Females have greater left ventricular twist mechanics than males during acute reductions to preload

Running title: Sex differences in LV twist during alterations to preload

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Abstract

Background—Compared to males, females have smaller left ventricular (LV) dimensions and volumes, higher ejection fractions (EF), and higher LV longitudinal and circumferential strain. LV twist mechanics determine ventricular function, and are preload-dependent. Therefore, the sex differences in LV structure and myocardial function may result in different mechanics when preload is altered. This study investigated sex differences in LV mechanics during acute challenges to preload.

Methods and Results—Using conventional and speckle-tracking echocardiography, LV structure and function were assessed in 20 males (24 ± 6.2 yr) and 20 females (23 ± 3.1 yr) at baseline and during progressive levels of lower body negative pressure (LBNP). Fourteen participants (8 males, 6 females) were also assessed following a rapid infusion of saline. LV end-diastolic volume, end-systolic volume, stroke volume (SV) and EF were reduced in both groups during LBNP ($p<0.001$). While males had greater absolute volumes ($p<0.001$) there were no sex differences in allometrically scaled volumes at any stage. Sex differences were not detected at baseline in basal rotation, apical rotation, or twist. Apical rotation and twist increased in both groups ($p<0.001$) with LBNP. At -60 mmHg, females had greater apical rotation ($p=0.009$), twist ($p=0.008$) and torsion ($p=0.002$), and faster untwisting velocity ($p=0.02$) than males. There were no differences in mechanics following saline infusion.

Conclusions— Females have larger LV twist and a faster untwisting velocity than males during large reductions to preload, supporting that females have a greater reliance on LV twist mechanics to maintain SV during severe reductions to preload.

New & Noteworthy

This is the first study to demonstrate sex differences in left ventricular twist mechanics during acute preload challenges. Our data demonstrate that females utilize larger left ventricular twist and faster untwisting velocity than males to maintain mean arterial pressure during severe reductions to preload.

Key words: Sex, mechanics, echocardiography

Introduction

It is well established that sex differences exist in left ventricular (LV) geometry and function (11, 12, 21, 33). For example, compared to males, females have smaller LV wall thicknesses, volumes and mass, and these differences often remain when indexed to body size (12, 33). The disparities in geometry are accompanied by differences in global LV function (i.e. systolic emptying and diastolic relaxation and filling). Despite lower absolute stroke volumes (SV), females have greater ejection fractions (EF) than males, such that they attain a lower LV end-systolic volume (ESV) and a greater SV for a given end-diastolic volume (EDV) at rest (11, 33). The differences in function may be attributed to intrinsic properties of the myocardium: the smaller female ventricle is "stiffer", with lower diastolic compliance and a greater reliance on contractility compared to males (11, 12, 21). Whether these differences in LV structure and global function are associated with sex differences in LV mechanics remains unclear. However, recent studies have reported greater longitudinal (LS) and circumferential strain (CS) in females compared to males (3, 24, 26), providing preliminary support that sex differences in these fundamental mechanics do exist.

Left ventricular mechanics characterize myocardial deformation throughout the cardiac cycle (2, 36), and are dynamically load-dependent (17, 24, 47). Opposite rotations of the LV base and apex result in LV twist (8, 36, 43), which supports ejection during systole, and subsequent untwisting during diastole. The regional shortening and re-lengthening of the myocardium are quantified as LV strain (35) (see (27) for more in-depth review of LV mechanics). During increases to preload, increased LVEDV and myocardial stretch increase SV via the Frank-Starling mechanism (6). Increasing preload

also results in increased apical rotation, twist, diastolic untwisting velocity, CS and LS (47). In contrast, during reductions to preload, lowered LVEDV and preload-recruitable stroke work lead to decreases in SV and LS (14, 19, 22, 37). However, reduction of preload also results in increased heart rate (HR) and adrenergic stimulation (19, 37), as well as increased apical rotation, twist and untwisting velocity (22, 23). Although these responses of LV function to altered preload are well established, there is a paucity of data regarding whether the male and female hearts respond differently to alterations in preload.

Studies utilizing lower body negative pressure (LBNP) and head-up tilt have reported greater increases in HR in females, but similar reductions in LVSV and cardiac output (Q) between the sexes (37, 48). However, females appear to have greater reductions to LVSV for a given decrease in LV filling pressure, and a steeper slope of the Frank-Starling relationship compared to males (19). These findings suggest that females may have a lower functional reserve to cope with changes to LV loading, and a potentially greater reliance on increasing contractility and chronotropy to support SV and Q during acute preload challenges.

It remains unknown whether sex differences in LV structure and global function during periods of altered preload are underpinned by sex differences in LV mechanics. Therefore, the aim of the present study was to investigate how sex affects LV mechanics in response to acute challenges to preload. It was hypothesized that females would have greater LV apical rotation and twist, and greater LS and CS than males during decreases and increases in preload.

Methods

Ethical approval and study participants

A total of 48 healthy individuals from the local university community, aged 18-39 years, volunteered and were enrolled in the study. Participants were excluded if they had a history of cardiovascular, metabolic or respiratory disease; were current smokers (or stopped smoking for <12 months) or had hypertension (≥140/90). Additionally, females were excluded if pregnant or nursing. The study was approved by the Institutional Clinical Research Ethics Board of the University of British Columbia and conformed to the standards set by the *Declaration of Helsinki*. Written informed consent was obtained from all participants.

Study design

Participants visited the laboratory on two occasions. Prior to each visit, participants were asked to refrain from caffeine, alcohol and exercise for a minimum of 24 hours. On *day 1*, participants were screened for their ability to tolerate -60 mmHg LBNP. On *day 2*, total blood volume was measured using the optimized carbon monoxide rebreathing technique. Participants then rested in a LBNP box, sealed at the level of the iliac crests with a velcro strap, and were angled toward the sonographer in a left lateral decubitus position. Following collection of baseline echocardiographic images, LBNP was applied at -20, -40 and -60 mmHg. Echocardiographic images were recorded at each level of LBNP, following stabilization of HR within 5 beats per minute, and MAP within 5 mmHg. Blood pressure and HR were continuously monitored using finger photoplethysmography (Finometer, Amsterdam, NL) and three-lead electrocardiography,

