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

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Key points

- 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 two-dimensional echocardiography and speckle tracking analysis, this study compared LV mechanics in males and females matched for LV length during post-exercise ischaemia (PEI) and β_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 β_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 and 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.

Abstract Sex differences in left ventricular (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 haemodynamics. Accordingly, this study aimed to investigate sex differences in LV mechanics with altered adrenergic stimulation achieved through post-handgrip-exercise ischaemia (PEI) and β_1-adrenergic receptor (AR) blockade. Twenty males (23 ± 5 years) and 20 females (22 ± 3 years) were specifically matched for LV length (males: 8.5 ± 0.5 cm, females: 8.2 ± 0.6 cm, $P = 0.163$), and two-dimensional speckle-tracking echocardiography was used to assess LV structure and function at baseline, during PEI and following administration of 5 mg bisoprolol (β_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 were not altered from baseline or different between the sexes; however, basal rotation increased in males ($P = 0.035$). During β_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 β_1-AR blockade but not females, resulting in lower apical rotation (males: 6.8 ± 2.1 deg, females: 8.8 ± 2.3 deg, $P = 0.007$) and twist (males: 8.6 ± 1.9 deg, females: 10.7 ± 2.8 deg, $P = 0.008$), and slower untwisting velocity (males: 68.2 ± 22.1 deg s^{-1}, females: 82.0 ± 18.7 deg s^{-1}, $P = 0.046$) compared to females. 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.

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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 ventriclular; 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 ischaemia; PWT, posterior wall thickness; $\dot{Q}$, cardiac output; SBP, systolic blood pressure; SV, stroke volume; TPR, total peripheral resistance.

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 an increased high frequency power component of heart rate variability (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 to be 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 ischaemia 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.

Study participants

Participants from the local university community, between the ages of 19 and 39 years were recruited for the study. Exclusion criteria included the following: a history of cardiovascular, respiratory, or musculoskeletal disease; a body mass index (BMI) greater than 30 kg m^{-2}; a resting blood pressure $\geq$ 140/90 or $<$ 110/60 mmHg and smoking (or smoking cessation $<$ 12 months). Given the potential influence of sex-related differences in LV size on LV twist mechanics, males and females were matched for LV length. More specifically, individuals were continually enrolled until a total of 20 males and 20 females were matched for LV length. Those that could not be matched for LV length (within $\pm$0.2 cm) to an individual of the opposite sex were excluded. To minimize the potential variability in

LV structure (Arbab-Zadeh *et al.* 2014; Weiner *et al.* 2015), mechanics (Baggish *et al.* 2008; Weiner *et al.* 2010*a*) and adrenergic control (Martin *et al.* 1991) associated with chronic endurance training, individuals performing $>$ 1 h of moderate-intensity training five times per week, or $\geq$ 3 bouts of high intensity training per week were also excluded from the study. Of the 21 males and 26 females enrolled, one male and two females were excluded in the first visit for poor imaging windows. Four females were further excluded at the conclusion of data collection, as a male participant matched for LV length was not enrolled in the study. A total of 20 males and 20 females completed the study and were included in the analysis.

Study design

Participants visited the laboratory on two separate occasions, and were asked to refrain from caffeine, exercise and alcohol for a minimum of 12 h prior to the first visit, and 24 h 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 min of quiet rest. Then, participants performed 3 min of isometric handgrip exercise, after which echocardiographic images were collected during the post-exercise ischaemic (PEI) period. Participants were then administered bisprolol, and a final set of images were collected 2.5 h 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 ischaemia. Participants performed three maximal handgrip efforts using their right hand to determine maximal voluntary contraction (MVC), with each trial separated by at least 1 min. An inflatable cuff was placed around the upper right arm, and participants performed isometric handgrip exercise at 35% MVC for 3 min, followed by 3–5 min of PEI to isolate the muscle metaboreflex (Mark *et al.* 1985). PEI was achieved by inflating the cuff to suprasystolic pressures (240 mmHg) 10 s 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 s following cuff inflation, and the cuff was released when imaging was complete (within approximately 3 min of cuff inflation).

