

Identification of physiologic treatment targets with favourable haemodynamic consequences in heart failure with preserved ejection fraction

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Abstract

Aims Heart failure with preserved ejection fraction (HFpEF) is characterized by complex pathophysiology including an impaired diastolic reserve. We recently showed that milrinone favourably modifies filling pressures at rest and during exertion in HFpEF patients; however, the responsible mechanism is uncertain. The objective of this study was to develop a clearer understanding of the acutely modifiable physiologic parameters that may be targeted in HFpEF.

Methods and results We conducted computer modelling simulations based on invasive haemodynamic assessments, by right heart catheterization, in HFpEF patients at baseline and in response to milrinone. Our aim was to develop a detailed understanding of the physiologic mechanisms, which accounted for the observed actions. The resultant circulatory model of HFpEF encompassed the left ventricular (LV) end-systolic and end-diastolic pressure–volume relations, together with stressed blood volume, heart rate, and arterial mechanics. To support the modelled action of milrinone, we conducted complementary LV conductance catheter and echocardiography studies in sheep to evaluate LV end-systolic and end-diastolic pressure–volume relations. In HFpEF patients, the acute haemodynamic effects of intravenous milrinone ($n = 10$) administration compared with placebo ($n = 10$) included significant reductions in right atrial pressure (7 ± 1 to 3 ± 1 mmHg, $P < 0.001$) and pulmonary capillary wedge pressure (13 ± 1 to 8 ± 1 mmHg, $P < 0.001$), while cardiac index increased (2.77 ± 0.19 to 3.15 ± 0.14 L/min/m², $P < 0.05$), and mean arterial pressure remained unchanged (95 ± 2 to 93 ± 3 mmHg, $P =$ not significant). Computer simulations showed that these haemodynamic effects were explained by a concomitant 31% reduction in stressed blood volume together with 44% increase in LV end-systolic elastance (LV E_{es}). Individually, changes in these parameters were not sufficient to explain the haemodynamic effects of milrinone. *In vivo* studies conducted in sheep ($n = 5$) showed that milrinone reduced LV filling pressure (8.0 ± 0.8 to 2.7 ± 0.6 mmHg, $P < 0.01$) and increased LV E_{es} (0.96 ± 0.07 to 2.07 ± 0.49 , $P < 0.05$), while no significant effect on LV stiffness was observed (0.038 ± 0.003 to 0.034 ± 0.008 , $P =$ not significant).

Conclusions These data demonstrate that stressed blood volume in HFpEF represents a relevant physiologic target in HFpEF; however, concomitant modulation of other cardiovascular parameters including LV contractility may be required to achieve desirable haemodynamic effects.

Keywords Heart failure with preserved ejection fraction; Haemodynamics; Circulatory modelling

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Introduction

Heart failure with preserved ejection fraction (HFpEF) has emerged as one of the commonest and most challenging problems in contemporary cardiovascular medicine. As yet

no specific therapy has been demonstrated to consistently improve all of the key treatment objectives, including quality of life, heart failure (HF) hospitalization, or survival.¹ To a certain extent, the challenges presented in identifying effective HFpEF therapies lie in the heterogeneous pathophysiological

nature of the disorder and in the frequent concurrence of co-morbidities that complicate therapy and the likelihood of benefit. Additionally, the complexities of accurate diagnosis in HFpEF using non-invasive means for the purposes of trial inclusion are recognized.

For HFpEF patients, exertional dyspnoea and corresponding exercise intolerance are cornerstone symptoms. Increased left atrial (LA) pressure, particularly during exertion, has been implicated as a principal determinant of functional capacity and potentially outcome.^{2–5} Accordingly, impaired left ventricular (LV) diastolic performance and its respective components have received particular attention as a key determinant of LA pressure. Recently, the pathophysiologic derangements that underpin HFpEF have been appreciated as being more complex and are likely to include contributions from reduced contractility, increased LA stiffness, volume overload, and dynamic mitral regurgitation,⁶ all of which influence LA pressure.

Previously, we investigated the effects of milrinone, a phosphodiesterase type III inhibitor, on exercise haemodynamics in HFpEF patients.⁷ Our study demonstrated favourable effects on pulmonary capillary wedge pressure (PCWP) at rest and during exertion; however, we did not determine the precise physiological basis for this observation. The beneficial haemodynamic actions of phosphodiesterase type III inhibition could be explained by physiologic actions including pulmonary and systemic vasodilation (of both arterial and venous beds), positive lusitropy, and positive inotropy. A clearer understanding of the mechanism could inform in the development of more effective treatments. Thus, the objective of the current study was to explore the physiological basis for the haemodynamic effects of milrinone in HFpEF using complementary clinical and experimental approaches. To obtain a comprehensive evaluation, complementary computer modelling and animal experimental studies were performed in patients with HFpEF and in an animal model. Computer models were derived using clinical data applied to a previously developed and validated cardiovascular simulation⁸ that was used to define the relative contributions of changes in contractility, diastolic properties, heart rate, and vascular tone.

