Left ventricular twist is augmented in hypoxia by ß$_1$-adrenergic-dependent and – independent factors, without evidence of endocardial dysfunction

Short title: Hypoxic modulation of ventricular twist mechanics

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Word count: 5999

Subject terms: echocardiography, physiology, contractile function

Abstract

Background. Left ventricular (LV) twist mechanics are augmented with both acute and chronic hypoxemia. Although the underlying mechanisms remain unknown, sympathetic activation and/or a direct effect of hypoxemia on the myocardium have been proposed, the latter of which may produce subendocardial dysfunction that is masked by larger subepicardial torque. This study therefore sought to i) determine the individual and combined influences of ß$_1$-adrenergic receptor (ß$_1$-AR) stimulation and peripheral O_2 saturation (SpO_2) on LV twist in acute and chronic hypoxia, and ii) elucidate whether endocardial versus epicardial mechanics respond differently to hypoxia. **Methods.** Twelve males (27±4yr) were tested near sea level (SL) in acute hypoxia (SpO_2=83±3%) and following 3-6 days at 5050m (HA; SpO_2=83±3%). In both settings, participants received infusions of ß$_1$-AR blocker esmolol and volume-matched saline (double-blind, randomized). LV mechanics were assessed with 2-dimensional speckle-tracking echocardiography, and region-specific analysis to compare subendocardial and subepicardial mechanics. **Results.** At SL, compared to baseline (14.8±3.0º) LV twist was reduced with esmolol (11.2±3.3º; p=0.007) and augmented during hypoxia (19.6±4.9º; p<0.001), while esmolol+hypoxia augmented twist compared to esmolol alone (16.5±3.3º; p<0.001). At 5050m, LV twist was increased compared to SL (19.5±5.4°; p=0.004), and reduced with esmolol (13.0±3.8°; p<0.001) and SpO_2 normalization (12.8±3.4°; p<0.001). Moreover, esmolol+normalized SpO_2 lowered twist further than esmolol alone (10.5±3.1°; p=0.036). There was no mechanics-derived evidence of endocardial dysfunction with hypoxia at SL or HA. **Conclusions**. These findings suggest LV twist is augmented in hypoxia via ß$_1$-AR-dependent and -independent mechanisms (e.g. α_1-AR stimulation), but does not appear to reflect endocardial dysfunction.

Clinical Perspective

Acute and chronic hypoxemia are prevalent in a myriad of clinical pathologies and cause significant physiological stress. The required response involves a complex coordination of cardiovascular adjustments to ensure adequate whole-body oxygen delivery, including to the myocardium itself. Left ventricular (LV) twist mechanically supports the efficient generation of stroke volume and cardiac output, and becomes elevated with acute and chronic hypoxic exposure. Increased twist in hypoxia has been proposed to reflect subendocardial dysfunction that is masked by a predominating greater torque or moment arm of the subepicardium. The potential for endocardial dysfunction is supported by *ex vivo* data where (often extreme) localized hypoxia impairs myocardial fiber contractility; however, human studies consistently report augmented LV systolic function in hypoxia. In the current study, advanced speckle-tracking analyses of LV mechanical data suggest that there is no divergence in endocardial versus epicardial responses to acute and chronic hypoxia. Our findings suggest that LV twist is augmented in hypoxia largely via $ß_1$-adrenergic stimulation. Elevated LV twist therefore appears to represent an adaptive rather than maladaptive response to lowered oxygen availability, and the intensity of hypoxia experienced near 5000m above sea level is not likely extreme enough to significantly impair myocardial function.

Introduction

Left ventricular (LV) twist mechanics are anatomically underpinned by the opposing helical orientations of subendocardial and subepicardial myofibers[1]. Myocardial mechanics are highly responsive to physiological and environmental stressors such as hypoxia, which augments LV twist with both acute (i.e. reduced fraction of inspired oxygen, FiO_2) and chronic (i.e. high altitude) exposure[2-4]. At the myofiber level, extremely low O_2 availability reduces contractile function in *ex vivo* cardiac muscle preparations[5], and this 'depressed' myocardial contractility would be expected to result in lowered LV twist in low-oxygen environments. Though LV twist mechanics are impaired in animal models of myocardial ischemia[6], the majority of studies examining humans at altitude report increased indices of LV systolic function[7-10] and augmented twist mechanics[2-4,11,12]. It therefore remains unclear whether hypoxia leads to improved versus impaired ventricular contractile function, and the mechanism(s) contributing to elevated LV mechanics in hypoxia have not been experimentally determined.

The key mechanisms that have been proposed to contribute to increased LV twist in hypoxia include: (i) a sympathetic-dependent increase in adrenergic stimulation and contractility via the peripheral chemoreceptors[13]; (ii) a sympathetic-independent mechanism (e.g. vagal withdrawal)[11,14]; and/or (iii) altered contributions of endocardial versus epicardial torque[3]. The first two mechanisms suggest that increased LV twist is compensatory in nature[15,16], augmenting cardiac output in acute hypoxia and defending stroke volume in chronic hypoxia where LV filling pressure is reduced. The third mechanism poses that the subendocardium, with a lower oxygen supply-to-demand than the subepicardium, has impaired function in hypoxia that is masked by a greater rotational moment arm and torque of the subepicardium[3]. Yet, this remains speculative as there are

limited experimental data to support the hypothesis of hypoxia-induced endocardial dysfunction. Therefore, the aims of the current study were twofold. First, to experimentally elucidate the independent and combined influences of cardiac-specific β_1-adrenergic receptor (β_1-AR) stimulation and altered peripheral O_2 saturation (SpO_2) on LV twist, we manipulated β_1-AR stimulation and SpO_2 in two sequential experiments: in acute hypoxia at sea level (SL; 344m, Kelowna) and chronic hypoxia at high altitude (HA; 5050m, Nepal). Utilizing the β_1-AR blocker esmolol and FiO_2 manipulations, it was hypothesized that alterations to both SpO_2 and β_1-AR would independently influence LV twist in acute (SL) and chronic (HA) hypoxia. Second, to evaluate whether 'masked endocardial dysfunction' occurs in hypoxia, we utilized advanced speckle tracking analysis to compare endocardial versus epicardial mechanics, and assess reverse twist in early systole (representative of early endocardial fibre shortening)[1] in acute and chronic hypoxia. It was hypothesized that endocardial mechanics would not be impaired in acute or chronic hypoxia compared to with normal SpO_2.

