Cardiac Performance with Chronic Hypoxia: Mechanisms regulating stroke volume

Mike Stembridge[1] and Benjamin Levine[2]

[1]Cardiff School of Sport and Health Sciences, Cardiff Metropolitan University, Cardiff, UK.
[2]Institute for Exercise and Environmental Medicine, Texas Health Presbyterian Hospital Dallas, The University of Texas Southwestern Medical Center, Dallas, Texas, US.

<u>Highlighted References:</u>

*Dedobbeleer C, Hadefi A, Pichon A, Villafuerte F, Naeije R & Unger P. (2015). Left ventricular adaptation to high altitude: speckle tracking echocardiography in lowlanders, healthy highlanders and highlanders with chronic mountain sickness. *Int J Cardiovasc Imaging* **31,** 743-752.
This work investigated the changes in cardiac function seen with high altitude exposure in health lowlanders and highlanders, as well as those suffering from chronic mountain sickness.

*Maufrais C, Rupp T, Bouzat P, Doucende G, Verges S, Nottin S & Walther G. (2016). Heart mechanics at high altitude: 6 days on the top of Europe. *Eur Heart J Cardiovasc Imaging*.
This study mapped the termporal changes in cardiac fucntion over the first week of exposure and the normalisation following return to sea level.

Pratali L, Allemann Y, Rimoldi SF, Faita F, Hutter D, Rexhaj E, Brenner R, Bailey DM, Sartori C, Salmon CS, Villena M, Scherrer U, Picano E & Sicari R. (2013). RV contractility and exercise-induced pulmonary hypertension in chronic mountain sickness: a stress echocardiographic and tissue Doppler imaging study. *JACC Cardiovasc Imaging* **6, 1287-1297.
This elegant non-invasive study provides evidecne that right heart function is maintained at rest and in response to exercise at high altitude, even in those suffering from chronic mountain sickness and exhibiting an exegerated pulmonary pressure reponse to exercise.

**Stembridge M, Ainslie PN, Boulet LM, Anholm J, Subedi P, Tymko MM, Willie CK, Cooper SM & Shave R. (2018). The independent effects of hypovolemia and pulmonary vasoconstriction on ventricular function and exercise capacity during acclimatisation to 3800 m. *J Physiol*.
The authors employed a double-blind, randomised placebo-controlled trial to independently manipulate two of the mechanisms responsible for the decrease in stroke volume at high altitude. The decrease in blood volume had the largest effect on stroke volume, but increased pulmonary artery pressure also plays a role in reducing stroke volume in chronic hypoxia.

*Stembridge M, Ainslie PN, Hughes MG, Stohr EJ, Cotter JD, Nio AQ & Shave R. (2014). Ventricular structure, function and mechanics at high altitude: chronic remodelling in Sherpa verses short-term lowlander adaptation. *J Appl Physiol (1985)* **117,** 334-343.

This study was the first to apply speckle-tracking imaging techniques to the assessment of cardiac fucntion in chronic hypoxia, and showed an increase in the mechanical function of the heart that underpinns the decrease in end-systolic volume previously reported.

Abstract

When humans are exposed to high altitude hypoxia for a sustained period, the cardiac stroke volume is reduced. The changes in cardiac performance seen at high altitude are a result of complex and concomitant changes in preload, afterload and contractility, although the precise mechanisms underpinning the decrease in stroke volume are not known despite being of scientific interest for over fifty years. In this review, we briefly revisit the seminal work performed in the area before focusing on recent developments that have applied mechanistic experimental models and novel imaging technologies to further understand why stroke volume is decreased in chronic hypoxia. First, the review focuses on systolic contractile function before considering the role of diastolic function and ventricular filling.

Introduction

Afterload, preload and contractility (the latter mediated in the chronic state primarily by autonomic control) are the three fundamental influences on stroke volume, all of which change with acclimatisation to chronic hypoxia. For example, (i) the afterload on the left and right ventricles is mildly (Parati *et al.*, 2012) and moderately-severely (Penaloza & Arias-Stella, 2007) increased compared to sea level, respectively; (ii) sympathetic activation (Hansen & Sander, 2003; Lundby *et al.*, 2017) and parasympathetic withdrawal (Siebenmann *et al.*, 2015) together increase inotropy and chronotropy and (iii) right atrial pressure and left ventricular filling are both reduced (Alexander & Grover, 1983; Reeves *et al.*, 1990). The consequence is a cardiac output that is the same as it is at sea level, but achieved via a higher heart rate and a lower stroke volume; a pattern that remains evident during exercise for a given oxygen uptake or absolute workload (Sutton *et al.*, 1992). The physiological mechanisms underpinning the decrease in stroke volume have been of scientific interest for over 50 years, but no unifying theory has been established. The concomitant decrease in ejection and filling has stimulated the investigation of both systolic and diastolic function as potential underlying causes. This review will focus on the recent developments that have attempted to shed light on this phenomenon by first focusing on the changes in contractile function that occur in chronic hypoxia, followed by a discussion of the factors that influence ventricular filling and how these may translate to a decrease in stroke volume.

