The influence of adrenergic stimulation on sex differences in left ventricular twist mechanics

Running title: Sex differences in LV twist during altered adrenergic stimulation

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Key points summary

- Sex differences in left ventricular (LV) mechanics occur during acute physiological challenges, however it is unknown whether sex differences in LV mechanics are fundamentally regulated by differences in adrenergic control.
- Using 2-dimensional echocardiography and speckle tracking analysis, this study compared LV mechanics in males and females matched for LV length during post exercise ischemia (PEI) and $ß_1$-adrenergic receptor blockade.
- Our data demonstrate that while basal rotation was increased in males, LV twist was not significantly different between the sexes during PEI. In contrast, during $ß_1$-adrenergic receptor blockade LV apical rotation, twist and untwisting velocity were reduced in males compared to females.
- Significant relationships were observed between LV twist with LV internal diameter and sphericity index in females, but not males.
- These findings suggest that LV twist mechanics may be more sensitive to alterations in adrenergic stimulation in males, but more highly influenced by ventricular structure and geometry in females.

Abbreviations. A, atrial diastolic inflow velocity; β_1-AR, β_1-adrenergic receptor; BMI, body mass index; BSA, body surface area; DBP, diastolic blood pressure; E, early diastolic inflow velocity; EDV, end-diastolic volume; EF, ejection fraction; ESV, end-systolic volume; HR, heart rate; IVST, intraventricular septal wall thickness; $Length_d$, length at end-diastolic; LV, left ventricle; $LVID_d$, left ventricular end-diastolic internal diameter; $LVID_s$, left ventricular end-systolic internal diameter; MAP, mean arterial pressure; MVC, maximal voluntary contraction; PEI, post-exercise ischemia; PWT, posterior wall thickness; Q, cardiac output; SBP, systolic blood pressure; SV, stroke volume; TPR, total peripheral resistance.

Abstract

Background. Sex differences in LV mechanics exist at rest and during acute physiological stress. Differences in cardiac autonomic and adrenergic control may contribute to sex differences in LV mechanics and LV hemodynamics. Accordingly, this study aimed to investigate sex differences in LV mechanics with altered adrenergic stimulation achieved through post handgrip exercise ischemia (PEI) and $ß_1$-adrenergic receptor (AR) blockade.

Methods and Results. 20 males (23±5 yr) and 20 females (22±3 yr) were specifically matched for LV length (males: 8.5±0.5 cm, females: 8.2±0.6 cm, p=0.163), and 2-dimensional speckle-tracking echocardiography was used to assess LV structure and function at baseline, during PEI and following administration of 5mg bisoprolol ($ß_1$-AR antagonist). During PEI, LV end-diastolic volume and stroke volume were increased in both groups (p<0.001), as was end-systolic wall stress (p<0.001). LV twist and apical rotation weren't altered from baseline or different between the sexes, however basal rotation increased in males (p=0.035). During $ß_1$-AR blockade, LV volumes were unchanged but blood pressure and heart rate were reduced in both groups (p<0.001). LV apical rotation (p=0.036) and twist (p=0.029) were reduced in males with $ß_1$-AR blockade but not females, resulting in lower apical rotation (males: 6.8±2.1º, females: 8.8±2.3º, p=0.007) and twist (males: 8.6±1.9º, females: 10.7±2.8º, p=0.008), and slower untwisting velocity (males: 68.2±22.1º·s^{-1}, females: 82.0±18.7º·s^{-1}, p=0.046) compared to females.

Conclusions. LV twist mechanics are reduced in males compared to females during reductions to adrenergic stimulation, providing preliminary evidence that LV twist mechanics may be more sensitive to adrenergic control in males than in females.

Introduction

Left ventricular (LV) mechanics are fundamental to ventricular function, as LV twist supports the production of stoke volume (SV) during ejection, and diastolic untwisting drives early filling during diastole (Notomi *et al.*, 2007; Stohr *et al.*, 2011). Previous studies have identified sex differences in LV mechanics, where females have greater LV longitudinal and circumferential strain at rest (Lawton *et al.*, 2011; Augustine *et al.*, 2013). Our group has also identified that females have greater LV twist and faster untwisting than males during large reductions to preload (Williams *et al.*, 2016). It is currently unknown what structural differences or regulatory mechanisms are responsible for these sex differences in LV mechanics. However, it is feasible that differences in LV size or adrenergic stimulation may play a contributing role (Notomi *et al.*, 2007).

Females have been reported to have larger chronotropic responses to periods of acute physiological stress (Fu *et al.*, 2004; Williams *et al.*, 2016), as well as having a increased high frequency power component of HRV (Gregoire *et al.*, 1996; Ramaekers *et al.*, 1998; Barantke *et al.*, 2008), both of which are believed to reflect greater vagal control in females (Shoemaker *et al.*, 2001; Fu *et al.*, 2004). These findings are in contrast to males who commonly have a larger ratio of low-to-high frequency power which is believed to reflect greater sympathetic (adrenergic) control (Ryan *et al.*, 1994; Gregoire *et al.*, 1996; Kuo *et al.*, 1999; Barantke *et al.*, 2008). These potential sex differences in cardiac adrenergic stimulation are especially relevant to differences in LV mechanics, as altered adrenergic stimulation is reported to impact LV twist (Rademakers *et al.*, 1992; Dong *et al.*, 1999; Notomi *et al.*, 2007). Specifically, the administration of β_1 adrenergic receptor (β_1-AR) agonists produces increases in SV and may even double LV

twist and peak untwisting velocity (Moon *et al.*, 1994; Akagawa *et al.*, 2007; Notomi *et al.*, 2007). In contrast, β_1-AR blockade results in reductions in LV twist, peak untwisting velocity (Notomi *et al.*, 2007) and strain (Thorstensen *et al.*, 2011). The changes to LV twist predominantly result from alterations to apical rotation, which is likely reflective of a greater β-AR density at the apex compared to the base (Mori *et al.*, 1993; Lyon *et al.*, 2008). However, given that these previous studies have involved exclusively male cohorts, it remains unknown how regional adrenergic control differs between the sexes to ultimately regulate LV twist mechanics. Therefore, the aim of this study was to investigate sex differences in LV mechanics with altered adrenergic stimulation, using activation of the muscle metaboreflex with post-exercise ischemia and β_1-AR blockade (bisoprolol) to augment and attenuate adrenergic stimulation, respectively. It was hypothesized that 1) during increases to adrenergic stimulation, LV twist and untwisting velocity would be lower in females than males, and 2) during reductions to adrenergic stimulation, females would have greater twist and faster untwisting than males.

Methods

Ethical approval

All procedures for the study were approved by the University of British Columbia clinical research ethics board (H13-03472) and conformed to the standards set by the *Declaration of Helsinki*. Written informed consent was obtained from all participants.