Methods

Study participants and ethical approval

Twelve young, healthy males (age 27±4 yrs, body mass index 23±2 $kg \cdot m^{-2}$) consented to participate in the study. Participants had no history of cardiovascular, respiratory, or musculoskeletal disease, were not current smokers and did not utilize any cardiorespiratory medications throughout the study, including acetazolamide. The study was approved by The University of British Columbia Clinical Research Ethics Board (H16-01028) and conformed to the Declaration of Helsinki, apart from being registered as a trial. Data

supporting the findings of this study will be made available from the corresponding author upon reasonable request.

Study design

Participants visited the laboratory on two occasions, visit 1 near SL (344m, Kelowna, Canada) and visit 2 at HA (5050m, Ev-K2-CNR Pyramid Laboratory, Khumbu Valley, Nepal). For all participants, visit 1 at SL was completed between September 5th and 9th, 2016, and visit 2 at HA was completed within 3-6 days following arrival to the Ev-K2-CNR Laboratory, a cumulative 12-16 days' exposure to ≥3000m. Individuals were instructed to refrain from caffeine, exercise and alcohol for ≥24hr prior to both visits.

A double-blind, randomized crossover design was utilized for experiments at SL and HA, where participants received intravenous infusions of β_1-AR antagonist esmolol (Brevibloc, Baxter Healthcare Corporation) or placebo (volume-matched saline). During both visits, an 18-20-gauge catheter was inserted into a forearm vein, and infusions of esmolol or volume-matched saline began after ≥10 minutes quiet rest (ALARIS^{TM} PC Pump 8100, CareFusion, San Diego, USA). Esmolol was infused with an initial bolus of 500 μg/kg in the first minute, and continuously at 150 μg/kg/min thereafter[17]. This dosing regime was well tolerated by all participants without adverse effects at both altitudes. Participants were instrumented a finger pulse oximeter (7500FO, Nonin Medical Inc., Plymouth, MN, USA) to monitor SpO_2, and blood pressure was assessed with automated sphygmomanometer (HEM-775CAN, Omron Healthcare, USA).

During visit 1 at SL, participants first breathed room air and baseline echocardiographic images were collected 5 minutes following the initial bolus infusion (esmolol or placebo). Participants were then connected to a dynamic end-tidal forcing

system for acute poikilocapnic hypoxia, with the partial pressure of end-tidal O_2 controlled at 45 mmHg[18]. Echocardiographic images were collected 10 minutes following onset of hypoxia, after which infusion ended and participants returned to breathing room air. Participants rested ≥45 minutes (>5 half-lives of esmolol[19]) before the protocol was repeated for the opposite condition (i.e. esmolol or placebo). During visit 2 at HA, infusions of esmolol and placebo were performed identically to visit 1. Baseline echocardiographic images were collected while participants breathed room air. Participants were then fitted with an open circuit, non-rebreather face mask, and low flow 100% O_2 was titrated to normalize SpO_2 to SL ranges (i.e. ≥97%). Images were collected 10 minutes after normalized SpO_2 was achieved, after which participants returned to breathing room air. Participants rested ≥45 minutes before repeating the protocol for the opposite condition. None of the participants showed symptoms of acute mountain sickness as assessed by the Lake Louise Questionnaire during visit 2.

Specific methodology

Transthoracic echocardiography. A single experienced sonographer performed all imaging for the study using a commercially available ultrasound system (Vivid E9 at 344m, Vivid q at 5050m, GE Healthcare, Fairfield, CT, USA) and 1.5-4.6 MHz transducers (M5S and 4V probes). Two-dimensional and pulsed Doppler recordings were acquired at end-expiration with participants in a left-lateral position for assessments of LV structure and function in accordance with current guidelines[20]. LV volumetric and structural indices including end-diastolic volume (EDV), end-systolic volume (ESV), stroke volume (SV), ejection fraction (EF), posterior (PWT) and septal wall thicknesses (SWT), LV end-diastolic internal diameter ($LVID_d$), and end-diastolic length ($length_d$) were performed as

outlined previously[21]. Parasternal long-axis images were analyzed during systole for PWT_s, SWT_s and $LVID_s$ for estimation of end-systolic wall stress (see *analysis of hemodynamics*). LV sphericity index was calculated as $length_d/LVID_d$, and relative wall thickness as $2 \cdot PWT/LVID_d$. All echo-derived data were averaged over three cardiac cycles.

Speckle-tracking analysis. A single experienced sonographer performed all analyses and was blinded to experimental condition (i.e. esmolol vs placebo) for SL and HA data. Images for speckle-tracking analysis were acquired at 70-90 frames/sec. Measurements of LV rotation, circumferential strain (CS) and longitudinal strain (LS) were performed for the entire myocardial region of interest as previously described[21]. Additionally, strain measures were assessed separately for the endocardial and epicardial regions (EchoPAC v.113, GE, Fairfield CT, USA). Images with inadequate tracking in ≥2 myocardial segments were excluded from analysis. For twist data, tracking was inadequate at the apex in one participant during SL baseline and one participant during esmolol at SL (n=10 for each condition). Speckle tracking measurements were performed for three cardiac cycles, and data were time-aligned and transformed to 1200 points using cubic spline interpolation (2D Strain Analysis Tool, Stuttgart, Germany). Twist data were calculated by subtracting time-aligned basal data from apical data, and torsion calculated as $twist/length_d$. Reverse twist was determined as the largest negative deflection of LV twist in early systole. Peak data represent maximum values across the cardiac cycle. The intra-observer coefficients of variation for speckle tracking analyses were 4.4% for twist and 4.7% for apical rotation, in agreement with previous reports[22].

