Reduced left ventricular filling following blood volume extraction does not result in compensatory augmentation of cardiac mechanics

Running title: **Reduced left ventricular filling and cardiac mechanics**

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- What is the central question of this study?

A reduction in left ventricular (LV) filling, and concomitant increase in heart rate, augments LV mechanics to maintain stroke volume (SV), however the impact of reduced LV filling in isolation on SV and LV mechanics is currently unknown.

- What is the main finding and its importance?

An isolated decrease in LV filling did not provoke a compensatory increase in mechanics to maintain SV; in contrast LV mechanics and SV were reduced. These data indicate that when LV filling is reduced without changes in HR, LV mechanics do not compensate to maintain SV.

Abstract

An acute non-invasive reduction in preload has been shown to augment cardiac mechanics to maintain stroke volume and cardiac output. Such interventions induce concomitant changes in heart rate (HR), whereas blood volume extraction reduces preload without HR changes. Therefore, the purpose of this study was to determine whether a preload reduction in isolation resulted in augmented stroke volume achieved via enhanced cardiac mechanics. Nine healthy volunteers (4 female, age 29 ± 11 years) underwent echocardiography for the assessment of left ventricular (LV) volumes and mechanics in a supine position at baseline and end-extraction following the controlled removal of 25% of total blood volume (1062 ± 342 ml). Arterial blood pressure was monitored continuously by a pressure transducer attached to an indwelling radial artery catheter. HR and total peripheral resistance were unchanged from baseline to end extraction, but systolic blood pressure was reduced (148 to 127 mmHg). LV end diastolic volume (89 to 71 ml) and stroke volume (56 to 37 ml) were significantly reduced from baseline to end extraction; however, there was no change in LV twist, basal or apical rotation. In contrast, LV longitudinal strain (-20 to -17%) and basal circumferential strain (-22 to -19%) were significantly reduced from baseline to end extraction. In conclusion, a preload reduction during blood volume extraction does not result in compensatory changes in stroke volume or cardiac mechanics. Our data suggest that LV strain is dependent on LV filling and consequent geometry whereas LV twist could be mediated by heart rate.

Introduction

Acute hypovolemia resulting from blood loss, such as that experienced during haemorrhage, provokes a reduction in central venous pressure (CVP) and hence left ventricular preload. If this loss is gradual, mean arterial pressure (MAP), pulse pressure and cardiac output are either maintained, or are modestly reduced, prior to rapid haemodynamic decompensation when blood loss becomes critical (Kirwan *et al.*, 1981; Johnson *et al.*, 2014; Rickards *et al.*, 2015). Maintenance of cardiac output in the presence of a diminishing preload, and in the absence of increased heart rate (Kirwan *et al.*, 1981; Little *et al.*, 1995), requires an augmented stroke volume, which may be achieved through either enhanced systolic or diastolic function that is mediated through altered cardiac mechanics.

Previously, lower body negative pressure (LBNP) (Johnson *et al.*, 2014; Rickards *et al.*, 2015; Williams *et al.*, 2016), glyceryl trinitrate administration (GTN) (Burns *et al.*, 2010a; Burns *et al.*, 2010b) and dehydration (Stohr *et al.*, 2011) have been used to examine the left ventricular response to a reduction in preload. Using these techniques, previous studies have shown augmented systolic ventricular mechanics alongside a reduction in LV end diastolic volume (EDV), indicative of a decreased preload (Burns *et al.*, 2010a; Burns *et al.*, 2010b; Stohr *et al.*, 2011). Importantly, however, these interventions increase heart rate (HR) (Burns *et al.*, 2010a; Johnson *et al.*, 2014; Rickards *et al.*, 2015) and sympathetic nerve activity (SNA) (Johnson *et al.*, 2014; Rickards *et al.*, 2015) and likely enhance LV function independent of changes in preload. Investigating the impact of preload in isolation is therefore challenging; however, blood volume extraction provides an experimental model where HR and blood pressure remain relatively constant and SNA increases to a lower magnitude compared to LBNP (Johnson *et al.*, 2014). Employing this model, Saygisunar and colleagues performed a modest blood volume extraction (450ml) and reported a small reduction in LV filling that was associated with a *decrease* in systolic ventricular mechanics (Saygisunar *et al.*, 2016). Whilst this study provides some insight into the LV mechanical response to an isolated reduction in preload, removing 450ml (commonly associated with blood donation) does not represent the significant cardiovascular challenge associated with marked haemorrhage. Therefore, the aim of this study was to determine whether stroke volume is maintained in response to severe blood volume extraction, and if so, how this is supported by changes

in left ventricular mechanics. We hypothesised that blood volume extraction would decrease LV filling, but stroke volume would be maintained through enhanced LV systolic mechanics.