115 **Study participants**

116 Participants from the local university community, between the ages of 19-39 were
117 recruited for the study. Exclusion criteria included: a history of cardiovascular,
118 respiratory, or musculoskeletal disease; a body mass index (BMI) greater than 30 kg/m^2;
119 a resting blood pressure ≥140/90 or <110/60 mmHg and smoking (or smoking cessation
120 <12 months). Given the potential influence of sex-related differences in LV size on LV
121 twist mechanics, males and females were matched for LV length. More specifically,
122 individuals were continually enrolled until a total of 20 males and 20 females were
123 matched for LV length. Those that could not be matched for LV length (within ± 0.2 cm)
124 to an individual of the opposite sex were excluded. To minimize the potential variability
125 in LV structure (Arbab-Zadeh *et al.*, 2014; Weiner *et al.*, 2015), mechanics (Baggish *et*
126 *al.*, 2008; Weiner *et al.*, 2010*a*) and adrenergic control (Martin *et al.*, 1991) associated
127 with chronic endurance training, individuals performing >1 hour of moderate-intensity
128 training five times per week, or ≥3 bouts of high intensity training per week were also
129 excluded from the study. Of the 21 males and 26 females enrolled, 1 male and 2 females
130 were excluded in the first visit for poor imaging windows. Four females were further
131 excluded at the conclusion of data collection, as a male participant matched for LV length
132 was not enrolled in the study. A total of 20 males and 20 females completed the study and
133 were included in the analysis.
134

135 **Study design**

136 Participants visited the laboratory on two separate occasions, and were asked to
137 refrain from caffeine, exercise and alcohol for a minimum of 12 hours prior to the first

visit, and 24 hours prior to the second visit. During visit 1, participants were assessed for resting blood pressure, adequate imaging windows and LV length. During visit 2, baseline echocardiographic images were collected following 15 minutes of quiet rest. Then, participants performed 3 minutes of isometric handgrip exercise, after which echocardiographic images were collected during the post-exercise ischemic (PEI) period. Participants were then administered bisprolol, and a final set of images were collected 2.5 hours later. To minimize differences in relative hormone levels and fluid shifts in the second visit, females who were not using combined oral contraceptives were tested in the early follicular phase of their menstrual cycles (days 3-6), and females using combined oral contraceptives were tested during the placebo or pill-free interval.

Specific methodology

Isometric handgrip and post-exercise ischemia. Participants performed three maximal handgrip efforts using their right hand to determine maximal voluntary contraction (MVC), with each trial separated by at least one minute. An inflatable cuff was placed around the upper right arm, and participants performed isometric handgrip exercise at 35% MVC for 3 minutes, followed by 3-5 minutes of PEI to isolate the muscle metaboreflex (Mark *et al.*, 1985). PEI was achieved by inflating the cuff to suprasystolic pressures (240 mmHg) ten seconds prior to handgrip release, and handgrip force was continuously recorded and displayed on a screen visible to the participant for visual feedback during the exercise. Collection of echocardiographic images began 30 seconds following cuff inflation, and the cuff was released when imaging was complete (within approximately 3 minutes of cuff inflation).

β_1-AR blockade. Following PEI, participants rested for >15 minutes, until blood pressure and HR had returned to resting values. Participants were administered an oral 5 mg dose of bisoprolol (β_1-AR antagonist), and returned to rest approximately 2.5 hours post-administration (time of peak plasma concentrations (Leopold, 1986)) and a final set of echocardiographic images were collected after 15 minutes of quiet rest. In the time between bisoprolol administration and imaging, participants remained seated in the laboratory, and refrained from the consumption of food, but were able to drink small quantities of water ad libitum.

Blood pressure and heart rate. Beat-to-beat blood pressure data were continually recorded during baseline, handgrip exercise and PEI using finger photoplethysmography (Finometer, Amsterdam, NL). Manual measurements of blood pressure were additionally taken immediately following echocardiographic imaging in each experimental phase. Heart rate was monitored using three-lead electrogradiography in all phases.

2D and triplane transthoracic echocardiography. Echocardiographic images were acquired with a commercially available ultrasound system (Vivid E9, GE, Fairfield, CT) using M5S 1.5-4.6 MHz and 4V 1.5-40 MHz transducers, and saved for offline analysis at a later date (EchoPAC v.113, GE, Fairfield, CT). All images were acquired by a single trained sonographer, with participants in the left lateral decubitus position, and at end-expiration for the assessment of LV structure global function and mechanics in accordance with current guidelines (Lang *et al.*, 2015). LV parasternal long-axis images were analyzed for intraventricular septal (IVST) and posterior wall thickness (PWT), and internal diameter at end-diastole ($LVID_d$) and end-systole ($LVID_s$). LV length at end-diastole ($LV\ length_d$) was determined as the mean length from the mitral plane to the

apical subendocardium in the apical two- and four-chamber views. Pulsed Doppler recordings were performed in the apical 4-chamber view, and analyzed for LV early (E) and atrial (A) diastolic inflow velocities. End-systolic volume (ESV), end-diastolic volume (EDV), SV and ejection fraction (EF) were determined using a modified Simpson's technique in triplane recordings of the apical 2-, 3- and 4-chamber views. All morphological, volume, and Doppler-derived data represent averages of three cardiac cycles. Relative wall thickness was calculated as $2 \cdot PWT/LVID_d$, and sphericity index was calculated as LV $length_d/LVID_d$. To account for sex-related differences LV morphology, LV dimensions and volumes were allometrically scaled to body surface area $(BSA)^{0.5}$ and $BSA^{1.5}$, respectively (Batterham *et al.*, 1997).

Images for speckle tracking analysis were acquired at a rate of 70-90 frames$\cdot s^{-1}$. Parasternal short-axis images were acquired at the base with leaflets of the mitral valve visible, for the assessment of basal rotation and circumferential strain. Parasternal short-axis images were acquired at the apex just proximal to end-systolic luminal obliteration (van Dalen *et al.*, 2008), for the assessment of apical rotation and circumferential strain. Apical 4-chamber images were analyzed for longitudinal strain.