120 respectively. Manual measurements of blood pressure were additionally taken at the end
121 of each stage, using a stethoscope and sphygmomanometer.
122 Following LBNP, a subset of 14 individuals (8 male, 6 female) received a rapid
123 infusion of saline. An 18- or 20-guage intravenous cannula was inserted into the
124 antecubital vein, and warm isotonic saline (~12 $ml \cdot kg^{-1}$ bodyweight) was infused at a rate
125 of ~100-200 $ml \cdot min^{-1}$, using a pneumatic sleeve to compress the solution (20). Following
126 the initial rapid infusion, saline was continually infused at a rate of 10 $ml \cdot min^{-1}$ to
127 maintain cardiac filling pressures (20), and echocardiographic images were recorded.
128 **Specific methodology**
129 **Screening and familiarization.** To screen for tolerance of LBNP, participants
130 were exposed to -10 mmHg increments each minute up to -60 mmHg, which was then
131 maintained for three minutes or until signs of pre-syncope. Pre-syncope was defined as a
132 decrease in systolic blood pressure (SBP) below 80 mmHg, or a decrease in SBP below
133 90 mmHg accompanied by lightheadedness, nausea, or tunnel vision. Participants unable
134 to tolerate up to or including -60 mmHg were excluded from the study.
135 **Total blood volume.** Total blood volume was measured using the carbon
136 monoxide rebreathing technique, as previously described (34). Briefly, participants were
137 fitted with a noseclip, and a custom-made glass spirometer (Blood tec, GbR, Germany)
138 attached to a 5 L reservoir bag of 100% O_2 gas. Participants were instructed to inhale
139 maximally, as a calculated amount of carbon monoxide (0.8 $ml \cdot kg^{-1}$ for males, 0.6 $ml \cdot kg^{-1}$
140 for females) was simultaneously administered to the rebreathing apparatus. Subjects held
141 a full lung volume for 10 s, and then rebreathed from the apparatus until 2 minutes, after
142 which they breathed room air. Venous blood was drawn from the antecubital vein at

143 baseline and 7 minutes following the onset of rebreathing for measurement of total
144 hemoglobin and carboxyhemoglobin (ABL 90, Radiometer, Denmark). Portable carbon
145 monoxide analyzers (Dräger Pac 3500, Draeger Safety Inc., Texas, USA) were used to
146 account for expired carbon monoxide at baseline, and at 4 and 7 minutes following the
147 onset of rebreathing.

148 **Transthoracic echocardiography.** Echocardiographic images were recorded on
149 a commercially available ultrasound system (Vivid-q, GE Healthcare, Little Chalfont,
150 UK) using a 1.5 to 4-MHz-phased array transducer, and saved for offline analysis
151 (EchoPAC, GE Healthcare). A trained sonographer acquired images at end-expiration for
152 the assessment of LV structure and function, in accordance with current guidelines (25).
153 During each stage of LBNP, small adjustments were made to the transducer position to
154 ensure optimal transducer angle and image quality. LV parasternal long-axis images were
155 analyzed for septal (IVST) and posterior wall thickness (PWT), and diastolic internal
156 diameter ($LVID_d$). LV length was measured at end-diastole from the mitral plane to the
157 apex in the apical two-chamber view. Pulsed Doppler recordings were performed in the
158 apical four-chamber view, with the sample volume at the tip of the mitral valve, and
159 analyzed for LV inflow early (E) and atrial (A) waves. Tissue Doppler recordings were
160 performed in the apical four-chamber view at the septal annulus, and analyzed for
161 myocardial velocities during systole (S'), and early (E') and atrial (A') diastole. LVESV,
162 EDV, SV and EF were measured using Simpson's biplane method. Relative wall
163 thickness was calculated as $2 \cdot PWT/LVID_d$. Sphericity index was calculated as LV
164 length/$LVID_d$ (45). Morphological, volume, and Doppler-derived data were averaged
165 over three cardiac cycles. To account for sex differences in anthropometric and cardiac

sizes, LV dimensions and volumes were scaled allometrically to $BSA^{0.5}$ and $BSA^{1.5}$, respectively (16).

Images for speckle tracking analysis were acquired at a rate of 70-90 frames·s^{-1}, in the following views: parasternal short-axis at the base, with leaflets of the mitral valve visible; parasternal short-axis at the apex, just proximal to end-systolic luminal obliteration (46); and apical four-chamber.

Speckle tracking analysis. An experienced sonographer blinded to group allocation and condition performed all analyses. The analysis of LV rotation and strain was performed using speckle tracking software (EchoPAC, GE Healthcare), as previously described (38, 42). The endocardial border was manually traced in digital 2D images, and the width of the region of interest was adjusted to include the full myocardial area (excluding trabeculations and papillary muscles). The software provided feedback on the reliability of tracking across six segments of the myocardium, and the observer visually confirmed adequate tracking. Segments without acceptable tracking were excluded from the analysis. For the selected region of interest, frame-by-frame curves for rotation and strain were generated by the software. Raw data were time-aligned and transformed to 1200 points using cubic spline interpolation (2D Strain Analysis Tool, Stuttgart, Germany). Twist data were calculated by subtracting time-aligned basal data from apical data. Torsion was calculated as twist/LV length. Peak twist, rotation, strain and untwisting velocity were defined as the maximal respective values across the cardiac cycle. Values were averaged across three cardiac cycles. Speckle tracking data represent average values over the entire myocardial region of interest (i.e. across all individual segments). The coefficient of variation of the sonographer for measuring twist and

189 untwisting velocity were 8.1% and 11%, respectively (39), which are in agreement with
190 previously published data (30, 31, 40).
191 **Analysis of LV hemodynamics.** Mean arterial pressure (MAP) was calculated as
192 1/3·SBP + 2/3·diastolic blood pressure (DBP). Q was calculated as the product of
193 LVSV·HR. Total peripheral resistance (TPR) was calculated as the quotient of MAP/Q.
194 **Statistical analysis and power calculation**
195 Data are presented as means ± standard deviation (SD). All dependent variables
196 were assessed using a 2 (group) x 4 (LBNP level) ANOVA for LBNP, and a 2 (group) x
197 2 (pre and post) ANOVA for the rapid saline infusion. When a positive effect was
198 detected, a Fisher's least significant difference test was used to determine pairwise
199 differences. These statistical analyses were performed using STATISTICA (version 8.0;
200 StatSoft, Tulsa, OK) with α set *a priori* to 0.05.
201 Linear regression analysis was used to assess the Frank-Starling relationship,
202 ΔLVSV/ΔEDV, for each participant during LBNP and saline infusion, and mean slopes
203 were compared using an independent samples t-test (19). Linear regression was also used
204 to assess the relationship between LV twist and untwisting velocity. Non-linear
205 regression analysis using a second order polynomial (quadratic) least squares fit was used
206 to assess the relationships between LV twist and torsion to LV absolute and
207 allometrically scaled volumes, respectively. The coefficients in the quadratic equation
208 $y=b0+b1x+b2x^2$ were calculated for each participant, and mean coefficients for male and
209 females were compared using an independent samples t-test. Regression analyses were
210 performed using GraphPad Prism (version 6.0f; GraphPad Software, Inc., La Jolla, CA).
211 No study has previously determined sex differences in cardiac mechanics during

