forward and the reflected waves in the peripheral vasculature. It is also possible that extensive pharmacological treatment of our cohort for heart failure and dyslipidemia may have resulted in pre-CRT improvement of vascular endothelial function. Statins, for instance, are known to cause a reduction in aortic AI_x .²² Angiotensin-converting enzyme inhibitors (ACE-I) also significantly reduce AI_x ,²³ and 91% of our cohort were receiving ACE-I or angiotensin receptor blocker. It is also possible, given the observation of a (non-significant) 20% improvement on vascular responsiveness to salbutamol and GTN post-CRT, that a small improvement in these parameters might have been obscured via type II error. Indeed, the probability of detecting a change of less than 0.5 SD of baseline values would have been less than 80%. In the case of responses to salbutamol, for example, an absolute improvement of less than 5% might have gone undetected. In addition, the use of β -adrenoceptor antagonists would potentially blunt the effect of salbutamol on AI_x , thus obscuring the effect of CRT. Interestingly, the study cohort included 24% of patients who were not receiving β -adrenoceptor antagonists presumably because of contraindications and/or prior adverse effects. However at baseline, there was no significant difference in AI_x response to salbutamol between patients receiving and not receiving β -adrenoceptor antagonists.

The lack of effect of CRT on either basal or NO-stimulated AI_x was somewhat different from the results of previous studies that utilized FMD as a criterion of NO-mediated arterial vasomotion. All of these have shown some evidence of improvement in FMD. Akar *et al.*⁷ observed that although patients with worse FMD of the brachial artery responded better to CRT and that these responders had improvement in FMD at 90 days, the overall improvement was not statistically significant ($P = 0.12$).

In a subsequent study of 57 patients, Santini *et al.*⁸ found that in a CRT cohort, significant improvement in FMD occurred in both responders and non-responders at 3, 6, and 12 months although with somewhat greater improvement in the responder group than in the non-responder group, with these results being only of borderline statistical significance. A recent study by Warriner *et al.*⁹ also found that lower FMD predicts response to CRT at 12 months. However, in their cohort, CRT did not result in significant improvement in FMD in either the responder group or the non-responder group. It is not completely clear to what extent the reported differential FMD responses in the Santini study represent the phenomenon of regression to the mean. In any case, we are unable to make direct comparisons in the current study. We did not measure FMD, essentially because AI_x is a more reproducible parameter than FMD.^{24,25} Importantly, in our

study, CRT did not affect ADMA concentrations also indicating that NO generation via endothelial NO synthase activation was likely to be unaffected. In one previous study, high ADMA concentrations tended to predict poor responses to endothelial NO synthase activation by FMD.²⁶ A further caveat concerning the current findings is that evaluation of changes in platelets and blood may not be fully representative of changes within the myocardium.

It might have been expected that CRT would tend to normalize patterns of arterial distention and thus decrease expression of TXNIP given that the latter is shear stress-dependent.²⁷ However, no change in platelet TXNIP content occurred. In retrospect, there have been no previous direct studies of effects of CRT on arterial shear stress, and it may be that reversal of LV dyssynchrony has minimal effects on this parameter. Furthermore, platelet TXNIP expression is suppressed by NO signalling, such as are induced by ACE inhibitor therapy.²⁸

A notable exception to the overall negative data in this study was the observed fall in plasma SDMA concentrations (Figure 3). This is most unlikely to be a 'false positive' given first, the $P = 0.013$ value. SDMA, unlike ADMA, is only a very weak inhibitor of NO synthase, but it is a substantial mediator of inflammatory change.²⁹ This could not be explained by improved renal function, an important consideration because SDMA is excreted (mainly) unchanged in the urine.³⁰ A fall in SDMA concentrations independent of alterations in renal function post-CRT may have reflected either decreased protein catabolism (representing the mechanism of formation of SDMA via the enzyme, protein arginine methyltransferase type II). Importantly, although there was no significant fluctuation in other inflammatory markers in the cohort as a whole, patients with a reduction in SDMA levels also had significant reductions in high-sensitivity C-reactive protein relative to those without a fall in SDMA, which further supports the concept that amelioration of inflammation activation may occur post-CRT. Interestingly, a fall in SDMA concentrations also predicted increases in VO_2 max (Figure 3E)

In summary, in an extensively medically treated cohort of patients, CRT did not significantly alter markers of NO signalling. It is therefore unlikely that changes in NO signalling significantly contribute to the salutary effects of CRT within 6 months. However, there is some evidence that CRT may lead to suppression of inflammatory activation within the systemic circulation.