Speckle tracking and torsional shear analysis. All analyses were performed by a single experienced sonographer who was blinded to the participant sex and the specific experimental condition. Analysis of LV rotation and strain parameters were performed using speckle tracking software (EchoPAC, GE Healthcare), and raw data were time-aligned and transformed (2D Strain Analysis Tool, Stuttgart, Germany), as previously described (Stöhr *et al.*, 2012; Stembridge *et al.*, 2014; Williams *et al.*, 2016). Images with inadequate tracking in ≥2 segments were excluded from analysis. Speckle-tracking data

represent averages across all myocardial segments, and averages of three cardiac cycles. Twist data were calculated by subtracting time-aligned basal data from apical data. Torsion was calculated as LV twist/length$_d$. Torsional shear angle was calculated as previously reported by Aelen et al. (Aelen *et al.*, 1997) as $((\phi_{apex} - \phi_{base})\ (r_{apex} + r_{base}))/2D$ where Φ is the rotation, r is the radius and D is LV length at end-systole. The coefficient of variation of the sonographer for LV twist was 9.2%, in agreement with previous reports (Stembridge *et al.*, 2015; Williams *et al.*, 2016).

LV hemodynamics. Cardiac output (Q) was calculated as SV·HR. Mean arterial pressure (MAP) was calculated as 1/3·systolic blood pressure (SBP)+2/3·diastolic blood pressure (DBP). Total peripheral resistance (TPR) was calculated as MAP/Q. End-systolic wall stress was estimated as surrogate for LV afterload, and calculated as 0.9·SBP ·(end-systolic cavity area/end-systolic myocardial area)(modified from (Haykowsky *et al.*, 2001)). End-systolic cavity area and myocardial area were calculated as $\pi\cdot(LVID_s/2)^2$ and $[\pi\cdot((PWT_s+LVID_s+IVST_s)/2)^2 - \pi\cdot(LVID_s/2)^2]$, respectively, under the assumption of a circular ventricular cavity just distal to the papillary muscles.

Statistical analysis and sample size calculation

Independent of analysis used, data are presented as mean ± standard deviation (SD) for clarity of interpretation. Normality of distribution was assessed using the Shapiro–Wilk test. For all dependent variables, normally distributed data were assessed using an independent *t*-test to detect differences between the sexes in each condition. A one-way repeated measures ANOVA was used to detect within-group differences, and a Fisher's least significant difference test was used to determine pairwise differences when

a positive effect was detected. When the normality test failed, a Mann-Whitney test was used to detect sex differences in each condition for nonparametric data. A Friedman one-way repeated measures ANOVA on ranks was also used to detect within-group differences, and the Wilcoxon matched pairs test was used to determine pairwise differences. All statistical analyses were performed using STATISTICA (version 8.0; StatSoft, Tulsa, OK) with α set *a priori* to 0.05.

Linear least-squares regression was used to assess the relationships of LV twist mechanics with LV structure and geometry, and LV volumes in both sexes (inclusive of data from baseline, PEI and β_1-AR blockade). Regression was additionally used to assess the relationship between LV twist and untwisting velocity. Pearson correlation and Spearman rank correlation were used to assess the relationships for normally distributed and nonparametric data, respectively. For clarity of interpretation, all correlation coefficients are presented as r. When a significant relationship was detected in both sexes, slopes of the regression were compared using the Extra Sum of Squares test.

No previous studies have investigated sex differences in LV twist with altered adrenergic stimulation, however previous work from Dedobbeleer et al. (Dedobbeleer *et al.*, 2013) reported a standard deviation (SD) of 2.3° in twist during β_1-AR blockade. Utilizing this SD and an α=0.05, it was determined that 20 participants per group would allow us to detect a difference of 2.0º in LV twist between the sexes with a β=0.80.

Results

Baseline characteristics, LV structure and hemodynamics

Baseline characteristics are summarized in Table 1. MVC and thus 35% MVC were greater in males (199±52N) than females (132±34N; $p<0.001$ for both)(Table 1). Males had larger BMI ($p=0.045$) and BSA ($p<0.001$) than females. As per the study design, LV $length_d$ was not different between the sexes ($p=0.163$). Despite the matching of LV $length_d$ between the sexes, $LVID_d$ was larger in males ($p<0.001$), resulting in sex differences in sphericity index ($p=0.005$). However, males had larger LV volumes ($p<0.001$) and SV ($p=0.017$) than females (Table 3), but allometrically scaled EDV and SV did not differ between the sexes at baseline. In contrast, scaled ESV was smaller in females at baseline ($p=0.034$), reflective of a greater EF in females ($p=0.001$). Blood pressure and HR did not differ between the sexes. There were additionally no baseline sex differences in relative wall thickness, or in scaled $LVID_d$, PWT, IVST. E was greater in females (F=0.94±0.15m·s^{-1}, M=0.82±0.14m·s^{-1}, $p=0.01$), however A (F=0.38±0.07m·s^{-1}, M=0.39±0.11m·s^{-1}), and E/A (F=2.58±0.70, M=2.31±0.76) did not differ between the sexes.

LV mechanics in response to altered adrenergic stimulation

Table 2 summarizes peak LV mechanics parameters. At baseline, there were no sex differences in LV twist mechanics (twist, torsion, apical rotation, basal rotation and untwisting velocity). However, circumferential strain at the base and longitudinal strain were higher in females compared to males ($p=0.025$ and $p=0.015$, respectively).

Post exercise ischemia. There were no changes from baseline in LV apical rotation, twist, untwisting velocity (Figure 1) or strain in either group. However, basal rotation was increased in males (p=0.037). Nonetheless, there were no sex differences in twist during PEI. Longitudinal strain remained higher in females, although circumferential strain at the base was not different between the sexes, and circumferential strain at the apex tended to be higher in females (p=0.055). There was also no difference between the sexes for torsional shear.

β_1-AR blockade. In females, LV twist mechanics did not differ from baseline, although there was a trend to reduced LV twist (p=0.063). In males, LV twist and torsion were reduced (p=0.029 and p=0.032, respectively), due to a significant reduction to apical rotation (p=0.036) and a trend to reduction in basal rotation (p=0.09)(Figure 1). Untwisting velocity also tended to be reduced in males compared to baseline (p=0.075). As a result, males had lower LV apical rotation (p=0.007), twist (p=0.008) and torsion (p=0.004), and slower untwisting velocity compared to females (p=0.046) after bisoprolol. LV strain parameters were not changed from baseline, such that longitudinal strain (p<0.001) and circumferential strain at the base (p=0.02) remained higher in females. Torsional shear was significantly reduced in males compared to females (p=0.022) but there were no sex differences in basal rotation or apical circumferential strain during β_1-AR blockade.