212 alterations in preload. However, it was determined *a priori* that with 20 participants per
213 group, we were powered to detect a difference of 4.4 degrees in LV twist between the
214 groups, utilizing a SD of 5 degrees obtained from the literature (22), an α=0.05 and a
215 β=0.80.

Results

Participant characteristics

Of the 48 individuals enrolled, a total of 20 males and 20 females completed the study. Seven individuals (5 females, 2 males) were unable to tolerate -60 mmHg during the familiarization, and were excluded. Additionally, one male was excluded for a previous cardiac condition. Participant characteristics and baseline cardiac and hemodynamic parameters are presented in **Table 1.** Females had smaller absolute LV dimensions compared to males, but there were no sex differences in allometrically scaled dimensions. Moreover, relative wall thickness and sphericity did not differ between groups. Total blood volume was greater in males, but normalized blood volume did not differ between the groups. There were no differences in baseline MAP or HR.

LV mechanics and geometry during altered preload

LV twist increased in both groups with progressive LBNP ($p<0.001$), and resulted predominantly from increases in apical rotation ($p<0.001$) (**Table 3 and Figure 1**). LV twist ($p=0.008$) and apical rotation ($p=0.009$) were significantly larger in females compared to males at -60 mmHg. Relative to LV length, LV torsion increased in both groups during LBNP, and was greater in females during -40 ($p=0.01$) and -60 mmHg ($p=0.002$) (**Figure 2)**. Untwisting velocity was greater in females compared to males at -60 mmHg ($p=0.02$). There was a significant relationship between LV twist and untwisting velocity in all participants ($r^2=0.46$, $p<0.001$), and slopes of the regression did not differ between the sexes. LS and CS declined in both groups with LBNP, but LS was greater in females at -60 mmHg ($p=0.002$).

LV length and $LVID_d$ decreased in both groups with progressive LBNP but were larger in men in all conditions (*p<0.001*). There were no sex differences in scaled dimensions, scaled wall thicknesses or relative wall thickness at any stage. However, females had a larger sphericity index than males at -60 mmHg (*p=0.007*) (**Table 3**).

In the cohort receiving the rapid saline infusion, there were no sex differences in LV apical rotation, twist, torsion, untwisting velocity or sphericity index at baseline or following saline infusion. These parameters were unchanged in both groups following infusion. LV length and $LVID_d$ were also unchanged following infusion.

Figure 3 illustrates the relationships between twist and LV volumes, which are presented using absolute and scaled data. A trend for greater *b1* and *b2* coefficients in females was observed for the relationship of twist-to-LVSV (*b1*: males -0.17 ± 0.44 vs. females -0.56 ± 0.60, *p=0.073; b2*: males 0.007 ± 0.016 vs. females 0.020 ± 0.023, *p=0.074*), and the relationship of torsion to allometrically scaled SV (*b1*: males -0.65 ± 1.50 vs. females -1.85 ± 1.96, *p=0.077; b2*: males 0.008 ± 0.019 vs. females 0.024 ± 0.028, *p=0.073*). *b0*, *b1* and *b2* coefficients did not differ between the sexes for the relationships of LV twist and torsion to LVEDV and allometrically scaled EDV, respectively.

LV volumes and hemodynamics during altered preload

LVEDV (*p<0.001*), ESV (*p<0.001*) and SV (*p<0.001*) gradually decreased with progressive levels of LBNP, and while absolute volumes were larger in males at all stages, there were no sex differences in allometrically scaled EDV, ESV or SV (**Table 2**). The relative decrease in LVEDV (males 34 ± 7%; females 37 ± 9%), ESV (males 25 ± 9%; females 23 ± 13%), and SV (males 41 ± 9%; females 46 ± 10%) from baseline to -60

mmHg did not differ between the sexes. However, the mean slope of the Frank-Starling relationship (ΔLVSV/ΔEDV) was greater in females (0.76 ± 0.09) compared to males (0.68 ± 0.09) *(p=0.02*). LVEF decreased in both groups with LBNP, but was higher in females at baseline (*p=0.01)* and at -40 mmHg (*p=0.02)*.

HR increased in both groups with LBNP, and was higher in females at -40 (*p=0.004)* and -60 mmHg (*p<0.001*) (**Table 2**). Q was reduced with LBNP in both groups, yet MAP did not change with LBNP and was not different between the sexes. TPR increased in both groups with LBNP (*p<0.001*), but did not differ between the sexes.

In both groups, E decreased from baseline to -40 mmHg, but was not significantly reduced further at -60 mmHg. The reduction to E, and trend of increasing A (*p=0.06*) resulted in a reduced E/A ratio from baseline to -60 mmHg in both groups (males 2.09 ± 0.58 to 1.36 ± 0.28; females 2.17 ± 0.71 to 1.13 ± 0.20; *p<0.001* for both). Diastolic filling velocities did not differ between the sexes during any stage. Diastolic tissue velocities decreased in both groups with LBNP (*p<0.001* for both), but females had greater A' (6.1 ± 1.4 $m \cdot s^{-1}$ vs. 4.9 ± 0.9 $m \cdot s^{-1}$; *p=0.002*) than males during -60 mmHg. Systolic tissue velocity (S') decreased in males from baseline to -60 mmHg (8.6 ± 1.6 $m \cdot s^{-1}$ to 7.3 ± 1.5 $m \cdot s^{-1}$; *p<0.001*), but was unchanged in females, resulting in a greater S' in females than males at -60 mmHg (8.3 ± 1.1 $m \cdot s^{-1}$ vs. 7.3 ± 1.5 $m \cdot s^{-1}$; *p=0.03*).