Methods

Clinical study

Patients referred for haemodynamic evaluation of patients with known HFpEF or suspected HFpEF were recruited into the study ($n = 20$). HFpEF patients were included on the basis of the presence of signs and/or symptoms consistent with a diagnosis of HF (New York Heart Association II–III), an LV ejection fraction $> 50\%$, and a haemodynamic profile that included PCWP ≥ 15 mmHg at rest or ≥ 25 mmHg during symptom limited exercise.^{9,10} In the HFpEF cohort, patients were excluded

in the presence of diagnoses including known coronary disease requiring revascularization, infiltrative cardiomyopathy, constrictive pericarditis, and moderate or greater valvular heart disease. Aspects of this study have been previously published in brief form, with a focus on the influence of milrinone on exercise haemodynamics.⁷ The study was approved by the Alfred Hospital Research and Ethics Committee, and all participants provided written informed consent.

Cardiac catheterization protocol

Arterial and central haemodynamics were measured as previously described.² In brief, patients were evaluated in the non-fasted, medicated state. Haemodynamic evaluation was conducted using a 7 Fr thermodilution catheter advanced via a brachial or right internal jugular sheath. Right atrial (RA), right ventricular (RV), pulmonary arterial (PA), and pulmonary capillary wedge pressures were measured at end expiration. Following assessment of baseline haemodynamics, diagnostic supine cycle ergometry was conducted to confirm the presence of HFpEF. Subjects were randomized according to a computer-generated random order, with study drug allocation assigned using sealed envelopes opened following confirmation of HFpEF. Patients received either milrinone (50 $\mu\text{g}/\text{kg}$ over 10 min) or an equivalent volume of saline ($n = 10$ per group). All investigators except for the study nurse were blinded to group allocation. Haemodynamic evaluation was performed 10 min after the conclusion of the infusion. Pulmonary and systemic arterial compliances (PAC and SAC) were calculated as the ratio of thermodilution-derived stroke volume to the pulmonary and systemic arterial pulse pressure, respectively. Systemic arterial elastance (E_a) was calculated as $0.9 \times$ systemic systolic blood pressure/stroke volume, and pulmonary arterial elastance (E_{pa}) was calculated as the PA systolic pressure divided by stroke volume.

Mathematical modelling of clinical data

In order to investigate the physiological basis of the haemodynamic effects of milrinone observed in HFpEF patients, we employed a comprehensive cardiovascular simulation, 'Harvi',¹¹ which has been described in detail previously⁸ and which has been used to understand and explain various aspects of HF physiology.¹² In brief, this models the entire circulatory system as a series of resistance and capacitive elements, with heart chambers modelled as time-varying elastances and valves modelled as diodes. The time-varying elastance model allows for characterization of systolic and diastolic chamber properties. Details of this model are provided in detail in the Appendix. This simulation has a 'patient fitting algorithm', which employs a custom-designed parameter search routine that simultaneously adjusts multiple model parameter values to reproduce the desired haemodynamic state including heart rate, cardiac output, ventricular sizes,

and central and systemic arterial and venous blood pressures. As detailed in the Appendix, RV and LV contractilities [indexed by end-systolic elastances (E_{es})] and diastolic stiffnesses (indexed by an α constant), SAC and PAC, and *stressed blood volume* (described in the next paragraph) are among the key parameters that are adjusted to achieve the fit. Total systemic and pulmonary vascular resistances (SVR and PVR) were set according to measured values, but the distribution of resistances across the circulation was allowed to vary to recreate pulse pressures. Heart rate was also set to directly measured values. Resultant haemodynamic signals (pressure–volume loops and time-domain pressure and volume signals) are provided by the simulation and can be used to infer pathophysiologic status or effect of devices and drugs.¹³

As noted previously, stressed blood volume is a key parameter that is varied to optimize the simulation fit of real data. Despite the fact that the concepts of stressed and unstressed blood volumes are fundamental to understanding regulation of cardiovascular performance,^{12,14} they are not widely appreciated within the clinical community. Generally speaking, unstressed blood volume is the amount of blood required to fill the dead space in the vascular system, above which vascular wall tension and intravascular pressures start to rise. The analogy can be made to an unfilled balloon; a certain amount of air is required to just start generating positive wall stress and balloon pressure. Stressed blood volume is simply the volume above the stressed blood volume that actually contributes to generating active pressure within the vessels. Very importantly, the partitioning of blood between stressed and unstressed compartments is functional, not anatomical in nature. Every vascular structure and cardiac chamber has its own unstressed volume; the unstressed volume of the entire circulation is the sum of unstressed volumes of all vascular structures included in the circulation. Total blood volume is the sum of stressed and unstressed blood volumes. However, with changes in vascular tone (e.g. by changes in autonomic tone or administration of vasoactive drugs), there can be a redistribution of blood between functional compartments. Thus, despite constant blood volume, change in stressed blood volume has the potential to exert powerful control of cardiovascular performance.