Ventricular Contractile Function

The reduction in stroke volume was first definitively described by Alexander *et al.* (1967) who used the direct Fick method to determine cardiac output at rest and during exercise at sea level and following 10 days at 3100 m. The authors proposed six potential mechanisms to explain the reduction in stroke volume, including the possible impairment of myocardial contraction due to the lower arterial oxygen tension. However this hypothesis was simply refuted by observations that acute restoration of arterial oxygen tension via 30 minutes of oxygen breathing did not increase stroke volume (Saltin *et al.*, 1968). These findings suggest any impairment was either not related to oxygen tension, or was not reversible in the relatively short time-frame.

Subsequent work by this same group of researchers utilised an elegant animal model to assess stroke volume (aortic electromagnetic flow transducer) and left ventricular pressure development (ventricular pressure transducer) in six goats before and after 14 days in hypobaric hypoxia equivalent to an altitude of 4300 m (Tucker *et al.*, 1976) under conditions of pacing (to keep heart rate constant) and beta blockade. The authors reported a decrease in stoke volume and contractile function in hypoxia, that was normalised after at least 48 hrs following return to sea level, but not with acute hypoxic reversal while exposed to hypobaric hypoxia.

This question of rest and exercise stroke volume was examined invasively in autonomically intact humans in the seminal series of studies entitled Operation Everest II. Here, Reeves *et al.* (1987) demonstrated that stroke volume was well preserved for any given right atrial or wedge pressure, even at a simulated barometric pressure near the limits of human

tolerance (barometric pressure = 240 Torr). To date, this study provides the best evidence of preserved cardiac contractile function in hypobaric hypoxia using gold-standard techniques. These invasive measurements were also complimented by the use of two-dimensional echocardiography during maximal exercise. Specifically, Suarez *et al.* (1987) reported a progressive decrease in end-diastolic volume and stroke volume with increasing altitude, but importantly also a decrease in end-systolic volume which emphasized that end-systolic elastance and contractility (estimated from end-systolic pressure/volume) was well preserved even at maximal exercise in extreme hypoxia in healthy humans. Furthermore, Fowles and Hultgren (1983) normalised markers of systolic function to the decrease in LV end-diastolic diameter during altitude acclimatization and concluded that contractile function was actually enhanced. Collectively, these findings indicate that despite a decrease in preload, and presumably Frank-Starling mediated contractility, systolic function is maintained or enhanced in chronic hypoxia in healthy individuals.

The seminal work performed in the latter half of the 20th century has stood the test of time, as modern imaging techniques have since confirmed and extended these findings through the interrogation of myocardial mechanics. 'Left ventricular mechanics' is an umbrella term for the assessment of systolic and diastolic function through the determination of the deformation (strain) and movement (rotation) of the heart. By assessing the counter-directional rotation that occurs during systolic contraction at the base and apex of the left ventricle, 'LV twist' can be measured and applied as a metric of systolic function (for a comprehensive review on this technique, please see Sengupta *et al*. (2008). The rate of relaxation or 'untwisting' can also be assessed (Notomi *et al.*, 2008), and this will be discussed in the subsequent section in the context of diastolic function. The rotational movement and multi-plane deformation are a consequence of the unique fibre alignment within the heart, and together allow a detailed and sensitive interrogation of myocardial movement throughout different phases of the cardiac cycle (Marwick, 2006). The first assessments of left ventricular mechanics at high altitude have revealed that the decreased end-systolic volume and maintained ejection fraction were supported by a progressive increase in LV twist both at rest (Stembridge *et al.*, 2014; Osculati *et al.*, 2015; Maufrais *et al.*, 2016) and during exercise in most (Stembridge *et al.*, 2015), but not all studies. (Dedobbeleer *et al.* (2015) (summarised in Figure 1).

The increase in LV twist observed with chronic altitude exposure has multiple possible interpretations. The most straightforward and widely accepted (Stembridge *et al.*, 2014; Stembridge *et al.*, 2015; Maufrais *et al.*, 2016) is that the increase in twist is simply compensatory in nature, to support the maintenance of ejection fraction as the heart starts from a smaller end-diastolic volume. However, (Osculati *et al.*, 2015) raised the possibility that the increase in twist is reflective of a combination of geometric changes to the LV and subendocardial dysfunction. The latter speculation is based on the theory that the inner subendocardial layer acts as the braking force on the twisting motion, and is the layer most affected by decreased oxygen tension due to regional disparity in oxygen distribution across the myocardial wall (Decking & Schrader, 1998). However, the analysis technique used by the investigators to justify this conclusion (multiple linear regression) is indirect, and it is the opinion of the authors that this hypothesis is unlikely to be true for two reasons. Firstly, whilst oxygen availability may be lower in the subendocaridal layer, a decrease in myocardial oxygen supply has been shown to actually *decrease* twist (Zhou *et al.*, 2013).