Methods

Ethical Approval

Written informed consent was obtained and ethics approval was granted by Duke Health Institutional Review Board and conformed to the standards set by the Declaration of Helsinki, except for registration in a database.

Study Design

Participants attended the laboratory once and underwent comprehensive echocardiographic scans for the assessment of left ventricular volumes, function and mechanics prior to and following removal of an estimated 25% of total blood volume. Nine individuals (5 male, 4 female, body mass 73.3 ± 14.2 kg, height 171 ± 12 cm, age 29 ± 11 years) were recruited and volunteered to take part in the study. Participants self-reported: no known cardiovascular disease, no prescribed medications and no comorbidities or early family history of cardiovascular disease. Participants were screened for hypertension and were all normotensive (SBP 121 ± 15 mmHg, DBP 79 ± 6 mmHg).

Protocol

Once positioned supine, local anesthetic (1% lidocaine) was injected into the surrounding tissue of the radial artery and internal jugular vein under the guidance of a portable 13 MHz ultrasound unit (Nanomaxx, Sonosite, Washington, USA). A 20-gauge catheter (Arrow, Markham, Ontario, Canada) was placed into the radial artery and attached to a pressure transducer that was positioned at the level of the right atrium in the midaxillary line for the measurement of beat-to-beat arterial blood pressure. A 5 Fr central venous catheter (Edwards PediaSat Oximetry Catheter, Edwards, Irvine, CA, USA) was placed under sterile conditions in the right internal jugular vein under ultrasound guidance and directed caudad into the superior vena cava. Following internal jugular and radial artery catheterization, subjects rested for 30 min. For each participant, the total blood volume was estimated as 70 ml/kg (males) and 65 ml/kg (females) (Pham & Shaz, 2013). The target blood volume to be removed was derived as 25% of the total blood volume based upon Gilcher's Rule of Five. Blood removal into sterile transfusion bags

was achieved passively under the influence of gravity by placement of transfusion bags onto a clean sheet upon the floor. The rate of target blood volume removal was approximately 10 ml/min/m^2, based upon body surface area. If participants demonstrated symptoms of pre-syncope, HR >110 bpm or SBP < 80 mmHg, extraction was terminated early and participants were stabilized using a 10^o head down tilt and then returned to supine for the end extraction data collection.

Echocardiograms were acquired over a 5-minute period immediately prior to and immediately after blood volume extraction. All echocardiographic images were acquired using a commercially available ultrasound system (Vivid Q, GE Medical, Horten, Norway) with a 1.5-4 MHz phased array transducer. HR was monitored using a 3-lead electrocardiogram. Images were obtained by a single experienced sonographer (MS) with the participants supine. Haemodynamics, including systolic (SBP) and diastolic blood pressure (DBP) were measured continually and mean arterial pressure calculated as 1/3.SBP + 2/3.DBP. HR was monitored using 3-lead ECG.

LV internal dimension in diastole (LVIDd) and LV posterior wall thickness (LVPWT) were assessed from a parasternal long axis to allow calculation of relative wall thickness as (2 * LVPWT)/LVIDd. LV end diastolic (EDV) and systolic (ESV) volumes and LV length were estimated using Simpsons biplane methodology allowing the calculation of systolic functional parameters SV and ejection fraction (EF). Q was calculated as HR*SV and total peripheral resistance (TPR) was calculated as the quotient of MAP/Q. LV diastolic function was assessed using trans-mitral Doppler, providing peak velocities in early (E) and late diastole (A) and their ratio (E/A). Pulsed wave tissue Doppler imaging (TDI) assessment of the lateral and septal annulus was also employed to provide peak myocardial velocities in systole (S'), early diastole (E') and late diastole (A').

