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Title: Reduced blood flow through intrapulmonary arteriovenous anastomoses at rest and during exercise in lowlanders during acclimatization to high altitude.

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Running Title: Exercise-induced intrapulmonary shunt at altitude

Abstract: Blood flow through intrapulmonary arteriovenous anastomoses (ĊIPAVA) is elevated during exercise at sea level (SL) and at rest in acute normobaric hypoxia. Following high altitude (HA) acclimatization, resting ĊIPAVA is similar to SL, but it is unknown if this is true during exercise at HA. We hypothesized that exercise at HA (5,050 m) would exacerbate ĊIPAVA due to heightened pulmonary arterial pressure. Using a supine cycle ergometer, seven healthy adults free from intracardiac shunts underwent an incremental exercise test at SL (25, 50, 75% of SL V̇O2peak) and at HA (25, 50% of SL V̇O2peak). Echocardiography was used to determine cardiac output (Ċ) and pulmonary artery systolic pressure (PASP) and agitated saline contrast was used to determine ĊIPAVA (bubble score; 0-5). The principle findings were: (1) Ċ was similar at SL-rest (3.9 {plus minus} 0.47 l min-1) compared to HA-

rest (4.5 {plus minus} 0.49 l min-1; P=0.382), but increased from rest during both SL and HA exercise (P<0.001); (2) PASP increased from SL-rest (19.2 {plus minus} 0.7 mmHg) to HA-rest (33.7 {plus minus} 2.8 mmHg; P=0.001) and, compared to SL, PASP was elevated during HA exercise (P=0.003); (3) ȮIPAVA was increased from SL-rest (0) to HA-rest (median=1; P=0.04) and increased from resting values during SL exercise (P<0.05), but were unchanged during HA exercise (P=0.91), despite significant increases in Ȯ and PASP. Theoretical modeling of microbubble dissolution suggests that the lack of ȮIPAVA in response to exercise at HA is unlikely caused by saline contrast instability.

New Findings: What is the central question of this study? To determine whether exercise following 4-7 days at 5,050 m would augment blood flow through intrapulmonary arteriovenous anastomoses (ȮIPAVA) compared to sea level exercise using the technique of agitated saline contrast echocardiography. What is the main finding and its importance? Despite a significant increase in both cardiac output and pulmonary pressure during exercise at high altitude, there is very little ȮIPAVA at rest or during exercise following 4-7 days of acclimatization. Mathematical modeling suggests bubble instability at high altitude is an unlikely explanation for the reduced ȮIPAVA.

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NEW FINDINGS

- **What is the central question of this study?** To determine whether exercise following 4-7 days at 5,050 m would augment blood flow through intrapulmonary arteriovenous anastomoses ($\dot{Q}_{IPAVA}$) compared to sea level exercise using the technique of agitated saline contrast echocardiography.

- **What is the main finding and its importance?** Despite a significant increase in both cardiac output and pulmonary pressure during exercise at high altitude, there is very little $\dot{Q}_{IPAVA}$ at rest or during exercise following 4-7 days of acclimatization. Mathematical modeling suggests bubble instability at high altitude is an unlikely explanation for the reduced $\dot{Q}_{IPAVA}$.

ABSTRACT

Blood flow through intrapulmonary arteriovenous anastomoses ($\dot{Q}_{IPAVA}$) is elevated during exercise at sea level (SL) and at rest in acute normobaric hypoxia. Following high altitude (HA) acclimatization, resting $\dot{Q}_{IPAVA}$ is similar to SL, but it is unknown if this is true during exercise at HA. We hypothesized that exercise at HA (5,050 m) would exacerbate $\dot{Q}_{IPAVA}$ due to heightened pulmonary arterial pressure. Using a supine cycle ergometer, seven healthy adults free from intracardiac shunts underwent an incremental exercise test at SL (25, 50, 75% of SL $\dot{V}O_{2peak}$) and at HA (25, 50% of SL $\dot{V}O_{2peak}$). Echocardiography was used to determine cardiac output ($\dot{Q}$) and pulmonary artery systolic pressure (PASP) and agitated saline contrast was used to determine $\dot{Q}_{IPAVA}$ (bubble score; 0-5). The principle findings were: (1) $\dot{Q}$ was similar at SL-rest (3.9 ± 0.47 l min^{-1}) compared to HA-rest (4.5 ± 0.49 l min^{-1}; P=0.382), but increased from rest during both SL and HA exercise (P<0.001); (2) PASP increased from SL-rest (19.2 ± 0.7 mmHg) to HA-rest (33.7 ± 2.8 mmHg; P=0.001) and, compared to SL, PASP was elevated during HA exercise (P=0.003); (3) $\dot{Q}_{IPAVA}$ was increased from SL-rest (0) to HA-rest (median=1; P=0.04) and increased from resting values during SL exercise (P<0.05), but were unchanged during HA exercise (P=0.91), despite significant increases in $\dot{Q}$ and PASP. Theoretical modeling of microbubble dissolution suggests that the lack of $\dot{Q}_{IPAVA}$ in response to exercise at HA is unlikely caused by saline contrast instability.

Key words: intrapulmonary arteriovenous anastomoses, exercise, high altitude, and contrast dissolution.

INTRODUCTION

Intrapulmonary arteriovenous anastomoses (IPAVA) are present throughout the lung in many mammals including humans and range in size from 25-500 μm (Lovering *et al.* 2007; Tobin, 1966). Though the pulmonary circulation facilitates gas exchange between the blood and alveoli, blood flow through IPAVA ($\dot{Q}_{IPAVA}$) is believed to bypass sites of gas exchange hindering pulmonary gas exchange efficiency (Stickland *et al.* 2004). $\dot{Q}_{IPAVA}$ is normally assessed using the technique of agitated saline contrast echocardiography in participants negative for intracardiac shunts and without pulmonary arteriovenous malformations (Lovering & Goodman, 2012). In these individuals, $\dot{Q}_{IPAVA}$ increases in an exercise-intensity dependent manner (Eldridge *et al.* 2004; Elliott *et al.* 2014b; Norris *et al.* 2014) and is exacerbated with acute normobaric hypoxia (Lovering *et al.* 2008b). For example, $\dot{Q}_{IPAVA}$ is non-detectable at rest in most healthy humans at both sea level (SL) (Elliott *et al.* 2013) and high altitude (HA) (Foster *et al.* 2014), but is present in >90% of participants during normoxic exercise and increases to 100% during hypoxic exercise (Elliott *et al.* 2011; Lovering *et al.* 2008b). Given this interaction between exercise intensity and hypoxia it seems logical that $\dot{Q}_{IPAVA}$ would be exacerbated when exercising at HA.

The primary purpose of the current study was to determine if $\dot{Q}_{IPAVA}$ is increased during exercise while acclimatizing to 5,050 m. Interestingly, previous reports have shown that $\dot{Q}_{IPAVA}$ is minimized during exercise when hyperoxia [fraction of inspired O_2 (F_IO_2) = 1.0] is administered (Lovering *et al.* 2008a). Therefore, the secondary purpose of this study was to identify if hyperoxia regulates $\dot{Q}_{IPAVA}$ during exercise at HA similar to sea-level reports. Finally, we conducted theoretical modeling and previously reported

bubble diameter distributions at SL to estimate the impact of HA on saline contrast stability (Feinstein *et al.* 1984; Vuille *et al.* 1994). We hypothesized that $\dot{Q}_{IPAVA}$ would be greater during exercise at HA compared to similar workloads at SL and hyperoxia would reduce $\dot{Q}_{IPAVA}$. We also hypothesized that the effect of HA on contrast stability would be negligible across the transit times expected during exercise from point of injection to detection in the left ventricle.