Moreover, after the initial few days of exposure, arterial oxygen content is normalised via haemoconcentration meaning myocardial oxygen delivery could be achieved with the same coronary blood flow. Recent studies of LV mechanics at high altitude have also been performed at rest in young healthy individuals who will possess a substantial ability to vasodilate so that delivery can easily meet demand (Duncker & Bache, 2008). Secondly, it is unlikely that changes in LV geometry secondary to decreased LV filling results in such marked changes in LV twist, as even during extraction of 25% blood volume, twist remains unchanged (Lord *et al.*, 2018). Moreover, when plasma volume is restored to sea level values at high altitude via saline infusion, LV twist remains elevated from sea level (Stembridge *et al.*, 2018). Given LV twist is known to be highly dependent on inotropic state (Hansen *et al.*, 1991; Dong *et al.*, 1999), and sympathetic nervous system activity is elevated at high altitude (Hansen & Sander, 2003; Lundby *et al.*, 2017), a more plausible explanation is that a sympathetically-mediated increase in contractility results in a smaller end-systolic volume and thus increasing twist. This hypothesis has recently been tested, where the effects of increased adrenergic stimulation were blunted with a beta-adrenergic receptor (β_1-AR) antagonist and oxygen content was normalised with supplementary oxygen. LV twist was reduced at high altitude following β_1-AR antagonist and further attenuated with combined β_1-AR antagonist and oxygen supplementation (Williams *et al.*, 2017). These findings suggest that the increase in LV twist in chronic hypoxia is primarily regulated by β_1-AR activation.

Much of the work in high altitude physiology has focused on the LV, partially due to the relative ease of measurement with non-invasive techniques compared to the right side. However, it is the right ventricle that experiences the most substantial change in afterload during chronic hypoxia, with anywhere between a 50-300% increase in pulmonary pressure from sea level, from hypoxic pulmonary vasoconstriction (HPV) (Swenson, 2013). Despite the pulmonary hypertension and the particular afterload sensitivity of the right ventricle, the majority of the literature describes preserved right ventricular function assessed via traditional echocardiographic indices (Huez *et al.*, 2009; Maufrais *et al.*, 2016), although RV longitudinal strain derived via speckle-tracking echocardiography was mildly reduced in one study of lowlanders at 5050 m (Stembridge *et al.*, 2014). Decreased RV function has also been shown at rest in high altitude natives diagnosed with chronic mountain sickness; however, upon exercise, the RV contractile reserve (estimated from end-systolic pressure-area relationship) was the same as in healthy participants (Pratali *et al.*, 2013).

Left and Right Ventricular Filling

Within the first few days of exposure to high altitude, LV end-diastolic volume is decreased relative to sea level both at rest (Alexander & Grover, 1983; Boussuges *et al.*, 2000; Stembridge *et al.*, 2014) and during exercise (Stembridge *et al.*, 2015). The decrease in volume is accompanied by a reduction in the ratio of early to late transmitral filling (E/A ratio), indicative of reduced atrial-ventricular pressure gradients at the onset of mitral valve opening (Boussuges *et al.*, 2000). The two-dimensional echocardiographic techniques previously employed on high altitude expeditions are not well suited to the geometric assessment of the right ventricle, and as such no accurate volume data exist. Surrogates for volume show that right ventricular area is maintained (Stembridge *et al.*, 2014) and diameter mildly increased (Boussuges *et al.*, 2000) when assessed in the supine position. Right atrial pressure is decreased from sea level to 6100 m at rest and during exercise (Reeves *et al.*, 1990) when measured in a seated position.

Plasma volume contraction occurs upon exposure to high altitude (Singh *et al.*, 1990) and remains lower than at sea level even after 4-6 months above 4000 m (Pugh, 1964). Given that there is no meaningful change in red cell volume within the initial time period (Ryan *et al.*, 2014), absolute blood volume is decreased. Alexander *et al.* (1967) were among the first to postulate that the decrease in blood volume could be the cause of the lower stroke volume, and tested their hypothesis via intravenous dextran infusion in two subjects at high altitude. To the authors' surprise, restoration of plasma volume only restored stroke volume in one subject, despite normalisation of right atrial pressure in both. Over the years this theory has been tested numerous times with varying results. For example, Grover *et al.* (1976) kept five participants normovolemic by preventing hypocapnia-related alkalosis with supplementary CO_2 (3.77%) in the atmosphere. After five days, stroke volume was maintained in the experimental group (with supplementary CO_2) and decreased in the control group. In contrast, Calbet *et al.* (2004) observed an increase in heart rate and no change in stroke volume following plasma volume expansion after 9 weeks at 5260 m. Siebenmann *et al.* (2013) reported that plasma volume expansion restored stroke volume after 25 days at altitude to sea level values, regardless of body posture (head down vs head up tilt). Most recently, Stembridge *et al.* (2018) also restored plasma volume following acclimatisation to 3800 m. Plasma volume expansion restored left ventricular end-diastolic volume, transmitral filling velocity and stroke volume in 10 participants. Although some degree of ambiguity still exists, it seems highly likely that the decrease in plasma volume with acclimatisation to high altitude plays a considerable but not exclusive role in the decrease in left ventricular filling.