Analysis of hemodynamics. Mean arterial pressure (MAP) was calculated as $1/3 \cdot$systolic blood pressure (SBP)$+2/3 \cdot$diastolic blood pressure (DBP). Cardiac output (Q) was calculated as $SV \cdot HR$, and total peripheral resistance as MAP/Q. LV end-systolic wall

stress (surrogate estimate of afterload) was calculated as 0.9·SBP·[end-systolic cavity area/end-systolic myocardial area](modified from Haykowski *et al.* 2001[23]).

Statistical analysis and power calculation

Data are presented as mean±standard deviation (SD). To assess the effects of ß$_1$-blockade and SpO_2 on dependent variables, data from SL and HA were analyzed using a repeated-measures ANOVA with two independent factors (esmolol×hypoxia). *Post hoc* pairwise comparisons were performed with Bonferroni correction to adjust for multiple testing (six comparisons) with adjusted *P* values reported. Baseline data were compared between SL and HA using paired *t*-tests. To compare the endocardial versus epicardial responses to hypoxia, strain data were analyzed using a repeated measures ANOVA with factors for myocardial region (region$_\varepsilon$) and hypoxia (region$_\varepsilon$×hypoxia). All analyses were performed using STATA IC v12.1 (StataCorp, College Station, TX).

Stembridge et al.[2] have previously reported a 4.5° difference in LV twist between SL (344m) and HA (5050m), and pooled SD of 3.5°. With a power of 0.80 and alpha of 0.05, we required a minimum of 10 individuals to detect this effect.

Results

Effects of esmolol and hypoxia at sea level (344 m)

LV twist mechanics at sea level. During hypoxia alone, twist ($p<0.001$), torsion ($p<0.001$), apical rotation ($p<0.001$), basal rotation ($p=0.012$) and untwisting velocity ($p=0.003$) were increased compared to baseline (Figure 1A, Table 1). During esmolol (ß$_1$-AR blockade) alone, LV twist ($p=0.007$), torsion ($p=0.008$) and basal rotation ($p=0.032$)

were reduced and apical rotation tended to be lower (p=0.07) compared to baseline, though untwisting velocity was unchanged. During ß$_1$-blockade combined with hypoxia (esmolol+hypoxia), twist mechanics were not different from baseline; however, twist, torsion, apical rotation and basal rotation were all augmented compared to esmolol alone ($p \leq 0.001$ for all), suggesting that LV twist mechanics were augmented via a β_1-AR-independent pathway during hypoxia. There was no effect of hypoxia on initial reverse twist (p=0.28), supporting that endocardial mechanics were not impaired with reduced SpO_2 at SL (Figure 2B).

LV strain at sea level. During hypoxia alone, apical CS (p=0.004) and LS (p=0.047) increased but basal CS was unchanged (Table 1). With esmolol alone, basal and apical CS were not altered, though LS was lower than baseline (p=0.023). During esmolol+hypoxia, CS and LS were not different from baseline or esmolol alone. There was no significant interaction for region$_\varepsilon$×hypoxia, indicating that hypoxia did not influence endocardial versus epicardial mechanics differently at SL (Figure 2A).

LV and global hemodynamics at sea level. During hypoxia alone, Q became augmented (p<0.001) via an increase in HR (p<0.001), while LV volumes and EF were unaltered (Table 2). During esmolol alone, only EF was reduced compared to baseline (p=0.002) whereas LV volumes, HR, blood pressure and Q were unaffected. The addition of acute hypoxia (esmolol+hypoxia) led to reductions in EDV (p=0.016) and ESV (p=0.008). Although SV and EF were not significantly altered, Q was elevated (p=0.001) compared to esmolol alone.

LV structure and geometry at sea level. There were no effects for esmolol or hypoxia on LV length, $LVID_d$, relative wall thickness or sphericity index (Table 3).

Effects of esmolol and normalized SpO_2 at high altitude (5050 m)

LV twist mechanics at high altitude. At HA, baseline twist (p=0.004), torsion (p=0.006), apical rotation (p=0.013) and basal rotation (p=0.007) were all augmented compared to SL baseline (Figure 1A, Table 1). Normalizing SpO_2 lowered twist and torsion (p<0.001), apical rotation (p<0.001), basal rotation (p=0.007) and untwisting velocity (p=0.005). Similarly, esmolol alone reduced twist, torsion and apical rotation (p<0.001), basal rotation (p=0.03) and untwisting velocity (p=0.007) compared to baseline. The combination of normalized SpO_2 with esmolol resulted in a further reduction of LV twist (p=0.036), torsion (p=0.022), and apical rotation (p=0.023) compared to esmolol alone, suggesting that SpO_2 influenced LV twist mechanics via a β_1-AR-independent pathway. There was no effect of hypoxia on initial reverse twist, supporting that endocardial mechanics were not impaired with reduced SpO_2 at HA (Figure 1B).

LV strain at high altitude. At HA baseline, basal CS was increased (p=0.025), while LS and apical CS were unaltered compared to SL baseline (Table 1). Normalizing SpO_2 lowered basal CS (p<0.003), apical CS (p=0.002) and LS (p<0.001) compared to HA baseline. Esmolol reduced basal CS (p=0.026), apical CS (p<0.001) and LS (p<0.001). The combination of normalized SpO_2 with esmolol further reduced LS and tended to lower basal CS (p=0.055) compared to esmolol alone. There was no significant $region_{\varepsilon}$×hypoxia interaction, indicating that hypoxia did not have divergent influences on endocardial versus epicardial mechanics (Figure 2A).