Prior to and following saline infusion, absolute LV volumes were larger in males, but there were no sex differences in allometrically scaled volumes. Following saline infusion, LVEDV increased in males (*p=0.003*) but not in females **(Table 2**). However, scaled EDV increased in both groups. Absolute and scaled LVESV were unchanged in both groups. Absolute and scaled LVSV increased in both groups (*p<0.001*), but EF was

284 unchanged. E and E' increased in both groups following infusion ($p<0.01$), but there

285 were no sex differences in filling or septal tissue velocities.

Discussion

This is the first study to investigate sex differences in cardiac mechanics during acute alterations to preload. In support of our hypothesis, LV apical rotation, twist and LS were all greater in females than males, but only at higher levels of LBNP. In contrast, circumferential strain was not significantly different between the sexes. The sex differences in LV twist coincided with differences in LV geometry and chronotropy, as LV sphericity index and HR were greater in females at higher levels of LBNP.

Sex differences in LV responses to altered preload

LV twist was greater in females compared to males at -60 mmHg, and this resulted primarily from greater rotation at the apex. Due to the shorter LV length in females, LV torsion was also greater during -40 and -60 mmHg LBNP, demonstrating that females have greater twist for a given LV length compared to males. While previous reports have demonstrated similar reductions to LVEDV, ESV and SV (19), and increases to LV apical rotation and twist (22, 47) during LBNP, our data specifically demonstrate that females rely on greater apical rotation and LV twist than males during large challenges to preload.

Greater responsiveness at the apex in comparison to the base has been highlighted in previous investigations amongst male-only or mixed-sex cohorts (1, 22, 23, 41, 47), and has been suggested to help maintain the base-to-apex intraventricular pressure gradients that drive effective filling and ejection. Specifically, as LV systolic twist results in the storage of potential energy, the subsequent release of this energy in early diastole produces a rapid recoil or “suction” effect (29). In the current study, the greater apical rotation and twist in females were accompanied by a greater untwisting velocity during -

60 mmHg LBNP, supporting the notion that increased apical rotation contributed to greater systolic twist and diastolic untwist mechanics in females. In accordance with previous studies, we found a strong relationship between LV twist and untwisting velocity, which supports the important role of LV twist in generating the appropriate intraventricular pressure gradients required for diastolic filling (15, 29, 41). In the current study, early filling velocity was maintained in both groups between -40 and -60 mmHg; therefore, the greater untwisting velocity in females during -60 mmHg suggests that greater systolic twist and diastolic untwisting are required in the smaller female LV to generate adequate intraventricular pressure gradients, maintain passive filling and ultimately protect SV during challenges to preload.

Sex differences in LV adrenergic stimulation

During higher levels of LBNP, HR was higher in females than in males. This elevated chronotropic response in females is commonly observed during reductions to preload (19, 37, 48), and has been proposed to reflect sex differences in sympathovagal balance. It has been proposed that compared to males, females respond with more prominent vagal withdrawal (13, 19, 37). In the current study, MAP was maintained in both groups despite reductions to Q during progressive LBNP. The higher HR during high levels of LBNP may reflect a lower SV reserve in females, requiring greater vagal withdrawal, or increased sympathetic drive, to increase HR, maintain Q and prevent reductions to MAP.

The concurrent increases to LV twist, untwisting velocity and HR have been suggested to reflect increased inotropy during reductions to preload (22, 23). Indeed, this is supported by the fact that LV twist and untwisting velocity are increased following

administration of inotropic agents (1, 7, 17, 18, 29, 32). Therefore, relatively higher adrenergic stimulation or contractility may have contributed to the greater LV apical rotation, twist, and untwisting velocity in females during high levels of LBNP in this study. This is further supported by the greater systolic tissue velocity observed in females at -60 mmHg. It has also been reported that females have increased HR responsiveness to inotropic agents than males (13). Although regional adrenergic receptor densities have not been compared between the sexes, the LV apex is typically more responsive to acute stressors than the base, which likely reflects regional differences in adrenoreceptor density and sensitivity (1, 41). In the current study, relatively greater adrenergic stimulation at the apex likely contributed to the greater LV apical rotation and twist in females during high levels of LBNP.

Sex differences in LV geometry

Our participants demonstrated classic sex differences in LV geometry at baseline (28), with larger absolute LV dimensions and volumes in males than females. Nonetheless, relative wall thickness and sphericity index were similar, and allometrically scaled volumes and dimensions did not differ between the sexes, suggesting that LV morphology was relatively similar at baseline. With progressive LBNP, LV volumes and dimensions decreased, whereas sphericity index increased in both groups and was greater in females at -60 mmHg. In the LV wall, myocardial fibers are arranged in oblique orientations, and progressively change from a right-handed helix in the subendocardium to a left-handed helix in the subepicardium (35, 44). This continuum of helical fiber arrangement functionally underpins the generation of LV twist and shear strain (4, 5). When LV shape and helix angle are changed, the distributions of sarcomere length,

355 passive fiber stress, and active fiber stress may be altered within the myocardium (4, 10),
356 and subsequently impact LV twist (44, 45). In the current study, the reductions to LV
357 volumes and increased sphericity index during LBNP likely coincided with altered LV
358 helix angles in both males and females. However, the higher sphericity index and greater
359 ellipsoid shape in females during -60 mmHg could reflect an altered fiber configuration
360 that would result in larger apical rotation and twist in females.

361 **Sex differences in the Frank-Starling relationship**

362 A significantly higher slope of the Frank-Starling relationship (ΔLVSV/ΔEDV)
363 was observed in females compared to males. These findings agree with observations of
364 Fu et al. (19), who reported a steeper maximal slope for ΔLVSV for any given LV
365 pulmonary wedge pressure in females. Previous reports have suggested that the smaller
366 female ventricle has lower diastolic compliance and greater elastance than that of males
367 (12, 21). Accordingly, during reductions to preload, the less compliant female LV will
368 store less elastic energy during diastole than that of males. A reduction in stored potential
369 energy will subsequently result in a lowered capacity to utilize passive end-diastolic
370 tension and the Frank-Starling mechanism. Therefore, females may require greater
371 increases to LV twist and/or contractility than males to support SV.

