The influence of increased venous return on right ventricular dyssynchrony during acute and sustained hypoxemia

Michiel Ewalts[1,2], Tony Dawkins[1], Lindsey M Boulet[3], Dick Thijssen[2] and Mike Stembridge[1].

Affiliations

1. Cardiff School of Sport and Health Sciences, Cardiff Metropolitan University, Cardiff, UK.
2. Department of Physiology, Radboudumc, Nijmegen, The Netherlands
3. Centre for Heart Lung and Vascular Health, University of British Columbia, Kelowna, BC, Canada.

Running Title

Right ventricular dyssynchrony in hypoxia

Key words

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Corresponding Author:

Dr Mike Stembridge

Cardiff Metropolitan University

Cyncoed Road

Cardiff

CF23 6XD

New Findings

What is the central question of this study?

Right ventricular dyssynchrony is a marker of function that is elevated in healthy individuals exposed to acute hypoxia. This study aimed to determine whether (i) it can be reduced by

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augmenting venous return and (ii) if it persists during sustained exposure to high altitude hypoxia?

What is the main finding and its importance?

For the first time, we demonstrate that (i) increasing venous return in acute hypoxia restores the synchrony of right ventricular contraction and (ii) dyssynchrony is evident after acclimatisation to high altitude, and remains sensitive to changes in venous return. Therefore, the interpretation of right ventricular dyssynchrony requires consideration the prevailing haemodynamic state.

Abstract

Regional heterogeneity in timing of right ventricular (RV) contraction (RV dyssynchrony; RVD) occurs when pulmonary artery systolic pressure (PASP) is increased during acute hypoxia. Interestingly, RVD is not observed during exercise, a stimulus that increases both PASP and venous return. Therefore, we hypothesized that RVD in healthy humans is sensitive to changes in venous return, and examined whether (i) increasing venous return in acute hypoxia lowers RVD and (ii) if RVD is further exaggerated in sustained hypoxia, given increased PASP is accompanied by decreased ventricular filling at high altitude. RVD, PASP and RV end diastolic area (EDA) were assessed using transthoracic two-dimensional and speckle-tracking echocardiography during acute normobaric hypoxia (F_iO_2 = 0.12) and sustained exposure (5-10 days) to hypobaric hypoxia (3800m). Venous return was augmented with lower body positive pressure at sea level (LBPP; +10 mmHg) and saline infusion at high altitude. PASP was increased in acute hypoxia (20±6 vs. 28±7, P<0.001) concomitant to an increase in RVD (18±7 vs. 38±10, P<0.001); however, the addition of LBPP during hypoxia decreased RVD (38±0 vs. 26±10, P<0.001). Sustained hypoxia increased PASP (20±4 vs. 26±5, P=0.008) and decreased RV EDA (24±4 vs. 21±2, P=0.042), with RVD augmented (14±5 vs. 31±12, P=0.001). Saline infusion increased RV EDA (21±2 vs. 23 ± 3, P=0.008) and reduced RVD (31±12 vs. 20±9, P=0.001). In summary, an increase in PASP secondary to acute and sustained exposure to hypoxia augments RVD, which can be at least partly reduced via increased venous return.

Introduction

The pulmonary circulation is a high-flow, low-pressure circuit designed to optimize gas exchange (Naeije & Chesler, 2012). The pulmonary circulation receives forward flow from the right ventricle, that has evolved to be a thin-walled flow generator (Naeije & Dedobbeleer, 2013). As such, the right ventricle has a reduced capacity to accommodate for changes in pressure compared to the thick-walled left ventricle (La Gerche *et al.*, 2011). Historically, examining RV function has proved problematic due to the complex RV anatomy (Armour & Randall, 1970; Weyman *et al.*, 1976; Berger *et al.*, 1978; Barnard & Alpert, 1987; Incalzi *et al.*, 1999). Recently, speckle-tracking echocardiography has been used to detect subtle changes in function, whereby the regional heterogeneity in timing of contraction of the different RV segments, called RV dyssynchrony (RVD) (Kalogeropoulos *et al.*, 2008; Pezzuto *et al.*, 2018). RVD appears to show prognostic significance in patients with elevated pulmonary artery pressure (Lopez-Candales *et al.*, 2005a; Lopez-Candales *et al.*, 2005b; Marcus *et al.*, 2008; Smith *et al.*, 2014; Badagliacca *et al.*, 2015a; Badagliacca *et al.*, 2015b; Badagliacca *et al.*, 2017; Murata *et al.*, 2017), but the haemodynamic factors that influence this marker remain to be determined.

In a study of healthy volunteers, Pezzuto *et al.* (2018) have shown that RVD occurs during acute hypoxia (F_iO_2 = 0.12), a stimulus known to increase pulmonary artery pressure (Pezzuto *et al.*, 2018). Interestingly however, RVD did not occur during exercise, despite observing a greater increase in pulmonary pressure during exercise than during acute hypoxia (Pezzuto *et al.*, 2018). The absence of RVD during exercise was attributed to systemic factors associated with global hypoxemia. However, a change in RV geometry and increase in contractility that would result from the augmentation of venous return during exercise (Naeije & Badagliacca, 2017) may better explain the homogenous contraction, although this is yet to be investigated. Moreover, little is known about whether RVD occurs in healthy individuals when the hypoxic exposure is sustained, such as during sojourn to high altitude. In this setting, as well as the increase in pulmonary pressure, venous return will be simultaneously decreased due to plasma volume constriction (Stembridge *et al.*, 2019), and may exacerbate RVD if venous return does indeed influence the timing of regional contraction in the right ventricle. We therefore sought to examine the independent and combined effects of increased pulmonary artery pressure and venous return on RVD in healthy volunteers by performing two separate studies; one in (i) acute and another in (ii) sustained hypoxia (5-10 days), both with and without augmented RV venous return. We hypothesised that increased pulmonary artery pressure in hypoxia would increase RVD in both the (i) acute and (ii) sustained setting. Furthermore, RVD will be reduced by increasing

venous return in (iii) acute and (iv) sustained hypoxic exposure. This work will provide novel insight into the mechanisms contributing to RVD, thus enhancing its utility as a marker of right heart function.

Methods

Ethical approval

Study 1 was approved by the Natural Sciences Board of the Cardiff School of Sport and Health Sciences Research Ethics Committee (CSS REC PGR-860) and *Study 2* was granted approval by the Clinical Research Ethics Board at the University of British Columbia. Both studies conformed the Declaration of Helsinki, except for registration in a database, and all participants provided informed consent prior to participation.

Study 1: Acute normobaric hypoxia

Study population

15 participants aged 25 ± 4 years old (12 M and 3 F) were enrolled. All participants were normotensive, non-smokers with no history of cardiorespiratory disease and did not take any medication for cardiovascular risk factors. Participants were recruited based on their optimal echocardiographic windows, to ensure measurements of tricuspid regurgitant velocity to estimate pulmonary artery pressure.