LV circumferential strain and strain rate in systole (SSR) and early diastole (DSR), rotation and twist were assessed from short-axis images at the level of the LV base and apex. LV longitudinal strain, SSR and DSR were assessed from an apical 4 chamber view. Offline analysis and interpolation of raw data to provide 600 points for systole and diastole (Oxborough *et al.*, 2011) allowed the assessment of peak global longitudinal LV strain and peak global LV circumferential strain as an average of 6 myocardial segments at both basal and apical levels, representing systolic LV strain mechanics. Peak basal and apical rotation and rotation rates in systole and early and late diastole were obtained to allow the calculation of peak twist and twist and untwist rates as the net difference between basal and apical

rotation and rotation rate respectively. LV systolic twist parameters comprised twist, basal and apical rotation and systolic twist and rotation rates, whereas untwist rate and diastolic rotation rates were used as markers of diastolic function. All Images were recorded to DVD in raw DICOM format and data were analysed offline using commercially available software (EchoPac version 13, GE Medical, Horten, Norway).

Statistical Analysis

Data were analysed across two time points; baseline and end extraction using paired samples T-tests. Bonferroni adjustment was applied based on two major data comparisons of variables grouped under two categories: haemodynamic and LV functional parameters. Statistical significance was set as the adjusted $P < 0.025$. Based on the only previous study to examine the cardiac response to blood volume extraction (Saygisunar *et al.*, 2016), a power calculation was performed *a priori* to determine our required sample size. LV internal dimension was the only measure of LV filling used by Saygisunar and colleagues and was therefore used to determine that with 4 participants, we were powered to detect a difference of 5.8mm in LV internal dimension, with an $\alpha = 0.05$ and a $\beta = 0.8$.

Results

Effects of intervention

The intervention employed resulted in a total blood volume extraction of 1062 ± 342 ml with extraction time of 55 ± 18 minutes. Although the target blood volume was only achieved in 2 out of 9 participants, the actual extracted blood volume was equivalent to an average of 20% of the total estimated blood volume. Early termination occurred due to pre-syncopal symptoms (4 participants), systolic BP < 80mmHg (2 participants) and HR above 110bpm (1 participant).

Haemodynamics and LV volumes

Systolic BP was significantly reduced at end extraction compared to baseline ($P = 0.01$, see Table 1), but MAP, HR, TPR and diastolic BP were unchanged ($P > 0.05$, see Table 1 and Figure 1). Cardiac

output, LV EDV , LV length and SV were significantly lower at end extraction compared to baseline ($P < 0.006$, see Table 1 and Figure 1), however LV ESV was unchanged ($P > 0.05$, see Table 1). Relative wall thickness was increased at end extraction ($P < 0.02$, see Table 1).

LV systolic function

LV EF and S' were unchanged from baseline to end extraction ($P > 0.05$, see Table 2), in contrast there was a significant reduction in LV longitudinal and basal circumferential strain ($P < 0.025$, see Table 2 and Figure 1). Despite this, all systolic rotational parameters and strain rates were unchanged from baseline to end extraction ($P < 0.05$, see Table 2).

LV diastolic function

Although LV E ($P = 0.008$, see Table 2), E/A ($P = 0.046$, see Table 2) and LV longitudinal DSR were significantly reduced from baseline to end extraction ($P = 0.001$, see Table 2), LV A, E', and A' were all unchanged ($P > 0.05$, see Table 2). Similar to systolic mechanics, diastolic rotation rates and untwist rate were unchanged from baseline to end extraction ($P > 0.05$, see Table 2), as were diastolic circumferential strain rates.