MATERIALS AND METHODS

All experimental procedures and protocols were submitted to, and approved by the Clinical Research Ethics Board at the University of British Columbia and the Nepal Health Research Council, and conformed to the Canadian Government Tri-Council Policy Statement for research involving humans and the Declaration of Helsinki. All participants provided written informed consent prior to participation in this study. This study was part of a larger research expedition conducted in April-June in 2012 (Foster *et al.* 2014; Lewis *et al.* 2014; Smirl *et al.* 2014; Smith et al., 2014; Stembridge *et al.* 2015; Willie *et al.* 2014; Willie *et al.* 2015). As such, participants took part in a number of studies conducted in Kelowna, BC (344 m) and during three weeks at the Ev-K2-CNR Pyramid Laboratory located near Mt. Everest base camp at 5,050 m. Participants involved in this study had a minimum of 48 hours between studies to mitigate any concern of cross-contamination from pharmaceutical interventions.

Participants.

17 healthy participants volunteered for this study and were tested in Kelowna, British Columbia, Canada near SL (elevation = 344m). From this group of volunteers, seven (age = 33.1 ± 7.8 yrs; one female) were negative for a patent foramen ovale (PFO-) based upon transthoracic agitated saline contrast echocardiography (Marriott *et al.* 2013). Since the purpose of this study was to determine $\dot{Q}_{IPAVA}$, participants were excluded if they had a PFO at rest or with a provocative maneuver (*see Agitated Saline Contrast Echocardiography*). These seven participants were studied at SL and HA. All participants studied met the inclusion criteria including 18-49 years of age, non-smoking,

and free from any known history of neurological, respiratory and cardiovascular diseases. Participants were asked to refrain from caffeine, alcohol and vigorous exercise at least 12 hours prior to experimentation. Participants were not born at altitudes >1,200 m and had not travelled to HA (>2,500m) within six months prior to SL testing. In addition, participants were excluded if they were hypertensive (>140/90 mmHg), had poor pulmonary function as determined by spirometry (i.e. forced expiratory volume in 1s/forced vital capacity < 0.75), or did not present with tricuspid regurgitation for the estimation of pulmonary artery systolic pressure (PASP). The results presented, unless otherwise specified, are based on seven participants.

Experimental Protocol.

Participants were studied on three separate occasions. First, participants attended the laboratory in Kelowna and were assessed for inclusion/exclusion criteria and completed an incremental exercise test to exhaustion (*see specific methods below*). Height and weight were measured and spirometry (Vmax Spectra 20, Sensormedics, Yorba Linda, California) was conducted in accordance with the standards outlined by the American Thoracic Society and the European Respiratory Society (Miller *et al.* 2005). Participants returned within one week to complete the SL submaximal exercise protocol. During this visit, participants were instrumented with an intravenous catheter at the antecubital fossa while resting supine on a hospital bed. Participants were then asked to lie in the supine position on a custom built, supine cycle ergometer. Following 5 minutes of rest, the participant was positioned in the left lateral decubitus position and, three consecutive blood pressure measurements were manually obtained from the right upper arm using a

sphygmomanometer and stethoscope for auscultation of Korotkoff sounds. Also, at this time, peripheral arterial oxyhaemoglobin saturation (SpO_2; Model 3100, WristOx, Nonin Medical Inc., MN, USA) was estimated by pulse oximetry of the right index finger. Next baseline images were collected for stroke volume (SV; ml), cardiac output ($\dot{Q}$; l min^{-1}) and pulmonary artery systolic pressure (PASP; mmHg) by echocardiography. HR was measured by standard lead two electrocardiogram. Next, an apical 4-chamber view of the heart was acquired and $\dot{Q}_{IPAVA}$ was determined by the technique of agitated saline contrast echocardiography. These experimental measurements were repeated during submaximal exercise stages (5 min each) equivalent to 25, 50, and 75% of SL $\dot{V}O_{2peak}$. Following the 75% $\dot{V}O_{2peak}$ submaximal exercise stage at SL, participants were given a 10 minute period of rest and then the stage was repeated while the participant was administered hyperoxic gas (F_IO_2 = 0.5). The F_IO_2 used to test hyperoxic reversibility of $\dot{Q}_{IPAVA}$ at SL (0.5) and HA (1.0) were selected to match a similar inspired PO_2 of approximately 350 mmHg.

Participants then travelled to Kathmandu, Nepal (1,400 m), and following a week in Kathmandu, underwent an 8-10 day trek to the Ev-K2-CNR Pyramid Laboratory located at 5,050 m in the Himalayan Mountains in Nepal under prophylactic acetazolamide administration (125 mg 2x per day). Acetazolamide was discontinued 24 hours prior to ascending from Pheriche (4,300 m) to the laboratory (5,050 m). Four-to-seven days after arrival at 5,050 m participants conducted two submaximal exercise stages corresponding to 25 and 50% of SL $\dot{V}O_{2peak}$. Following the last stage of exercise, participants rested for 10 minutes prior to repeating the final stage in hyperoxic gas (F_IO_2 = 1.0).

Measurements.

Maximal Exercise Testing. Using a custom built, supine cycle ergometer, participants underwent an incremental exercise test to exhaustion in normoxia to obtain relative peak power outputs at SL. The maximal exercise protocol was developed for individual participants to reach their maximum workload capacity in approximately 6-8 minutes. The protocol began at 50 watts and workload increased by 50 watts every minute until participants reached volitional exhaustion. During the maximal exercise protocol expired gas was analyzed for minute ventilation ($\dot{V}_E$), O_2 uptake ($\dot{V}O_2$), CO_2 production ($\dot{V}CO_2$), and the respiratory exchange ratio (RER) (Vmax 29C, SensorMedics, Yorba Linda, CA), and heart rate was determined by electrocardiograph (CardioSoft, GE Healthcare, Waukesha, WI).

Pulmonary Haemodynamics. All echocardiography measurements were performed using the same commercially available ultrasound system (Vivid Q, 3.5 MHz transducer, GE Healthcare, Piscataway, NJ, USA) and by the same sonographer (M.S.) with several years of experience (Stembridge *et al.* 2015). First, the diameter of the left ventricular outflow tract at the level of the aortic annulus was determined from the parasternal long axis view. Measurements were taken at the end of systole and the average of three cardiac cycles taken as the diameter of the aorta. Then, the velocity time integral of the left ventricular outflow tract was obtained from an apical five-chamber view by placing a pulsed wave Doppler sample volume just within the aortic valve. SV was calculated as the product of the velocity time integral and aortic cross-sectional area, and $\dot{Q}$ was

obtained by multiplication with HR. These methods have been previously described and validated against thermodilution and direct Fick (Christie *et al.* 1987). Tricuspid regurgitation peak velocity was measured by continuous-wave Doppler ultrasound using color flow imaging from the apical four-chamber view. The pulmonary artery pressure gradient was then estimated from the simplified Bernoulli equation and PASP could be estimated by addition of right atrial pressure. Right atrial pressure was estimated from collapse of the IVC during inspiration as recommended by the American Society of Echocardiography (Rudski *et al.* 2010). The collapsibility index was calculated as the percentage of difference between maximal and minimal size of IVC. This method has been validated against right atrial pressure obtained directly by right heart catheterization (Yildirimturk *et al.* 2011). All pulmonary haemodynamic measurements were made on three cardiac cycles and averaged to provide a single value.