Experimental design

Following initial screening, experimental testing was performed during a single visit to the cardiovascular laboratory at Cardiff Metropolitan University. Participants were asked to abstain from strenuous exercise, alcohol and caffeine consumption for 12 hours before testing. The experimental design allowed the examination of cardiac response to an increase in pulmonary artery pressure (afterload) and RV venous return (preload) achieved via acute normobaric hypoxia and lower body positive pressure (LBPP), respectively. The experimental design yielded four distinct conditions: (i) normoxia baseline, (ii) normoxia with increased venous return (LBPP), (iii) hypoxia baseline and (iv) hypoxia with increased venous return (LBPP). All conditions consisted of a sphygmomanometer blood pressure (BP) recording followed by an echocardiographic evaluation while simultaneously recording heart rate (HR) and peripheral oxygen saturation (SpO_2). Measurements during LBPP were

started after two minutes wash-in at the desired positive pressure and a 30 minute hypoxic wash in period was applied before hypoxic measurements were taken.

Lower body positive pressure

Participants assumed the supine position with their lower torso and legs in a custom-made box sealed by a flexible neoprene cover around the waist, enclosed at the level of the iliac crest. The pressure in the box was increased to 10 mmHg, based on previous research demonstrating a 5.9 mmHg increase in central venous pressure at this level of LBPP (Norsk *et al.*, 1986). Participants were asked to keep their legs relaxed to prevent any discrepancies in venous return due to leg movement.

Hypoxic breathing

Hypoxia was administered by a hypoxic generator (PowerBREATHE Altitude Systems, High Performance Pro System, POWERbreathe International Ltd., Warwickshire, UK), producing a fraction of inspired oxygen (F_iO_2 FiO_2) of 12%. This degree of hypoxia corresponds to an altitude of 4500 m and has been shown to be well tolerated with minimal changes in arterial PCO_2 (Pezzuto *et al.*, 2018).

Study 2: Sustained hypobaric hypoxia

To examine RVD in sustained hypoxia, a retrospective analysis was performed on data collected in the autumn of 2015. Some of the data reported herein have been reported previously (Stembridge *et al.*, 2019); however, a distinct research question is addressed with a novel primary outcome variable (RVD) that has not been previously reported.

Study population

Ten male participants, aged 27 ± 6, were recruited from the expedition team who were normotensive, non-smokers with no previous history of cardiovascular or respiratory diseases and were taking no prescription medications. At the time of testing, none of the participants were experiencing any symptoms of acute mountain sickness, as assessed by the Lake Louise questionnaire, when at high altitude (3800 m).

Experimental design

The data was collected during two experimental visits; one at sea level (Kelowna, British Columbia; 344 m) and one at high altitude after 5-10 days acclimatisation (The Barcroft Laboratory, White Mountain, California; 3800 m). This experimental design allowed the examination of RVD at baseline, under sustained hypoxia with and without plasma volume expansion to increase venous return (Guerin *et al.*, 2015).

Plasma volume expansion

Saline infusions were performed using an infusion pump following cannulation of a peripheral vein. The volume infused was tailored to each individual in order to restore absolute blood volume to sea level values assessed via carbon monoxide rebreathing, as previously used by the research team (Williams *et al.*, 2016). The average infusion volume required to restore plasma volume was 398 ± 248 ml, as previously described in detail (Stembridge *et al.*, 2019). Infusion duration varied between individuals (20-40 minutes) and haemoglobin concentration was checked following infusion to ensure sufficient volume had entered the vascular space to normalise plasma volume to sea level.

Experimental Measures: Study 1 and Study 2

All measurements were recorded continuously, with the exception of echocardiography assessment and manual blood pressure assessment in *study 1*. SpO_2 was monitored via fingertip pulse oximetry (Choice Mmed, MD300C2, Beijing Choice Electronic Technology Co Ltd, Beijing, China) and heart rate via a 3-lead ECG (ML132, ADInstruments, Colorado Springs CO, USA). In study 1, manual sphygmomanometer BP recordings were obtained at the beginning of each condition before the echocardiographic assessment and in *study 2*, continuous beat-to-beat measures of arterial BP (finger photoplethysmography; Finapres Medical Systems, Biomedical Instruments, The Netherlands) were recorded. Inspired and expired O_2 and CO_2 were assessed continuously by breath-by-breath online gas analysis measured in all participants in study 2 but only fourteen participants in study 1 due to a technical error (n=1) (Oxycon Mobile, Carefusion, San Diego, CA, USA).

Echocardiography

Conventional measurements

Echocardiographic evaluation was performed with a 4.5-MHz phased array transducer using a commercially available ultrasound system (GE, Vivid E9, GE Healthcare, Chalfont St Giles, Bucks, UK). Digital grayscale two-dimensional (2D) and tissue Doppler cine loops from five consecutive beats were obtained at functional residual capacity, with a brief relaxed end-expiratory breath hold. Cardiac parameters were obtained according to the American Society of Echocardiography guidelines (Rudski *et al.*, 2010; Lang *et al.*, 2015) and were taken by two highly trained cardiac sonographers (MS and TD). The conventional parameters of right ventricular function were acquired via 2D and Doppler imaging. Peak systolic right ventricle–right atrium pressure gradient was calculated according to the simplified Bernoulli equation (4V2, where V is peak systolic velocity of tricuspid regurgitant flow in continuous wave Doppler) (Rudski *et al.*, 2010). Right atrial pressure was estimated by diameter and collapsibility of the inferior vena cava, according to Rudski *et al.* (2010). sPAP was estimated

by adding the peak systolic right ventricle–right atrium pressure gradient to right atrial pressure; mPAP was estimated from sPAP using Chemla's formula (0.61 × sPAP + 2 mmHg) (Chemla *et al.*, 2004; Rudski *et al.*, 2010). Pulmonary vascular resistance was calculated according to the Abbas's formula [(peak velocity of the tricuspid regurgitant jet/RV outflow tract velocity–time integral) × 10 + 0.16] (Abbas *et al.*, 2003). RV stroke volume (SV) was calculated from the RV outflow tract diameter (RVOTd) and RV outflow tract velocity-time integral (RVOT VTI) with the formula $SV=0.785 \times RVOTd^2 \times$ RVOT VTI (Zoghbi *et al.*, 2017). RV end diastolic (EDA) and end systolic area (ESA) were determined from a modified apical four-chamber view in line with the RV American Society of Echocardiography guidelines for the assessment of the right heart (Rudski *et al.*, 2010).