Animal procedures

Studies performed in animals were approved by the Animal Ethics Research Committee, Victorian Institute of Animal Science (VIAS), and the animals were handled in accord with the National Institutes of Health guidelines on the Care and Use of Laboratory Animals. Five adult cross-bred sheep were included in the study. Evaluation of cardiac function was performed under individually titrated anaesthesia (comprising isoflurane and propofol) using echocardiography and LV conductance catheterization, as previously performed by us.¹⁵

Echocardiography, haemodynamics, and conductance catheterization

Echocardiographic images were obtained from short-axis and long-axis views to obtain 2D measurements of the left ventricle to estimate LV volumes. A 7 Fr pressure–volume catheter (Model CA-71103-PN; CD Leycom, the Netherlands) was advanced into the LV cavity under fluoroscopic guidance via an 8 F right carotid arterial sheath. In conjunction, a 14 Fr Fogarty catheter (Model 62080814F; Edwards Lifesciences, Irvine, CA, USA) was positioned in the inferior vena cava via the right internal jugular vein. Pressure–volume loops were recorded under stable haemodynamic conditions and then obtained during inferior vena cava balloon occlusion to derive the end-systolic and end-diastolic pressure–volume relationships (ESPVR and EDPVR). Following baseline measurement, milrinone (50 $\mu\text{g}/\text{kg}$) was infused over 10 min, and assessment of LV pressure–volume relationships was again obtained. Offline analysis of obtained data was performed using Conduct NT software (version 2.8.1; CD Leycom) with volume calibration performed using echocardiographic estimates of LV volumes. The ESPVR was identified to derive the E_{es} . Evaluation of diastolic performance was conducted by assessing the time constant of isovolumetric relaxation (τ) and the minimal rate of LV pressure change (dp/dt_{\min}) and by determining LV stiffness from the EDPVR noted previously. Given the curvilinear nature of the EDPVR, we derived an index of LV stiffness from the relationship: $\alpha = (\log_e 2.33 \times P_{ed})/V_{edr}$, based on Mirsky and Parmley.¹⁶

Statistical analysis

Data are presented as mean \pm standard error of the mean. Within-subject comparisons were performed using a paired Student's *t*-test or Wilcoxon test as appropriate. A *P* value of <0.05 was considered to be statistically significant. Statistical analysis was performed using IBM SPSS Statistics version 25 (IBM, Armonk, NY).

Results

The study comprised 20 patients (12 female and 8 male), aged 68 ± 2 years, with an LV ejection fraction $64\% \pm 2\%$ and body mass index $31 \pm 1 \text{ kg}/\text{m}^2$. Background medications included angiotensin-converting enzyme/angiotensin receptor blockers (85%), beta-blockers (30%), spironolactone (15%), and calcium channel blockers (40%). There were no significant differences between patients randomized to milrinone or vehicle. N terminal pro brain natriuretic peptide levels and echocardiography were not obtained at the time of the study.

Baseline haemodynamics and response to intravenous milrinone in heart failure with preserved ejection fraction

As shown in *Table 1*, HFpEF patients subsequently randomized to either vehicle or milrinone were well matched at baseline. In response to milrinone, there were statistically significant reductions in the PCWP (13 ± 1 to 8 ± 1 mmHg, $P < 0.001$), RA pressure (7 ± 1 to 3 ± 1 mmHg, $P < 0.001$), SVR (16.6 ± 1.3 to 14.8 ± 1.1 mmHg/L/min, $P < 0.05$), and pulmonary artery mean pressure (23 ± 2 to 19 ± 3 mmHg, $P < 0.01$), while there were modest increases in heart rate (70 ± 3 to 77 ± 4 b.p.m., $P < 0.01$) and cardiac index (2.77 ± 0.19 to 3.15 ± 0.14 L/min/m², $P < 0.05$) as detailed in *Table 2*. There were no significant changes in LV or RV stroke work index. Milrinone was also without any effect on PVR or PAC and SAC and elastances.