Agitated Saline Contrast Echocardiography. The presence of PFO was determined under resting conditions and during a release from Valsalva maneuver using the technique of agitated saline contrast echocardiography (Marriott *et al.* 2013). Two, 5 ml syringes were connected by two three-way stopcocks connected in line with each other and to a 22-gauge cannula placed in the antecubital vein. One syringe contained 4 ml of sterile saline and the other contained 0.5 ml of air. The two syringes were flushed back and forth rapidly and forcefully, creating microbubbles within the agitated saline solution prior to injection. Using an apical 4-chamber view with harmonic imaging, microbubbles could be visualized traveling through the heart immediately after rapid injection of the agitated contrast. If a negative test was identified, a Valsalva maneuver was used to

further assess the patency of the foramen ovale. A PFO was identified if contrast appeared in the left ventricle <5 cardiac cycles after the contrast filled the right atrium. After all contrast injections, a minimum of 20 cardiac cycles was recorded. In the event that a participant was identified as being PFO negative they were then studied for resting $\dot{Q}_{IPAVA}$. An IPAVA was defined when contrast appeared in the left ventricle six or more cardiac cycles after the contrast appeared in the right atrium. This technique has been used to investigate $\dot{Q}_{IPAVA}$ in participants at SL during rest and exercise breathing room air or acute normobaric hypoxia (Elliott *et al.* 2014a; Elliott *et al.* 2014b; Elliott *et al.* 2015; Kennedy *et al.* 2012; Laurie *et al.* 2012; Stickland *et al.* 2004; Tremblay *et al.* 2014). In addition, this technique has been used to investigate $\dot{Q}_{IPAVA}$ at HA during rest (Foster *et al.* 2014). A previously published scoring system was used to determine the severity of $\dot{Q}_{IPAVA}$ based on the greatest density and spatial distribution of microbubbles in the left ventricle of a single cardiac cycle during the subsequent 20 cardiac cycles (Laurie *et al.* 2010). This 0-5 scoring system assigns a '0' for no microbubbles; '1' for 1-3 microbubbles; '2' for 4-12 microbubbles; '3' for greater than 12 microbubbles bolus; '4' for greater than 12 microbubbles heterogeneously distributed; and a '5' for greater than 12 microbubbles homogeneously distributed. This scaling system has been validated for reproducibility against independent blinded observers (Laurie *et al.* 2010).

Model of Contrast Bubble Dissolution. Eqn. 1, derived by Solano-Altamirano and Goldman (2014), provides the dissolution time for a gas bubble of a given radius in a fluid medium. The underlying model has three regions: a bubble, a diffusive region that surrounds the bubble, and a well-mixed region that surrounds the diffusive region. The

bubble is spherical, and both the diffusive and the well-stirred regions are spherical shells. Diffusion is the only from of transport that is explicit in the model. Convection is considered indirectly and qualitatively, by using a three-region model, and the parameter lambda (λ). When λ=1, the diffusion shell thickness is equal to 0, such that convection is the dominant transport mechanism; as λ approaches infinity, the model reduces to a two-region model, wherein diffusion is the only transport mechanism [see Solano-Altamirano and Goldman (2014)].

$$t = -\frac{(R^2 - R_O^2)(\lambda-1)}{2D\lambda d(1-f)} + \frac{2(2f+1)\gamma(R-R_O)(\lambda-1)}{3D\lambda d(1-f)^2 P_e} - \left\{\frac{4(2f+1)\gamma^2(\lambda-1)}{3D\lambda d(1-f)^3 P_e^2} \cdot \ln\left(\frac{(1-f)P_e R+2\gamma}{(1-f)P_e R_O+2\gamma}\right)\right\}.$$

Equation 1.

Using this model, time to bubble dissolution (t) can be estimated as a function of initial bubble radius (R_0) by setting the final bubble radius (R) to zero and solving the remaining constants as appropriate for the environmental conditions at SL and HA during rest and exercise. Table 1 provides a summary of key constants and parameters used to solve Eqn 1. Diffusivity (D) was calculated using the Stokes-Einstein equation (Eqn 2; Kholodenko & Douglas, 1995) using body temperature at rest (T, 310.15 K) and exercise (311.15 K), a Boltzman constant (K_B) of 1.38 x 10^{-23} J/K, the molecular radius of air (r; calculated as the fractional sum of the molecule radius of O_2, CO_2, and N_2) and, finally, the average blood viscosity (η) of a healthy human with a haematocrit level of 46.7% at SL (2.94 cP) and 49.4% at HA (3.15 cP) (Hoffman, 2011; Foster *et al.* 2014).

$$D = \frac{k_B T}{6\pi\eta r}$$

Equation 2.

In Eqn 1. the external pressure (P_e) is assumed to be the sum of atmospheric pressure (P_{atm}) and PASP during each condition (PASP values measured in this study were used for the bubble dissolution model). The *f* ratio in Eqn 1 is the ratio of the sum of all partial pressures of dissolved air in mixed venous blood (i.e. $PvN_2 + PvO_2 + PvCO_2 + PvH_2O$) to P_e. The *f* ratio varies according to the difference in P_{atm}, PASP, and the partial pressure of constituent gases in mixed venous blood in different environmental and physical conditions (see Table 1). For example, at SL, P_e is 780 mmHg (P_{atm}=760 mmHg and PASP=20 mmHg) and at rest, the partial pressure of nitrogen, oxygen, carbon dioxide and water vapor in mixed venous blood are 573 mmHg, 36 mmHg, 39 mmHg and 47 mmHg respectively (George & Kinasewitz, 1983; Wagner *et al*. 1986), resulting in a *f* ratio of 0.891. Surface tension (γ) of the bubbles in blood is assumed to be 0.5 μatm at both SL and HA (Burkard, 1995). The term λ is a constant used to account for the mixing and convection effects of the downstream compartment and has been set to a value of 2, similar to previous predictions involving gas emboli in circulating blood (Solano-Altamirano and Goldman, 2015). Finally, d represents the Henry's solubility constant and is defined by RT/K_H where *R* is gas constant, T is body temperature during rest and exercise and K_H is Henry's Law constant. Henry's law constant for air in water is first temperature corrected using the van't Hoff equation (Atkins & de Paula, 2006) and determined to be 1735 l.atm/mol at rest and 1775 l.atm/mol during exercise. Bubble dissolution time was calculated for bubble radii ranging in size from 0-20μm by completing Eqn 1 for resting conditions and exercise conditions expected at SL and HA. For each condition, contrast lost over time was determined by generating decay curves based on a random normal distribution of bubble radii which had a mean and standard

deviation based on two individual published reports (8.0 ± 6.5 & 19.0 ± 7.5 μm) (Feinstein *et al.* 1984; Vuille *et al.* 1994). Using the dissolution time for each bubble within each distribution, contrast decay curves were generated to illustrate the time dependent instability of saline contrast in all conditions.

Statistical Analysis.

Statistical comparisons and calculations were made using statistical software (R, http://cran.r-project.org). Two-way repeated-measures analyses of variance were used to test for differences in $\dot{Q}$, PASP, SpO_2, and HR between rest and multiple exercise stages for both SL and HA. Bonferroni-corrected post-hoc tests were used for pair-wise comparisons. Friedman's test with Conover post-hoc test was used to test for differences in bubble scores between rest and exercise stages for both SL and HA. Correlation of shunt scores to PASP, and $\dot{Q}$ were conducted using Spearman's rank order correlation coefficient. For all tests, significance was assumed at $P<0.05$.