β_1-AR blockade. Following PEI, participants rested for $>$ 15 min, until blood pressure and heart rate (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 h post-administration (time of peak plasma concentrations; Leopold, 1986) and a final set of echocardiographic images were collected after 15 min 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, Netherlands). 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, USA) using M5S 1.5–4.6 MHz and 4 V 1.5–40 MHz transducers, and saved for offline analysis at a later date (EchoPAC v.113, GE). 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 analysed for intraventricular septal thickness (IVST) and posterior wall thickness (PWT), and internal diameter at end-diastole ($LVID_d$) and internal diameter at 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 four-chamber view, and analysed 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 two-, three- and four-chamber views. All morphological, volume, and Doppler-derived data represent means of three cardiac cycles. Relative wall thickness was calculated as $2 \times PWT/LVID_d$, 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 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 analysed 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 two or more 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.* 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 haemodynamics. Cardiac output ($\dot{Q}$) 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/$\dot{Q}$. End-systolic wall stress was estimated as a surrogate for LV afterload, and calculated as 0.9SBP × (end-systolic cavity area/end-systolic myocardial area) (modified from Haykowsky *et al.* 2001). End-systolic cavity area and myocardial area were calculated as $\pi(LVID_s/2)^2$ and $\{\pi[(PWT_s + LVID_s + IVST_s)/2]^2 - \pi(LVID_s/2)^2\}$, 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 Student's *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 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 non-parametric data. Friedman's one-way repeated measures ANOVA on ranks was also used to detect within-group differences, and Wilcoxon's matched pairs test was used to determine pairwise differences. All statistical analyses were performed using Statistica (version 8.0; StatSoft, Tulsa, OK, USA) 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's correlation and Spearman's rank correlation were used to assess the relationships for normally distributed and non-parametric 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.* (2013) reported an SD of 2.3 deg in twist during β_1-AR blockade. Utilizing this SD and $\alpha = 0.05$, it was determined that 20 participants per group would allow us to detect a difference of 2.0 deg in LV twist between the sexes with $\beta = 0.80$.

Results

Baseline characteristics, LV structure and haemodynamics

Baseline characteristics are summarized in Table 1. MVC and thus 35% MVC were greater in males (199 ± 52 N) than females (132 ± 34 N; $P < 0.001$ 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 2), 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

Table 1. Baseline characteristics, LV haemodynamics, structure and geometry

	Males ($n = 20$)	Females ($n = 20$)
Participant characteristics		
Age (years)	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 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 haemodynamics		
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$_d$ × BSA$^{-0.5}$ (cm m^{-1})	6.15 (0.29)	6.37 (0.38)#
LVID$_d$ (mm)	45.1 (3.2)	40.9 (3.1)#
LVID$_d$ × BSA$^{-0.5}$ (mm 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)

Values are means (SD). BMI, body mass index; BSA, body surface area; DBP, diastolic blood pressure; EF, ejection fraction; HR, heart rate; Length$_d$, end-diastolic length; LVID$_d$, left ventricular internal diameter during diastole; MVC, maximal voluntary contraction; SBP, systolic blood pressure; MAP, mean arterial pressure. *$P < 0.05$ *vs.* males; #$P < 0.01$ *vs.* males.

wall thickness, or in scaled LVID$_d$, PWT or IVST. E was greater in females (females (F) = 0.94 ± 0.15 m s^{-1}, males (M) = 0.82 ± 0.14 m s^{-1}, $P = 0.01$); however, A ($F = 0.38 \pm 0.07$ m s^{-1}, $M = 0.39 \pm 0.11$ m s^{-1}) and E/A ($F = 2.58 \pm 0.70$, $M = 2.31 \pm 0.76$) did not differ between the sexes.

LV mechanics in response to altered adrenergic stimulation

Table 3 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 ischaemia. There were no changes from baseline in LV apical rotation, twist, untwisting velocity (Fig. 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 ($P = 0.09$) (Fig. 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 ($P = 0.046$) during β1-AR blockade. 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.