Acknowledgements

The authors wish to thank Jeanette Stansborough for her help with the logistics of the research and the 6 min walk distance. The authors thank Irene Stafford for ensuring the

laboratory bench ran smoothly and also thank Adrian Elliott for initial assistance with performing cardiopulmonary exercise tests.

Conflict of interest

None declared.

Funding

This research was supported by The Queen Elizabeth Hospital Research Foundation, APA Grant to C.N.A. by the University of Adelaide, and the National Health and Medical Research Council of Australia (NHMRC) postgraduate scholarship to C.-R.C. (APP1075767).

References

- Bristow MR, Saxon LA, Boehmer J, Krueger S, Kass DA, De Marco T, Carson P, DiCarlo L, DeMets D, White BG, DeVries DW, Feldman AM. Cardiac-resynchronization therapy with or without an implantable defibrillator in advanced chronic heart failure. *N Engl J Med* 2004; **350**: 2140–2150.
- Abraham WT, Fisher WG, Smith AL, Delurgio DB, Leon AR, Loh E, Kocovic DZ, Packer M, Clavell AL, Hayes DL, Ellestad M, Trupp RJ, Underwood J, Pickering F, Truex C, McAtee P, Messenger J. Cardiac resynchronization in chronic heart failure. *N Engl J Med* 2002; **346**: 1845–1853.
- Cleland JGF, Daubert J-C, Erdmann E, Freemantle N, Gras D, Kappenberger L, Tavazzi L. The effect of cardiac resynchronization on morbidity and mortality in heart failure. *N Engl J Med* 2005; **352**: 1539–1549.
- Fornwalt BK, Sprague WW, BeDell P, Suever JD, Gerritse B, Merlino JD, Fyfe DA, León AR, Oshinski JN. Agreement is poor among current criteria used to define response to cardiac resynchronization therapy. *Circulation* 2010; **121**: 1985–1991.
- Tesselaar E, Schiffer A, Widdershoven J, Broers H, Hendriks E, Luijten K, Creusen J. Effect of cardiac resynchronization therapy on endothelium-dependent vasodilatation in the cutaneous microvasculature. *Pacing Clin Electrophysiol* 2012; **35**: 377–384.
- Enomoto K, Yamabe H, Toyama K, Matsuzawa Y, Yamamuro M, Uemura T, Morihisa K, Iwashita S, Kaikita K, Sugiyama S, Ogawa H. Improvement effect on endothelial function in patients with congestive heart failure treated with cardiac resynchronization therapy. *J Cardiol* 2011; **58**: 69–73.
- Akar JG, Al-Chekakie MO, Fugate T, Moran L, Froloshki B, Varma N, Santucci P, Wilber DJ, Matsumura ME. Endothelial dysfunction in heart failure identifies responders to cardiac resynchronization therapy. *Heart Rhythm* 2008; **5**: 1229–1235.
- Santini L, Capria A, Di Molfetta A, Mahfouz K, Panattoni G, Minni V, Sergi D, Forleo GB, Romeo F. Endothelial dysfunction is a marker of systemic response to the cardiac resynchronization therapy in heart failure. *J Card Fail* 2013; **19**: 419–425.
- Warriner DR, Lawford P, Sheridan PJ. Measures of endothelial dysfunction predict response to cardiac resynchronization therapy. *Open Heart* 2016; **3**: e000391. <https://doi.org/10.1136/openhrt-2015-000391>
- ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med* 2002; **166**: 111–117.
- Balady GJ, Arena R, Sietsema K, Myers J, Coke L, Fletcher GF, Forman D, Franklin B, Guazzi M, Gulati M, Keteyian SJ, Lavie CJ, Macko R, Mancini D, Milani RV. Clinician's guide to cardiopulmonary exercise testing in adults: a scientific statement from the American Heart Association. *Circulation* 2010; **122**: 191–225.
- Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, Flachskampf FA, Foster E, Goldstein SA, Kuznetsova T, Lancellotti P, Muraru D, Picard MH, Rietzschel ER, Rudski L, Spencer KT, Tsang W, Voigt Jens-Uwe. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging* 2015; **16**: 233–271.
- Pitzalis MV, Iacoviello M, Romito R, Massari F, Rizzon B, Luzzi G, Guida P, Andriani A, Mastropasqua F, Rizzon P. Cardiac resynchronization therapy tailored by echocardiographic evaluation of ventricular asynchrony. *J Am Coll Cardiol* 2002; **40**: 1615–1622.
- Kelly R, Hayward C, Avolio A, O'Rourke M. Noninvasive determination of age-related changes in the human arterial pulse. *Circulation* 1989; **80**: 1652–1659.
- Hayward CS, Kraidly M, Webb CM, Collins P. Assessment of endothelial function using peripheral waveform analysis: a clinical application. *J Am Coll Cardiol* 2002; **40**: 521–528.
- Procter N, Goh V, Mahadevan G, Stewart S, Horowitz J. Platelet reactivity is independent of left atrial wall deformation in patients with atrial fibrillation. *Mediators Inflamm* 2016; **2016**: 9754808.
- Heresztyn T, Worthley MI, Horowitz JD. Determination of L-arginine and NG, NG- and NG,NG'-dimethyl-L-arginine in plasma by liquid chromatography as AccQ-Fluor™ fluorescent derivatives. *J Chromatogr B* 2004; **805**: 325–329.
- Chirkov YY, Holmes AS, Chirkova LP, Horowitz JD. Nitrate resistance in platelets from patients with stable angina pectoris. *Circulation* 1999; **100**: 129–134.
- Abraham WT, Young JB, León AR, Adler S, Bank AJ, Hall SA, Lieberman R, Liem LB, O'Connell JB, Schroeder JS, Wheelan KR. Effects of cardiac resynchronization on disease progression in patients with left ventricular systolic dysfunction, an indication for an implantable cardioverter-defibrillator, and mildly symptomatic chronic heart failure. *Circulation* 2004; **110**: 2864–2868.
- Linde C, Leclercq C, Rex S, Garrigue S, Lavergne T, Cazeau S, McKenna W, Fitzgerald M, Deharo Jean-Claude, Alonso C, Walker S, Braunschweig F, Bailleul C, Daubert Jean-Claude. Long-term benefits of biventricular pacing in congestive heart failure: results from the Multisite STimulation in cardiomyopathy (MUSTIC) study. *J Am Coll Cardiol* 2002; **40**: 111–118.
- Brenyo A, Barsheshet A, Rao M, Huang DT, Zareba W, McNitt S, Hall W, Peterson D, Solomon S, Moss A, Goldenberg I. Brain natriuretic peptide and cardiac resynchronization therapy in patients with mildly symptomatic heart failure. *Circ Heart Fail* 2013; **6**: 998–1004.
- Sahebkar A, Pecin I, Tedeschi-Reiner E, Derosa G, Maffioli P, Reiner Z. Effects of statin therapy on augmentation index as a measure of arterial stiffness: a systematic review and meta-analysis. *Int J Cardiol* 2016; **212**: 160–168.
- Jiang XJ, Li QY, Zhang YQ, Liu GZ, Liu LS. The comparison of the effect of enalapril and indapamide on the peripheral blood pressure and central blood pressure through pulse wave analysis. *Zhonghua Xin Xue Guan Bing Za Zhi* 2005; **33**: 885–888.