RESULTS

Participant characteristics and maximal exercise data are presented in Table 2. Participants had normal pulmonary function and average exercise capacity. No participants included in this study had a patent foramen ovale at rest or during a release from a Valsalva maneuver.

Figure 1 illustrates the changes in pulmonary artery systolic pressure, heart rate, and cardiac output during supine cycle exercise at SL and at HA. Resting heart rate and cardiac output were similar at SL and HA. During exercise, heart rate and cardiac output increased with increasing exercise intensity similarly between altitudes. PASP was measured in all participants at SL but at HA a high drive to breathe during exercise impacted image quality. As a result, PASP was only measureable in 6 of 7 at 25% SL $\dot{V}O_{2peak}$ and 3 of 7 at 50% SL $\dot{V}O_{2peak}$. Resting PASP was increased at HA by 6.4 ± 2.4 mmHg ($P<0.05$). During exercise, PASP increased modestly with increasing exercise intensity whereas the increase in PASP at HA was substantial reaching levels of 22.8 ± 2.4 mmHg and 26.2 ± 3.3 mmHg above SL resting values ($P<0.05$) during 25% and 50% SL $\dot{V}O_{2peak}$, respectively. At SL, hyperoxia had no appreciable effect on PASP, heart rate, or $\dot{Q}$. At HA, hyperoxia trials were not conducted because there was little evidence of $\dot{Q}_{IPAVA}$ (*see below*). SpO_2 was not changed by exercise at SL (SpO_2: Rest = 97 ± 0.4%; 75% SL $\dot{V}O_{2peak}$ = 96 ± 0.6%) but was reduced at HA and with HA exercise (SpO_2: Rest = 81 ± 2%; 50% SL $\dot{V}O_{2peak}$ = 74 ± 2%).

Bubble score was zero in all participants at rest at SL. At rest at HA, with significantly elevated PASP and reduced SpO_2, bubble score was measured to be zero in two participants, one in three participants, and two in two participants, with a median

score of 1 (Figure 2). As expected, bubble score increased with increasing exercise intensity at SL with median values reaching a score of three at 75% SL $\dot{V}O_{2peak}$. Interestingly, hyperoxia only reduced bubble score in two participants. At HA, bubble scores were not increased with increasing exercise intensity. Due to the lack of response with increasing exercise, hyperoxia trials were not conducted at HA. Figure 3 shows the relationship between bubble score and $\dot{Q}$ and PASP during exercise at SL and HA. At SL a significant Spearman's rank order correlation coefficient (r_s) was found for $\dot{Q}$ (r_s= 0.558, P<0.001) and PASP (r_s = 0.732, P<0.001). Such relationships were absent at HA.

The model of saline bubble dissolution time suggested that at SL, bubbles survive approximately 24% longer than at HA over a bubble radii range of 8 to 19 μm, representing mean radii measured in previous investigations (Fig. 4a) (Feinstein *et al.* 1984; Vuille *et al.* 1994). Based on a mean bubble radius of 8 μm from Feinstein *et al* (1984), the estimated time to dissolution was 4.2s at SL and 3.2s at HA in resting state. Bubbles with a mean bubble radius of 19 μm reported by Vuille *et al* (1994) were found to have an estimated time to dissolution of 49.5s at SL compared to 37.7s at HA in a resting physiological state. In addition, the bubble stability is reduced under maximal exercise conditions at HA (Fig.4a). As a result, exercise at HA gives the shortest dissolution time due to the increase in pulmonary pressure and the decreased *f* ratio, which is ~3.1 or 38.3s for mean radii proposed by Feinstein *et al* (1984) and Vuille *et al* (1994), respectively. Figure 4b illustrates the decay of contrast over time where it is observed that time to dissolution is not only dependent upon the initial size of the bubble distribution but also on the altitude and physiological state (i.e. rest or exercise). However, the effect of the lost contrast is amplified at greater transit time (time from

point of contrast creation to its visualization in the left ventricle). Hence, near maximal exercise, when transit time is least, the amount of contrast lost to dissolution is much less than in the resting state.

DISCUSSION

In this study, we measured $\dot{Q}_{IPAVA}$ during sub-maximal exercise at sea-level and during acclimatization to high-altitude (5,050m) using the technique of agitated saline contrast echocardiography. The three major findings of this study are: i) the lack of change in $\dot{Q}_{IPAVA}$ from rest to 50% of SL $\dot{V}O_{2Peak}$ at HA, ii) the loss of relationship between PASP and $\dot{Q}$ with $\dot{Q}_{IPAVA}$ with increasing exercise intensity at HA, and iii) the time to dissolution model suggests that at near maximal exercise the destabilizing effect of HA on saline bubbles is an unlikely explanation for the lack of $\dot{Q}_{IPAVA}$ during exercise at HA. These data suggest an overall lack of $\dot{Q}_{IPAVA}$ during exercise following 4-7 days of HA. This finding is in contrast to previous reports at sea-level that show an increase in $\dot{Q}_{IPAVA}$ during exercise in hypoxic conditions, suggesting that hypobaria or prolonged hypoxia may be an important regulator of $\dot{Q}_{IPAVA}$.

Though anatomical evidence confirms the existence of IPAVA in humans, it remains unclear if they are actively or passively regulated. Graded exercise at SL typically results in an increase in $\dot{Q}_{IPAVA}$ in nearly all participants (Eldridge *et al.* 2004; Kennedy *et al.* 2012; Stickland *et al.* 2004) as does acute normobaric hypoxia (Bates *et al.* 2014; Elliott *et al.* 2011; Laurie *et al.* 2010; Lovering *et al.* 2008b; Norris *et al.* 2014), suggesting that the increase in $\dot{Q}$ and PASP may contribute to IPAVA recruitment. This mechanical hypothesis suggests that IPAVAs could serve as a pressure relief system to protect the pulmonary capillaries from high perfusion pressure associated with acute increases in cardiac output. In this scenario, shear stress could serve as a stimulus that actively governs $\dot{Q}_{IPAVA}$, or $\dot{Q}_{IPAVA}$ could be passively regulated through increases in pulmonary perfusion causing recruitment of under perfused regions of the lungs that

contain IPAVA. Studies that involve a pharmacological increase in cardiac output show a concomitant increase in $\dot{Q}_{IPAVA}$ (Bryan *et al.* 2012; Elliott *et al.* 2014a; Laurie *et al.* 2012). Our SL data are consistent with this hypothesis; we found a positive correlation between $\dot{Q}_{IPAVA}$ and both $\dot{Q}$ and PASP (Fig. 3). The increases in $\dot{Q}_{IPAVA}$ were mitigated by administering hyperoxia at SL (Fig. 2), which is consistent with previous data, providing evidence for a possible second regulatory mechanism of a humoral nature that responds to circulating PO_2 (Duke *et al.* 2016; Elliott *et al.* 2014b; Tremblay *et al.* 2014). Interestingly, the relationships between $\dot{Q}_{IPAVA}$ and $\dot{Q}$ and PASP were absent following 4-7 days of acclimatization at 5,050 m (Fig. 3); median bubble scores were 1 at rest and Q_{IPAVA} did not increase with exercise (Fig. 2) despite similar $\dot{Q}$ compared to SL and substantially augmented PASP (Fig. 2). These data are in contrast to previous work and suggest an added level of complexity in IPAVA regulation possibly involving pulmonary vascular remodeling in response to prolonged hypoxia (Stenmark *et al.* 2006). Alternatively, a serious limitation regarding the agitated saline contrast methodology could be involved if the stability of this contrast agent was greatly reduced at 5,050 m. Theoretical modeling data suggest that contrast instability is unlikely to account for the lack of $\dot{Q}_{IPAVA}$ during exercise and a large portion of the discussion will be dedicated to the factors contributing to microbubble stability in the hypobaric environment.