2D speckle tracking

Peak strain and time to peak strain from each of the six segments were determined from speckle tracking analysis of greyscale 2D images acquired at the highest frame rate possible (70-90 frames per second). A region of interest (ROI) was traced on the endocardial and epicardial border of the right ventricle. Natural acoustic markers, or speckles within the ROI were tracked over the cardiac cycle. Where data were missing from sub-optimal speckle-tracking as indicated by the software algorithm and/or visual inspection, the same variable was then omitted for that participant for the remaining conditions. The sample number reported for each variable can be found in the tables. Longitudinal strain was calculated as the change in length/initial length of RV myocardium within the ROI. In the longitudinal view, myocardial shortening was represented as negative strain and myocardial lengthening as positive strain. The software automatically divided the RV image into six standard segments and generates individual strain–time waveforms for basal, mid and apical septal wall, and basal, mid and apical free wall segments (EchoPac, Version 112, GE Healthcare). Images were not blinded for analysis as speckle-tracking indices are derived from automated analysis. Longitudinal strain analyses were performed including time to peak strain, defined as the timing of maximal systolic strain. RV longitudinal strain was considered as global longitudinal strain (average strain of all RV segments), and as longitudinal strain of RV mid and basal 2D strain of the free wall (RVFW) and of the interventricular septum (IVS), similar to the study by Pezzuto et al. 2018. Right ventricular dyssynchrony (RV-SD4) was calculated by taking the standard deviation of the times to peak-systolic strain for the four mid-basal RV segments, corrected to the R–R interval between two QRS complexes, according to Bazett's formula, and called RV-SD4 (Kalogeropoulos *et al.*, 2008; Badagliacca *et al.*, 2015a; Badagliacca *et al.*, 2015b; Badagliacca *et al.*, 2017; Pezzuto *et al.*, 2018). The two apical segments were excluded because of their excessive variability, not only in pulmonary hypertension patients but also in healthy volunteers, as is conventional (6-9, 11, 22). The

time to peak contraction response was determined by combining the basal and mid segments of the RV free wall and the interventricular septal wall. Our intra-observer coefficients for speckle-tracking echocardiography have previously been reported for the LV (Stembridge *et al.*, 2015) and range from 3.4-6.6% for RV areas and 4.7-6.0 for RV strain and strain rate. For visual representation, mean segmental strain curves were generated for each condition using cubic spline interpolation, as described previously (Stembridge *et al.*, 2014).

Statistical analysis

The data that support the findings of this study are openly available on Figshare at DOI: 10.25401/cardiffmet.13049699. All data of study 1 and 2 were analysed using SPSS (version 22, IBM, Surrey, UK) and expressed as means ± SD. Statistical significance was defined as $P \leq 0.05$ and distribution normality was confirmed using repeated Shapiro–Wilk W tests.

Study 1: The effects of hypoxia and LBPP on RVSD4 were assessed by a two-way repeated measures ANOVA. A Student's paired, two-tailed t-test or a Wilcoxon signed rank test, when not normally distributed, was used to assess the difference between the measures in the normoxia baseline, normoxia lower body positive pressure, hypoxia baseline and hypoxia lower body positive pressure. Cohen's D was used to assess the differences between time to peak contraction.

Study 2: Differences in RV-SD4 between sea level, high altitude and high altitude combined with plasma volume expansion were analysed using a one-way repeated measures ANOVA. A post hoc Tukey test was used to determine the difference in RV-SD4 between sea level, high altitude and high altitude combined with plasma volume expansion. Student's paired, two-tailed t-test or the Wilcoxon signed rank test when not normally distributed was used to assess the difference between the measures in sea level, high altitude and high altitude combined with plasma volume expansion. Cohen's D was used to assess the differences between time to peak contraction.

Results

Study 1- Acute Hypoxia

Haemodynamic response

Hypoxia elicited a significant increase in HR and mPAP compared to normoxia, with no change in RVEDA (Figure 1). LBPP in hypoxia further increased mPAP compared to hypoxia baseline, simultaneous to a significant increase in RVEDA (Figure 1). LV eccentricity index was increased in hypoxia but unchanged by LBPP (Table 1).

RVSD4 response

RVSD4 was significantly increased in hypoxia compared to normoxia, but was significantly lower in hypoxia + LBPP compared to hypoxia alone (Figure 2). Time to peak contraction of the RV free wall and interventricular septal wall was significantly longer (i.e. prolonged contraction) during hypoxia and hypoxia + LBPP compared to normoxia. Moreover, the time to peak contraction was significantly prolonged in the interventricular septal wall during hypoxia + LBPP compared to hypoxia alone, with no change in the RV free wall (Figure 2). This suggests the increase in RV filling was altering septal and not restoring RV free wall function, despite the difference in time to peak contraction between normoxia and hypoxia being larger in the RV free wall (Figure 2). These changes happened independent to changes in RV strain rate, which remained unaltered in response to either LBPP or hypoxia.

Study 2- Sustained Hypoxia

Haemodynamic response

HR and mPAP both significantly increased at high altitude compared to sea level (Figure 4). RVEDA significantly decreased at high altitude compared to sea level, but was restored at high altitude following plasma volume expansion (Figure 3) to be comparable to sea level values. In contrast to our preload intervention in Study 1 (LBPP), mPAP was not increased following plasma volume expansion (Figure 3). LV eccentricity index increased at high altitude compared to sea level, and remained elevated following plasma volume expansion. Further haemodynamic responses are depicted in Table 2.

RVSD4 response

RVSD4 was significantly increased at high altitude compared to sea level, but was lowered once blood volume was restored at high altitude via infusion (Figure 4). Time to peak contraction of the RV free wall was significantly longer at high altitude compared to sea level, and significantly shorter at high altitude with volume expansion compared to high altitude alone. The time to peak contraction of the interventricular septal wall was significantly longer at high altitude with volume expansion compared to sea level (Figure 4), but in contrast to acute hypoxia, the reduction in RVD with volume expansion was likely mediated by a decrease in time to peak in the RV free wall. Similar to acute hypoxia, there were no significant changes in RV strain rate in response to either LBPP or hypoxia.

Discussion

This study is the first to examine the effect of changes in venous return on RVD in acute and sustained hypoxia. In relation to our four hypotheses, our primary novel findings are: both (i) acute and (ii) sustained hypoxia increased RVSD4, but the increase was attenuated by

elevated ventricular filling in both (iii) acute and (iv) sustained hypoxia suggesting that RVD is dependent on both afterload and preload in healthy human participants.

The influence of increased pulmonary artery pressure in hypoxia on RVD

Pezzuto *et al.* (2018) were the first to show that an increase in RV afterload secondary to acute hypoxia increases RVD in healthy volunteers (Pezzuto *et al.*, 2018). Herein, we confirm and extend these findings by demonstrating that both acute and sustained hypoxia result in a robust increase in RVD in healthy volunteers. The results of this study are also in line with earlier work demonstrating that RVD is elevated in conditions with a chronic increase in right ventricular afterload in pulmonary hypertension patients (Kalogeropoulos *et al.*, 2008; Badagliacca *et al.*, 2015a; Badagliacca *et al.*, 2015b; Badagliacca *et al.*, 2017).