Given that the heart works as two pumps in series which share a ventricular wall and are linked by horizontal ventricular interaction (Glantz *et al.*, 1978; Beyar *et al.*, 1993), any impairment to right ventricular ejection will impair left ventricular filling. This process of ventricular interaction was first discussed over a century ago (Henderson & Prince, 1914) and occurs with pulmonary hypertension where both restriction of right ventricular output and a leftward shift of the interventricular septum have been shown to independently decrease left ventricular filling (Lumens *et al.*, 2010). Septal deviation towards the left ventricle has previously (Stembridge *et al.*, 2014) but not always been observed at high altitude (Huez *et al.*, 2009), and may only occur at more extreme altitudes due to the

progressive rise in RV pressure with ascent (Boussuges *et al.*, 2000). Recently, the influence of RV pressure on LV filling has been investigated experimentally through the reversal of the HPV response at 3800 m with administration of the phosphodiesterase-5 inhibitor, Sildenafil. Compared to placebo, Sildenafil reduced pulmonary pressure and increased LV filling to some extent (Stembridge *et al.*, 2018), suggesting that increased RV afterload may reduce RV stroke volume and, in turn, contribute at least in part to left ventricular filling. The same study also combined the sildenafil administration with a saline infusion (300-500 ml) but observed no further increase in LV end-diastolic volume. It is possible that, due to the increase in RV pressure, right ventricular volume is increased. If this is the case, then LV volume would be reduced via pericardial constraint with total cardiac volume remaining constant (Atherton *et al.*, 1997).

Similar to systolic function in chronic hypoxia, it has also been suggested that diastolic relaxation may be impaired when oxygen availability is limited. However, this notion is often based on the fact that traditional markers of LV diastolic function are reduced at high altitude, such as E/A ratio (Holloway *et al.*, 2011), and the somewhat distant link to animal models of chronic disease reporting increased LV stiffness (Gomez & Mink, 1986) or *in vitro* myocyte preparations demonstrating altered calcium reuptake (Silverman *et al.*, 1997). The reduction in E/A ratio observed at high altitude could purely reflect the lower filling pressure, and therefore does not directly indicate an impairment of myocardial relaxation. Moreover, if LV relaxation were to impair filling, then one would expect an increase in left atrial pressure; however, pulmonary capillary wedge pressure (as a surrogate of left atrial pressure) is not elevated at high altitude (Reeves *et al.*, 1987).

The rate of ventricular recoil (i.e. untwisting) during early diastole plays a pivotal role in the generation of atrioventricular pressure gradients that facilitate fast and efficient LV filling (Rademakers *et al.*, 1992). Recently, a number of groups have tried to quantify how chronic hypoxemia influences the rate of diastolic relaxation using LV untwisting velocity derived from speckle tracking echocardiography. Whilst untwist velocity is load-dependent, and will be markedly influenced by gravitational forces (Negishi *et al.*, 2017), it does correlate with invasive measurements of LV stiffness and pressure decay (Zhou *et al.*, 2013) and remains unchanged following a marked reduction in end-diastolic volume (Lord *et al.*, 2018). Virtually all investigators report an *increase* in LV untwist velocity despite the decrease in LV filling (Dedobbeleer *et al.*, 2015; Osculati *et al.*, 2015; Stembridge *et al.*, 2015). This increase suggests a maintained or enhanced LV relaxation that is likely a consequence of increased sympathetic activation (Rademakers *et al.*, 1992).

Conclusions

Collectively, recent and historical data consistently report a decrease in stroke volume at high altitude that is a consequence of the integrative changes in afterload, preload and contractility, modulated by autonomic control (Figure 2). The reduction in left ventricular filling is most likely due to decreased blood volume, although other factors such as the increase in right ventricular afterload also play a role. In response to the decrease in LV filling, contractility is increased and end-systolic volume subsequently reduced to maximise ejection. On balance, it is unlikely that the degree of chronic hypoxia tolerated by healthy