Modelling the circulatory effects of milrinone

To investigate the physiological basis for the effects of milrinone in individuals with HFpEF, we modelled the group average resting and post-milrinone haemodynamics in the cardiovascular simulator. Measured parameters, including heart rate, SVR, and PVR, were set according to measured values. Values of other key model parameters were adjusted to optimize the concordance between measured and simulated cardiovascular variables. As detailed in *Table 3*, the simulation recreated the average patient data with high degree of accuracy, precisely recreating cardiac output and simulating pressures within 1–2 mmHg for all measurements. Under baseline conditions, key parameters characterizing ventricular properties included an LV E_{es} of 2.09 mmHg/mL, an LV

stiffness constant, α , of 0.030 mL⁻¹, an RV E_{es} of 0.35 mmHg/mL, an RV stiffness of 0.022 mL⁻¹, and a stressed blood volume of 1539 mL. Additional details of model parameter values are provided in *Table A1*. The resultant baseline LV pressure–volume loops with constraining ESPVR and EDPVR are presented in *Figure 1*.

The same process was repeated for haemodynamics during milrinone infusion, with resulting parameters and pressure–volume loops also shown in *Table 3* and *Figure 1A*, respectively. As for the baseline state, the simulation reproduced cardiac output and all pressures with a high degree of accuracy. As measured clinically, there was no significant change in PVR, but SVR decreased from 15.95 to 14.42 Wood units (mmHg·min/L). As shown, the simulation suggests that there were increases of contractility with LV and RV E_{es} values increasing to 3.00 and 0.46 mmHg/mL, respectively. However, there were no significant changes in LV or RV stiffness, but there was a significant reduction of stressed blood volume to 1066 mL. The predicated LV pressure–volume loop during milrinone infusion is also shown in *Figure 1*.

To investigate the relative contribution of each parameter affected by milrinone, we introduced the changes of parameter values obtained by fitting the haemodynamics during milrinone exposure on an individual basis (*Figure 1B–E* and *Table 4*). Compared with the baseline state, the observed increase in the heart rate from 70 to 77 b.p.m. and the reduction in SVR from 15.95 to 14.42 mmHg/L/min exerted very little influence on the LV pressure–volume loop or haemodynamic parameters (*Figure 1B,C*). Increases in ventricular contractility resulted in increases in blood pressure and cardiac output beyond those observed in the clinical setting, and there was no reduction of filling pressures (*Figure 1D*). In contrast, the reduction of stressed blood volume from 1539 to

Table 1 Baseline haemodynamic parameters

	Vehicle ($n = 10$)	Milrinone ($n = 10$)	P value
Heart rate (b.p.m.)	67 ± 5	70 ± 3	0.55
Systolic blood pressure (mmHg)	155 ± 7	149 ± 4	0.47
Mean arterial pressure (mmHg)	101 ± 4	95 ± 2	0.11
Right atrial pressure (mmHg)	6 ± 1	7 ± 1	0.43
PA systolic pressure (mmHg)	31 ± 3	36 ± 4	0.30
PA mean pressure (mmHg)	20 ± 2	23 ± 2	0.25
PCWP (mmHg)	11 ± 1	13 ± 1	0.33
Cardiac index (L/min/m ²)	2.7 ± 0.2	2.8 ± 0.2	0.74
Derived cardiac indices			
LVSWI (g/m ² /beat)	80.0 ± 4.8	73.3 ± 5.4	0.36
RVSWI (g/m ² /beat)	8.1 ± 0.9	8.7 ± 0.9	0.65
Derived vascular indices			
SVR (mmHg/L/min)	20.1 ± 1.7	16.6 ± 1.3	0.11
PVR (mmHg/L/min)	1.8 ± 0.2	1.9 ± 0.3	0.78
SA compliance (mL/mmHg)	1.02 ± 0.12	1.00 ± 0.09	0.96
PA compliance (mL/mmHg)	4.19 ± 0.38	4.19 ± 0.53	1.0
SA elastance (mmHg/mL)	1.90 ± 0.16	1.75 ± 0.11	0.43
PA elastance (mmHg/mL)	0.40 ± 0.03	0.47 ± 0.06	0.34

LVSWI, left ventricular stroke work index; PA, pulmonary artery; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RVSWI, right ventricular stroke work index; SA, systemic arterial; SVR, systemic vascular resistance.