There are three main mechanisms that could cause a delay of cardiac contraction: (i) an increased QRS duration (Badagliacca *et al.*, 2015a; Morita *et al.*, 2019), (ii) the nonuniform distribution of wall stress due to the geometric inhomogeneous nature of the RV (Badagliacca *et al.*, 2015a; Morita *et al.*, 2019) and (iii) pressure overload of the RV (Badagliacca *et al.*, 2015b; Morita *et al.*, 2019). Considering (i), given that there is no relationship between the QRS duration and the delay in contraction of the RV free wall or the QRS duration with the onset of the RV contraction in pulmonary patients or in healthy volunteers (Marcus *et al.*, 2008; Pezzuto *et al.*, 2018), one could assume that a delay in QRS duration is not involved in RVD. In relation to geometric changes (ii), we found no evidence that the changes in RVD were linked to septal flattening, as eccentricity index increased in hypoxia but remained elevated during LBPP and plasma volume expansion despite RVD decreasing. Therefore, whilst changes in RV geometry occur in hypoxia, such alterations do not appear to result in dyssynchronous contraction *per se*. Therefore, the most likely explanation for the increase in RVD during an increase in hypoxia is an increase and nonuniform distribution of wall stress, induced by an increase in RV pressure and workload (Badagliacca *et al.*, 2015a; Badagliacca *et al.*, 2015b; Morita *et al.*, 2019). The importance of wall stress on timing of the contraction of myocytes has been described by van Heuningen et al. (1982), who showed that the contraction of rat myocytes was slower when wall stress was increased (van Heuningen *et al.*, 1982). Thus, when RV afterload is increased, wall stress is elevated in specific segments and causes a slower region-specific contraction, resulting in a dyssynchronized contraction. This is supported by the observation in the current study and previous work in pulmonary hypertension patients that demonstrates time to peak contraction of the RV free wall to be delayed more than the interventricular-septal wall (Lopez-Candales *et al.*, 2005a; Marcus *et al.*, 2008).

The influence of increased venous return on RVD in acute and sustained hypoxia

For the first time, we demonstrate that the hypoxia-induce increase in RVD can be attenuated in healthy volunteers when venous return is augmented, both in the acute and sustained hypoxemia. Thus, RVD is related to both RV preload and afterload, rather than solely RV afterload, and may explain why Pezzuto *et al.* (2018) did not report an increase in RVD during exercise, despite higher pulmonary artery pressures than during hypoxia.

Although RVD was decreased when right ventricular venous return was increased in both acute and sustained hypoxia, the way in which it was achieved differed. Increasing venous return via LBPP in acute hypoxia increased the time to peak contraction in the interventricular septal wall so that it was comparable with the RV free wall, thereby reducing dyssynchrony. This suggests that increasing RV venous return when PAP is elevated lengthens the time to peak contraction in the septum. In contrast to acute hypoxia, restoring venous return via saline in sustained hypoxia did in fact decrease the time to peak contraction in the RV free wall, lowering RVD through a direct effect on RV function. The contrasting findings may, in part, be due to the nature of our two scenarios. During acute hypoxia prior to LBPP, no change in ventricular volumes were observed, nor would it expected to be (Boulet *et al.*, 2016). With sustained hypoxic exposure, however, it is well established that ventricular volumes decrease (Stembridge & Levine, 2019), especially in the left heart. The saline infusion used in the current study was designed only to normalise ventricular volumes to sea level values, whereas LBPP increased ventricular filling beyond baseline levels. Nevertheless, in both scenarios, increasing RV filling decreased RVD, demonstrating the interaction between RVD and filling.

Clinical perspective

In conditions of chronically elevated pulmonary artery pressure, RVD is regarded as a marker of both the functional capacity and prognostic outcome (Lopez-Candales *et al.*, 2005a; Lopez-Candales *et al.*, 2005b; Marcus *et al.*, 2008; Smith *et al.*, 2014; Badagliacca *et al.*, 2015a; Badagliacca *et al.*, 2015b; Badagliacca *et al.*, 2017; Murata *et al.*, 2017). Based on the current study, one could argue that RVD may be related to the prevailing haemodynamics and not just the pulmonary pressure. Moreover, further information is required on how changes in RV structure with disease progression influence RVD. For example, RV coupling (contractility to arterial afterload) can be maintained in patients with pulmonary hypertension via an increase in workload (Vonk Noordegraaf *et al.*, 2017). In the early stages of the disease, RV uncoupling is prevented by an increase in RV contractility,

facilitated by remodelling of RV wall thickness and altering muscular properties of the myocardium (Vonk Noordegraaf *et al.*, 2017). When the RV is no longer able to adapt to the increase in workload by cardiac remodelling, the RV will dilate to increase preload to maintain coupling (Sano *et al.*, 2007; Vonk Noordegraaf *et al.*, 2017). Only in advanced stages of pulmonary hypertension will uncoupling occur, when the adaptability of the RV is insufficient to cope with an increase in workload (Trip *et al.*, 2015).

Limitations

The number of participants in this study is relatively small, especially in our study conducted at high altitude. Expeditions of this nature are financially and logistically difficult to organise, and small sample sizes are often unavoidable (Ainslie, 2014). Notwithstanding, all participants showed a similar response to the conditions of this study, and we report individual data points for transparency. Similarly, our method for assessing right atrial and right ventricular pressure was indirect, but invasive measures were not possible, especially in remote locations used in the sustained hypoxia trial. Despite both males and females being actively recruited for our studies, only three females volunteered for our sea level study and no female members of the expedition team were able to participate due to competing demands from other experiments. Therefore, regrettably, we report data on a sample that consists of predominantly males. Despite the small number of females, their RVD response was similar compared to males, indicating that sex has no influence on RVD response to alterations in preload and afterload. The stimuli used to increase preload differed between the acute and sustained setting. However, both stimuli sufficiently increased preload in our study, as well as in previous work (Greenway & Lautt, 1986; Norsk *et al.*, 1986; Grimminger *et al.*, 2017; Pezzuto *et al.*, 2018). The method employed to detect RV dyssynchrony (RVSD4) does not discriminate between systolic and post-systolic contraction. As the occurrence of post-systolic shortening is an important determinant in clinical prognosis in pulmonary hypertension patients, the timing of contraction should be considered when determining RV function (Badagliacca *et al.*, 2015a; Badagliacca *et al.*, 2015b). Lastly, speckle tracking is an analysis method that is subjective to human variability, as the tracking area has to be set manually. To ensure reproducibility of the tracking area, all tracking was performed by the same researcher (ME).

Conclusion

Collectively, our findings demonstrate the dependence of RVD on ventricular filling as well as afterload, and highlight the need of haemodynamic state and stage of structural remodelling to be considered in the interpretation of RV function in health and disease.

Competing Interests

We have no conflicts of interest to declare.

Author contribution

M.E., T.D. and M.S. conceived and designed and performed the experiments of research study 1 and L.M.B. and M.S. conceived and designed and performed the experiments of research study 2; M.E., D.T. and M.S. interpreted the results of study 1 and 2; M.E. and M.S. analysed the data and prepared the figures; M.E. and M.S. drafted the manuscript; and T.D., L.M.B and D.T. edited and revised the manuscript. All authors approved final version of manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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n/a

Tables

Table 1: Acute hypoxia - haemodynamic response during normoxia and hypoxia with and without lower body positive pressure.