humans at high altitudes has a negative effect on cardiac systolic or diastolic performance directly.
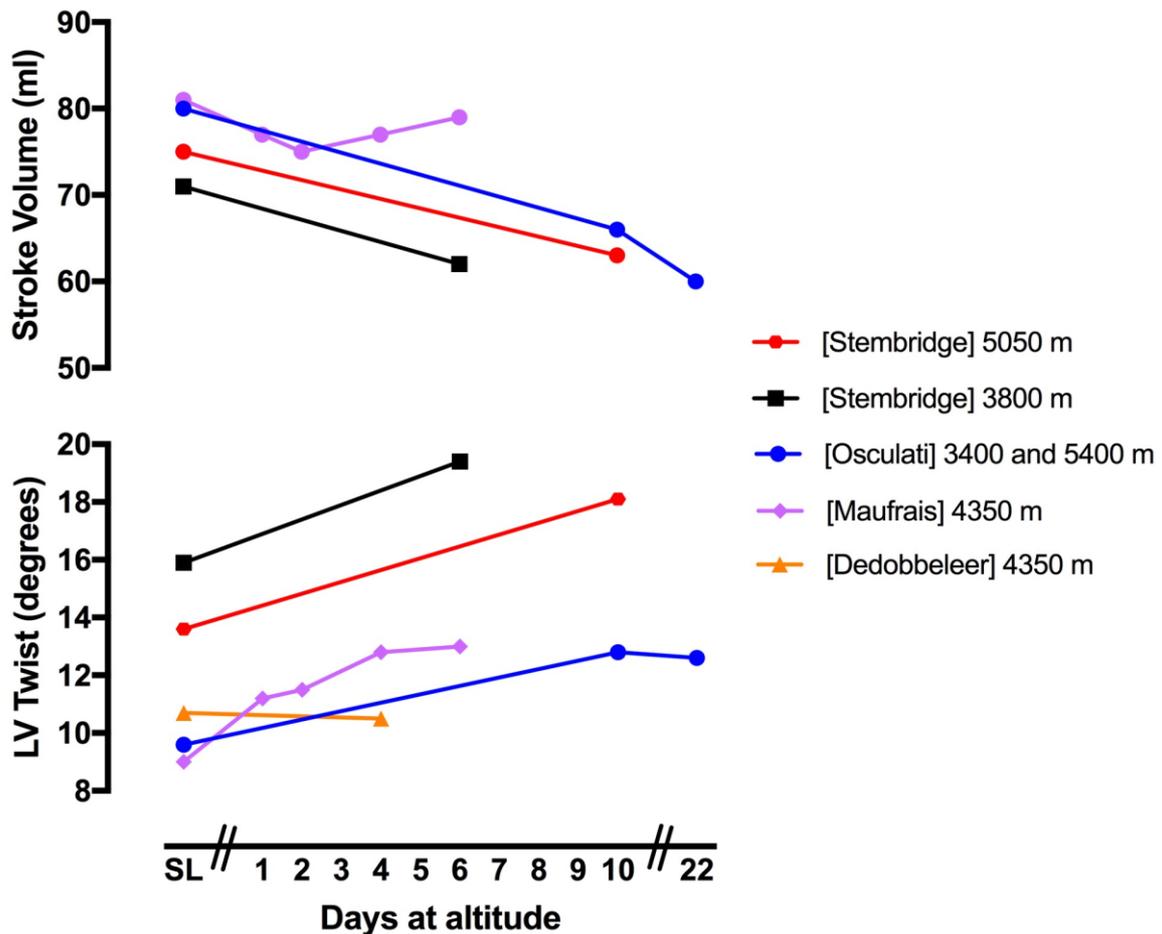
Figures

Figure 1- Summary of the changes in stoke volume and left ventricular twist during the first 22 days of acclimatisation to altitudes between 3400 m and 5400 m above sea level. The decrease in stroke volume has been consistently described in the literature over the last fifty years, but recent evidence shows an increase in LV twist mechanics that is compensatory in nature, helping to maintain stroke volume via a decrease in end-systolic volume when ventricular filling is decreased.
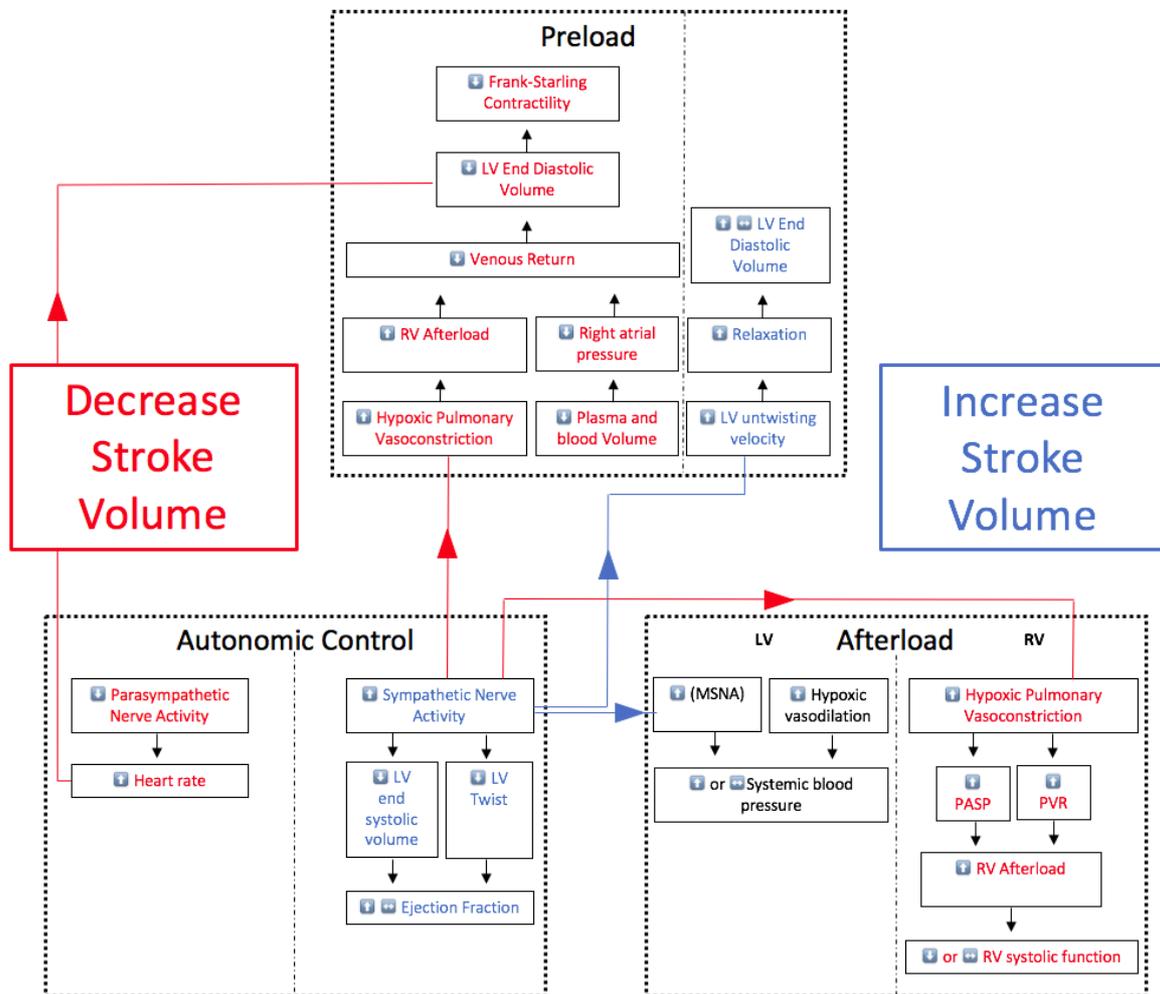
Figure 2- The integrative cardiac response to chronic hypoxia. Changes in afterload, preload and autonomic control all act to both increase (blue) and decrease (red) stroke volume at high altitude, with the net result being a decrease in stroke volume compared to sea level. Supporting literature is denoted by numbers.