Table 2 Effects of milrinone on resting haemodynamics

	Placebo (n = 10)	Milrinone (n = 10)	P value
Heart rate (b.p.m.)	-0.4 ± 1.2	6.9 ± 1.5	0.001
Systolic blood pressure (mmHg)	0.6 ± 3.6	3.5 ± 3.2	0.55
Mean arterial pressure (mmHg)	-0.5 ± 2.3	-1.6 ± 1.6	0.70
Right atrial pressure (mmHg)	-0.3 ± 0.4	-3.2 ± 0.6	0.001
PA systolic pressure (mmHg)	-0.5 ± 1.1	-4.4 ± 1.8	0.09
PA mean pressure (mmHg)	-1.0 ± 1.0	-4.3 ± 1.3	0.04
PCWP (mmHg)	0.1 ± 0.5	-5.6 ± 1.1	<0.001
Cardiac index (L/min/m ²)	-0.1 ± 0.1	0.4 ± 0.1	0.004
Derived cardiac indices			
LVSWI (g/m ² /beat)	-1.2 ± 2.4	7.4 ± 4.7	0.12
RVSWI (g/m ² /beat)	-0.8 ± 0.5	0.1 ± 1.1	0.46
Derived vascular indices			
SVR (mmHg/L/min)	-1.8 ± 0.8	1.0 ± 0.8	0.02
PVR (mmHg/L/min)	-0.2 ± 0.2	0.0 ± 0.2	0.67
SA compliance (mL/mmHg)	-0.10 ± 0.07	-0.06 ± 0.05	0.60
PA compliance (mL/mmHg)	0.68 ± 0.66	0.36 ± 0.35	0.67
SA elastance (mmHg/mL)	0.07 ± 0.06	-0.03 ± 0.08	0.32
PA elastance (mmHg/mL)	0.02 ± 0.03	-0.08 ± 0.03	0.02

LVSWI, left ventricular stroke work index; PA, pulmonary artery; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RVSWI, right ventricular stroke work index; SA, systemic arterial; SVR, systemic vascular resistance.

Table 3 Simulation input and output parameters

Haemodynamic parameter	Baseline		Milrinone	
	Observed	Computer simulation	Observed	Computer simulation
Arterial BP (mmHg)	149/68 (95)	149/65 (94)	153/64 (94)	153/63 (92)
Heart rate (b.p.m.)	70	70	77	77
Cardiac output (L/min)	5.6	5.6	6.3	6.3
RA pressure (mmHg)	7	8	3	4
PA pressure (mmHg)	36/14 (23)	37/15	32/11 (19)	31/11 (17)
PCWP (mmHg)	14	14	8	8
Model parameter				
LV E_{es} (mmHg/mL)		2.09		3.00
LV stiffness (mL ⁻¹)		0.030		0.028
LVEDV (mL)		135		116
LVEDP (mmHg)		18		9
LVESV (mL)		60		38
LVESP (mmHg)		126		114
RV E_{es} (mmHg/mL)		0.35		0.46
RV stiffness		0.022		0.018
SVR (mmHg·min/L)		15.95		14.42
PVR (mmHg·min/L)		1.66		1.69
Stressed blood volume (mL)		1539		1066

BP, blood pressure; LV, left ventricular; LV E_{es} , left ventricular end-systolic elastance; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume; LVESP, left ventricular end-systolic pressure; LVESV, left ventricular end-systolic volume; PA, pulmonary artery; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RA, right atrial; RV, right ventricular; RV E_{es} , right ventricular end-systolic elastance; SVR, systemic vascular resistance.

1036 mL caused marked reductions of mean RA pressure and PCWP, in line with those observed in patients, albeit at the cost of systemic hypotension (Figure 1E).

Effects of milrinone on left ventricular performance in experimental animals

To complement the simulation studies, we examined the effect of milrinone on LV performance in sheep by conductance

catheterization. As demonstrated in Table 5, similar effects to those seen in HFpEF patients were observed. Specifically, there was a significant reduction in LV end-diastolic pressure with preservation of the systolic pressure. Acute infusion of milrinone was associated with an increase in LV E_{es} . A detailed examination of the effects of milrinone on diastolic performance showed no effect on the time constant of relaxation, τ . As shown in Figure 2, infusion of milrinone was associated with a downward and leftward shift of the diastolic portion of the pressure–volume loop but along a

Figure 1 (A) Computer-simulated left ventricular (LV) pressure–volume loops derived from group average data obtained in heart failure with preserved ejection fraction patients at baseline (red) and in response to milrinone (blue). (B–E) Impact of individual alterations (*Table 4*) in key physiologic parameters (E_{es} , LV systolic elastance; HR, heart rate; SBV, stressed blood volume; SVR, systemic vascular resistance) on the LV pressure–volume loops. Black lines indicate resultant LV pressure–volume loop, compared with baseline (red) and milrinone (blue).