Table 1- Haemodynamic and left ventricular volume responses to blood volume reduction

Variable	Baseline	End extraction	*P* value
MAP (mmHg)	94 ± 5	84 ± 15	0.039
	(90 - 102)	(80 - 98)	
Systolic BP (mmHg)	148 ± 13	127 ± 19*	**0.003**
	(133 - 162)	(115 - 149)	
Diastolic BP (mmHg)	71 ± 3	66 ± 12	0.184
	(68 - 73)	(62 - 78)	
TPR (mmHg.L.min^{-1})	29.1 ± 4.9	33.6 ± 7.1	0.108
	(22.7 - 34.6)	(29.6 - 42.8)	
LV ESV (ml)	34 ± 9	30 ± 4	0.191
	(25 - 41)	(26 - 34)	
LV length (mm)	91 ± 7	87 ± 7*	**0.009**
	(85 - 96)	(81 - 94)	
Relative wall thickness	0.35 ± 0.04	0.50 ± 0.10*	**0.020**
	(0.32 - 0.38)	(0.42 - 0.58)	

∗ denotes significant difference from baseline to end extraction, data presented as mean ± SD (95% confidence intervals)

Table 2- Left ventricular functional and mechanical responses to blood volume reduction

Variable	Baseline	End extraction	*P* value
Systolic Function			
LV EF (%)	64 ± 7	58 ± 4	0.075
	(58 - 71)	(52 - 63)	
LV S' ($cm.s^{-1}$)	11 ± 1	12 ± 2	0.402
	(10 - 12)	(11 - 13)	
Twist (^{o})	22 ± 9	22 ± 7	0.871
	(12 - 31)	(16 - 28)	
Twist rate ($^{o}.s^{-1}$)	118.31 ± 54.16	140.24 ± 32.76	0.354
	(57.03 - 195.52)	(113.79 - 179.06)	
Basal rotation (^{o})	-6 ± 3	-6 ± 3	0.800
	(-9 - -4)	(-9 - -4)	
Basal systolic rotation rate ($^{o}.s^{-1}$)	-61.88 ± 24.15	-76.81 ± 22.72	0.115
	(-92.99 - -38.18)	(-100.68 - -65.68)	
Apical rotation (^{o})	17 ± 8	17 ± 5	0.918
	(7 - 25)	(10 - 23)	
Apical systolic rotation rate ($^{o}.s^{-1}$)	89.31 ± 32.99	102.23 ± 19.67	0.263
	(41.62 - 121.08)	(86.30 - 123.10)	
Apical circumferential strain (%)	-25 ± 4	-27 ± 5	0.341
	(-30 - -21)	(-33 - -26)	
Basal circ SSR ($l.s^{-1}$)	-1.25 ± 0.20	-1.22 ± 0.15	0.399
	(-1.54 - -1.09)	(-1.39 - -1.06)	
Apical circ SSR ($l.s^{-1}$)	-1.68 ± 0.58	-2.03 ± 0.55	0.134
	(-2.54 - -1.12)	(-2.79 - -1.67)	
Longitudinal SSR ($l.s^{-1}$)	-1.05 ± 0.10	-1.04 ± 0.16	0.889
	(-1.16 - -0.91)	(-1.22 - -0.88)	
Diastolic Function			
LV E/A	1.95 ± 0.40	1.63 ± 0.45	**0.046**
	(1.54 - 2.34)	(1.01 - 1.94)	

LV E ($m.s^{-1}$)	0.86 ± 0.16	0.66 ± 0.13*	0.008
	(0.75 - 1.07)	(0.52 - 0.83)	
LV A ($m.s^{-1}$)	0.46 ± 0.11	0.42 ± 0.10	0.273
	(0.36 - 0.62)	(0.39 - 0.56)	
LV E' ($cm.s^{-1}$)	17 ± 2	14 ± 4	0.035
	(15 - 19)	(10 - 19)	
LV A' ($cm.s^{-1}$)	9 ± 2	8 ± 2	0.305
	(7 - 11)	(6 - 11)	
Untwist rate ($^{o}.s^{-1}$)	-151.80 ± 69.81	-165.80 ± 53.43	0.768
	(-232.27 - -66.81)	(-224.43 - -102.43)	
Basal diastolic rotation rate ($^{o}.s^{-1}$)	73.84 ± 30.04	76.48 ± 27.93	0.611
	(48.15 - 116.01)	(45.08 - 109.18)	
Apical diastolic rotation rate ($^{o}.s^{-1}$)	-104.69 ± 51.72	-102.07 ± 30.01	0.859
	(-157.19 - -39.76)	(-122.89 - -62.06)	
Basal circumferential DSR ($l.s^{-1}$)	2.03 ± 0.38	1.85 ± 0.25	0.089
	(1.68 - 2.43)	(1.56 - 2.03)	
Apical circumferential DSR ($l.s^{-1}$)	2.92 ± 0.76	2.52 ± 0.68	0.222
	(2.32 - 3.94)	(2.47 - 3.34)	
Longitudinal DSR ($l.s^{-1}$)	1.96 ± 0.42	1.41 ± 0.43*	0.001
	(1.42 - 2.47)	(0.91 - 1.78)	