Haemodynamic responses to altered adrenergic stimulation

Post-exercise ischaemia. 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 in males (Table 2). HR increased in males ($P = 0.022$) and tended to increase in females ($P = 0.08$); however, HR was not different between the sexes. LVEDV increased ($P < 0.001$ for both) but ESV was unchanged, resulting in an augmentation of both LVSV and $\dot{Q}$ in both sexes ($P < 0.001$). There were no sex differences in scaled LVEDV or SV, but scaled ESV tended to be lower in females ($P = 0.08$). Thus, while EF was increased in males ($P < 0.001$) and tended to increase in females ($P = 0.07$), EF remained greater in females compared to males ($P = 0.025$). End-systolic wall stress increased in both sexes ($P < 0.001$), and was greater in males compared to females ($P = 0.013$). Although TPR increased in males ($P = 0.014$), it was unchanged in females and not different between the sexes. E increased in males (0.87 ± 0.19 m s^{-1}, $P = 0.012$), and A increased in both sexes during PEI ($F = 0.44 \pm 0.17$ m s^{-1}, $M = 0.42 \pm 0.10$ m s^{-1}, $P < 0.05$). E/A, however, was unchanged and there were no sex differences in these parameters.

β_1-AR blockade. Blood pressure, HR and $\dot{Q}$ were reduced from baseline in both groups ($P < 0.001$). Both DBP ($P = 0.012$) and MAP ($P = 0.002$) were higher in males. However, the reduction to HR was greater in females (−12 ± 6 bpm) compared to males (−8 ± 5 bpm,

Table 2. LV haemodynamics during altered adrenergic stimulation

		Baseline	Post-exercise ischaemia	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)$^{\ddagger,\#}$	75 (11)$^{\ddagger,\#}$
SBP (mmHg)	M	120 (8)	160 (13)‡	109 (9)‡
	F	115 (9)	145 (17)$^{\ddagger,\#}$	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)$^{\ddagger,\#}$	92 (13)$^{\#}$
EDV (ml 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 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)$^{\ddagger,\#}$	55 (9)$^{\#}$
SV (ml m^{-3})	M	24 (4)	27 (5)‡	24 (4)
	F	25 (4)	27 (4)‡	25 (4)
$\dot{Q}$ (l min^{-1})	M	3.66 (0.70)	4.30 (0.62)‡	3.26 (0.67)‡
	F	3.25 (0.41)*	3.68 (0.50)$^{\ddagger,\#}$	2.69 (0.45)$^{\ddagger,\#}$
$\dot{Q}$ (l min^{-1} 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 l^{-1} 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 (kdyn 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; $\dot{Q}$, cardiac output; SV, stroke volume; TPR, total peripheral resistance. See Tables 1 and 3 for additional abbreviations. $^{*}P < 0.05$ *vs.* males; $^{\#}P < 0.01$ *vs.* males; $^{\dagger}P < 0.05$ *vs.* baseline; $^{\ddagger}P < 0.01$ *vs.* baseline.

$P = 0.023$). LV volumes and EF were not different from baseline in either group. Similar to baseline, scaled LVEDV and SV did not differ between the sexes, but scaled ESV was smaller ($P = 0.01$) and EF was greater ($P < 0.001$) in females. End-systolic wall stress was reduced in both groups ($P < 0.001$ for both), but was not different between the sexes. TPR was unchanged and did not differ between the sexes. E was unchanged from baseline in both sexes; however, A was reduced in females (0.31 ± 0.06 m s^{-1}, $P < 0.001$) but not in males (0.35 ± 0.10 m s^{-1}). Thus, E/A was increased in females (2.99 ± 0.16, $P < 0.001$) but not males (2.56 ± 0.87, $P = 0.072$).

LV structure and geometry during altered adrenergic stimulation

There were no changes from baseline in absolute or scaled wall thicknesses and $LVID_d$ in either sex, during any stage (Table 4). IVST ($P < 0.05$) and $LVID_d$ ($P < 0.001$) were larger in males during all stages, and PWT was larger in males ($P < 0.05$) except during β_1-AR blockade ($P = 0.096$). Nonetheless, scaled wall thicknesses and scaled $LVID_d$ were not different between the sexes in any stage. Relative wall thickness and sphericity index were also unchanged in both sexes, during either intervention. Relative wall thickness did not differ between the sexes; however, sphericity index was greater in females at baseline ($P = 0.005$), during PEI ($P = 0.007$) and during β_1-AR blockade ($P = 0.003$). LV $length_d$ increased in males ($P < 0.001$) and tended to increase in females ($P = 0.07$) during PEI, but was unchanged during β_1-AR blockade in either sex. LV $length_d$ was not different between the sexes during either intervention, but tended to be smaller in females during β_1-AR blockade ($P = 0.052$).