In interpreting our data, it is tempting to attribute the lack of $\dot{Q}_{IPAVA}$ to a destabilizing effect associated with the low-density air within the microbubble in the HA environment. Measuring $\dot{Q}_{IPAVA}$ using a peripheral injection of agitated saline contrast is based on the assumption that as a bolus travels through the pulmonary circulation there are only two possible fates: (1) the bubbles travel through alveolar capillaries where

microbubbles dissolve and equilibrate with the alveolar gasses in the lung, or (2) the bubbles travel through an IPAVA and are detectable in the left atrium (Lovering & Goodman 2012). This oversimplified model does not account for the limited stability of the microbubbles within a blood vessel, which are susceptible to changes in blood gas concentrations, blood pressure, blood viscosity, bubble gas density, and blood gas solubility (Epstein & Plesset, 1950).

In the theoretical model of bubble stability presented here, the three major factors contributing to the difference of dissolution time between experimental conditions are diffusivity, ambient pressure and saturation ratio (*f*). Figure 4a illustrates that the decrease in diffusivity, primarily driven by a decrease in ambient pressure causes an overall decrease in bubble stability. This instability at HA would be greater if not for the 2.7% increase in blood haematocrit (7% increase in blood viscosity) expected following 2-3 weeks of exposure at 5,050m of altitude (Foster *et al*. 2014). Comparing rest and exercise states at SL and HA illustrates the importance the saturation factor (*f*) and the change in external pressure (P_e; P_{atm} + PASP) have on bubble stability. The exponential increase in time to dissolution with bubble radius clearly suggests that the initial bubble size within a bolus of agitated saline contrast is the major governor of stability. This phenomenon is further illustrated in figure 4b where it is apparent that there is a much slower rate of contrast decay in a bolus with large mean bubble sizes. Although the time dependent decay of contrast and the contrast instability caused by hypobaria are important factors to consider when quantifying $\dot{Q}_{IPAVA}$ (Hackett *et al*. 2016), it seems unlikely that during exercise when transit time is short (~2-5s) that bubble instability would be of sufficient magnitude to observe no contrast in the left ventricle. For

example, model data presented in Figure 4B would suggest that, at a transit time of 3s, the amount of contrast surviving its detection in the left ventricle would be 69.3 % at SL and 64.0% at HA using the bubble diameter distributions reported by Feinstein *et al.* (1984). This difference is negligible if using the bubble diameters reported by Vuille *et al.* (1994). Given the fact that microbubbles are nearly as stable at HA compared to SL, it must be assumed that there is some physiological phenomenon that arrests the recruitment of IPAVA at HA during exercise.

Though this model has been shown to accurately predict bubble characteristics within the cardiovascular system (Solano-Altamirano & Goldman, 2014), it does not consider normal physiological changes in blood pressure or flow. Once infused, the bubble encounters a number of different pressure environments while travelling from peripheral vein to its detection in the left ventricle, which could impact its stability. We assumed a constant pressure equal in magnitude to the pulmonary artery systolic pressure. Since high pressures decrease bubble stability, our model is a conservative estimate of bubble stability. We acknowledge that the pulsatile nature of pulmonary arterial blood pressure could also threaten bubble stability, though previous work in an in vitro model determined that the oscillations in pressure were secondary to changes in peak pressure when determining stability (Padial *et al.* 1995). Finally, it has been confirmed that increases in flow shorten the lifespan of a saline bubble (Yang *et al.* 1971). Our model does not account for this flow effect, though it seems unlikely that the lack of contrast observed at HA can be attributed to flow given the similar cardiac outputs at SL and HA (see Figure 1). Based upon this theoretical evidence, the lack of $\dot{Q}_{IPAVA}$ observed in our study during exercise at HA appears to be a true physiological

phenomenon associated with exposure to hypobaric hypoxia or as a result of the HA acclimatization process.

Our results are in contrast to our hypothesis and do not appear to be the result of altered saline contrast stability. In this section, we offer some speculation into the physiological underpinnings for the lack of $\dot{Q}_{IPAVA}$ at HA during exercise. Previous reports (Lovering *et al.* 2015) have speculated that IPAVA have greater distribution in the under-perfused apex of the lung, and that IPAVA may offer pressure relief in response to rising mean pulmonary artery pressure during hypoxic pulmonary vasoconstriction (HPV). In the case of severe hypoxia, such as hypobaric exercise, multiple inert gas elimination technique has shown that ventilation-perfusion matching is impaired, possibly due to heterogeneity in the HPV response (Torre-Bueno *et al.* 1985) which may discriminately divert cardiac output away from IPAVA. However, the discrepancy between acute hypoxia induced $\dot{Q}_{IPAVA}$ and the lack thereof at high altitude (Foster *et al.* 2014) potentially implicates hypobaria, *per se*, as a regulator of IPAVA tone. Evidence that the pulmonary vasculature is responsive to hypobaria independent of O_2 status is available in the literature. For example, Levine *et al.* (1988) reported increased pulmonary vascular resistance in sheep decompressed to 6,400 m while the F_IO_2 was increased to keep arterial PO_2 at normoxic levels. Likewise, estimates of total pulmonary resistance by echocardiography in humans suggests an independent effect of hypobaria. In a group of 8 subjects, total pulmonary resistance (PASP/$\dot{Q}$) was equal to 4.8 ± 1.7 mmHg/l/min in normobaria but when decompressed to 5,260 m while adding oxygen to maintain inspired PO_2, the pulmonary resistance increased to 6.2 ± 1.8 mmHg/l/min *(unpublished observations, A. Lovering, personal communication).* While it

is possible that hypobaria itself may directly effect pulmonary vascular tone or IPAVA patency, the specific mechanism is unknown. Finally, pulmonary vascular remodeling in response to prolonged HPV and increased pulmonary vascular pressure may impact $\dot{Q}_{IPAVA}$ measured following prolonged hypoxia. Pulmonary vascular remodeling occurs within 3 days of high altitude exposure in animals through the proliferation of smooth muscle cells in normally thin-walled small pulmonary arteries and the thickening of media and adventitia in larger pulmonary arteries (Stenmark *et al.* 2006). It is not fully understood when pulmonary vascular remodeling begins in humans but the inability to reverse HPV through the administration of hyperoxia, a diagnostic test for vascular remodeling, suggests it can begin as early as 24 hours post exposure (Kronenberg *et al.* 1971; Maggiorini *et al.* 2001). While our data clearly cannot implicate any of these hypotheses, they do offer future avenues for research.

We were able to study a limited number of participants (n = 7) at SL and HA, and this could be viewed as a limitation. However, 50% of sea-level $\dot{V}O_{2peak}$ is nearly maximal at 5,000 m, and is therefore a sufficiently potent stimulus which at sea level would evoke a substantial increase in $\dot{Q}_{IPAVA}$. Furthermore, a post hoc power calculation of our result using the Wilcoxon-Mann-Whitney test suggests that our study was sufficiently powered (>80%) to detect a difference in bubble score at this exercise intensity, given our sample size.