LV and global hemodynamics at high altitude. Compared to SL baseline, EDV, ESV and SV were reduced (p<0.001), while EF was unchanged at HA (Table 2). HR was increased (p=0.006), but Q was not different from SL baseline. SBP (p=0.011), DBP and MAP (p<0.001) were augmented, while end-systolic wall stress was not different between

HA and SL. With normalized SpO_2, HR ($p<0.001$), Q ($p<0.001$) and EDV were reduced compared to HA baseline ($p=0.039$), while ESV, SV and EF were not altered. During esmolol alone, HR ($p<0.001$), Q ($p=0.007$), EF ($p=0.028$) and Q ($p=0.007$) were reduced, while LV volumes were unchanged from HA baseline. The combination of normalized SpO_2 with esmolol led to further reductions in EDV ($p=0.023$) and Q ($p=0.028$), whereas all other hemodynamics were unaltered compared to esmolol alone.

LV structure and geometry at high altitude. There were no effects for esmolol or hypoxia on LV length, $LVID_d$, relative wall thickness or sphericity index (Table 3).

Discussion

This study aimed to determine the individual and combined influences of β_1-AR stimulation and SpO_2 on LV twist mechanics in hypoxia. In agreement with our primary hypothesis, our data support that both β_1-AR-dependent and -independent pathways modulate LV twist, alone and in combination, with hypoxic exposure (Figure 3). While reduced SpO_2 will augment sympathetic activation and β_1-AR stimulation, these data specifically demonstrate that acute alterations in SpO_2 influence LV twist mechanics via a separate mechanism independent of β_1-AR activation. Furthermore, utilizing novel analysis techniques to extract regional mechanics, we have demonstrated that endo- and epicardial strain respond similarly to alterations in SpO_2, and initial reverse twist is not impaired with hypoxia. These findings support our secondary hypothesis and suggest that endocardial dysfunction does not occur in hypoxia, nor does it appear to be masked by a greater contribution of epicardial mechanics.

β_1-AR-dependent and -independent modulation of LV twist with hypoxia

A key finding of the current study is that, despite β_1-AR blockade, LV twist mechanics are further modified by reductions to SpO_2. Specifically, during esmolol infusion, acute hypoxia still augmented twist at SL, and normalizing SpO_2 further lowered twist at 5050m. These observations provide strong evidence for β_1-independent modulation of LV mechanics in hypoxia. The notion of an SpO_2-related mechanism, independent of sympathetic or neurohormonal increases to β_1-AR stimulation, is supported by observations that 48-hour administration of β_1-blocker bisoprolol does not prevent the augmented LV mechanics with acute exposure to hypoxia[11]. One possible β_1-AR-independent modulator of LV twist is vagal activation, though most studies examining vagal cardiac control in hypoxia have solely focused on cardioacceleration. Siebenmann and colleagues[24,25] have reported that β-blockade with propanolol does not prevent hypoxia-related increases to HR in acute or chronic hypoxia, whereas muscarinic blockade prevents cardioacceleration. In the current study, esmolol also did not attenuate hypoxic cardioacceleration at SL; however, it seems improbable that vagal withdrawal contributes to augmented LV twist in hypoxia for several reasons. First, muscarinic receptor densities are higher in human atria versus ventricles[26], and cholinergic innervation is substantially greater in atrial and nodal tissues versus the ventricles as well as at the LV base versus apex[27]. These regional heterogeneities reflect a stronger vagal control of nodal firing and conduction[28], and relatively weaker effects on LV contractility and potentially twist. Second, our group has recently demonstrated only small increases to LV twist during vagal blockade that solely resulted from increased basal rotation while apical rotation remained unaltered[29]. Similarly, Dedobeleer et al.[11] reported increases to only basal rotation during vagal blockade in normoxia without changes to apical rotation or twist. Thus, it seems unlikely that vagal withdrawal is a key modulator of LV twist in general, and in acute and

chronic hypoxia. Another potential β_1-AR-independent modulator of LV twist in hypoxia is the activation of cardiac α_1-ARs[14], which are bound by post-synaptic norepinephrine from sympathetic neurons[30] as well as circulating catecholamines. The agonism of α_1-ARs increases LV contractility in humans[31], and thus may augment LV twist in hypoxia independent of β_1-AR-specific modulation. An intriguing characteristic of α_1-ARs is their activation increases cardiac contractility, while blockade does not impact basal contractile function[32]. If α_1-ARs are important regulators of LV twist in hypoxia, they may augment LV contractility and twist to a greater extent in acute hypoxia, compared to following acclimatization to chronic hypoxia. The specific role of α_1-ARs in modulating LV twist merits further investigation.

Preserved endocardial function with hypoxia

A recent hypothesis has proposed that augmented LV twist in hypoxia reflects endocardial dysfunction that is masked by mechanically greater subepicardial torque[3]. This postulate is based on observations in isolated cardiac muscle preparations that suggest lowered O_2 availability (1% or 10 Torr) impairs contractility[5]. Indeed, *in vivo* studies have shown that acute coronary artery ligation impairs LV contractility and reduces apical rotation and twist[6,33]. Though these *ex vivo* and *in vivo* data support that extreme, localized hypoxia can reduce myocardial contractility, it must be considered that those models isolate the myocardium from the highly integrative hemodynamic and neurohormonal responses to global hypoxia. Specifically, they do not account for sympathoexcitation driven by peripheral chemoreceptors during lowered SpO_2[13], which ultimately augments adrenergic stimulation and likely LV twist. Moreover, the local ischemia during occlusion studies would reduce myocardial oxygen delivery substantially compared to exposure to global

hypoxia as in our study. Even at barometric pressures near the summit of Mount Everest (240 Torr), LVSV is preserved for a given filling pressure[34], and the vast majority published data in the last fifty years indicate that contractile function is preserved at high altitude[35].