Relationships of LV mechanics with structure and geometry. There was a significant relationship between LV twist and untwisting velocity in both males ($r = -0.58$,

Table 3. LV mechanics during altered adrenergic stimulation

		Baseline	Post-exercise ischaemia	β_1-AR blockade
Twist (deg)	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 (deg 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 (deg 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 rotation (deg)	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 rotation (deg)	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 (deg)	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. $n = 20$ 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; $^{\dagger}P < 0.05$ *vs.* baseline; $^{\ddagger}P < 0.01$ *vs.* baseline.

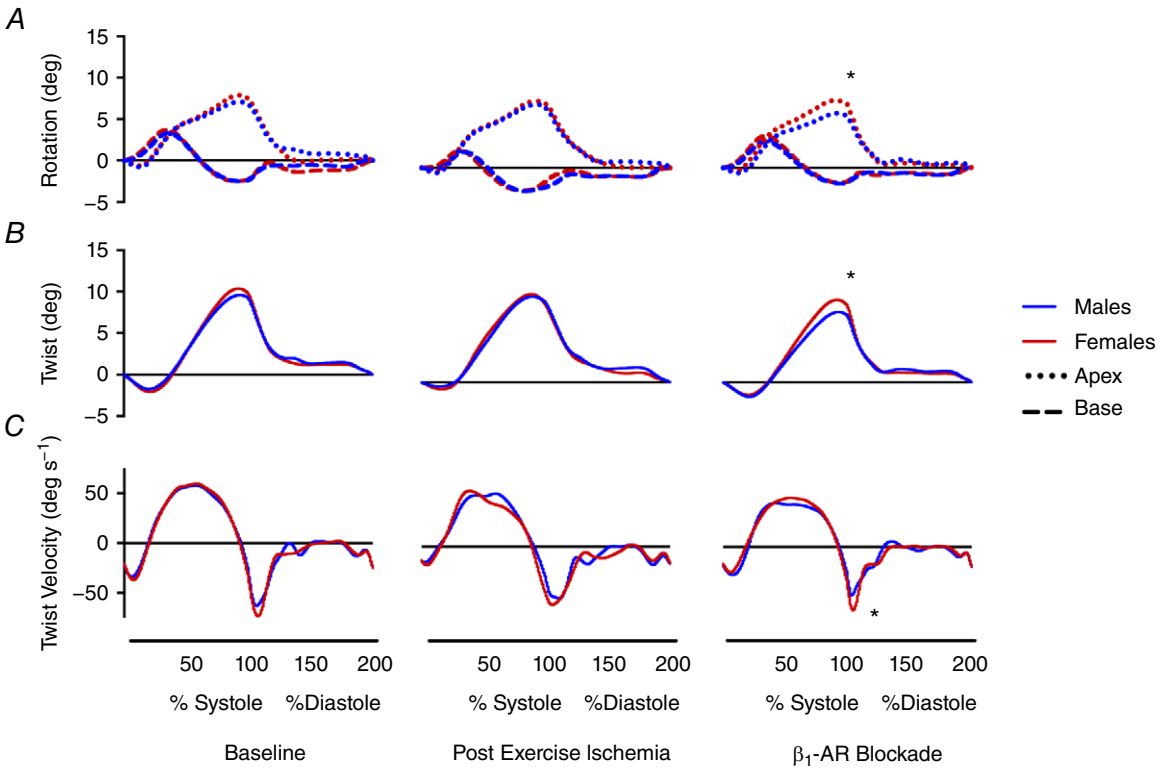

Figure 1. Graphical representation of mean left ventricular (LV) twist mechanics at baseline and during post-exercise ischaemia and β_1-AR blockade (bisoprolol)
Blue and red lines represent mean data for males and females, respectively. *A*, dotted and dashed lines represent rotations of the LV apex and base, respectively. *B*, continuous lines represent LV twist. *C*, continuous lines represent twist and untwisting velocities. SD values are provided in Table 3. $^{*}P < 0.05$ males *vs.* females. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 4. LV structure and geometry during altered adrenergic stimulation

		Baseline	Post-exercise ischaemia	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; $^{\dagger}P < 0.05$ *vs.* baseline; $^{\ddagger}P < 0.01$ *vs.* baseline.