References

Alexander JK & Grover RF. (1983). Mechanism of reduced cardiac stroke volume at high altitude. *Clinical cardiology* **6,** 301-303.

Alexander JK, Hartley LH, Modelski M & Grover RF. (1967). Reduction of stroke volume during exercise in man following ascent to 3,100 m altitude. *J Appl Physiol* **23,** 849-858.

Atherton JJ, Moore TD, Lele SS, Thomson HL, Galbraith AJ, Belenkie I, Tyberg JV & Frenneaux MP. (1997). Diastolic ventricular interaction in chronic heart failure. *Lancet* **349,** 1720-1724.

Beyar R, Dong SJ, Smith ER, Belenkie I & Tyberg JV. (1993). Ventricular interaction and septal deformation: a model compared with experimental data. *Am J Physiol* **265,** H2044-2056.

Boussuges A, Molenat F, Burnet H, Cauchy E, Gardette B, Sainty JM, Jammes Y & Richalet JP. (2000). Operation Everest III (Comex '97): modifications of cardiac function secondary to altitude-induced hypoxia. An echocardiographic and Doppler study. *Am J Respir Crit Care Med* **161,** 264-270.

Calbet JA, Radegran G, Boushel R, Sondergaard H, Saltin B & Wagner PD. (2004). Plasma volume expansion does not increase maximal cardiac output or VO2 max in lowlanders acclimatized to altitude. *Am J Physiol Heart Circ Physiol* **287,** H1214-1224.

Decking UK & Schrader J. (1998). Spatial heterogeneity of myocardial perfusion and metabolism. *Basic Res Cardiol* **93,** 439-445.

Dedobbeleer C, Hadefi A, Pichon A, Villafuerte F, Naeije R & Unger P. (2015). Left ventricular adaptation to high altitude: speckle tracking echocardiography in lowlanders, healthy highlanders and highlanders with chronic mountain sickness. *Int J Cardiovasc Imaging* **31,** 743-752.

Dong SJ, Hees PS, Huang WM, Buffer SA, Jr., Weiss JL & Shapiro EP. (1999). Independent effects of preload, afterload, and contractility on left ventricular torsion. *Am J Physiol* **277,** H1053-1060.

Duncker DJ & Bache RJ. (2008). Regulation of coronary blood flow during exercise. *Physiol Rev* **88,** 1009-1086.

Fowles RE & Hultgren HN. (1983). Left ventricular function at high altitude examined by systolic time intervals and M-mode echocardiography. *Am J Cardiol* **52,** 862-866.

Glantz SA, Misbach GA, Moores WY, Mathey DG, Lekven J, Stowe DF, Parmley WW & Tyberg JV. (1978). The pericardium substantially affects the left ventricular diastolic pressure-volume relationship in the dog. *Circ Res* **42,** 433-441.