CONCLUSION

Despite significant elevations in $\dot{Q}$ and PASP during exercise at HA, $\dot{Q}_{IPAVA}$ did not increase from resting levels. Given that our theoretical model suggests that bubble stability is not sufficiently altered at HA compared to SL during the transit times of

interest, our findings suggest that IPAVA recruitment at HA is not similar to their recruitment during SL exercise or with acute normobaric hypoxia. Using our experimental design, we are not able to identify the specific physiological mechanism for the lack of exercise-induced $\dot{Q}_{IPAVA}$ at high altitude. Future studies may consider manipulating the time of high altitude exposure or the level of hypobaria to help delineate if hypobaria or pulmonary vascular remodeling are responsible for the lack of IPAVA recruitment with HA exposure.

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AUTHOR CONTRIBUTIONS

(1) Conception and design of the experiments: ATL, PNA, GEF; (2) collection, analysis and interpretation of data: LMB, ATL, MMT, TAD, MS, TAN, PNA, GEF (3) Drafting the article or revising it critically for important intellectual content: LMB, ATL, MMT, TAD, MS, TAN, PNA, GEF.

DISCLOSURES

None

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TABLES

Table 1. Summary of mixed venous blood gases and model constants used to solve Eqn 1.

	Sea Level		High Altitude	
	Rest	Exercise	Rest	Exercise
P_{atm}, mmHg	760	760	413	413
PASP, mmHg	20	26	34	52
P_e, mmHg	780	786	447	465
PvN_2, mmHg	573	573	278	278
PvO_2, mmHg	36	19	27	13
$PvCO_2$, mmHg	39	63	34	42
Pv_{H2O}, mmHg	47	51	47	51
f	0.891	0.898	0.864	0.826
D, $\mu m^2/s$	683.3	686.8	637.8	641.0
d	0.015	0.015	0.015	0.014

Definition of abbreviations: P_{atm}, atmospheric pressure; PASP, pulmonary artery systolic pressure; P_e, external pressure; PvN_2, mixed venous partial pressure of nitrogen; PvO_2, mixed venous partial pressure of oxygen; $PvCO_2$, mixed venous partial pressure of carbon dioxide; PvH_2O, water vapour pressure in mixed venous environment; f, ratio of the sum of all mixed venous gases and the ambient pressure; D, diffusivity; d, Henry's solubility constant for an ideal gas. P_{atm} and PASP are measured in the current study while the partial pressure of mixed venous nitrogen, oxygen, and carbon dioxide are based on published reports at similar altitudes (George & Kinasewitz, 2005; Fenn et al. 1946; Wagner et al. 1986).

Table 2. Participant characteristics, resting pulmonary function and maximal exercise data measured at SL.

Variable	MEAN ± SD
Age, yr	33 ± 8
Height, m	1.76 ± 0.08
Weight, kg	81.7 ± 12.5
BMI, kg m^{-2}	26.4 ± 2.9
FVC, l	5.0 ± 0.9
(% predicted)	(99 ± 11)
$FEV_{1.0}$, l	4.14 ± 0.71
(% predicted)	(105 ± 13)
$FEV_{1.0}$/FVC, %	82 ± 2
(% predicted)	(106 ± 3)
$\dot{V}O_{2peak}$, ml kg^{-1} min^{-1}	45 ± 9
Power at $\dot{V}O_{2peak}$, W	292 ± 66
Maximal HR, bpm	173 ± 17

Data are mean ± SD. Maximal exercise data is measured at sea level. Definition of abbreviations: BMI, body mass index; FVC, forced vital capacity; $FEV_{1.0}$, forced expiratory volume in one second; $\dot{V}O_{2peak}$, peak oxygen consumption; HR, heart rate.

FIGURE LEGENDS

Figure 1: Pulmonary artery systolic pressure (PASP; A), heart rate (B), and cardiac output ($\dot{Q}$; C) at rest and during supine cycle exercise at sea level and 5,050m. Data are means ± SEM. *P<0.05 compared to rest. †P<0.05 compared to sea level. Definitions of abbreviations: % SL $\dot{V}O_{2peak}$: percent of peak oxygen consumption determined during a maximal supine cycle exercise test conducted at sea level.

Figure 2: The bubble score at rest, during exercise, and with hyperoxia while at sea-level (A) and high altitude (B). Individual participant bubble scores are provided at each stage of exercise. The line indicates the median value. *P<0.05 compared to rest. Definition of abbreviations: % SL $\dot{V}O_{2peak}$: percent of peak oxygen consumption determined during a maximal supine cycle exercise test conducted at sea level.

Figure 3. The relationship between bubble score and cardiac output (A) and pulmonary artery systolic pressure (PASP; B) at sea level and high altitude. At sea level, a significant spearman rank order coefficient was found relating bubble scores with cardiac output and PASP. At high altitude this correlation was lost. Definition of abbreviations: SL: sea level; HA: high altitude; r_s = Spearman rank order correlation coefficient; PASP: pulmonary artery systolic pressure.

Figure 4. Theoretical model of microbubble stability. **A.** The time to bubble dissolution as a function of bubble radius in blood during rest and exercise at sea level and following 4-7 days of acclimatization to 5,050m. **B.** The estimated percent of contrast conserved within a bolus of agitated saline contrast over time for bubbles in the pulmonary circulation during rest and exercise at sea level and following 4-7 days of acclimatization at 5,050 m. Data are calculated based on the curves presented in panel A and a random normal bubble diameter distribution based on published reports of bubble diameter in agitated saline made at sea-level (Feinstein *et al.* 1984; Vuille *et al.* 1994). Hatched box represents the range of expected transit times from rest (~9.3s) to exercise (~3.0s) (Hopkins *et al*. 1996).

Figure 1.