$P < 0.001$) and females ($r = -0.57$, $P < 0.001$), and this was not different between the sexes. In females, there was a significant relationship for $LVID_d$ with LV apical rotation ($r = -0.30$, $P = 0.02$), and twist ($r = -0.35$, $P = 0.013$) (Fig. 2). Additionally, there were significant relationships for sphericity index with apical rotation ($r = 0.32$, $P = 0.019$) and twist ($r = 0.35$, $P = 0.012$), in females but not males. There were no relationships for LV $length_d$ with twist or rotation in either group. No relationships between LVEDV or SV with LV apical 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 β_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 ischaemia 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.* (2003, 2006) who have demonstrated that SV increases to ∼130% of baseline during PEI in an all-male cohort. In females, 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, suggesting that increased atrial contraction and filling potentially contributed to increasing EDV. Increases to central venous pressure also occur during PEI (Shoemaker *et al.* 2007; Marongiu *et al.* 2013) and are likely to have increased venous return, thus explaining the higher LVEDV in the current study.

Given that increases to adrenergic stimulation and LV preload can each independently increase LV twist, the concomitant increases to LVEDV and SV during PEI would be expected to accompany increases to LV twist. While LVEDV and SV were increased in this study, neither males nor females had alterations to LV apical rotation or twist during PEI. Given that increases to afterload reduce LV twist, especially at the apex (Gibbons Kroeker *et al.* 1995; Dong *et al.* 1999; Weiner *et al.* 2012), the increased end-systolic wall stress during PEI may have countered any potential increases to apical rotation and thus LV twist in both groups. Finally, although increases to LV preload are reported to augment LV twist mechanics (Weiner *et al.* 2010*b*), the increases to LVEDV of ∼5–7 ml in the current study probably were not enough to increase LV twist. This is supported by prior investigations from our group (Williams *et al.* 2016) and others (Burns *et al.* 2010) in which small increases (∼10 ml) to LVEDV and LVSV did not produce significant alterations to LV twist.

While twist was not significantly altered in either sex during PEI, males did have a small but significant increase to basal rotation, but this did not result in significant sex differences in LV rotation or twist. Nonetheless, the increased basal rotation in males could provide some evidence that the responses of LV mechanics may differ between the sexes with increased adrenergic stimulation. The increases to basal rotation but not apical rotation in males may reflect greater receptor sensitivity at the base. However, this seems unlikely as greater receptor densities

and augmented responsiveness to adrenergic stimulation have been demonstrated at the apex compared to the base (Mori *et al.* 1993; Akagawa *et al.* 2007). Additionally, while we theorized that males would have a larger increase in apical rotation and thus twist than females, it is possible that this effect was countered by the significantly greater LV afterload (as determined by end-systolic wall stress) observed in males during PEI.

Recently, Balmain *et al.* (2016) used PEI in an attempt to discriminate between the contributions of increased afterload and chronotropy that occur during static handgrip exercise. In contrast to our findings, they observed reductions to LV apical rotation, twist and untwisting velocity during PEI, without changes to LVEDV, ESV and SV. While the authors proposed that large increases to LV afterload attenuated LV twist, the haemodynamic data are not entirely consistent with increased afterload, given that increases to LVESV and reductions to SV would be expected to occur when EDV is unchanged. The reduction to LV twist is thus surprising given that increases to sympathetic activation and LV contractility occur during PEI (Victor *et al.* 1988; Crisafulli *et al.* 2006). In the current study, the increase to EF in males and the lack of change to ESV in both sexes suggests that an increase in LV contractility maintained LV twist and offset the increased LV afterload.