Hemodynamic responses to altered adrenergic stimulation

Post exercise ischemia. Blood pressure increased from baseline in both groups (p<0.001 for both), and SBP (p=0.007), DBP (p=0.031) and MAP (p=0.006) were greater

296 in males. HR increased in males ($p=0.022$) and tended to increase in females ($p=0.08$),
297 however HR was not different between the sexes. LVEDV increased ($p<0.001$ for both)
298 but ESV was unchanged, resulting in an augmentation of both LVSV and Q in both sexes
299 ($p<0.001$). There were no sex differences in scaled LVEDV or SV, but scaled ESV
300 tended to be lower in females ($p=0.08$). Thus, while EF was increased in males ($p<0.001$)
301 and tended to increase in females ($p=0.07$), EF remained greater in females compared to
302 males ($p=0.025$). End-systolic wall stress increased in both sexes ($p<0.001$), and was
303 greater in males compared to females ($p=0.013$). Although TPR increased in males
304 ($p=0.014$), it was unchanged in females and not different between the sexes. E increased
305 in males ($0.87\pm0.19 m \cdot s^{-1}$, $p=0.012$), and A increased in both sexes during PEI
306 ($F=0.44\pm0.17 m \cdot s^{-1}$, $M=0.42\pm0.10 m \cdot s^{-1}$, $p<0.05$). E/A, however, was unchanged and there
307 were no sex differences in these parameters.

308 *β_1-AR blockade*. Blood pressure, HR and Q were reduced from baseline in both
309 groups ($p<0.001$). Both DBP ($p=0.012$) and MAP ($p=0.002$) were higher in males.
310 However, the reduction to HR was greater in females (-12±6 bpm) compared to males (-
311 8±5 bpm, $p=0.023$). LV volumes and EF were not different from baseline in either group.
312 Similar to baseline, scaled LVEDV and SV did not differ between the sexes, but scaled
313 ESV was smaller ($p=0.01$) and EF was greater ($p<0.001$) in females. End-systolic wall
314 stress was reduced in both groups ($p<0.001$ for both), but was not different between the
315 sexes. TPR was unchanged and did not differ between the sexes. E was unchanged from
316 baseline in both sexes, however A was reduced in females ($0.31\pm0.06 m \cdot s^{-1}$, $p<0.001$) but
317 not in males ($0.35\pm0.10 m \cdot s^{-1}$). Thus, E/A was increased in females (2.99 ± 0.16, $p<0.001$)
318 but not males (2.56 ± 0.87, $p=0.072$).

319

320 *LV structure and geometry during altered adrenergic stimulation*

321 There were no changes from baseline in absolute or scaled wall thicknesses and
322 $LVID_d$ in either sex, during any stage. IVST ($p<0.05$) and $LVID_d$ ($p<0.001$) were larger
323 in males during all stages, and PWT was larger in males ($p<0.05$) except during β_1-AR
324 blockade ($p=0.096$). Nonetheless, scaled wall thicknesses and scaled $LVID_d$ were not
325 different between the sexes in any stage. Relative wall thickness and sphericity index
326 were also unchanged in both sexes, during either intervention. Relative wall thickness did
327 not differ between the sexes, however sphericity index was greater in females at baseline
328 ($p=0.005$), during PEI ($p=0.007$) and β_1-AR blockade ($p=0.003$). LV $length_d$ increased in
329 males ($p<0.001$) and tended to increase in females ($p=0.07$) during PEI, but was
330 unchanged during β_1-AR blockade in either sex. LV $length_d$ was not different between the
331 sexes during either intervention, but tended to be smaller in females during β_1-AR
332 blockade ($p=0.052$).

333 *Relationships of LV mechanics with structure and geometry*. There was a
334 significant relationship between LV twist and untwisting velocity in both males ($r=-0.58$,
335 $p<0.001$) and females ($r=-0.57$, $p<0.001$), and this was not different between the sexes. In
336 females, there was a significant relationship for $LVID_d$ with LV apical rotation ($r=-0.30$,
337 $p=0.02$), and twist ($r=-0.35$, $p=0.013$)(Figure 2). Additionally, there were significant
338 relationships for sphericity index with apical rotation ($r=0.32$, $p=0.019$) and twist ($r=0.35$,
339 $p=0.012$), in females but not males. There were no relationships for LV $length_d$ with twist
340 or rotation in either group. No relationships between LVEDV or SV with LV apical
341 rotation, basal rotation or twist were observed for either sex.

Discussion

This is the first study to compare LV mechanics between males and females, matched for LV $length_d$, during altered adrenergic stimulation. In support of our hypothesis, females had greater LV twist and faster untwisting velocity than males during $ß_1$-AR blockade. However, in contrast, no sex differences in LV twist mechanics were observed with increased adrenergic stimulation during PEI.

Effects of post exercise ischemia on sex differences in LV mechanics

In the current study, PEI was used to activate the muscle metaboreflex, and effectively increase adrenergic stimulation independently of increases to HR (O'Leary, 1993; Nishiyasu *et al.*, 1994). During PEI, LVSV was increased in both males and females. However, contrary to our hypothesis, LV twist was not different from baseline or between the sexes. This occurred despite a small but significant increase to basal rotation in males during PEI. The increases to LVSV in our study are in agreement with Crisafulli et al. (Crisafulli *et al.*, 2003; 2006) who have demonstrated that SV increases to ~130% of baseline during PEI in an all male cohort. In females, Shoemaker et al. (Shoemaker *et al.*, 2007) have also reported a trend of elevated SV during PEI. In the current study, the elevations to LVSV resulted from increases to EDV, while ESV was unchanged, suggesting that the increases to LV contractility were enough to offset the pronounced increases in afterload as indicated by the elevated systolic wall stress. Both groups had increases to A filling velocity, therefore increased atrial contraction and filling potentially contributed to increasing EDV. Increases to central venous pressure

365 also occur during PEI (Shoemaker *et al.*, 2007; Marongiu *et al.*, 2013), and likely
366 increased venous return thus explaining the higher LVEDV in the current study.
367 Given that increases to adrenergic stimulation and LV preload can each
368 independently increase LV twist, the concomitant increases to LVEDV and SV during
369 PEI would be expected to accompany increases to LV twist. While LVEDV and SV were
370 increased in this study, neither males nor females had alterations to LV apical rotation or
371 twist during PEI. Given that increases to afterload reduce LV twist, especially at the apex
372 (Gibbons Kroeker *et al.*, 1995; Dong *et al.*, 1999; Weiner *et al.*, 2012), the increased end-
373 systolic wall stress during PEI may have countered any potential increases to apical
374 rotation and thus LV twist in both groups. Finally, although increases to LV preload are
375 reported to augment LV twist mechanics (Weiner *et al.*, 2010*b*), the increases to LVEDV
376 of ~5-7 mL in the current study likely were not enough to increase LV twist. This is
377 supported by prior investigations from our group (Williams *et al.*, 2016) and others
378 (Burns *et al.*, 2010) in which small increases (~10 mL) to LVEDV and LVSV did
379 produce significant alterations to LV twist.
380 While twist was not significantly altered in either sex during PEI, males did have
381 a small but significant increase to basal rotation, but this did not result in significant sex
382 differences in LV rotation or twist. Nonetheless, the increased basal rotation in males
383 could provide some evidence that the responses of LV mechanics may differ between the
384 sexes with increased adrenergic stimulation. The increases to basal rotation but not apical
385 rotation in males may reflect greater receptor sensitivity at the base. However, this seems
386 unlikely as greater receptor densities and augmented responsiveness to adrenergic
387 stimulation have been demonstrated at the apex compared to the base (Mori *et al.*, 1993;

388 Akagawa *et al.*, 2007). Additionally, while we theorized that males would have a larger
389 increase in apical rotation and thus twist than females, it is possible that this effect was
390 countered by the significantly greater LV afterload (as determined by end-systolic wall
391 stress) observed in males during PEI.