	N	Normoxia	Normoxia + LBPP	Hypoxia	Hypoxia + LBPP	FiO_2	ANOVA LBPP	FiO_2 * LBPP
Systemic haemodynamics								
Heart rate (BPM)	15	57 ± 9	57 ± 9	64 ± 9*	65 ± 10‡	**0.003**	0.922	0.670
MAP (mmHg)	15	92 ± 8	96 ± 7*	92 ± 8	96 ± 5†	0.863	**0.031**	0.949
SBP (mmHg)	15	128 ± 11	132 ± 8	128 ± 11	131 ± 9	0.912	0.229	0.784
DBP (mmHg)	15	75 ± 7	79 ± 7*	74 ± 7	79 ± 5	0.852	**0.013**	0.914
Oxygen Saturation (%)	15	96 ± 1	97 ± 1	79 ± 6*	79 ± 6‡	**<0.001**	0.592	0.763
$PETO_2$ (mmHg)	1	100 ± 5	101.2 ±	42.1 ± 4*	42.5 ±	**<0.00**	0.532	0.749

	4		5		5‡	**1**		
$PETCO_2$ (mmHg)	14	42.2 ± 4	41.8 ± 4	38.1 ± 3*	37.7 ± 3‡	**<0.001**	0.626	0.991
Pulmonary haemodynamics								
TR velocity (m/s)	15	2.0 ± 0.4	2.3 ± 0.2	2.4 ± 0.4*	2.6 ± 0.4‡	**<0.001**	0.064	0.795
sPAP (mmHg)	15	20 ± 6	24 ± 6*	28 ± 7*	31 ± 8‡†	**<0.001**	**0.045**	0.862
mPAP (mmHg)	15	14 ± 3	17 ± 4*	19 ± 4*‡	21 ± 5‡†	**0.045**	**<0.001**	0.862
PVR (WU)	14	1.34 ± 0.31	1.41 ± 0.28	1.41 ± 0.24	1.54 ± 0.28	0.270	0.119	0.566
RV dimensions								
RVEDA (cm^2)	15	25 ± 6	28 ± 7	25 ± 6	29 ± 8	0.058	0.613	0.791
RVESA (cm^2)	15	12 ± 3	14 ± 4	13 ± 4	13 ± 4	0.169	0.960	0.285
RVFAC (%)	15	50 ± 3	49 ± 7	48 ± 4‡	54 ± 3*	0.244	0.327	**0.040**
LVEID (%)	14	0.93 ± 0.08	0.93 ± 0.08	1.09 ± 0.07*	1.07 ± 0.08‡	**<0.001**	0.567	0.697
LVEIS (%)	14	0.92 ± 0.06	0.94 ± 0.07	1.11 ± 0.07*	1.08 ± 0.05‡	**<0.001**	0.667	0.213
RV SV (ml)	8	99 ± 36	98 ± 47	107 ± 47	98 ± 36	0.773	0.767	0.798
RV Strain								
Peak 2DS IVS mid (%)	15	-19.9 ± 2.2	-20.1 ± 2.5	-21.2 ± 2.7	-32.4 ± 7.6	0.067	0.847	0.872
Peak 2DS IVS bas (%)	15	-18.3 ± 2.0	-17.9 ± 2.1	-19.7 ± 3.0*	-26.1 ± 5.2‡	**0.019**	0.615	0.857
Peak 2DS RVFW mid (%)	15	-32.0 ± 7.2	-32.8 ± 6.0	-32.4 ± 6.5	-32.4 ± 7.8	0.951	0.824	0.804
Peak 2DS RVFW bas (%)	15	-24.4 ± 6.1	-25.8 ± 5.8	-25.5 ± 8.4	-26.3 ± 6.1	0.634	0.546	0.867
RV GLS (%)	15	-22.6 ± 3	-23.2 ± 3	-23.7 ± 4	-24.1 ± 4	0.217	0.597	0.812
RV GLSR (% S^{-1})	15	-1.1 ± 0.2	-1.1 ± 0.1	-1.2 ± 0.2	-1.2 ± 0.2	0.094	0.483	0.731
RVSD4 (ms)	15	18 ± 7	18 ± 7*	38 ± 10*‡	26 ± 10*‡†	**<0.001**	**0.003**	**<0.001**
TTP IVS 2DS (ms)	15	367 ± 30	373 ± 28	358 ± 20*	375 ± 36‡	**0.002**	0.257	0.286
TTP RVFW 2DS (ms)	15	395 ± 31	403 ± 30	409 ± 30*	413 ± 31‡	**<0.001**	0.743	0.862
TTP GLSR (ms)	15	221 ± 40	220 ± 35	175 ± 54*‡	172 ± 48*‡	**<0.001**	0.857	0.952

**=p value paired T-test versus normoxia baseline, ‡=p value paired T-test versus normoxia lower body positive pressure, †=p value paired T-test versus hypoxia baseline. BPM=beats per minute, MAP=mean arterial pressure, SBP=systolic blood pressure, DBP=diastolic blood pressure, Pulse oximeter=oxygen saturation, PETO2=partial end tidal oxygen pressure, PETCO2=partial end tidal carbon dioxide pressure, TR=tricuspid regurgitation, sPAP=systolic pulmonary artery pressure,*

mPAP=mean pulmonary artery pressure, PVR=pulmonary vascular resistance, RVEDA=right ventricular end diastolic area, RVESA=right ventricular end systolic are, RVFAC=right ventricular fractional area change, LVEID=left ventricular diastolic eccentricity index, LVEIS=left ventricular systolic eccentricity index, RV SV=right ventricular stroke volume, mls/min=millilitres per minute, IVS=intraventricular septum, RVFW=right ventricular free wall, mid=mid level, bas=basal level, GLS=global longitudinal strain, TTP=time to peak, diff=difference, RVSD4=right ventricular dyssynchrony index, GLSR=global longitudinal strain rate.

Table 2: Sustained Hypoxia – haemodynamic response during sea level, high altitude and volume expansion at high altitude.