To specifically examine whether there are divergent alterations to endo- versus epicardial mechanics in hypoxia, we performed speckle tracking analyses of those regions and found no significant interaction of myocardial region$_{\varepsilon}$×hypoxia, indicating that endo- and epicardial mechanics do not respond differently to hypoxia. Initial reverse twist, which is predominantly driven by the contraction of subendocardial fibers in early systole[1], was also not significantly influenced by SpO_2. Furthermore, subendocardial dysfunction would be expected to result in impaired relaxation and longitudinal mechanics[36], neither of which were apparent in the current or previous studies examining twist mechanics at high altitude[2-4] or acute hypoxia[11]. It has been relatively well-established that the myocardium has 'excellent' tolerance to hypoxic exposure[37], thus, based on our data, we believe it is unlikely that subendocardial dysfunction contributes to augmented LV twist during reductions to SpO_2. Clinically, hypoxemia can manifest in chronic pathologies that co-present with cardiac dysfunction, however the hypoxia-related elevations to LV twist appear to be adaptive rather than maladaptive in healthy participants, and the degree of hypoxemia experienced at 5000m is not likely extreme enough to cause myocardial dysfunction[34].

Potential influences of LV geometry, preload and afterload on LV twist in hypoxia

In addition to altered adrenergic stimulation and SpO_2, alterations to LV geometry and loading may influence twist in hypoxia and warrant consideration. LV geometry is an

important regulator of apical rotation and twist[38], and a larger sphericity index (i.e. more ellipsoid chamber) could contribute to increased twist in chronic hypoxia[3]. However, in the current study twist increased in hypoxia independent of any alterations to sphericity index, therefore hypoxia-related increases to twist likely were not related to LV geometry. Additionally, while reductions to preload (i.e., total blood volume and EDV[2,3,12]) could contribute to augmented LV twist at high altitude, Stembridge et al.[13] have demonstrated that normalization of total blood volume and EDV at high altitude does not restore twist to sea level values. Additionally, substantial reductions to blood volume (~25%) and preload alone do not significantly alter mechanics in the absence of sympathetic activation[39]. In the current study, we further observed that LV end-systolic wall stress, an index of afterload, was unchanged from baseline at both SL and HA, thus ventricular afterload does not appear to be a key modulator of the LV twist responses to hypoxia.

Limitations

While previous studies have utilized similar esmolol dosing protocols to block β_1-AR stimulation[17,40], we did not pharmacologically test the completeness of blockade, thus it is possible that altered SpO_2 could have influenced LV mechanics to a small degree during esmolol via β_1-AR-dependent pathways. However, given that our esmolol dosage neared the clinical limits (continuous infusion >200μg/kg/min is not recommended) and is known to block ~90% of β_1-AR stimulation[40], we are confident that a strong degree of β_1-AR blockade was achieved in the current study. Though we are not aware of any investigations that have utilized such an approach to assess the completeness of pharmacological blockade in humans, the addition of a β_1-specific agonist such as

dobutamine could be utilized in future experiments to determine the extent of β_1-AR blockade.

Conclusions

The findings from this study provide compelling evidence that both β_1-AR-dependent and -independent regulators augment LV twist mechanics in acute and chronic hypoxia. Even during β_1-AR blockade, changes in SpO_2 alter LV twist and hemodynamics. While the specific β_1-AR independent factor remains to be determined, it is possible that alpha-adrenergic stimulation regulates LV twist with hypoxia. Furthermore, for the first time, we have demonstrated that endocardial function does not appear to be impaired with lowered SpO_2, suggesting that augmented LV twist is not a result of a greater epicardial torque 'masking' endocardial dysfunction.

Funding. Funding was provided by the Natural Sciences and Engineering Research Council of Canada (P.N.A.), Canadian Research Chairs (P.N.A.) and The Physiological Society UK (A.M.W.).

Disclosures. None declared.

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534 Table 1. LV mechanics during β_1-AR blockade and altered SpO_2 at sea level and high altitude.

	Sea Level (344m)				***High Altitude (5050m)***			
	Baseline	*Esmolol*	*Hypoxia*	*Esmolol+ Hypoxia*	*Baseline*	*Esmolol*	*Norm. SpO_2*	*Esmolol+ Norm. SpO_2*
Twist (˚)	14.8±3.0	11.2±3.3*	19.6±4.9*†	16.5±3.3†‡	19.5±5.4§	13.0±3.8*	12.8±3.4*	10.5±3.1*†
Torsion (˚cm^{-1})	1.55±0.26	1.18±0.35*	2.07±0.48*†	1.75±0.32†‡	2.09±0.54§	1.41±0.41*	1.38±0.35*	1.13±0.33*†
Apical rotation (˚)	10.7±2.7	8.5±2.0	14.4±3.0*†	12.3±2.9†	14.0±4.6§	9.7±2.9*	9.2±2.8*	7.6±2.1*†
Basal rotation (˚)	-4.8±1.5	-3.7±1.9*	-6.0±1.5*†	-5.2±1.7†	-6.3±2.3§	-4.6±2.6*	-4.3±1.2*	-4.1±2.8*
Untwisting velocity (˚s^{-1})	-104±33	-87±30	-138±41*†	-102±30‡	-131±41	-99±38*	-98±33*	-84±25*
Longitudinal Strain (%)	-18.1±1.6	-16.8±1.5*	-19.2±1.7*†	-17.0±1.4‡	-18.7±1.3	-16.9±1.3*	-17.3±1.7*	-15.9±1.3*†‡
Circumferential Strain, Apex (%)	-24.6±6.7	-23.3±4.6	-30.6±5.2*†	-24.8±4.1‡	-29.2±5.2	-23.2±3.8*	-25.8±4.2*†	-22.2±5.1*‡
Circumferential Strain, Base (%)	-19.4±3.5	-18.2±2.4	-20.3±2.8	-18.7±3.6	-21.8±3.0§	-18.5±3.4*	-17.8±2.5*	-15.5±3.6*

535 Values are means±SD. Norm. SpO_2: normalized peripheral oxygen saturation. *p<0.05 vs baseline; †p<0.05 vs esmolol; ‡p<0.05 vs
536 hypoxia/norm. SpO_2; §p<0.05 vs SL baseline.
537