392 Recently, Balmain et al. (Balmain *et al.*, 2016) used PEI in an attempt to
393 discriminate between the contributions of increased afterload and chronotropy that occur
394 during static handgrip exercise. In contrast to our findings, they observed reductions to
395 LV apical rotation, twist and untwisting velocity during PEI, without changes to LVEDV,
396 ESV and SV. While the authors proposed that large increases to LV afterload attenuated
397 LV twist, the hemodynamic data aren't entirely consistent with increased afterload, given
398 that increases to LVESV and reductions to SV would be expected to occur when EDV is
399 unchanged. The reduction to LV twist is thus surprising given that increases to
400 sympathetic activation and LV contractility occur during PEI (Victor *et al.*, 1988;
401 Crisafulli *et al.*, 2006). In the current study, the increase to EF in males and the lack of
402 change to ESV in both sexes suggests that an increase in LV contractility maintained LV
403 twist and offset the increased LV afterload.
404
405 *Effects of ß$_1$-AR blockade on sex differences in LV mechanics*

406 The reduced LV twist mechanics in males compared to females during ß$_1$-AR
407 blockade predominantly resulted from reductions to LV apical rotation in males, whereas
408 LV rotation and twist were unchanged in females. The lower LV apical rotation, twist
409 and untwisting velocity in males during ß$_1$-AR blockade provide preliminary evidence for
410 sex-related differences in LV adrenergic control of LV twist mechanics, specifically

411 during reductions to adrenergic stimulation. Studies using HRV consistently report
412 greater low frequency power and low-to-high frequency ratios in males, compared to
413 females (Ryan *et al.*, 1994; Gregoire *et al.*, 1996; Kuo *et al.*, 1999; Barantke *et al.*, 2008)
414 suggesting that males are more sympathetically mediated than their female counterparts.
415 Our data support the contention that males are more sympathetically mediated as
416 reductions to adrenergic stimulation during $ß_1$-AR blockade resulted in significant
417 reductions to LV twist in males but not in females. The finding that torsional shear
418 (which controls for LV length and radius) is also reduced in males compared to females
419 suggests that sex differences in LV twist mechanics are mediated, in part, by mechanisms
420 independent of LV geometry. It is plausible that differences in the adrenergic control of
421 myocardial contractility might exist, whereby males might have greater $ß_1$-AR densities
422 at the apex compared to females, resulting in greater reductions to myofiber shortening in
423 comparison to females.

424 It has been proposed that changes to HR coincide with similar alterations to
425 contractility and LV twist mechanics (Hodt *et al.*, 2011). However, our data do not
426 support this mechanistic link between HR and twist in females, as they experienced a
427 greater reduction to HR without a significant reduction to LV twist. As both HR and twist
428 were reduced in males, this suggests that altered adrenergic stimulation may affect
429 chronotropy and twist differently between the sexes. This postulate is partially supported
430 by previous work that demonstrated a greater increase in HR with a $ß_1$-AR agonist in
431 females, but a greater increase to an index of contractility in males (Convertino, 1998;
432 Turner *et al.*, 1999). Likewise, data from Evans et al. (Evans *et al.*, 2001) reported
433 potentially greater reductions to HR in females than males during $ß_1$-AR blockade with

propranolol. Collectively, these data suggest that females have greater chronotropic responses to alterations in adrenergic stimulation whereas males may have greater inotropic responses. Thus, in the current study, it is possible that β_1-AR blockade reduced LV contractility in males and contributed to the attenuated LV twist mechanics compared to females, whereas females had greater reductions to HR but no alterations to LV twist.

Relationships between LV twist mechanics and chamber geometry

To our knowledge, this is the first study to match LV $length_d$ between the sexes, rather than scaling or indexing to LV dimensions or body size. First, we have demonstrated that for the same LV $length_d$, females have a smaller $LVID_d$ than males, resulting in a greater sphericity index, or a greater LV ellipsoid geometry compared to males. As a result, males have greater LV volumes than females for the same LV $length_d$. Second, we did not observe any associations between LV $length_d$, EDV or SV with apical rotation, basal rotation or twist in either sex. This confirms that sex differences in LV twist mechanics are likely not fundamentally determined by differences in LV size or volume. However, there was a negative relationship between $LVID_d$ and twist ($r=-0.35$, $p=0.013$), as well as a positive relationship for sphericity index with LV apical rotation ($r=0.32$, $p=0.019$) and twist ($r=0.35$, $p=0.012$) in females. In contrast, there were no relationships observed for LV structure or geometry with LV twist mechanics in males. Combined with the observed sex differences in LV twist during β_1-AR blockade, these data suggest that LV twist may be more sensitive to LV structure and geometry in females, but more sensitive to altered adrenergic stimulation in males.

We have previously demonstrated that females have greater LV twist and sphericity index than males during significant reductions to preload utilizing LBNP, despite similar relative reductions to LV volumes in both sexes (Williams *et al.*, 2016). In connection with the current findings, these sex differences may reflect a greater influence of LV geometry on twist in females. Given that LV deformation is primarily determined by interactions between myofibre layers (Rademakers *et al.*, 1994), alterations to LV shape and thus myofibre alignment can directly alter fibre mechanics and twist in various regions of the LV wall (Choi *et al.*, 2011). Compared to a spherical ventricle, a more ellipsoid shape favours increased active fibre shortening and ejection performance (Choi *et al.*, 2011). To that effect, LV sphericity index has been identified as a strong independent predictor of LV rotation and twist (Dalen *et al.*, 2010). Therefore, the observed sex differences in LV sphericity index in this study and our previous work (Williams et al., 2016) suggest that sex differences in LV fibre alignment may occur for a given LV $length_d$. This is supported by correlative data from this study that suggest LV twist mechanics may be more influenced by LV geometry in females compared to males. Intrinsic sex-related differences in myocardial structure and geometry could potentially contribute to sex differences in the dynamic responses of LV twist mechanics to acute stress.