* denotes significant difference from baseline to end extraction, data presented as mean ± SD (95% confidence intervals)

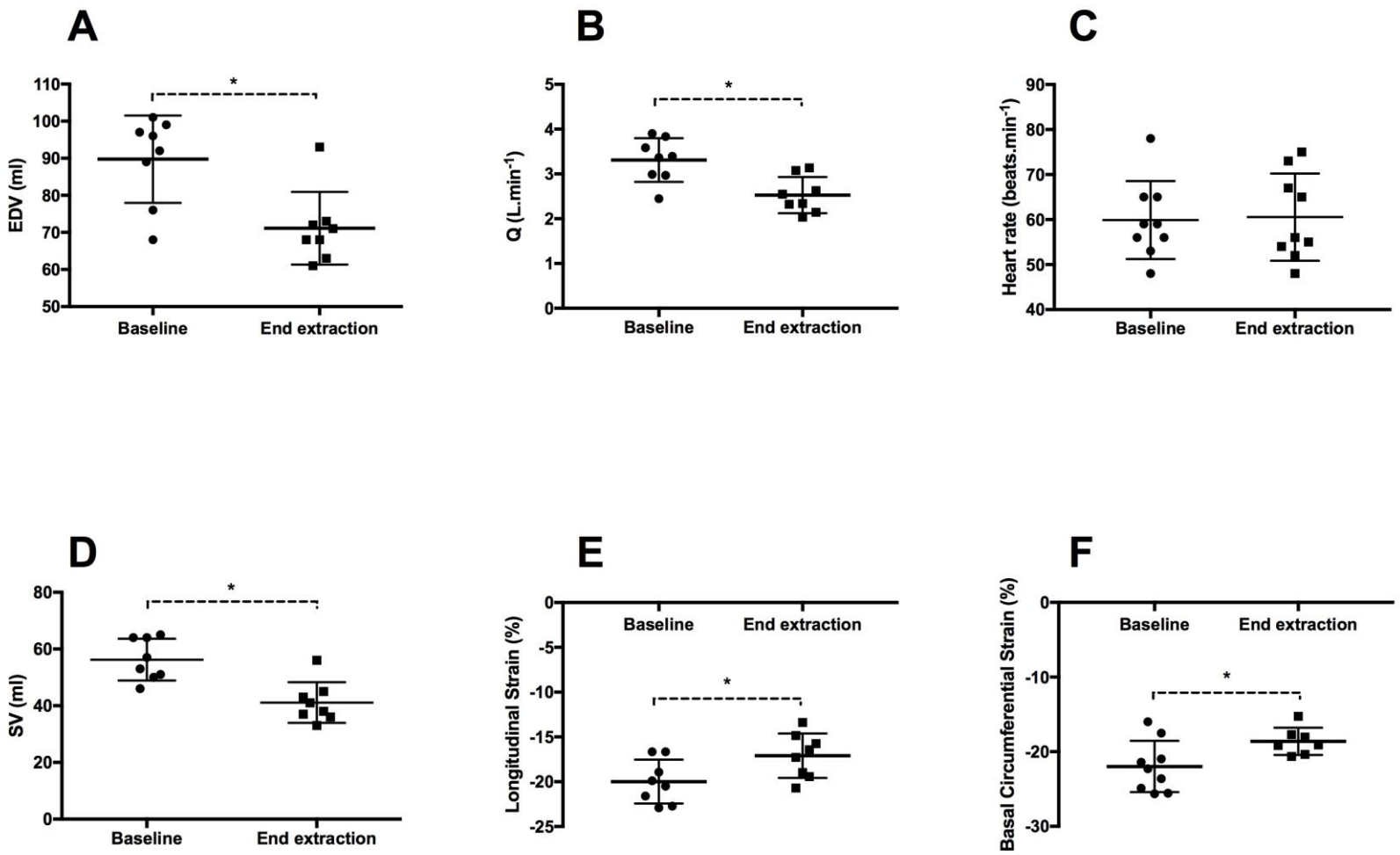

Figure 1 – Left ventricular response to blood volume extraction A) Left ventricular end diastolic volume, B) Cardiac output, C) heart rate, D) stroke volume, E) LV longitudinal strain and F) basal circumferential strain at baseline and following blood volume extraction. Statistical significance indicated with dashed lines and asterisk, $P < 0.025$. *EDV – end diastolic volume, Q – cardiac output, SV – stroke volume*

Discussion

This is the first study to assess the effects of significant blood volume extraction on stroke volume and the underpinning LV mechanics. The principle findings were; 1) blood volume extraction of 1062 ml resulted in a decrease in indices of LV filling and SV without changes in HR, and 2) counter to our hypothesis, SV was reduced without a compensatory increase in LV mechanics. LV longitudinal and basal circumferential strain and longitudinal DSR were significantly reduced following blood volume extraction. These data suggest that when LV filling is reduced without concomitant changes in HR, and likely SNA, LV mechanics do not compensate to maintain SV.

Differential response of LV strain and twist to blood volume extraction

The reduction in LV filling, secondary to blood volume extraction, resulted in a decrease in LV longitudinal and circumferential strain, whereas LV rotation and twist were maintained. The reduction in LV strain could potentially be explained by 1) a decrease in SNA, 2) lower Frank-Starling mediated contractility or 3) altered LV geometry. Firstly, as HR was similar following blood volume extraction, and previous studies have shown an increase in SNA in response to a similar stimulus (Rea *et al.*, 1991; Johnson *et al.*, 2014; Rickards *et al.