372 The relationships between twist and LV volumes are highlighted in Figure 3.
373 When plotted against absolute volumes, the curvilinear relationships of twist-to-LVEDV
374 and twist-to-LVSV are similar for both sexes, though the female curves extend to a lower
375 range of LV volumes and larger twist than males. However, when both twist and LV
376 volumes are scaled, the relationships of LV torsion to allometrically scaled SV and EDV
377 are visibly steeper in females compared to males. Thus, across a more comparable range

of volumes, females appear to operate on a "steeper" portion of the twist-to-volume relationship, similar to differences in the Frank-Starling relationship. Analysis of quadratic functions for these relationships revealed a trend of greater *b1* and *b2* coefficients in females for the relationships of twist-to-LVSV and torsion to allometrically scaled SV, which supports the contention that the amplitudes of LV twist for a given SV differ between the sexes during reductions to preload. The steeper slope of the curves in females may reflect a lower functional reserve, whereby if preload was challenged further, females may have a diminished reserve to further augment twist and maintain SV and MAP.

In an attempt to confirm that the differences observed in LV mechanics between the sexes were not due to differences in LV geometry or body size, we normalized our twist data to LV length (i.e. torsion) and scaled LV volumes to body surface area. Furthermore, we found no association between LV length and twist (males: r^2=0.002, *p=0.87;* females: r^2=0.06, *p=0.31*), LV length and the change in twist from baseline to -60 mmHg (males: r^2=0.12, *p=0.17;* females: r^2=0.13, *p=0.19*), or LVEDV and twist (males: r^2=0.06, *p=0.33;* females: r^2=0.004, *p=0.80*). Combining these findings, our data support that the differences observed in LV twist during reductions to preload are a true sex difference rather than a result of variations in LV size or volume. Nonetheless, future studies might consider matching males and females for LV length or EDV to further confirm these sex differences in LV mechanics.

Limitations

In the cohort that received the rapid saline infusion, there were no changes or sex differences in LV apical rotation, twist, and untwisting velocity. The responses of LV

401 mechanics to volume loading in prior investigations have been varied, with some
402 reporting increased (47) or unchanged (9) apical rotation and twist, despite significant
403 increases in EDV. These differences may be related to variations in volume loading
404 protocols (i.e. total volume delivered, speed of infusion). Nevertheless, our findings
405 demonstrate that changes in LVEDV within ± 10-20 ml from baseline with either LBNP
406 or saline infusion did not have a significant effect on LV mechanics. With more
407 substantial alterations to preload, we may have observed compensatory differences in LV
408 mechanics between the male and female groups.
409 While we have accounted for large differences in LV geometry using LV torsion
410 and allometrically scaled LV volumes, we are limited in our ability to determine the
411 physiological mechanisms responsible for the sex differences in LV mechanics observed
412 in this study. The sex differences in LV twist and untwisting might occur due to intrinsic
413 differences in the male and female hearts (LV size, geometry, properties of the
414 myocardium), or differences in the adrenergic or autonomic control of the heart. It is
415 likely that the combined influences of some or all of these factors contribute to the
416 findings of the current study. Future work should focus on determining the independent
417 effects of each of these factors in isolation and combination to better understand the
418 mechanisms responsible for these sex differences in LV mechanics.
419
420 **Conclusions**
421 During high levels of LBNP, LV twist is greater in females compared to males,
422 primarily as a result of greater apical rotation. In cases where passive tension and reliance
423 on the Frank-Starling mechanism are reduced, females utilize greater LV twist and may

424 rely on increasing contractility to a greater extent than males. While females have smaller
425 absolute LV volumes and dimensions during high levels of LBNP, these differences in
426 LV mechanics occur with similar relative reductions to LVEDV and SV in both sexes.
427 Compared to males, the combination of higher LV twist and HR in females appears to
428 protect SV and Q, and ultimately maintain MAP during reductions to preload. Overall,
429 our data have demonstrated that females utilize greater LV mechanics than males to
430 compensate during severe reductions to preload, and these sex differences may result
431 from differences in LV geometry, intrinsic properties of the myocardium, or adrenergic
432 stimulation.
433

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598

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Tables

Table 1. Baseline participant characteristics and echocardiographic measurements

	Males n=20	**Females** n=20
Resting characteristics		
Age (yrs)	24 (6.2)	23 (3.1)
Height (m)	1.79 (0.06)	1.66 (0.08)*
Weight (kg)	76.9 (11.7)	62.4 (8.6)*
BMI ($kg \cdot m^{-2}$)	23.8 (2.8)	22.6 (2.3)
MAP (mmHg)	76 (6)	74 (7)
HR (bpm)	56 (9)	64 (10)
Total blood volume (L)	7.20 (1.15)	5.24 (0.79)*
Normalized blood volume ($ml \cdot kg^{-1}$)	94.3 (12.0)	85.3 (16.2)
LV dimensions		
$Length_d$ (cm)	9.14 (0.69)	8.14 (0.44)*
$Length_d \cdot BSA^{-0.5}$ ($cm \cdot m^{-1}$)	6.55 (0.40)	6.27 (0.33)
$LVID_d$ (mm)	46.7 (3.2)	41.6 (3.7)*
$LVID_d \cdot BSA^{-0.5}$ ($mm \cdot m^{-1}$)	33.4 (2.0)	31.9 (2.0)
IVST (mm)	11.1 (1.5)	9.7 (1.5)*
$IVST \cdot BSA^{-0.5}$ ($mm \cdot m^{-1}$)	7.9 (0.9)	7.5 (1.2)
PWT (mm)	9.2 (1.2)	8.3 (1.2)*
$PWT \cdot BSA^{-0.5}$ ($mm \cdot m^{-1}$)	6.6 (0.8)	6.4 (1.0)
Relative wall thickness	0.40 (0.05)	0.40 (0.06)
Sphericity index	1.96 (0.16)	1.97 (0.14)

Values are means (SD). BMI: body mass index; MAP: mean arterial pressure; HR: heart rate; $Length_d$: end-diastolic length; $LVID_d$: left ventricular diastolic internal diameter; IVST: interventricular septum thickness; PWT: posterior wall thickness; BSA: body surface area. *$p<0.05$ vs males.