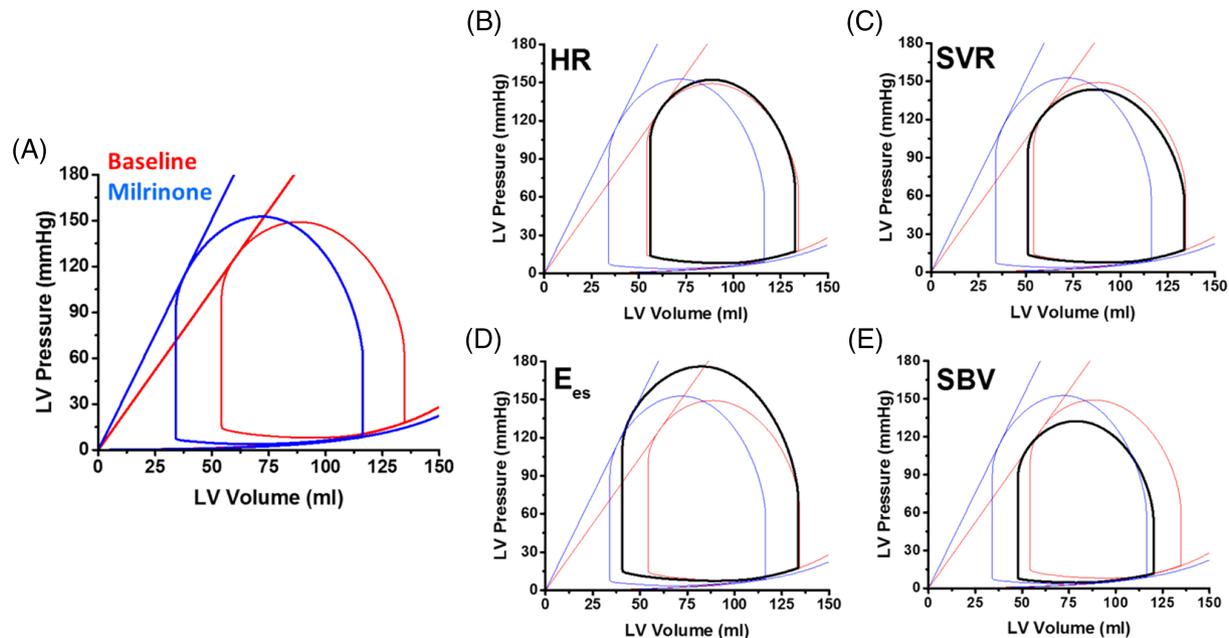


Table 4 Impact of changing one model parameter at a time on simulated haemodynamic parameters

Parameter	Units	Baseline	Milrinone	HR	SVR	RV and LV E_{es}	SBV
HR	b.p.m.	70	77	77	70	70	70
SVR	mmHg·min/L	15.95	14.42	15.95	14.42	15.95	15.95
LV E_{es}	mmHg/mL	2.09	3.00	2.09	2.09	3.00	2.09
RV E_{es}	mmHg/mL	0.35	0.46	0.35	0.35	0.46	0.35
SBV	mL	1539	1066	1539	1539	1539	1066
RA	mmHg	7	3	8	8	7	5
PA	mmHg	36/14 (23)	32/11 (19)	37/15 (22)	37/15 (21)	42/15 (23)	29/10 (15)
PCWP	mmHg	14	8	14	13	14	8
ABP	mmHg	149/68 (95)	153/64 (94)	152/70 (99)	144/58 (88)	176/74 (108)	132/56 (83)
CO	L/min	5.6	6.3	5.9	5.8	6.5	5.1

ABP, arterial blood pressure; CO, cardiac output; HR, heart rate; LV E_{es} , left ventricular end-systolic elastance; PA, pulmonary artery; PCWP, pulmonary capillary wedge pressure; RA, right atrial; RV E_{es} , right ventricular end-systolic elastance; SBV, stressed blood volume; SVR, systemic vascular resistance. Baseline and milrinone columns represent hemodynamic observations and the derived E_{es} and SBV values. Columns *HR*, *SVR*, *Ees* and *SBV* represent the expected hemodynamic effects on the relevant individual physiological manipulation (**in bold**).

common EDPVR assessed during preload manipulation by balloon occlusion of the inferior vena cava. In this analysis, milrinone caused a small, non-significant reduction in LV stiffness from 0.038 ± 0.003 to 0.034 ± 0.008 (*Table 5*).

Discussion

Elevated LA pressure is a hallmark feature of HFpEF, particularly in the context of physical activity.⁶ The extent of the rise has been associated with both functional capacity and outcomes.^{4,17} We previously showed that milrinone

administered intravenously was able to reduce the PCWP in HFpEF patients especially during exercise.⁷ In the current study, we sought to investigate physiologic mechanisms underlying this observation using both computer-based simulations and experimental *in vivo* studies. The results suggest that the actions of milrinone were predominantly explained by a concomitant reduction in stressed blood volume together with an enhancement of LV contractility.