	N	Sea Level	High altitude	High altitude + volume expansion	ANOVA
Systemic haemodynamics					
Heart rate (BPM)	7	56 ± 10	68 ± 16	64 ± 15	0.796
MAP (mmHg)	7	93 ± 12	102 ± 12	103 ± 10	0.212
SBP (mmHg)	7	129 ± 17	141 ± 16	139 ± 12	0.448
DBP (mmHg)	7	74 ± 11	80 ± 9	82 ± 11	0.345
Oxygen Saturation (%)	7	98 ± 1	88 ± 2*	90 ± 2*	**<0.001**
RV dimensions					
RVEDA (cm^2)	10	24 ± 4	21 ± 2	23 ± 3	0.111
RVESA (cm^2)	10	13 ± 2	11 ± 3	12 ± 2	0.474
RVFAC (%)	10	44 ± 6	46 ± 12	48 ± 7	0.542
LVEID (%)	10	1.05 ± 0.03	1.15 ± 0.07*	1.17 ± 0.06*	**<0.001**
LVEIS (%)	10	1.01 ± 0.06	1.05 ± 0.04	1.06 ± 0.06	0.061
RV SV (ml)	9	115 ± 43	117 ± 56	116 ± 48	0.996
Pulmonary haemodynamics					
TR velocity (m/s)	8	2.0 ± 0.3	2.4 ± 0.3*	2.4 ± 0.3*	**0.022**
sPAP (mmHg)	8	20 ± 4	26 ± 5*	26 ± 5*	**0.027**
mPAP (mmHg)	8	14 ± 3	18 ± 3*	18 ± 3*	**0.027**
PVR (WU)	8	1.21 ± 0.14	1.51 ± 0.25*	1.54 ± 0.2*	**0.006**
RV Strain					
Peak 2DS IVS mid (%)	10	-18.3 ± 1.9	-21.6 ± 3.5	-20.0 ± 3.7	0.240
Peak 2DS IVS bas (%)	10	-17.9 ± 3.3	-19.0 ± 3.1	-17.1 ± 2.3	0.390
Peak 2DS RVFW mid (%)	10	-31.1 ± 2.9	-30.1 ± 4.6	29.8 ± 4.1	0.749
Peak 2DS RVFW bas (%)	10	-23.5 ± 4.5	-22.1 ± 6.2	-21.3 ± 8.9	0.759
RV GLS (%)	10	-23.6 ± 1.9	-24.7 ± 3.1	-23.7 ± 3.3	0.658
RV GLSR (% S^{-1})	10	1.2 ± 0.2	1.3 ± 0.3	1.2 ± 0.1	0.212
RVSD4 (ms)	10	14 ± 5	31 ± 12*	20 ± 8.7*‡	**0.001**
TTP IVS (ms)	10	362 ± 21	338 ± 38	347 ± 24	0.476
TTP RVFW (ms)	10	379 ± 26	370 ± 28	379 ± 29	0.225
TTP GLSR (ms)	10	167 ± 57	170 ± 43	181 ± 52	0.819

*=p value paired T-test versus sea level, ‡=p value paired T-test versus high altitude. BPM=beats per minute, MAP=mean arterial pressure, SBP=systolic blood pressure, DBP=diastolic blood pressure, Pulse oximeter=oxygen saturation, PETO2=partial end tidal oxygen pressure, PETCO2=partial end tidal carbon dioxide pressure, TR=tricuspid regurgitation, sPAP=systolic pulmonary artery pressure, mPAP=mean pulmonary artery pressure, PVR=pulmonary vascular resistance, RVEDA=right ventricular end diastolic area, RVESA=right ventricular end systolic are, RVFAC=right ventricular fractional area change, LVEID=left ventricular diastolic eccentricity index, LVEIS=left ventricular systolic eccentricity index, RV SV=right ventricular stroke volume, mls/min=millilitres per minute, IVS=intraventricular septum, RVFW=right ventricular free wall, mid=mid level, bas=basal level, GLS=global longitudinal strain, TTP=time to peak, diff=difference, RVSD4=right ventricular dyssynchrony index, GLSR=global longitudinal strain rate, GLSR=global longitudinal strain rate.

Figure Legends

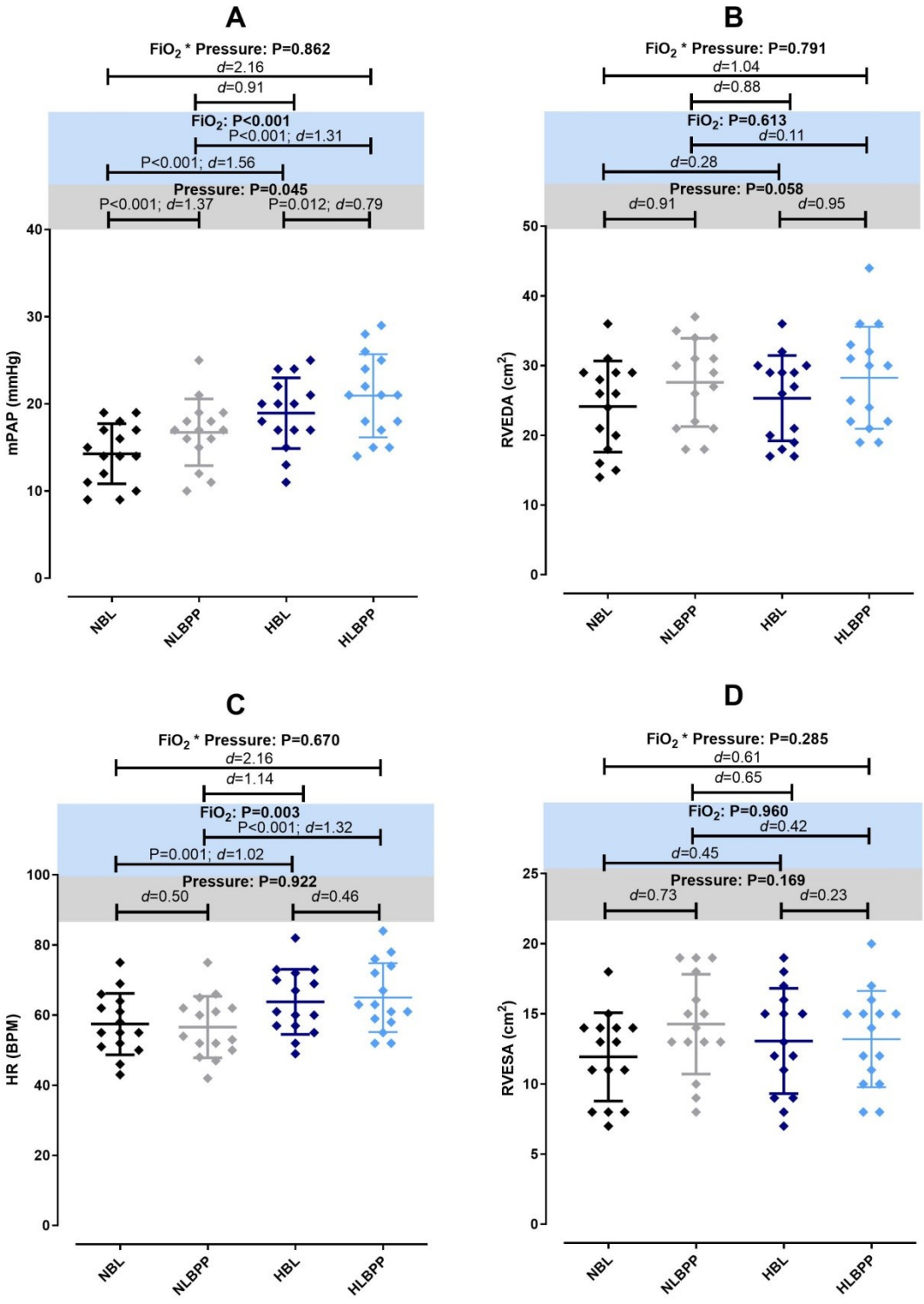

Figure 1: haemodynamic response during normoxia baseline (NBL), normoxia and lower body positive pressure (NLBPP), hypoxia baseline (HBL) and hypoxia and lower body positive pressure (HLBPP); P=p value of paired t test. A=mean pulmonary artery pressure (mPAP) in mmHg; B=Right ventricular end diastolic area (RVEDA) in cm^2; C=heart rate (HR) in beats per minute and D=right ventricular end systolic area (RVESA) in cm^2.