615 Table 2. Cardiovascular responses to altered preload.

	LBNP				Saline Infusion	
	Baseline	-20 mmHg	-40 mmHg	-60 mmHg	Pre	Post
HR (bpm)						
Males	56 (9)	58 (10)	66 (11)†‡	75 (12)†‡§	53 (9)	56 (11)
Females	64 (10)	65 (9)	75 (12)*†‡	95 (16)*†‡§	61 (9)	60 (10)
SBP (mmHg)						
Males	112 (5)	112 (8)	107 (11)†‡	101 (11)†‡§	114 (6)	120 (9)†
Females	107 (6)*	102 (7)*†	99 (7)*†‡	96 (7)*†‡	101 (2)*	108 (7)*
DBP (mmHg)						
Males	59 (8)	61 (9)	59 (11)	62 (13)	63 (7)	70 (4)†
Females	59 (9)	55 (7)	58 (10)	61 (13)	57 (6)	61 (12)*
MAP (mmHg)						
Males	76 (6)	77 (8)	74 (11)	75 (11)	79 (6)	86 (5)†
Females	74 (7)	70 (6)	71 (8)	72 (10)	71 (4)*	76 (10)*
EDV (ml)						
Males	106 (18)	95 (19)†	82 (18)†‡	70 (17)†‡§	106 (15)	117 (19)†
Females	77 (10)*	67 (9)*†	57 (8)*†‡	48 (7)*†‡§	76 (14)*	82 (15)*
ESV (ml)						
Males	48 (11)	44 (11)†	42 (11)†‡	35 (9)†‡§	47 (9)	49 (11)
Females	31 (5)*	29 (4)*†	27 (3)*†‡	24 (5)*†‡§	29 (6)*	30 (3)*
SV (ml)						
Males	59 (9)	51 (10)†	41 (9)†‡	35 (9)†‡§	59 (7)	69 (10)†
Females	45 (7)*	38 (7)*†	30 (6)*†‡	24 (4)*†‡§	46 (10)*	52 (12)*†

EDV·BSA$^{-1.5}$ ($ml \cdot m^{-3}$)						
Males	39 (6)	35 (6)†	30 (6)†‡	26 (5)†‡§	39 (6)	43 (6)†
Females	35 (5)	31 (4)†	26 (4)†‡	22 (3)†‡§	36 (5)	39 (4)†
ESV·BSA$^{-1.5}$ ($ml \cdot m^{-3}$)						
Males	17 (3)	16 (3)†	15 (3)†	13 (3)†‡§	17 (3)	18 (3)
Females	14 (2)	13 (2)†	12 (1)†‡	11 (2)†‡§	14 (2)	14 (1)
SV·BSA$^{-1.5}$ ($ml \cdot m^{-3}$)						
Males	22 (4)	19 (4)†	15 (3) †‡	13 (3) †‡§	22 (4)	25 (4)†
Females	21 (3)	17 (3)†	14 (3) †‡	11 (2) †‡§	22 (4)	24 (4)†
EF (%)						
Males	55 (4)	54 (4)	50 (4)†‡	49 (6)†‡	56 (4)	59 (4)
Females	59 (3)*	56 (4)†	53 (4)*†‡	51 (4)†‡§	61 (4)*	63 (4)
Q ($L \cdot min^{-1}$)						
Males	3.24 (0.65)	2.89 (0.60)†	2.65 (0.56)†‡	2.58 (0.71)†‡	3.09 (0.56)	3.87 (0.89)†
Females	2.92 (0.49)*	2.44 (0.38)*†	2.29 (0.37)†	2.29 (0.45)†	2.66 (0.30)	3.00 (0.43)
TPR ($mmHg \cdot L^{-1} \cdot min^{-1}$)						
Males	24.3 (5.1)	27.8 (6.2)†	29.1 (8.0)†	31.3 (9.5)†‡	23.1 (4.9)	26.1 (4.4)
Females	25.9 (4.8)	29.2 (4.6)†	31.7 (6.1)†	33.0 (8.5)†‡	25.7 (6.2)	25.9 (4.1)

Values are means (SD). SBP: systolic blood pressure; DBP: diastolic blood pressure; EDV: end-diastolic volume; ESV: end-systolic volume; SV: stroke volume; EF: ejection fraction; Q: cardiac output; TPR: total peripheral resistance. See Table 1 for additional

619 abbreviations. *p<0.05 vs males. †p<0.05 vs baseline. ‡p<0.05 vs -20 mmHg. §p<0.05 vs -40 mmHg. *n= 20* males, *n=20* females for

620 LBNP; *n=8* males, *n=6* females for rapid saline infusion.

621

622 Table 3. LV mechanics and geometry during altered preload.

	LBNP				Saline Infusion	
	Baseline	-20 mmHg	-40 mmHg	-60 mmHg	Pre	Post
Twist mechanics (peak)						
Apical rot (°)						
Males	10.6 (3.4)	9.9 (3.2)	10.7 (3.9)	13.1 (5.9)†	9.8 (3.8)	10.0 (3.5)
Females	12.4 (4.5)	11.4 (5.1)	13.6 (4.8)	18.0 (6.9)*†‡§	12.3 (5.1)	10.8 (1.2)
Basal rot (°)						
Males	-3.2 (2.7)	-3.0 (2.9)	-3.4 (2.7)	-3.6 (2.9)	-3.9 (2.1)	-3.3 (3.1)
Females	-4.1 (2.1)	-3.8 (2.9)	-4.2 (3.1)	-4.8 (2.7)	-4.1 (1.7)	-2.4 (2.5)
Twist (°)						
Males	13.6 (4.6)	12.7 (3.5)	13.4 (4.2)	15.8 (5.2)‡§	13.3 (5.3)	13.8 (4.6)
Females	16.1 (5.9)	14.4 (4.8)	17.4 (5.1)‡	21.4 (6.7)*†‡§	15.8 (4.6)	13.6 (1.6)
Untwisting velocity ($°\cdot s^{-1}$)						
Males	109 (28)	105 (29)	111 (40)	118 (45)	110 (29)	95 (33)
Females	126 (51)	123 (39)	140 (46)	152 (45)*‡	117 (53)	107 (18)
Strain mechanics (peak)						
LS (%)						
Males	-18.5 (2.2)	-16.5 (2.3)†	-15.4 (2.3)†	-14.4 (2.1) †‡§	-17.7 (2.7)	-18.9 (1.8)
Females	-20.6 (1.5)	-18.7 (2.3)†	-17.7 (2.1)†‡	-17.0 (2.2)*†‡	-21.0 (0.8)*	-21.5 (1.5)*
CS, base (%)						
Males	-19.5 (3.7)	-17.6 (3.7)†	-16.7 (4.1)†	-15.0 (3.6)†‡§	-19.8 (3.9)	-17.8 (2.7)
Females	-20.8 (3.4)	-20.2 (3.1)	-17.1 (3.0)†‡	-15.9 (4.1)†‡	-21.4 (1.7)	-18.4 (2.4)†
CS, apex (%)						