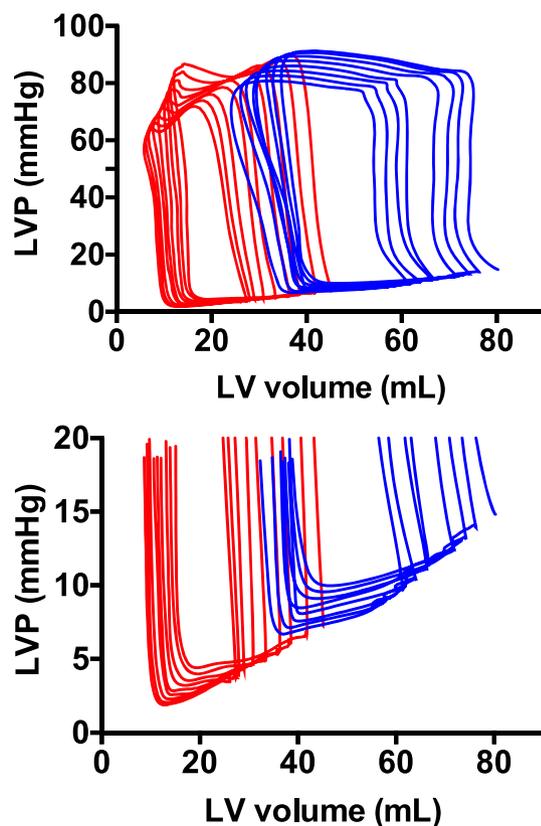
Given the central role of LA pressure elevation in the pathogenesis of HFpEF, advancement of the field and the development of effective HFpEF therapy requires a comprehensive consideration of the contributing mechanisms. LA pressure is determined by a complex interaction of factors

Table 5 Effects of milrinone on left ventricular mechanics in sheep

	Baseline	Milrinone	P value
LVESP (mmHg)	76 ± 2	65 ± 5	0.08
LVEDP (mmHg)	8.0 ± 0.8	2.7 ± 0.6	<0.01
LVEDV (mmHg)	75 ± 6	47 ± 3	<0.01
LVESV (mmHg)	29 ± 3	8 ± 3	<0.01
Heart rate (b.p.m.)	93 ± 6	106 ± 4	0.08
LV dp/dt_{min}	-1420 ± 109	-1069 ± 111	<0.05
LV τ	26.2 ± 1.0	28.6 ± 2.2	0.48
LV E_{es}	0.96 ± 0.07	2.07 ± 0.49	<0.05
LV stiffness (α)	0.038 ± 0.003	0.034 ± 0.008	ns

LV, left ventricular; LV E_{es} , left ventricular end-systolic elastance; LVEDP, left ventricular end-diastolic pressure; LVEDV, left ventricular end-diastolic volume; LVESP, left ventricular end-systolic pressure; LVESV, left ventricular end-systolic volume; ns, not significant.

Figure 2 Representative sequential pressure–volume loop from sheep at baseline (blue) and following milrinone (red) during unloading by vena caval occlusion. Top panel demonstrates full pressure–volume loops. Lower panel provides expanded scale view of the left ventricular (LV) diastolic pressure–volume relationship. LVP, left ventricular pressure.



including LV diastolic and systolic performance, LA mechanical properties, mitral valve function, extrinsic constraint, and the central blood volume. Ventricular diastolic function comprises an active relaxation phase followed by a passive phase defined by the EDPVR. The EDPVR has been shown to be correlated with the extent of myocardial fibrosis.^{18,19} Although abnormal diastolic reserve leading to an upward

and leftward shift of the EDPVR is considered prototypic in HFpEF, this is not a uniform finding, and in some situations, a rightward shift with larger volumes has been reported.^{20,21}