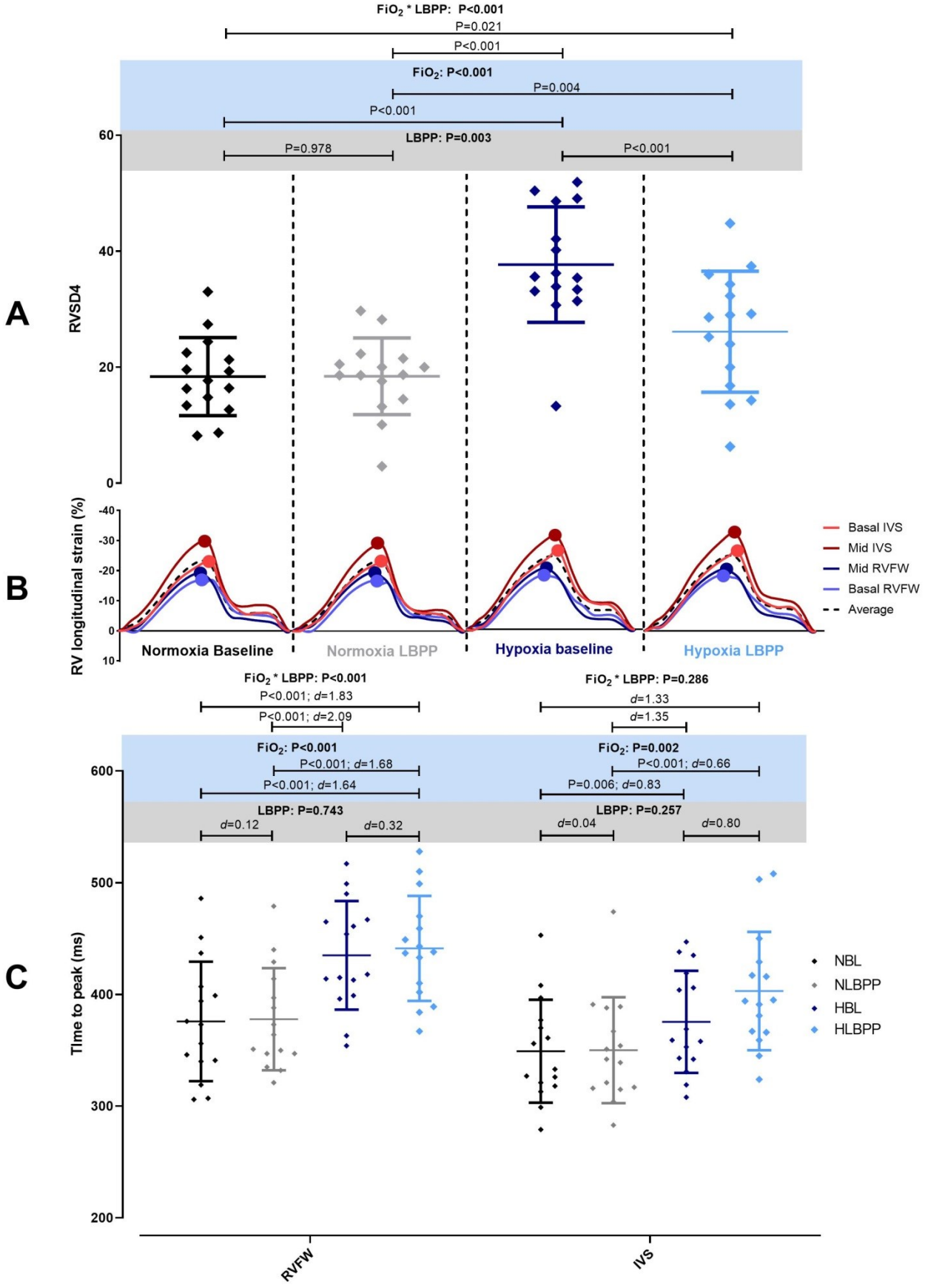

Figure 2: RV response during normoxia baseline (NBL), normoxia and lower body positive pressure (NLBPP), hypoxia baseline (HBL) and hypoxia and lower body positive pressure (HLBPP). Panel A = RVSD4 response; FiO2 is the effect for inspired air (normoxic or hypoxic) and LBPP is the effect for lower body positive pressure on RVSD4. Panel B = Interpolated segmental strain curves for the four right ventricular wall segments and their time to peak response (indicated with the closed dots; IVS=intraventricular septal wall; RVFW=right ventricular septal wall). Panel C = corrected time to peak (TTP) response in milliseconds of the different segments; d=Cohen's D; P=p value of ANOVA in figure A and paired t test in figure C; RVFW=combination of basal and mid segments of the right ventricular free wall and IVS= combination of basal and mid segments of the intraventricular septum.