Gomez A & Mink S. (1986). Increased left ventricular stiffness impairs filling in dogs with pulmonary emphysema in respiratory failure. *J Clin Invest* **78,** 228-240.

Grover RF, Reeves JT, Maher JT, McCullough RE, Cruz JC, Denniston JC & Cymerman A. (1976). Maintained stroke volume but impaired arterial oxygenation in man at high altitude with supplemental CO_2. *Circ Res* **38,** 391-396.

Hansen DE, Daughters GT, 2nd, Alderman EL, Ingels NB, Stinson EB & Miller DC. (1991). Effect of volume loading, pressure loading, and inotropic stimulation on left ventricular torsion in humans. *Circulation* **83,** 1315-1326.

Hansen J & Sander M. (2003). Sympathetic neural overactivity in healthy humans after prolonged exposure to hypobaric hypoxia. *J Physiol* **546,** 921-929.

Henderson V & Prince A. (1914). The systolic discharge and the pericardial volume. *Sam J Physiol* **35,** 116-118.

Holloway CJ, Montgomery HE, Murray AJ, Cochlin LE, Codreanu I, Hopwood N, Johnson AW, Rider OJ, Levett DZ, Tyler DJ, Francis JM, Neubauer S, Grocott MP & Clarke K. (2011). Cardiac response to hypobaric hypoxia: persistent changes in cardiac mass, function, and energy metabolism after a trek to Mt. Everest Base Camp. *FASEB J* **25,** 792-796.

Huez S, Faoro V, Guenard H, Martinot JB & Naeije R. (2009). Echocardiographic and tissue Doppler imaging of cardiac adaptation to high altitude in native highlanders versus acclimatized lowlanders. *Am J Cardiol* **103,** 1605-1609.

Lord R, MacLeod D, George K, Oxborough D, Shave R & Stembridge M. (2018). Reduced left ventricular filling following blood volume extraction does not result in compensatory augmentation of cardiac mechanics. *Exp Physiol* **103,** 495-501.

Lumens J, Blanchard DG, Arts T, Mahmud E & Delhaas T. (2010). Left ventricular underfilling and not septal bulging dominates abnormal left ventricular filling hemodynamics in chronic thromboembolic pulmonary hypertension. *Am J Physiol Heart Circ Physiol* **299,** H1083-1091.

Lundby C, Calbet J, van Hall G, Saltin B & Sander M. (2017). Sustained sympathetic activity in altitude acclimatizing lowlanders and high-altitude natives. *Scand J Med Sci Sports*.

Marwick TH. (2006). Measurement of strain and strain rate by echocardiography: ready for prime time? *J Am Coll Cardiol* **47,** 1313-1327.

Maufrais C, Rupp T, Bouzat P, Doucende G, Verges S, Nottin S & Walther G. (2016). Heart mechanics at high altitude: 6 days on the top of Europe. *Eur Heart J Cardiovasc Imaging*.

Negishi K, Borowski AG, Popovic ZB, Greenberg NL, Martin DS, Bungo MW, Levine BD & Thomas JD. (2017). Effect of Gravitational Gradients on Cardiac Filling and Performance. *J Am Soc Echocardiogr* **30,** 1180-1188.

Notomi Y, Popovic ZB, Yamada H, Wallick DW, Martin MG, Oryszak SJ, Shiota T, Greenberg NL & Thomas JD. (2008). Ventricular untwisting: a temporal link between left ventricular relaxation and suction. *Am J Physiol Heart Circ Physiol* **294,** H505-513.

Osculati G, Revera M, Branzi G, Faini A, Malfatto G, Bilo G, Giuliano A, Gregorini F, Ciambellotti F, Lombardi C, Agostoni P, Mancia G & Parati G. (2015). Effects of hypobaric hypoxia exposure at high altitude on left ventricular twist in healthy subjects: data from HIGHCARE study on Mount Everest. *Eur Heart J Cardiovasc Imaging* **17,** 635-643.

Parati G, Revera M, Giuliano A, Faini A, Bilo G, Gregorini F, Lisi E, Salerno S, Lombardi C, Ramos Becerra CG, Mancia G & Salvi P. (2012). Effects of acetazolamide on central blood pressure, peripheral blood pressure, and arterial distensibility at acute high altitude exposure. *Eur Heart J*.

Penaloza D & Arias-Stella J. (2007). The heart and pulmonary circulation at high altitudes: healthy highlanders and chronic mountain sickness. *Circulation* **115,** 1132-1146.

Pratali L, Allemann Y, Rimoldi SF, Faita F, Hutter D, Rexhaj E, Brenner R, Bailey DM, Sartori C, Salmon CS, Villena M, Scherrer U, Picano E & Sicari R. (2013). RV contractility and exercise-induced pulmonary hypertension in chronic mountain sickness: a stress echocardiographic and tissue Doppler imaging study. *JACC Cardiovasc Imaging* **6,** 1287-1297.

Pugh LG. (1964). Blood Volume and Haemoglobin Concentration at Altitudes above 18,000 Ft. (5500 M). *J Physiol* **170,** 344-354.