Males	-28.2 (4.8)	-27.1 (5.4)	-26.1 (5.5)†	-25.1 (6.6)†	-27.9 (4.1)	-27.6 (5.0)
Females	-27.6 (3.3)	-25.2 (5.6)	-27.0 (5.0)†	-26.8 (4.9)†	-27.6 (2.4)	-27.5 (1.4)
LV geometry						
Length$_d$ (cm)						
Males	9.14 (0.69)	8.96 (0.69)†	8.81 (0.69)†‡	8.57 (0.72)†‡§	9.10 (0.51)	9.02 (0.58)
Females	8.14 (0.44)*	7.99 (0.52)*†	7.73 (0.61)*†‡	7.58 (0.62)*†‡§	7.90 (0.39)*	7.96 (0.32)*
LVID$_d$ (mm)						
Males	46.7 (3.2)	45.2 (3.5)†	42.2 (4.2)†‡	39.5 (4.3)†‡§	46.2 (2.8)	46.3 (4.3)
Females	41.6 (3.7)*	39.7 (3.0)*†	37.2 (2.9)*†‡	32.3 (3.1)*†‡§	39.9 (3.7)*	40.8 (4.5)*
Sphericity						
Males	1.96 (0.16)	1.99 (0.20)	2.11 (0.24)†‡	2.17 (0.24)†‡§	1.98 (0.16)	1.96 (0.21)
Females	1.97 (0.14)	2.03 (0.16)	2.09 (0.17)†	2.36 (0.17)*†‡§	2.02 (0.10)	1.97 (0.19)

Values are means (SD). LBNP: lower body negative pressure; Rot: rotation; LV: left ventricle; LS: longitudinal strain; CS: circumferential strain; see Table 1 for additional abbreviations. *p<0.05 vs males. †p<0.05 vs baseline. ‡p<0.05 vs -20 mmHg. §p<0.05 vs -40 mmHg. *n=20* males, *n=20* females for LBNP; *n=8* males, *n=6* females for rapid saline infusion.

627 **Figure legends**

628

629 **Figure 1.** Graphical representation of mean LV twist mechanics during LBNP. Blue and
630 red lines represent mean data for males and females, respectively. ***Top panel****:* dotted and
631 dashed lines represent rotations of the LV apex and base, respectively. ***Middle panel****:*
632 solid lines represent LV twist. ***Lower panel****:* solid lines represent twist and untwisting
633 velocities. Standard deviations are provided in *Table 3*. *p<0.05 males vs. females.

634

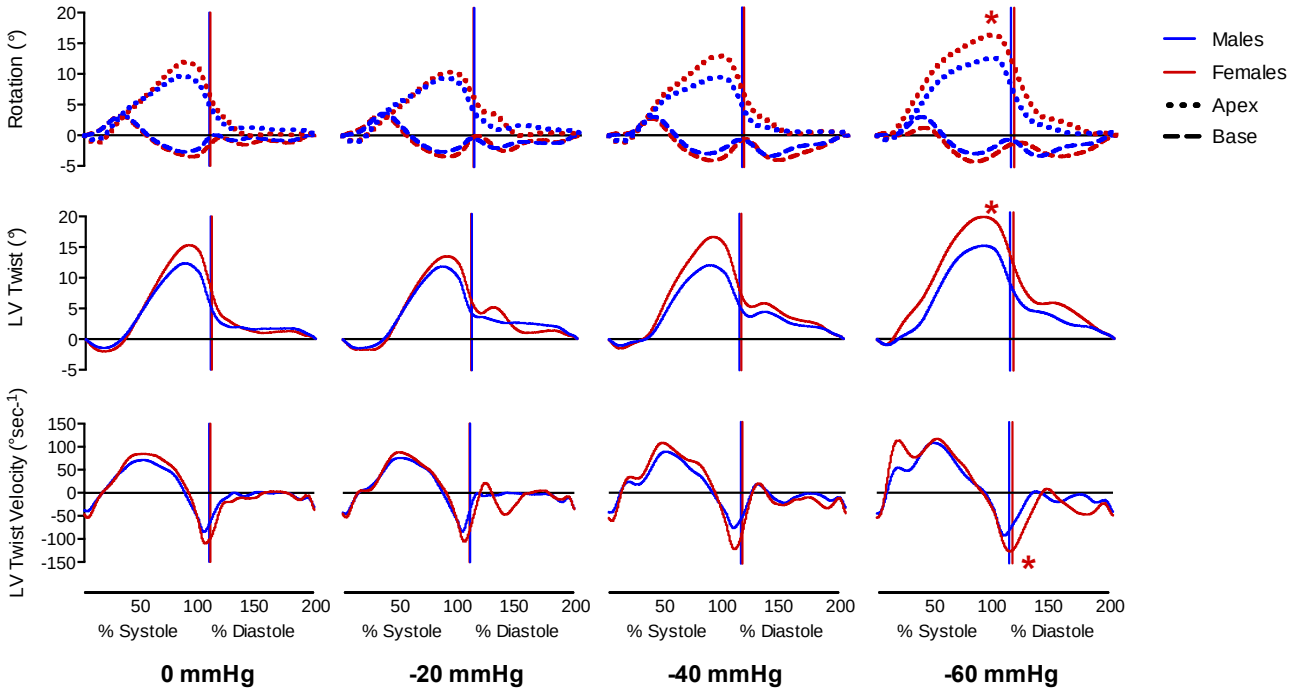
635 **Figure 2.** LV torsion in males (blue circles) and females (red circles) during LBNP.
636 Points represent means ± SD. *p<0.05 vs. males for given stage of LBNP.