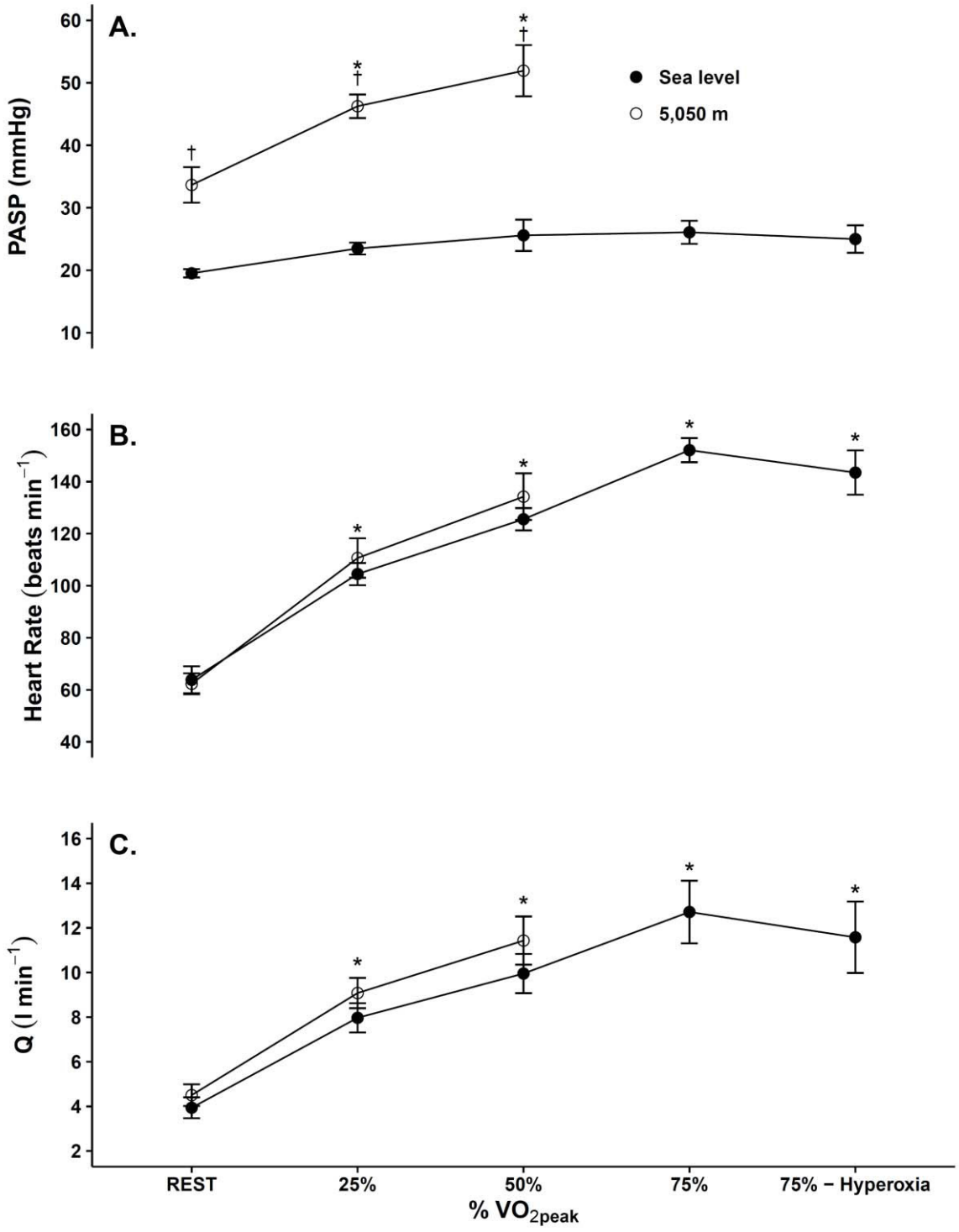

A.
PASP (mmHg)
60
50
40
30
20
10
Sea level
5,050 m
B.
Heart Rate (beats min^{-1})
160
140
120
100
80
60
40
C.
Q (l min^{-1})
16
14
12
10
8
6
4
2
REST
25%
50%
75%
75% − Hyperoxia
% VO$_{2peak}$

Figure 2.

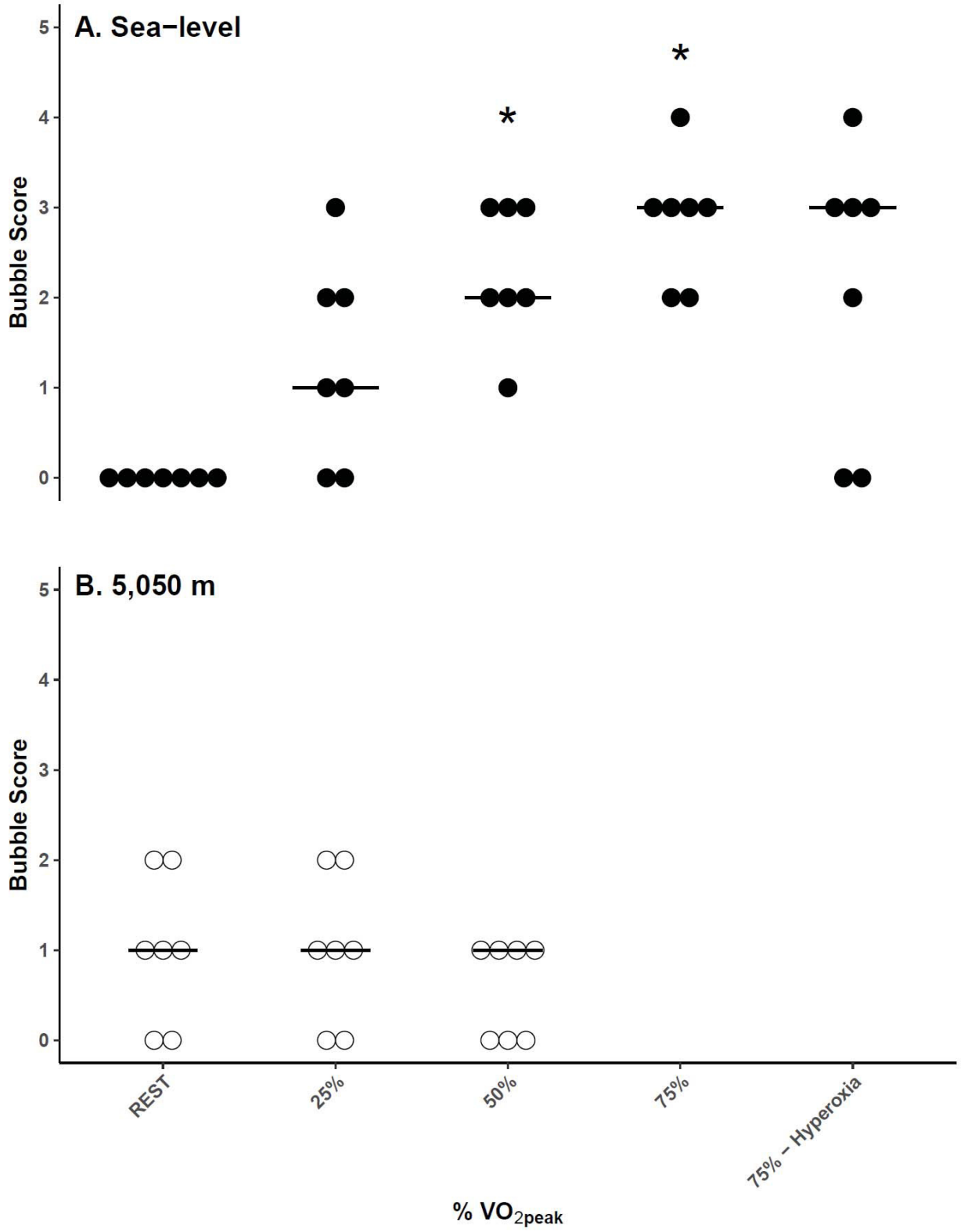

A. Sea−level
B. 5,050 m
Bubble Score
0
1
2
3
4
5
*
*
REST
25%
50%
75%
75% – Hyperoxia
% VO2peak

Figure 3.