Rademakers FE, Buchalter MB, Rogers WJ, Zerhouni EA, Weisfeldt ML, Weiss JL & Shapiro EP. (1992). Dissociation between left ventricular untwisting and filling. Accentuation by catecholamines. *Circulation* **85,** 1572-1581.

Reeves JT, Groves BM, Cymerman A, Sutton JR, Wagner PD, Turkevich D & Houston CS. (1990). Operation Everest II: cardiac filling pressures during cycle exercise at sea level. *Respir Physiol* **80,** 147-154.

Reeves JT, Groves BM, Sutton JR, Wagner PD, Cymerman A, Malconian MK, Rock PB, Young PM & Houston CS. (1987). Operation Everest II: preservation of cardiac function at extreme altitude. *J Appl Physiol* **63,** 531-539.

Ryan BJ, Wachsmuth NB, Schmidt WF, Byrnes WC, Julian CG, Lovering AT, Subudhi AW & Roach RC. (2014). AltitudeOmics: Rapid Hemoglobin Mass Alterations with Early Acclimatization to and De-Acclimatization from 5260 m in Healthy Humans. *PLoS One* **9,** e108788.

Saltin B, Grover RF, Blomqvist CG, Hartley L & Johnson RL, Jr. (1968). Maximal oxygen uptake and cardiac output after 2 weeks at 4,300 m. *Journal of Applied Physiology* **25**.

Sengupta PP, Tajik AJ, Chandrasekaran K & Khandheria BK. (2008). Twist mechanics of the left ventricle: principles and application. *JACC Cardiovasc Imaging* **1,** 366-376.

Siebenmann C, Hug M, Keiser S, Muller A, van Lieshout J, Rasmussen P & Lundby C. (2013). Hypovolemia explains the reduced stroke volume at altitude. *Physiol Rep* **1,** e00094.

Siebenmann C, Rasmussen P, Sorensen H, Bonne TC, Zaar M, Aachmann-Andersen NJ, Nordsborg NB, Secher NH & Lundby C. (2015). Hypoxia increases exercise heart rate despite combined inhibition of beta-adrenergic and muscarinic receptors. *Am J Physiol Heart Circ Physiol* **308,** H1540-1546.

Silverman HS, Wei S, Haigney MC, Ocampo CJ & Stern MD. (1997). Myocyte adaptation to chronic hypoxia and development of tolerance to subsequent acute severe hypoxia. *Circ Res* **80,** 699-707.

Singh MV, Rawal SB & Tyagi AK. (1990). Body fluid status on induction, reinduction and prolonged stay at high altitude of human volunteers. *International journal of biometeorology* **34,** 93-97.

Stembridge M, Ainslie PN, Boulet LM, Anholm J, Subedi P, Tymko MM, Willie CK, Cooper SM & Shave R. (2018). The independent effects of hypovolemia and pulmonary vasoconstriction on ventricular function and exercise capacity during acclimatisation to 3800 m. *J Physiol*.

Stembridge M, Ainslie PN, Hughes MG, Stohr EJ, Cotter JD, Nio AQ & Shave R. (2014). Ventricular structure, function and mechanics at high altitude: chronic remodelling in Sherpa verses short-term lowlander adaptation. *J Appl Physiol (1985)* **117,** 334-343.

Stembridge M, Ainslie PN, Hughes MG, Stohr EJ, Cotter JD, Tymko MM, Day TA, Bakker A & Shave RE. (2015). Impaired myocardial function does not explain reduced left ventricular filling and stroke volume at rest or during exercise at high altitude. *J Appl Physiol (1985)* **119,** jap 00995 02014.

Suarez J, Alexander JK & Houston CS. (1987). Enhanced left ventricular systolic performance at high altitude during Operation Everest II. *Am J Cardiol* **60,** 137-142.

Sutton JR, Reeves JT, Groves BM, Wagner PD, Alexander JK, Hultgren HN, Cymerman A & Houston CS. (1992). Oxygen transport and cardiovascular function at extreme altitude: lessons from Operation Everest II. *Int J Sports Med* **13 Suppl 1,** S13-18.

Swenson ER. (2013). Hypoxic pulmonary vasoconstriction. *High Alt Med Biol* **14,** 101-110.

Tucker CE, James WE, Berry MA, Johnstone CJ & Grover RF. (1976). Depressed myocardial function in the goat at high altitude. *J Appl Physiol* **41,** 356-361.

Williams AM, Ainslie PN, Anholm JD, Gasho C, Subedhi P & Stembridge M. (2017). Left ventricuar twist is influenced by both oxygen saturation and beta 1-adrenergic stimulation in acute and chronic hypoxia. In *20th International Hypoxia Symposia*. Lake Louise, Canada.

Zhou W, Benharash P, Ho J, Ko Y, Patel NA & Mahajan A. (2013). Left ventricular twist and untwist rate provide reliable measures of ventricular function in myocardial ischemia and a wide range of hemodynamic states. *Physiol Rep* **1,** e00110.