Limitations

An important limitation to this study was that we observed no increase in LV twist in either sex during PEI and were subsequently unable to investigate whether sex differences in LV twist occur with increased adrenergic stimulation. As blood pressure,

LVSV and EF increased with PEI, we are confident that this intervention augmented adrenergic stimulation and the unaltered LV twist was likely due to the concomitant increases to LV afterload. Future studies should consider administrating pharmacological ß$_1$-AR agonists (i.e. isoproterenol, dobutamine) to effectively augment LV twist (Moon *et al.*, 1994; Akagawa *et al.*, 2007) and to further examine whether sex differences in LV mechanics exist with increased adrenergic stimulation.

A limitation of measuring torsional shear using echocardiography is that the distance between measurement sites for basal and apical rotation cannot be accurately determined. As such we have measured LV length as the distance between the MV leaflets and apical endocardium. While this may underestimate torsional shear, this would be consistent for males and females, so we believe the significant sex differences observed in the present study are real.

Conclusion

In males and females matched for LV length$_d$, differences in LV twist mechanics occur during reductions to adrenergic stimulation. Females have greater LV apical rotation, twist, untwisting velocity and torsional shear than males during ß$_1$-AR blockade. The reductions to apical rotation and twist in males are suggestive of greater sympathetic-related adrenergic control of LV twist mechanics compared to females. Although sex differences in LV twist were not observed during increases to adrenergic stimulation with PEI, potentially greater increases to LV twist in males may have been countered by larger increases to afterload. In addition, the matching of LV length$_d$ has revealed marked sex differences in LV chamber geometry, which may contribute to differences in the

responses of LV twist to altered loading and adrenergic stimulation. Altogether, our data provide preliminary evidence that LV twist be more sensitive to alterations in adrenergic stimulation in males, but influenced to a greater extent by LV geometry in females.

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Additional Information

Competing interests. None declared.

Author contributions. All data collection and analysis were completed at the Center of Heart, Lung and Vascular Health, at The University of British Columbia's Okanagan Campus. A.M.W. contributed to the conception and design of the study, data collection, analysis, interpretation of the data and drafting of the manuscript. N.D.E. contributed to the conception and design of the study, analysis and interpretation of the data and drafting of the manuscript. R.E.S. contributed to the conception and design of the study, interpretation of the data and critical revision of the manuscript. W.S.C. contributed to the analysis and interpretation of the data and drafting of the manuscript. All authors have approved the final version of the manuscript, agree to be accountable for all aspects of the work, and qualify for authorship of the manuscript.

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706 **Tables**

707 Table 1. Baseline characteristics, LV hemodynamics, structure and geometry

	Males (*n=20*)	Females (*n=20*)
Participant characteristics		
Age (yr)	23 (5)	22 (3)
Height (m)	1.77 (0.05)	1.66 (0.07) #
Weight (kg)	72.4 (6.4)	60.3 (6.4) #
BMI ($kg \cdot m^{-2}$)	23.0 (2.0)	21.8 (1.5) *
BSA (m^2)	1.89 (0.10)	1.67 (0.12) #
MVC (N)	571 (150)	377 (98) #
Resting hemodynamics		
HR (bpm)	60 (10)	62 (8)
SBP (mmHg)	120 (8)	115 (9)
DBP (mmHg)	73 (9)	70 (8)
MAP (mmHg)	88 (8)	85 (7)
EF (%)	55 (3)	58 (3) #
Resting LV structure and geometry		
$Length_d$ (cm)	8.45 (0.45)	8.22 (0.55)
$Length_d \cdot BSA^{-0.5}$ ($cm \cdot m^{-1}$)	6.15 (0.29)	6.37 (0.38) #
$LVID_d$ (mm)	45.1 (3.2)	40.9 (3.1) #
$LVID_d \cdot BSA^{-0.5}$ ($mm \cdot m^{-1}$)	32.8 (2.2)	31.7 (2.2)
Sphericity index	1.88 (0.13)	2.02 (0.16) #
Relative wall thickness	0.45 (0.06)	0.45 (0.07)

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710 Values are means (SD). BMI: body mass index; BSA: body surface area; MVC: maximal

711 voluntary contraction; HR: heart rate; SBP: systolic blood pressure; DBP: diastolic blood

712 pressure; MAP: mean arterial pressure; EF: ejection fraction; $Length_d$: end-diastolic

713 length; $LVID_d$: left ventricular internal diameter during diastole. *$p<0.05$ vs. males.

714 #$p<0.01$ vs. males.

Table 2. LV mechanics during altered adrenergic stimulation.

		Baseline	*Post Exercise Ischemia*	*β_1-AR Blockade*
Twist (˚)	*M*	10.2 (2.5)	11.1 (3.2)	8.6 (1.9) †
	F	11.3 (3.1)	11.3 (2.4)	10.7 (2.8) *
Torsion (˚·cm^{-1})	*M*	1.20 (0.30)	1.28 (0.36)	1.02 (0.23) †
	F	1.38 (0.38)	1.37 (0.30)	1.33 (0.38) #
Untwisting velocity (˚·s^{-1})	*M*	-80.3 (25.3)	-77.6 (22.0)	-68.2 (22.1)
	F	-93.5 (22.6)	-79.4 (28.5)	-82.0 (18.7) *
Apical rot (˚)	*M*	7.8 (1.7)	8.4 (3.3)	6.8 (2.1) †
	F	8.7 (2.5)	8.9 (2.3)	8.8 (2.3) *
Basal rot (˚)	*M*	-3.1 (1.8)	-3.8 (1.9)†	-2.5 (1.1)
	F	-3.3 (2.0)	-3.3 (2.3)	-2.4 (1.7)
Longitudinal strain (%)	*M*	-17.5 (1.9)	-17.0 (1.7)	-17.2 (1.6)
	F	-19.0 (1.7) *	-19.5 (1.5) #	-19.0 (1.6) #
Circumferential strain, base (%)	*M*	-20.3 (3.3)	-20.2 (3.9)	20.2 (2.5)
	F	-22.3 (2.1) *	-22.0 (3.0)	-22.3 (3.0) *
Circumferential strain, apex (%)	*M*	-26.1 (3.7)	-25.5 (3.5)	-25.7 (2.5)
	F	-25.5 (3.4)	-27.6 (2.7)	-26.0 (2.7)
Torsional Shear (˚)	*M*	1.92 (0.50)	2.09 (0.54)	1.62 (0.37) †
	F	2.03 (0.55)	2.02 (0.44)	1.91 (0.38) *

Values are means (SD). All data represent peaks across the cardiac cycle. M: males; F: females; Rot: rotation. *n*=20 females, 20 males for all measures but apical rotation (female *n=19*), basal rotation (female *n=19*), twist and torsion (female *n*=18), torsional shear (female *n*=18). *p<0.05 vs. males. #p<0.01 vs. males. †p<0.05 vs. baseline. ‡p<0.01 vs. baseline.