*, 2015), it is unlikely that the decrease in LV strain is explained by reduced SNA. Secondly, if a reduction in ventricular stretch was responsible, according to Frank-Starling, both systolic strain rate and myocardial tissue velocity would also have been decreased, which was not the case. Accordingly, we propose that the change in strain is secondary to altered LV geometry; a lower EDV coupled with no change in trans-mural pressure dictates that LV length will reduce and wall thickness will increase due to the conservation of mass. With a thicker, shorter myocardium, the myocytes are under increased tension when relaxed (Choi *et al.*, 2010) and therefore the potential for further myocyte shortening is reduced, potentially due to actin-myosin overlap (Gordon & Pollack, 1980). The changes in LV shape under reduced filling, and the associated increase in myocyte tension at end diastole (Brutsaert *et al.*, 1971), likely reduce the functional shortening capacity of the myocardium (Spotnitz, 2000) and would therefore explain the reduction in LV strain. The maintenance of LV rotation and twist in the current study, alongside the reduced strain, suggests that changes in LV geometry, induced by decreased filling, do not have the same impact on LV twist. Although these changes in LV geometry impact the magnitude of myocardial shortening, the fibre

alignment and architecture that underpin LV rotational mechanics are unchanged (Spotnitz, 2000). Thus, when EDV is reduced through blood volume extraction, it is possible that the LV twist and strain response is not related, as previously observed during LBNP and dehydration (Stohr *et al.*, 2011; Williams *et al.*, 2016). Another potential explanation for the disparate response of LV twist and strain could be related to LV afterload. An increase in afterload impacts LV twist but not strain (Balmain *et al.*, 2016) and a decrease in afterload results in augmentation of both LV strain and twist (Burns *et al.*, 2010a; Burns *et al.*, 2010b). Although afterload is reduced in the current study following blood volume extraction, the magnitude of change is negligible in comparison to studies deliberately manipulating afterload which may explain why strain and twist are not increased. The previous studies also demonstrate a concomitant heart rate increase during changes in afterload which may mediate the LV strain and twist response.