538 Table 2. LV and global hemodynamics during β_1-AR blockade and altered SpO_2 at sea level and high altitude.

	Sea Level (344m)				***High Altitude (5050m)***			
	Baseline	*Esmolol*	*Hypoxia*	*Esmolol+ Hypoxia*	*Baseline*	*Esmolol*	*Norm. SpO_2*	*Esmolol+ Norm. SpO_2*
SpO_2 (%)	98±1	97±1	82±4*†	78±4*†‡	83±3§	82±5	98±1*†	98±1*†
HR (bpm)	54±9	52±10	63±11*†	61±11*†	64±10§	59±11*	58±11*	57±10*
MAP (mmHg)	79±6	77±6	81±5†	77±5	90±8§	88±7	87±7	85±6*
SBP (mmHg)	112±8	110±9	117±9*†	112±8‡	121±9§	115±8*	118±7	113±6*‡
DBP (mmHg)	63±6	61±5	64±5	62±4	76±8§	76±7	73±8	73±6
EDV (ml)	129±23	128±24	125±20	121±19*†	118±19§	118±19	113±18*	112±18*†
ESV (ml)	52±11	54±10	49±9†	50±9†	48±9§	51±10	46±8†	48±9
SV (ml)	77±13	74±15	76±13	71±12*‡	71±11§	67±11	67±12	64±11*
EF (%)	60±3	57±3*	61±4†	59±4	60±2	57±3*	59±3	57±3*
Q ($l \cdot min^{-1}$)	4.11±0.77	3.81±0.79	4.72±0.68*†	4.27±0.66†‡	4.43±0.63	3.97±0.73*	3.79±0.53*	3.56±0.49*†
End-systolic wall stress ($kdyne \cdot cm^{-2}$)	34.9±8.4	37.0±7.7	32.6±7.1	36.2±9.4	37.7±6.0	38.1±5.5	37.1±4.7	37.7±5.8
Total peripheral resistance ($mmHg \cdot l^{-1} \cdot min^{-1}$)	19.9±4.8	21.0±4.9	17.5±2.9*†	18.6±3.2†	20.7±3.7	23.0±4.9*	23.4±4.0*	24.3±4.1*

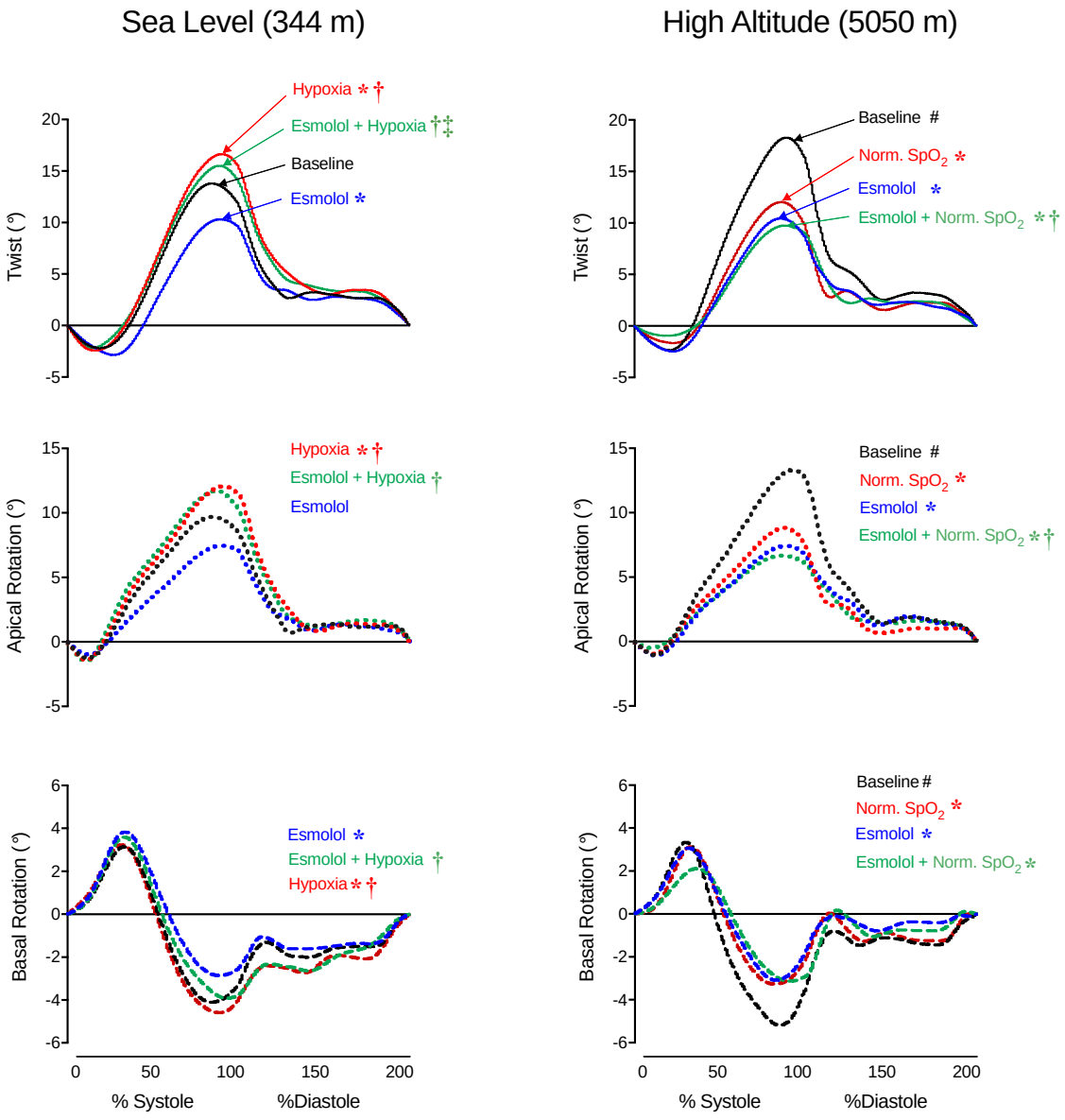
539 Values are means±SD. Norm. SpO_2: normalized peripheral oxygen saturation; HR: heart rate; MAP: mean arterial pressure; SBP:
540 systolic blood pressure; DBP: diastolic blood pressure; EDV: end-diastolic volume; ESV: end-systolic volume; SV: stroke volume;
541 EF: ejection fraction; Q: cardiac output. *$p<0.05$ vs baseline; †$p<0.05$ vs esmolol; ‡$p<0.05$ vs hypoxia/norm. SpO_2; §$p<0.05$ vs SL
542 baseline.