Table 3. LV hemodynamics during altered adrenergic stimulation.

		Baseline	*Post Exercise Ischemia*	*β_1-AR Blockade*
HR (bpm)	*M*	60 (10)	63 (10) †	52 (9) ‡
	F	62 (8)	65 (11)	50 (8) ‡
MAP (mmHg)	*M*	88 (8)	116 (10) ‡	81 (8) ‡
	F	85 (7)	107 (10) ‡#	75 (11) ‡#
SBP (mmHg)	*M*	120 (8)	160 (13) ‡	109 (9) ‡
	F	115 (9)	145 (17) ‡#	104 (11) ‡
DBP (mmHg)	*M*	73 (9)	95 (11) ‡	67 (9) ‡
	F	70 (8)	88 (9) ‡*	61 (12) ‡*
EF (%)	*M*	55 (3)	58 (4) ‡	55 (3)
	F	58 (3) #	60 (3) *	60 (3) #
EDV (ml)	*M*	113 (20)	120 (24) ‡	115 (19)
	F	91 (14) #	96 (16) ‡#	92 (13) #
EDV ($ml \cdot m^{-3}$)	*M*	43 (7)	46 (8) ‡	44 (7)
	F	42 (6)	45 (6) ‡	43 (6)
ESV (ml)	*M*	51 (10)	51 (12)	51 (9)
	F	38 (6) #	38 (7) #	37 (5) #
ESV ($ml \cdot m^{-3}$)	*M*	20 (3)	20 (4)	20 (3)
	F	18 (3) *	18 (2)	17 (3)*
SV (ml)	*M*	62 (12)	70 (14) ‡	63 (12)
	F	53 (9) *	58 (10) ‡#	55 (9) #
SV ($ml \cdot m^{-3}$)	*M*	24 (4)	27 (5) ‡	24 (4)
	F	25 (4)	27 (4) ‡	25 (4)
Q ($L \cdot min^{-1}$)	*M*	3.66 (0.70)	4.30 (0.62) ‡	3.26 (0.67) ‡
	F	3.25 (0.41) *	3.68 (0.50) ‡#	2.69 (0.45) ‡#
Q ($L \cdot min^{-1} \cdot m^{-3}$)	*M*	1.41 (0.24)	1.66 (0.26) ‡	1.26 (0.26) ‡
	F	1.52 (0.24)	1.73 (0.32) ‡	1.26 (0.22) ‡

TPR ($mmHg \cdot L^{-1} \cdot min^{-1}$)	*M*	25.1 (5.8)	27.6 (4.3)	26.2 (5.7)
	F	26.7 (4.9)	29.5 (4.9) *	28.8 (7.1)
End-systolic wall stress ($kilodyne \cdot cm^{-2}$)	*M*	39.1 (5.3)	54.7 (8.4) ‡	33.5 (6.9) ‡
	F	37.2 (4.4)	48.5 (5.2) ‡*	32.8 (6.0) ‡

Values are means (SD). EDV: end-diastolic volume; ESV: end-systolic volume; SV: stroke volume; Q: cardiac output; TPR: total peripheral resistance. See Table 1 and 2 for additional abbreviations. *p<0.05 vs. males. #p<0.01 vs. males. †p<0.05 vs. baseline. ‡p<0.01 vs. baseline.

Table 4. LV structure and geometry during altered adrenergic stimulation.

		Baseline	*Post Exercise Ischemia*	*β_1-AR Blockade*
$Length_d$ (mm)	*M*	84.5 (4.5)	85.7 (4.8) ‡	84.7 (4.7)
	F	82.2 (5.5)	83.0 (5.3)	81.6 (5.2)
$LVID_d$ (mm)	*M*	45.1 (3.2)	45.3 (2.7)	45.5 (3.3)
	F	40.9 (3.1) #	40.8 (3.9) #	40.3 (2.8) #
Sphericity index	*M*	1.88 (0.13)	1.89 (0.12)	1.88 (0.13)
	F	2.02 (0.16) #	2.05 (0.21) #	2.03 (0.17) #
Relative wall thickness	*M*	0.45 (0.06)	0.44 (0.06)	0.45 (0.07)
	F	0.45 (0.07)	0.45 (0.07)	0.48 (0.06)

Values are means (SD). See Table 1 for abbreviations. *p<0.05 vs. males. #p<0.01 vs. males. †p<0.05 vs. baseline. ‡p<0.01 vs. baseline.

Figure legends

Figure 1. Graphical representation of mean left ventricular (LV) twist mechanics at baseline, during post exercise ischemia and $ß_1$-AR blockade (bisoprolol). Blue and red lines represent mean data for males and females, respectively. Top: dotted and dashed lines represent rotations of the LV apex and base, respectively. Middle: solid lines represent LV twist. Bottom: solid lines represent twist and untwisting velocities. SD are provided in Table 2. *$p<0.05$ males vs. females.

Figure 2. Relationships for LV twist mechanics with chamber structure and geometry. Data include measures during baseline, post exercise ischemia and $ß_1$-AR blockade. Blue and red represent data for males and females, respectively. Top: closed circles represent LV twist. Bottom: open triangles and circles represent LV rotation at the apex and base, respectively. *Significant relationship ($p<0.05$).