24. Bots ML, Westerink J, Rabelink TJ, de Koning EJ. Assessment of flow-mediated vasodilatation (FMD) of the brachial artery: effects of technical aspects of the FMD measurement on the FMD response. *Eur Heart J* 2005; **26**: 363–368.
25. Arrebola-Moreno AL, Laclaustra M, Kaski JC. Noninvasive assessment of endothelial function in clinical practice. *Rev Esp Cardiol (Engl Ed)* 2012; **65**: 80–90.
26. Antoniadis C, Demosthenous M, Tousoulis D, Antonopoulos AS, Vlachopoulos C, Toutouza M, Marinou K, Bakogiannis C, Mavragani K, Lazaros G, Koumallos N, Triantafyllou C, Lympieriadis D, Koutsilieris M, Stefanadis C. Role of asymmetrical dimethylarginine in inflammation-induced endothelial dysfunction in human atherosclerosis. *Hypertension* 2011; **58**: 93–98.
27. Yamawaki H, Pan S, Lee RT, Berk BC. Fluid shear stress inhibits vascular inflammation by decreasing thioredoxin-interacting protein in endothelial cells. *J Clin Invest* 2005; **115**: 733–738.
28. Spindel ON, Burke RM, Yan C, Berk BC. Thioredoxin-interacting protein is a biomechanical regulator of Src activity: key role in endothelial cell stress fiber formation. *Circ Res* 2014; **114**: 1125–1132.
29. Schepers E, Barreto DV, Liabeuf S, Glorieux G, Eloot S, Barreto FC, Massy Z, Vanholder R. Symmetric dimethylarginine as a proinflammatory agent in chronic kidney disease. *Clin J Am Soc Nephrol* 2011; **6**: 2374–2383.
30. Ronden RA, Houben AJ, Teerlink T, Bakker JA, Bierau J, Stehouwer CD, De Leeuw PW, Kroon AA. Reduced renal plasma clearance does not explain increased plasma asymmetric dimethylarginine in hypertensive subjects with mild to moderate renal insufficiency. *Am J Physiol Renal Physiol* 2012; **303**: F149–F156.