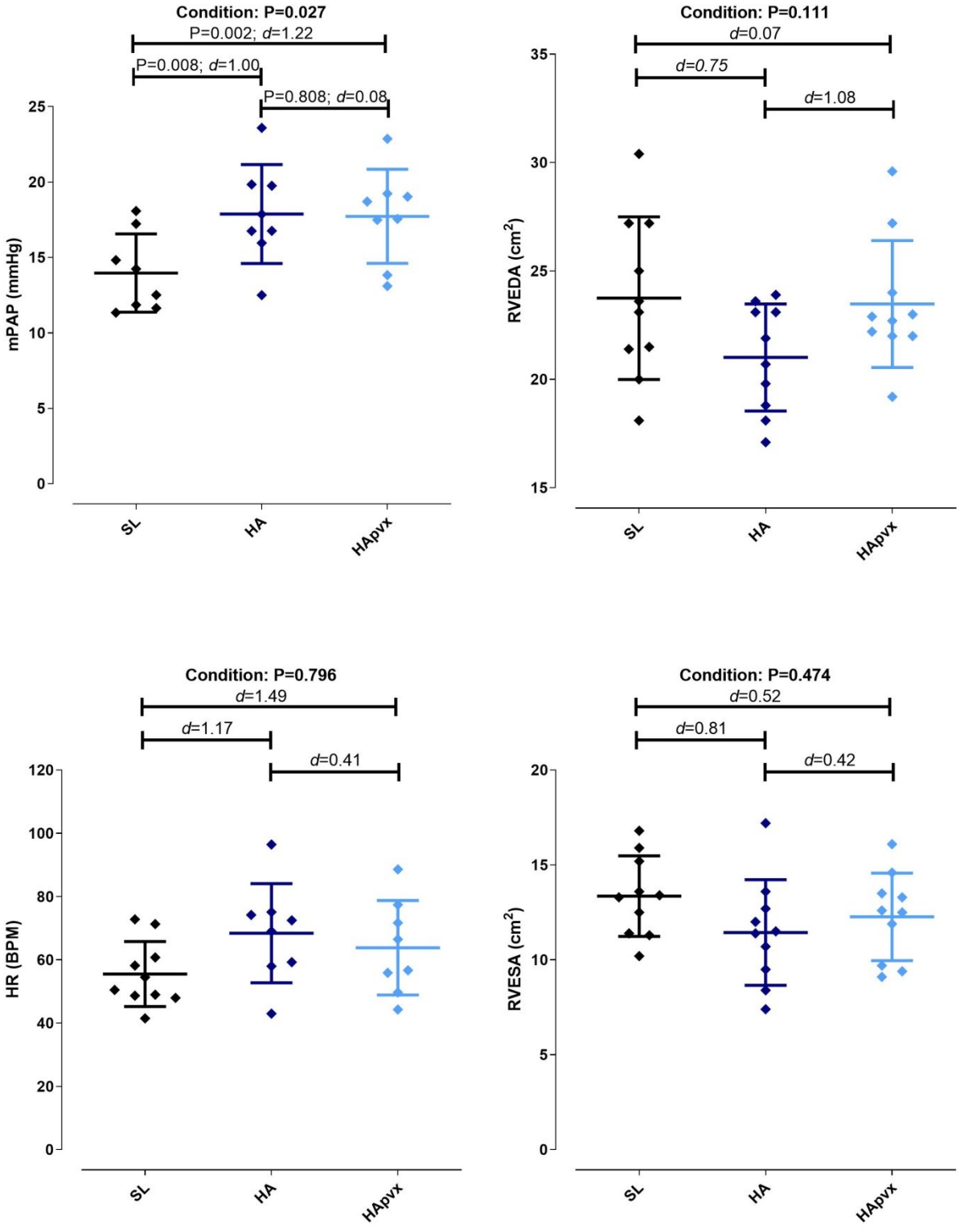

Figure 3: haemodynamic response at sea level (SL), high altitude (HA) and during high altitude with volume expansion (HApvx); P=p value of paired t test. A=mean pulmonary artery pressure (mPAP) in mmHg; B=Right ventricular end diastolic area (RVEDA) in cm^2; C=heart rate (HR) in beats per minute and D=right ventricular end systolic area (RVESA) in cm^2.

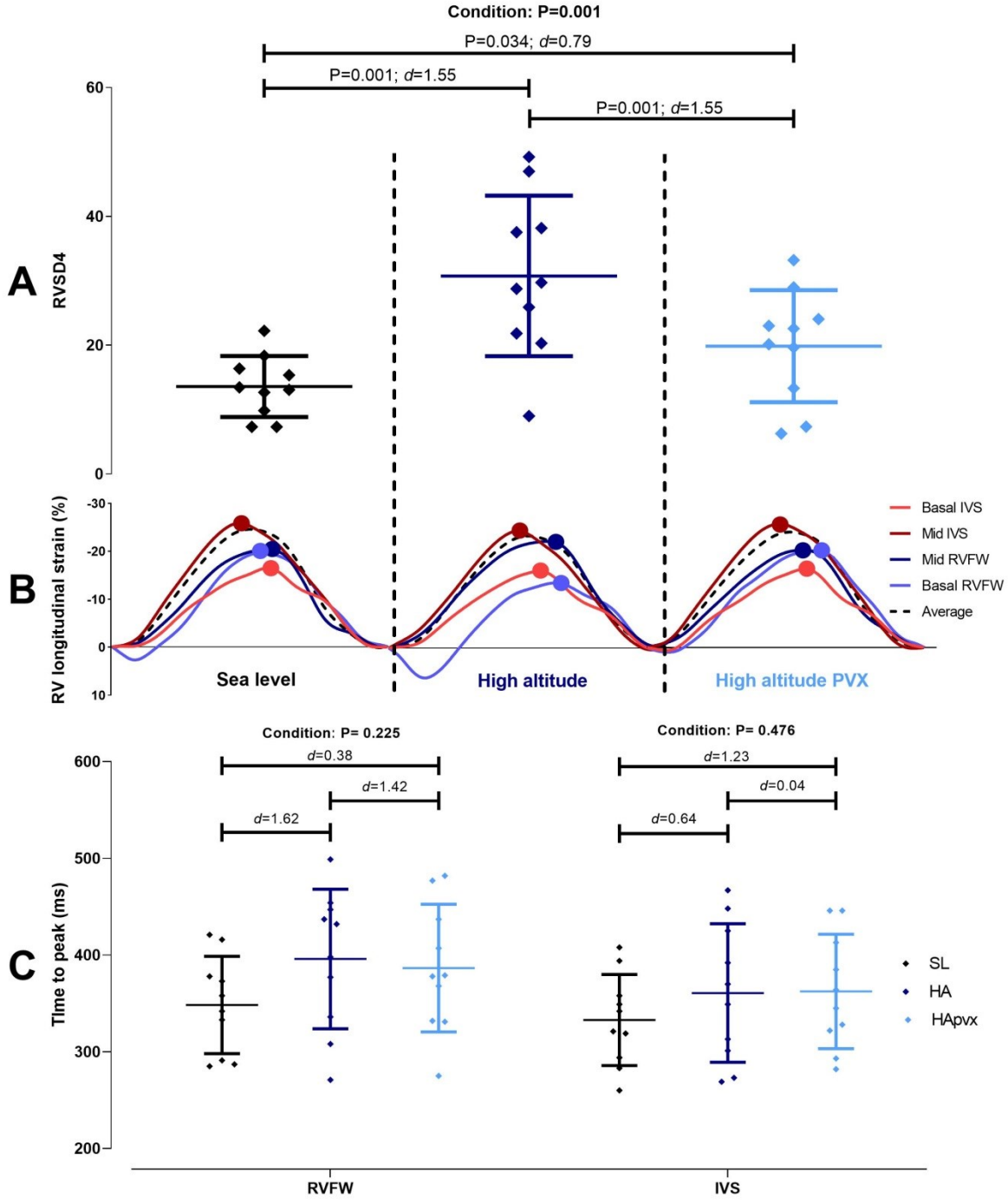

Figure 4: RV response at sea level (SL), high altitude (HA) and volume expansion at high altitude (HApvx). Panel A = RVSD4 response; condition indicates the effect of the different conditions on RVSD4. Panel B = Interpolated segmental strain curves for the four right ventricular wall segments and their time to peak response (indicated with the closed dots; IVS=intraventricular septal wall; RVFW=right ventricular septal wall). Panel C = corrected time to peak (TTP) response in milliseconds of the different segments during sea level (SL), high altitude (HA) and volume expansion at high altitude (HApvx); d=Cohen's D difference between the conditions; P=p value of ANOVA in figure A and paired t test in figure C; RVFW=combination of basal and mid segments of the right ventricular free wall and IVS= combination of basal and mid segments of the intraventricular septum.

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