Effects of β_1-AR blockade on sex differences in LV mechanics

The reduced LV twist mechanics in males compared to females during β_1-AR blockade predominantly resulted from reductions to LV apical rotation in males, whereas LV rotation and twist were unchanged in females. The lower LV apical rotation, twist and untwisting velocity in males during β_1-AR blockade provide preliminary evidence for sex-related differences in LV adrenergic control of LV twist mechanics, specifically during reductions to adrenergic stimulation. Studies using heart rate variability consistently report greater low frequency power and low-to high-frequency ratios in males, compared to females (Ryan *et al.* 1994; Gregoire *et al.* 1996; Kuo *et al.* 1999; Barantke *et al.* 2008) suggesting that males are more sympathetically mediated than their female counterparts. Our data support the contention that males are more sympathetically mediated as reductions to adrenergic stimulation during β_1-AR blockade resulted in significant reductions to LV twist in males but not in females. The finding that torsional shear (which controls for LV length and radius) is also reduced in males compared to females suggests that sex differences in LV twist mechanics are mediated, in part, by mechanisms independent of LV geometry. It is plausible that differences in the adrenergic control of myocardial contractility might exist, whereby males might have greater β_1-AR densities at the apex compared to females, resulting in greater reductions to myofibre shortening in comparison to females.

It has been proposed that changes to HR coincide with similar alterations to contractility and LV twist mechanics (Hodt *et al.* 2011). However, our data do not support this mechanistic link between HR and twist in females, as they experienced a greater reduction to HR without a significant reduction to LV twist. As both HR and twist were reduced in males, this suggests that altered

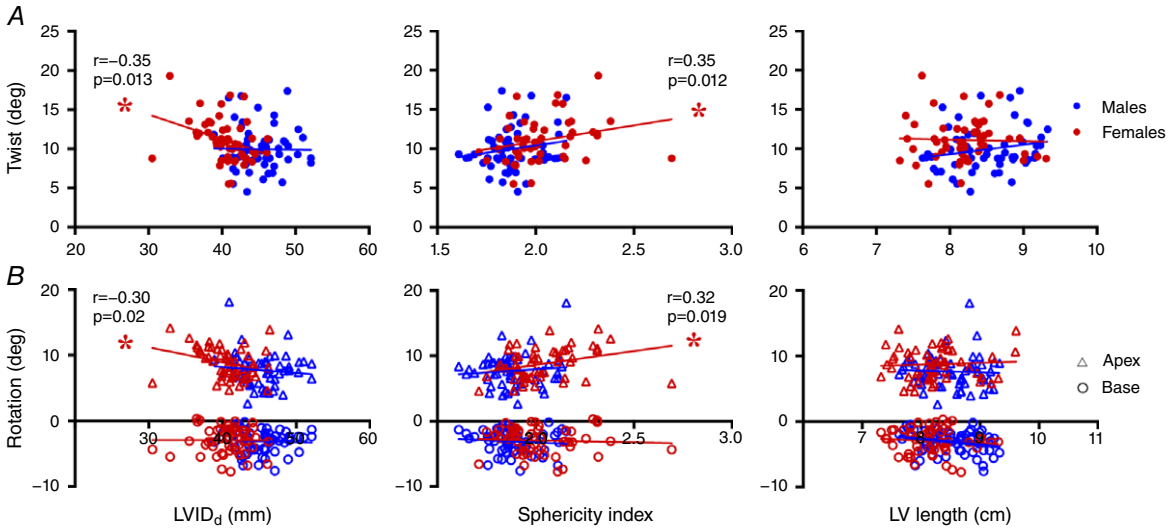

Figure 2. Relationships for LV twist mechanics with chamber structure and geometry
Data include measures during baseline, post-exercise ischaemia and β_1-AR blockade. Blue and red represent data for males and females, respectively. *A*, filled circles represent LV twist. *B*, open triangles and circles represent LV rotation at the apex and base, respectively. *Significant relationship ($P < 0.05$). [Colour figure can be viewed at wileyonlinelibrary.com]

adrenergic stimulation may affect chronotropy and twist differently between the sexes. This postulate is partially supported by previous work that demonstrated a greater increase in HR with a β_1-AR agonist in females, but a greater increase to an index of contractility in males (Convertino, 1998; Turner *et al.* 1999). Likewise, data from Evans *et al.* (2001) reported potentially greater reductions to HR in females than males during β_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 probably 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 to be due to the concomitant increases to LV afterload. Future studies should consider administrating pharmacological β_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 mitral valve 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 β_1-AR blockade. The reductions to apical rotation and twist in males are suggestive of greater sympathetically 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 may 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 Centre 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 and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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