Figure 1

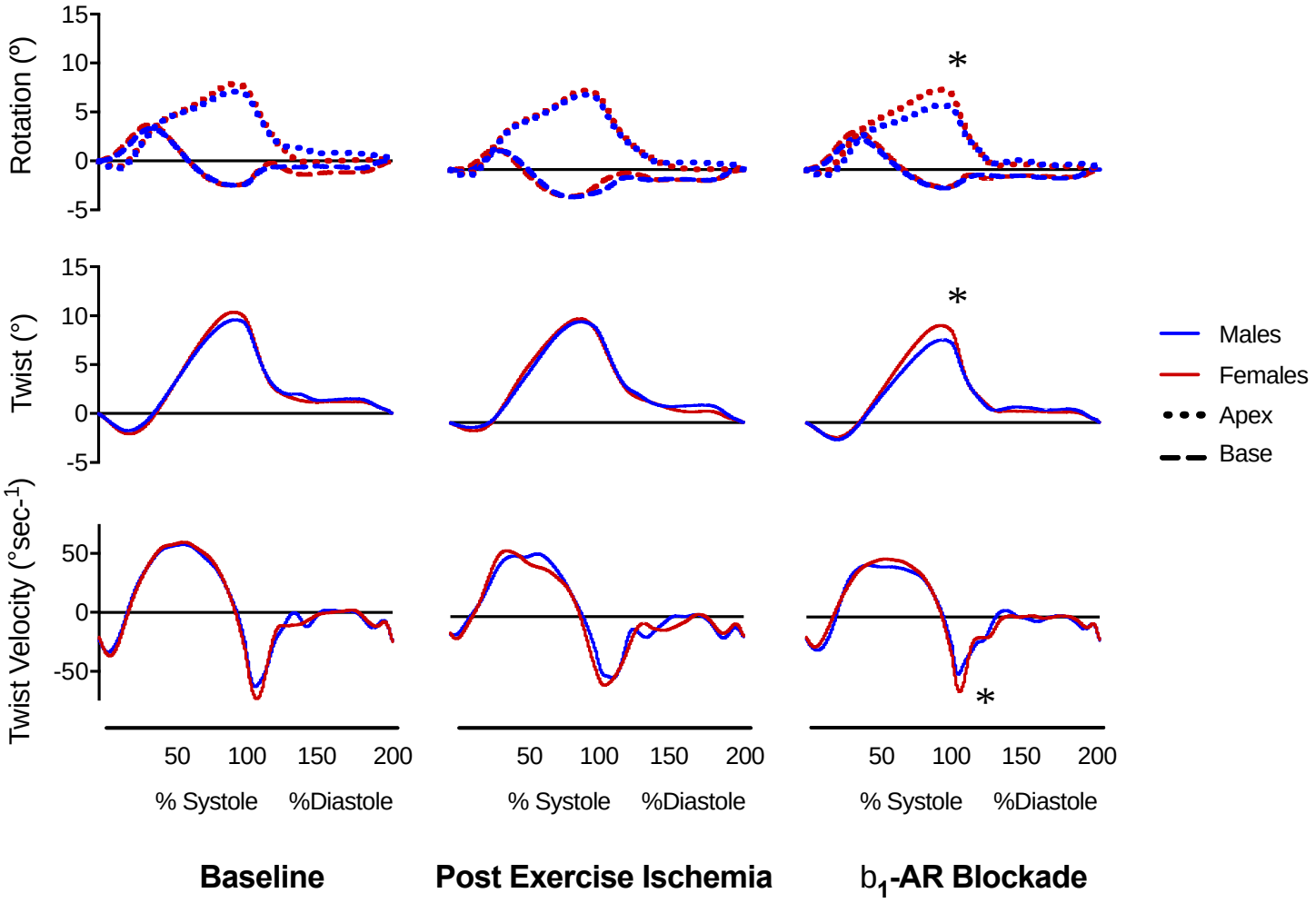
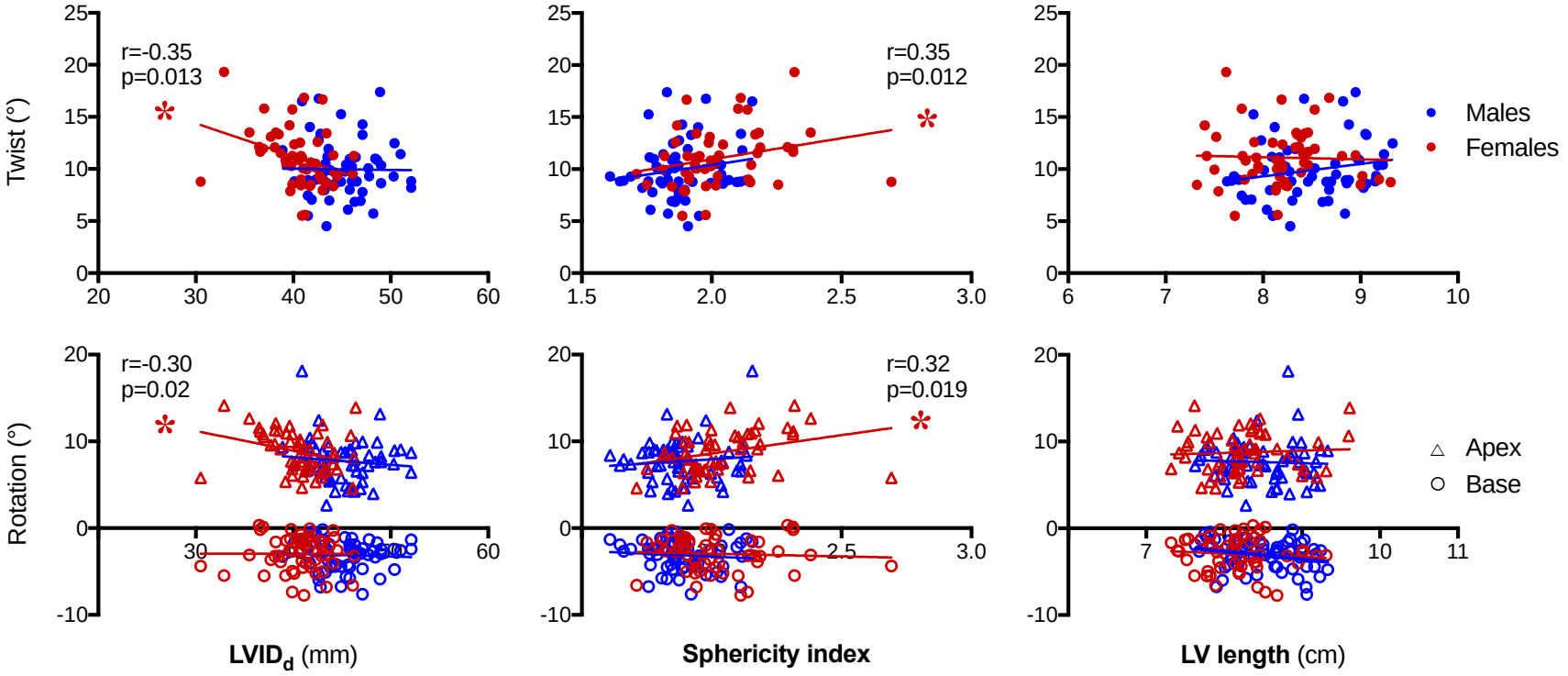

Rotation (°)
15
10
5
0
-5
Twist (°)
15
10
5
0
-5
Twist Velocity (°sec-1)
50
0
-50
*
*
*
50
100
150
200
% Systole
%Diastole
Baseline
Post Exercise Ischemia
b1-AR Blockade
Males
Females
Apex
Base

Figure 2


Twist (°)
Rotation (°)
r=-0.35
p=0.013
r=0.35
p=0.012
r=-0.30
p=0.02
r=0.32
p=0.019
Males
Females
Apex
Base
LVID$_d$ (mm)
Sphericity index
LV length (cm)