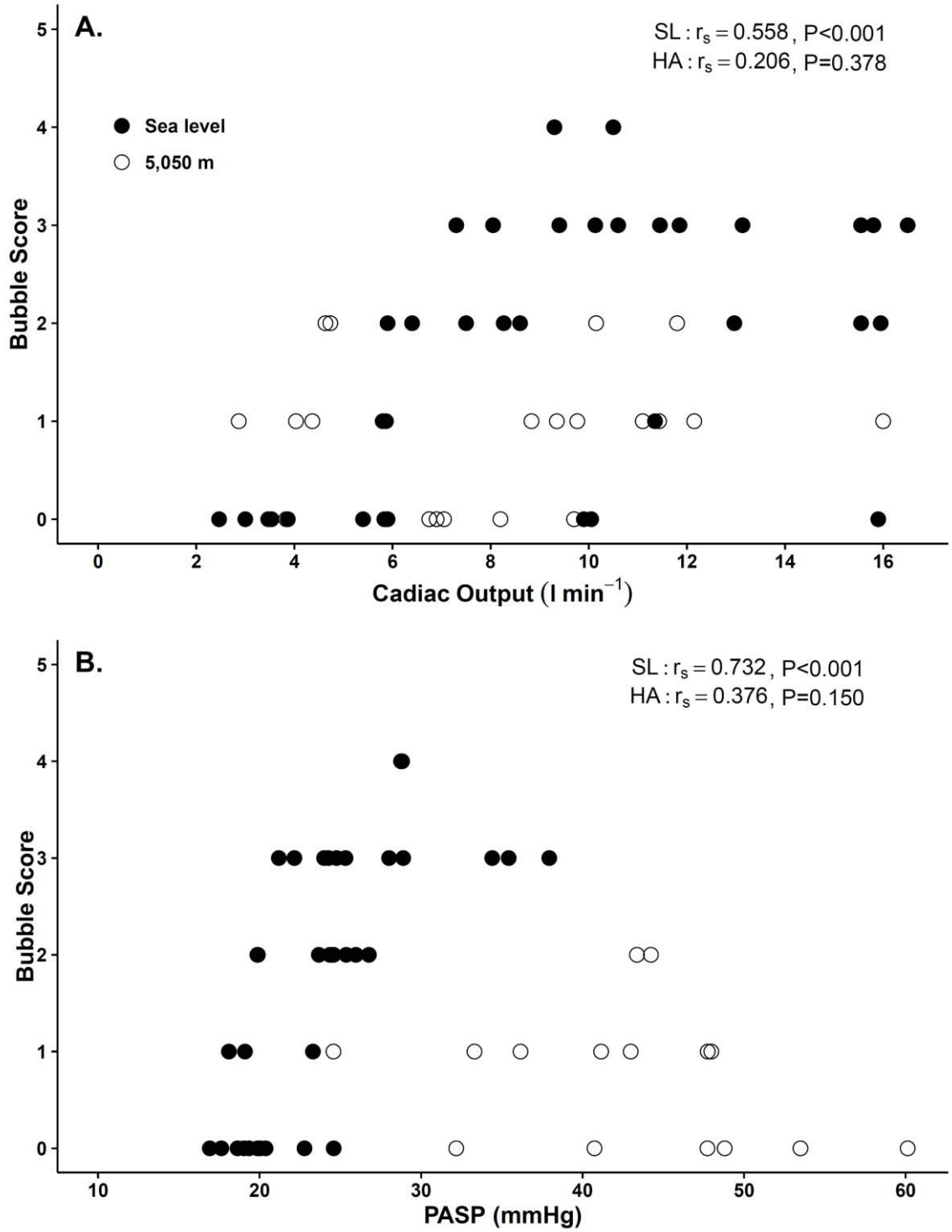
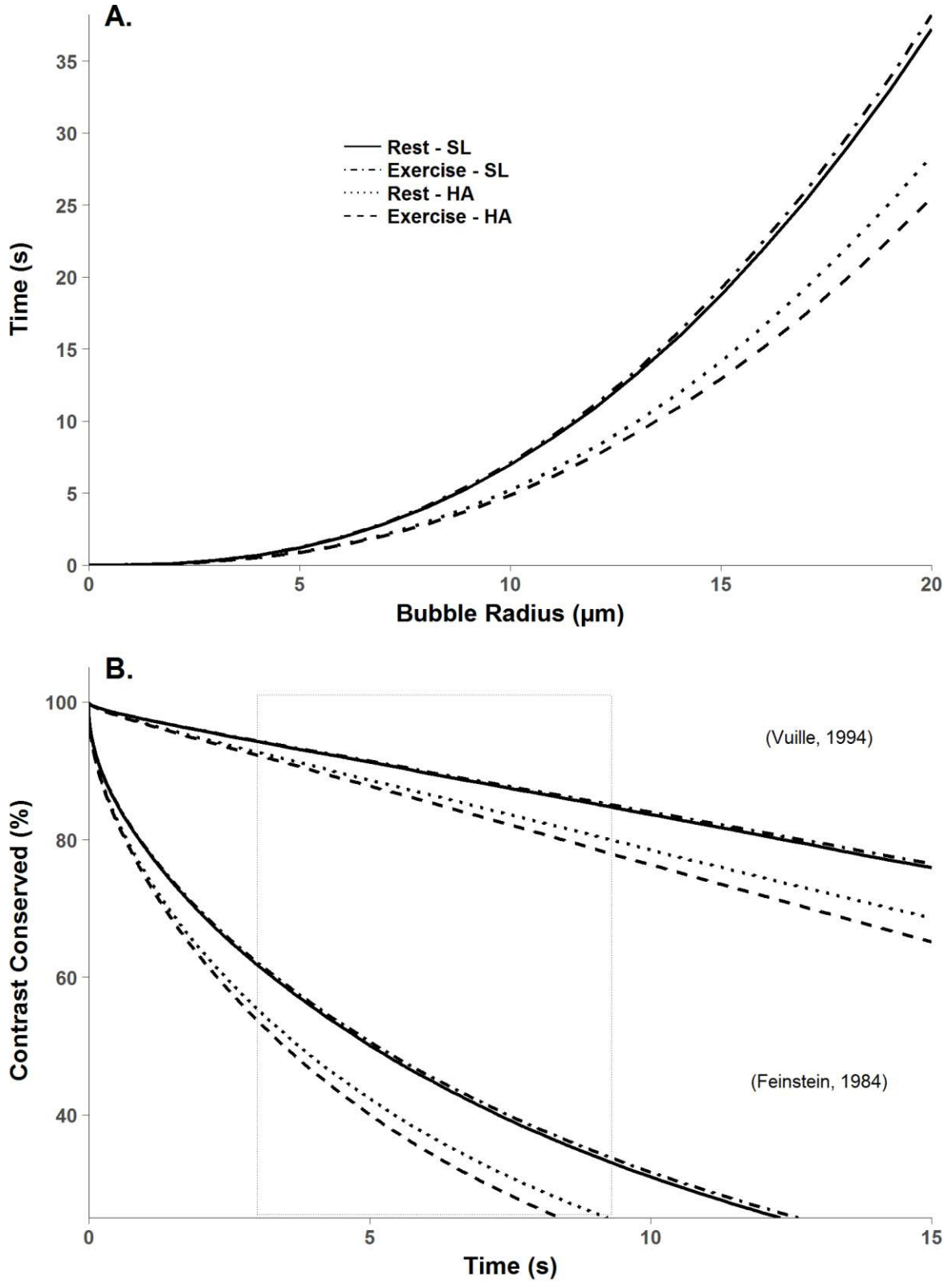

A.
SL : r_s = 0.558, P<0.001
HA : r_s = 0.206, P=0.378
Sea level
5,050 m
Bubble Score
Cadiac Output (l min^{-1})
B.
SL : r_s = 0.732, P<0.001
HA : r_s = 0.376, P=0.150
Bubble Score
PASP (mmHg)

Figure 4


A.
Rest - SL
Exercise - SL
Rest - HA
Exercise - HA
Time (s)
Bubble Radius (μm)
B.
(Vuille, 1994)
(Feinstein, 1984)
Contrast Conserved (%)
Time (s)