In our study, at the doses used, milrinone had no effect on the rate of isovolumic relaxation *in vivo*. In the ovine studies, milrinone did not significantly influence LV stiffness, which was consistent with modelling estimates of HFpEF patient data. The predominant effect of milrinone in both patients and sheep was to shift the pressure–volume loops downwards and to the left along a common EDPVR; in contrast, a primary reduction in LV stiffness would be expected to shift the EDPVR downwards and rightwards. Although we did not investigate the effects of milrinone on LV stiffness directly in the clinical study, the lack of effect of milrinone on the EDPVR suggests that it may not be acutely modifiable in HFpEF patients. This point requires further investigation in clinical studies, including the measurement of detailed pressure–volume analyses. This finding may be of relevance to ongoing interest in the role of titin and its phosphorylation status in the pathophysiology of HFpEF.²² Protein kinase A is known to phosphorylate titin with a resultant decrease in myofiber stiffness.²³ As a corollary, given its pharmacologic mode of action, it would be expected that milrinone would increase titin phosphorylation; however, we did not observe an alteration in LV stiffness. This finding could be explained by the overwhelming influencing of myocardial fibrosis or a lack of sensitivity of *in vivo* measures to detect subtle changes in myocardial stiffness *per se*. The clinical failure of other interventions expected to modify titin phosphorylation, such as protein kinase G activation,^{24,25} might also be explained by a lack of effect on LV stiffness, although this remains uncertain.

Left ventricular end-diastolic pressure is also dependent upon RV function and the central blood volume. The magnitude of a volume-mediated change in LV filling pressure is non-linear and depends on the prevailing filling status together with LV stiffness. Our modelling indicated that milrinone markedly reduced the stressed blood volume, potentially via acute splanchnic vasodilation, leading to lower filling pressures in both the right and left circulations (i.e. central venous pressure and PCWP) and potentially therefore leading to a lesser rise during physical exertion. Blood volume status in HFpEF has been reported to be expanded in patients with HFpEF when measured using radiotracer methodology,^{20,26} although this remains controversial when measured using other methods.²⁷ Our study suggests that stressed blood volume manipulation may be a valid therapeutic strategy. However, as illustrated by our modelling data, this approach alone may contribute to hypotension. Splanchnic nerve block has also been reported as a strategy by which to modulate stressed blood volume in HF patients.²⁸ BNP infusion has also been shown reductions in filling pressures,²⁹ potentially due to splanchnic vasodilation.³⁰ Alternately, pulmonary C-type natriuretic peptide (CNP) receptors might also play a role in haemodynamic modulation.³¹ Interestingly, a recent

mechanistic study of the effects of sacubitril/valsartan in HFpEF patients showed greater reductions in LV volumes and improved quality of life independent of a postulated change in afterload, raising the possibility of an effect on preload.³² From a therapeutic perspective however, it is of note that HFpEF patients with small LV cavities have been shown to have poorer exercise capacity,³³ and in the setting of significant hypertrophy, a risk of cavity obliteration during excessive intravascular volume depletion is of concern.

Considerable evidence has accumulated to suggest that LV contractility, as measured either by strain imaging or by invasive means, is impaired at rest in some HFpEF patients³⁴ and/or that there is also a failure of contractile recruitment in some HFpEF patients.³⁵ Importantly, reduced global longitudinal strain has also been associated with poorer outcome in HFpEF,³⁶ and we recently showed that failure of contractile recruitment is an independent contributor to the rise in LA pressure during exercise.³⁵ The finding that a key mode of action of milrinone was to enhance contractility is therefore of potential benefit. Nevertheless, further studies would be required to investigate the impact of a positive inotropic intervention in HFpEF on myocardial oxygen consumption and on the potential for arrhythmic events.

The modest effects of milrinone on SVR and heart rate were also investigated in the computer simulations. Neither was associated with clinically meaningful haemodynamic changes, suggesting that the observed effects of milrinone were not primarily mediated by changes in these parameters. Similarly, we did not observe substantial acute changes in arterial elastance or pulmonary vascular mechanics, suggesting that neither of these properties was the primary mechanism involved in the acute modulation of LA or RA pressure. Consistent with these findings, nitrite has been observed to significantly decrease filling pressures, while SVR and heart rate were relatively unchanged.³⁷ In the present HFpEF patient simulation, we also examined the influence of increasing the heart rate to 90 b.p.m. (data not shown), and this was also without influence on the predicted LV filling pressure. As such, it is unlikely that heart rate modification would be

solely mediated by cardiac output augmentation rather than filling pressure alteration. Clinical trials of atrial pacing are ongoing in HFpEF (NCT02145351).

Taken together, the current study highlights the complex interplay between derangements in fundamental physiologic parameters that lead to the haemodynamic features commonly observed in HFpEF. By characterizing these features, we have highlighted the potential utility of interventions that target systolic performance and stressed blood volume. These hypothesis-generating conclusions may help to inform the design of future studies, which will be required to investigate whether interventions that target these factors in a durable manner provide clinical benefit.

Conflict of interest

D.M.K. is co-founder and stockholder in Cardiora, which has developed an oral formulation of milrinone for heart failure with preserved ejection fraction. Cardiora played no role in the present study. D.B. is co-founder of PV Loops LLC, which developed and distributes the Harvi cardiovascular simulation.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure A1

Table A1 Model parameter values

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