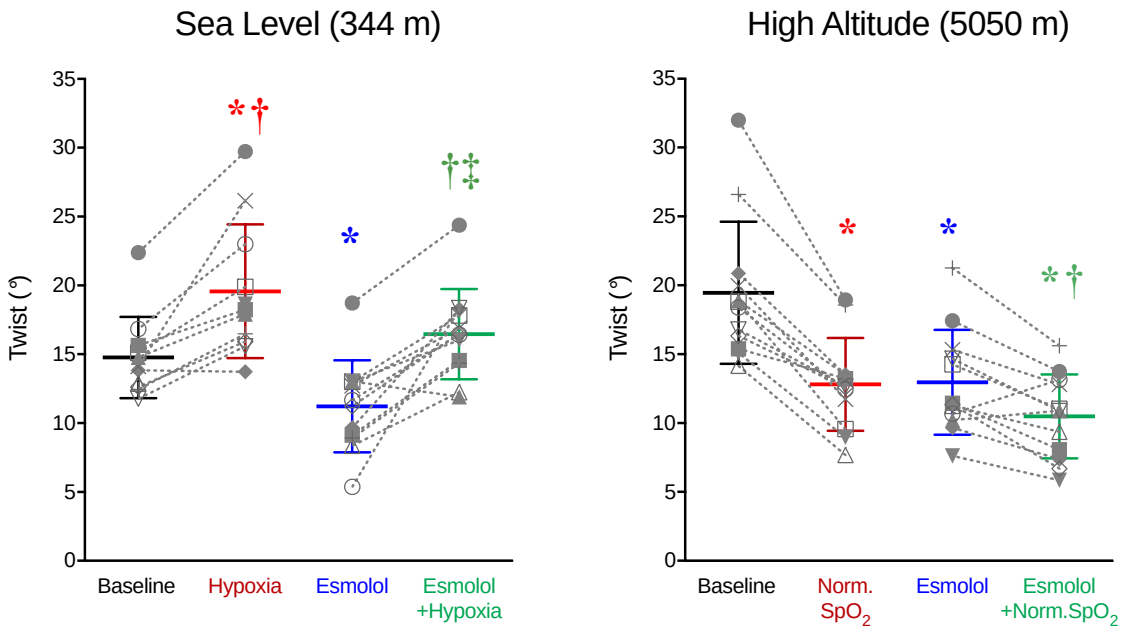
543 Table 3. LV structure and geometry during β_1-AR blockade and altered SpO_2 at sea level and high altitude.

	Sea Level (344m)				***High Altitude (5050m)***			
	Baseline	*Esmolol*	*Hypoxia*	*Esmolol+ Hypoxia*	*Baseline*	*Esmolol*	*Hyperoxia*	*Esmolol+ Hyperoxia*
$Length_d$ (cm)	9.5±0.6	9.5±0.7	9.4±0.6	9.4±0.6	9.3±0.6	9.2±0.5	9.3±0.6	9.3±0.6
$LVID_d$ (mm)	48±3	48±4	48±3	47±3	48±4	47±3	48±3	47±3
Sphericity index	1.97±0.09	1.98±0.15	1.97±0.12	2.00±0.14	1.97±0.17	1.97±0.14	1.95±0.13	1.98±0.16
Relative wall thickness	0.43±0.04	0.43±0.04	0.43±0.05	0.45±0.05	0.44±0.05	0.44±0.04	0.44±0.06	0.47±0.05

544 Values are means±SD.*p<0.05 vs baseline; †p<0.05 vs esmolol; ‡p<0.05 vs hypoxia/norm. SpO_2; §p<0.05 vs SL baseline.

Figures


Sea Level (344 m)
High Altitude (5050 m)
Twist (°)
Apical Rotation (°)
Basal Rotation (°)
Hypoxia * †
Esmolol + Hypoxia †‡
Baseline
Esmolol *
Baseline #
Norm. SpO2 *
Esmolol *
Esmolol + Norm. SpO2 * †
Esmolol + Hypoxia †
Esmolol + Norm. SpO2 * †
Esmolol + Norm. SpO2 *
% Systole
%Diastole



547
548 **Figure 1. A)** Graphical representation of mean LV twist mechanics across the cardiac cycle
549 (*x axis*) during at sea level (*left*), and high altitude (*right*). Data are shown for LV twist (*top*,
550 solid lines), apical rotation (*middle*, dotted lines) and basal rotation (*bottom*, dashed lines)
551 at baseline (*black*), during administration of esmolol (*blue*), altered SpO_2 (*red*) and
552 esmolol+altered SpO_2 (*green*). **B)** Individual peak LV twist data are shown at sea level
553 (*left*) and high altitude (*right*). Means and SD are represented by horizontal bars, and
554 participants are distinguished with individual symbols. *p<0.05 vs baseline; †p<0.05 vs
555 esmolol; ‡p<0.05 vs hypoxia/norm. SpO_2; §p<0.05 vs SL baseline.
556