Differences in the LV twist response to blood volume extraction and lower body negative pressure

Blood loss poses a complex physiological challenge that necessitates an integrated response that includes an elevation in SNA (Rea *et al.*, 1991; Johnson *et al.*, 2014; Rickards *et al.*, 2015). This increase in SNA during blood withdrawal is significantly lower compared to non-invasive simulation of blood loss such as LBNP, even when the preload reduction occurs over the same time course (Johnson *et al.*, 2014; Rickards *et al.*, 2015). Although we did not directly assess SNA, the lack of change in heart rate pre-to-post blood volume extraction strongly suggests there was minimal change in SNA. In contrast, during LBNP at -60mmHg, suggested to be equivalent to 25% of total blood volume loss (Johnson *et al.*, 2014), a significant increase in heart rate (Hodt *et al.*, 2011, 2015; Williams *et al.*, 2016) and SNA was evident (Johnson *et al.*, 2014; Rickards *et al.*, 2015) and accompanied by a marked increase in LV twist mechanics. This suggests that there may be a threshold at which increased SNA augments LV twist (Zaglia & Mongillo, 2017), and that the relatively slow withdrawal of blood, even to the point of pre-syncope, does not exceed this threshold. Further work examining *rapid* blood volume extraction, potentially a better model of haemorrhage, may invoke a greater SNA response and hence augment LV twist mechanics.

Maintenance of mean arterial pressure following blood volume extraction

Without compensatory changes in LV mechanics or HR, SV and Q were significantly reduced following blood volume extraction; despite this, MAP was maintained. As such, it is likely that peripheral vasoconstriction occurs, secondary to a mild increase in SNA (Johnson *et al.*, 2014; Rickards *et al.*, 2015). This response is markedly different to that seen with LBNP, where LV filling, SV, Q *and* MAP are all reduced. The differential response could be explained by the large volume of blood redistributed to the legs during LBNP. The artificial accumulation of blood in the lower limbs may prevent peripheral vasoconstriction and explain why MAP is reduced and indeed why SNA has been shown to be higher in previous studies comparing LBNP to blood volume extraction. The greater increase in SNA may reach the threshold proposed above, whereby HR and LV mechanics increase in an attempt to maintain MAP by increasing Q. Future work is required examining both the SNA and cardiac responses to both LBNP and blood volume extraction to answer this question.

Limitations

The current study used a relatively small sample size, however our *a priori* power analysis, based on the only study assessing cardiac responses to blood volume extraction (450 ml) (Saygisunar *et al.*, 2016), indicated that we were appropriately powered to detect changes in LV filling with 9 participants. Early termination occurred due to pre-syncope symptoms in 7 out of 9 participants; although the small sample size precluded any comparison with the 2 participants who reached target volume, the magnitude of change in EDV, SV and strain parameters was similar for all 9 participants, as evidenced by the individual plots within Figure 1. Although we did not directly assess SNA, we believe that our participants would have responded similarly to those in previous studies based on the similar HR and haemodynamic response. Sex differences have previously been identified in the LV mechanical response to reduced filling (Williams *et al.*, 2016). While we have combined male and female data in this study, the size of the sample precluded any comparison between sexes. The key findings from this study are based on the response of young healthy individuals to blood volume extraction which limits the generalization to broader populations.

Conclusion

A reduction in LV filling, secondary to blood volume extraction, does not result in compensatory changes in LV mechanics to maintain volumes. As a consequence of the reduction in SV and no change in HR, Q was significantly reduced. Despite the reduced Q, MAP was maintained, potentially mediated by an increase in peripheral vasoconstriction. The reduction in LV longitudinal and basal circumferential strain following blood volume extraction are likely explained by an amelioration in myocardial shortening capacity, secondary to changes in LV geometry with reduced LV filling. LV twist was unchanged despite reduced LV filling, suggesting LV twist is not affected by altered LV geometry during blood volume extraction. The absence of changes in HR during blood volume extraction, and the notion that LV twist may be HR dependent, likely explain why there was no augmentation in twist mechanics.

Competing interests

The authors have no competing interests to declare.

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Author contributions

DM, KG, DO, RS and MS contributed to conception or design of the work, DM, RL, RS and MS contributed to acquisition, analysis and interpretation of data for the work. RL, DM, KG, DO, RS and MS contributed to drafting the work or revising it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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