637

638 **Figure 3.** ***Upper panel:*** the relationship of LV twist to LVSV (***A***) and LV torsion to
639 $SV \cdot BSA^{-1.5}$ (***B***) ***Lower panel:*** the relationship of LV twist to LVEDV (***C***) and LV torsion
640 to $EDV \cdot BSA^{-1.5}$ (***D***). Points represent group means at baseline and LBNP (closed, *n=20*
641 males*, n=20* females) and following saline infusion (open, *n=8* males*, n=6* females). A
642 trend toward a difference between the sexes was observed in the relationships of twist to
643 LVSV (***A***) (*b1*, p=0.077; *b2*, p=0.073) and LV torsion to $SV \cdot BSA^{-1.5}$ (***B***) (*b1*, p=0.073; *b2*,
644 p=0.074).

645

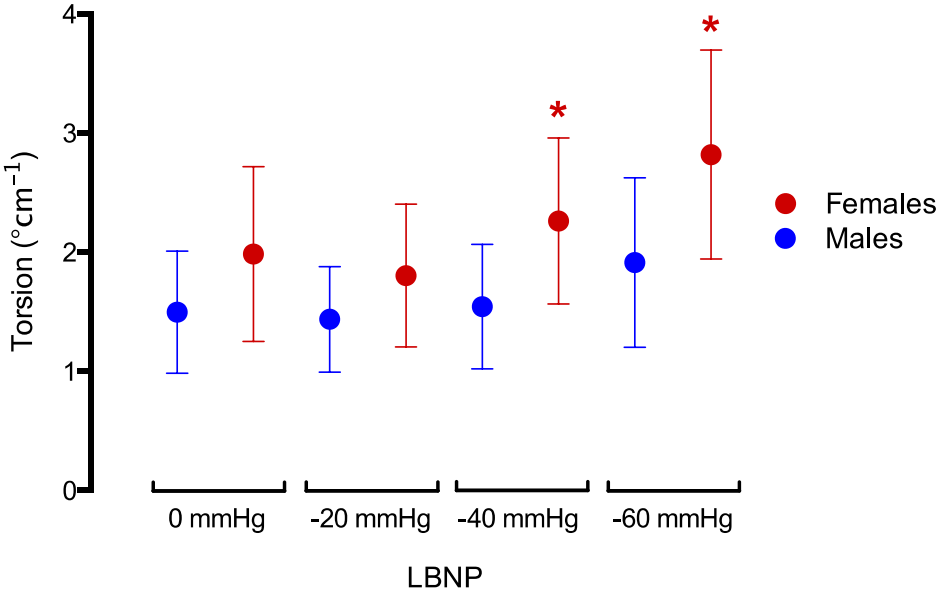
646 **Figure 1**



647

648

649 **Figure 2**



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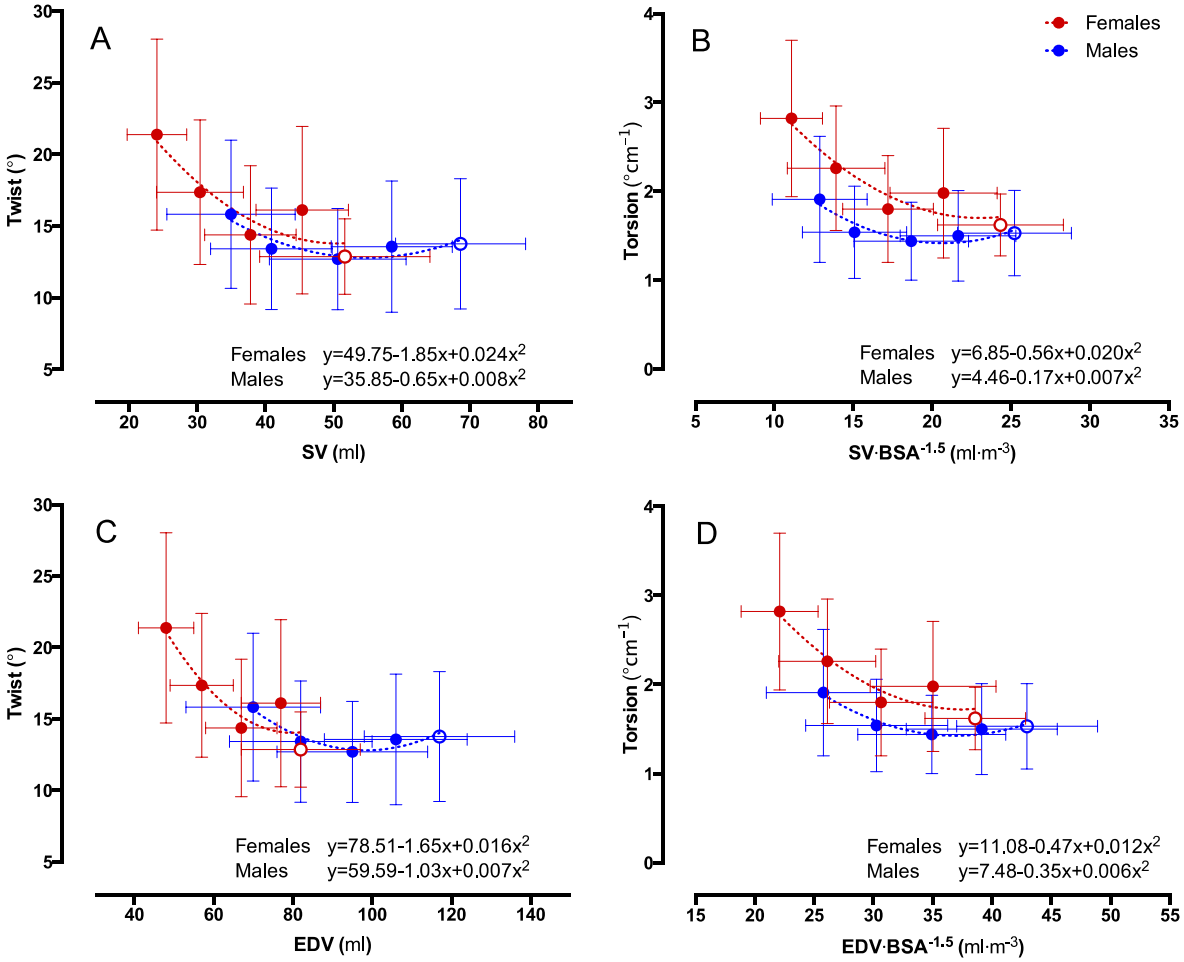
651

652 **Figure 3**



653