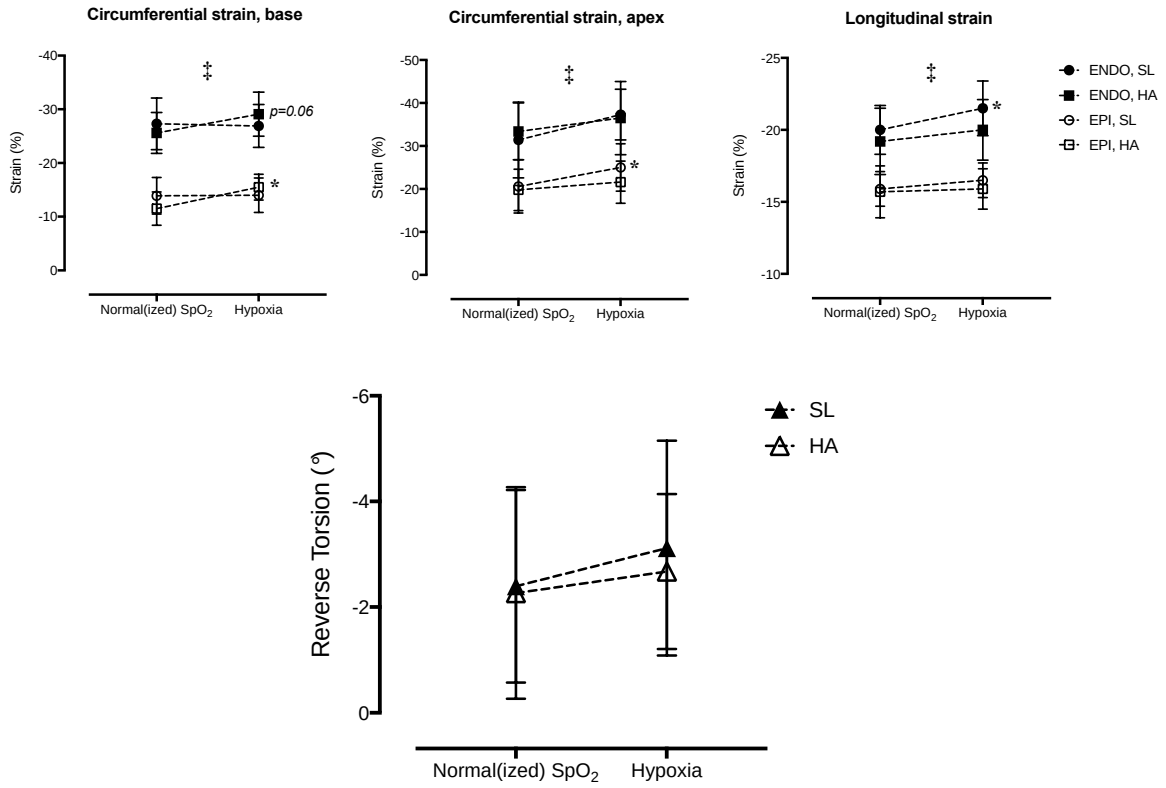
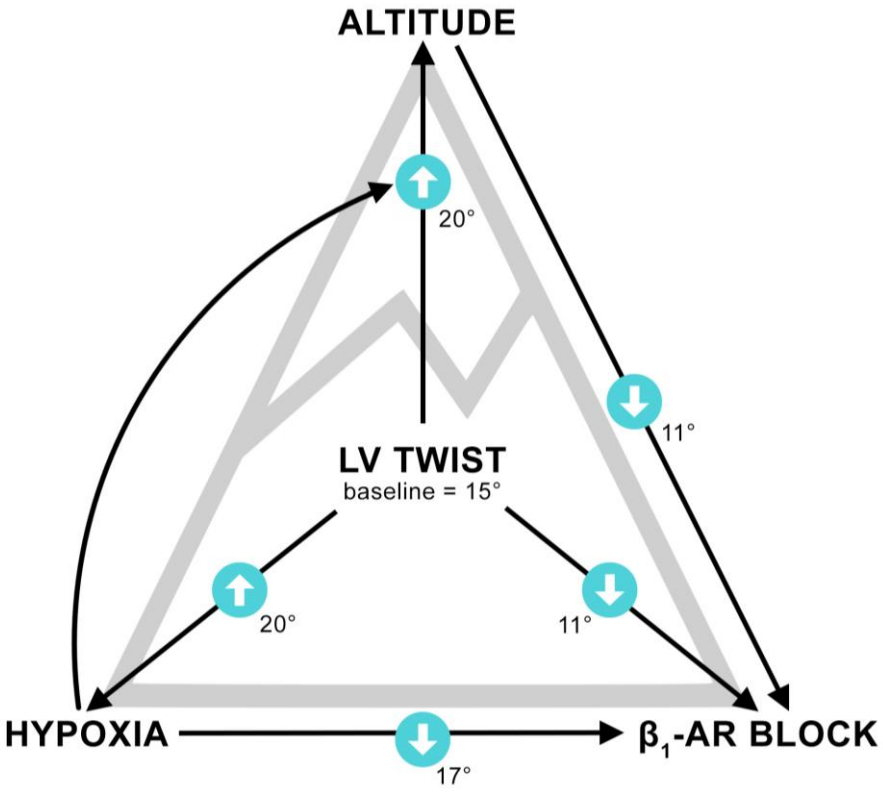


Figure 2. A) Alterations to endocardial (solid symbols) and epicardial (open symbols) measures of circumferential strain at the base (*left*) and apex (*middle*), and longitudinal strain (*right*) between normal/sea level SpO_2 and hypoxia, at sea level (SL, circles) and high altitude (HA, squares). Significant interactions for $region_m$×hypoxia were not detected. **B)** Peak data for reverse LV twist in early systole are shown during normal/sea level SpO_2 and hypoxia at SL and HA. Initial reverse twist was not significantly influenced by hypoxia. *$p<0.05$ vs normal(ized) SpO_2; ‡$p<0.05$ effect for hypoxia.



567

568 **Figure 3.** Schematic representation of the influences of hypoxia (acute and chronic/altitude
569 exposure) and ß$_1$-adrenergic blockade on LV twist mechanics. Numbers represent mean
570 LV twist data from the current study (see Table 1 for SD). Upward or downward arrow
571 icons indicate increases or reductions to LV twist, respectively.