Title: Resting pulmonary haemodynamics and shunting: a comparison of sea-level inhabitants to high altitude Sherpas.

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Key points summary:

- Evolutionary pressure to improve gas exchange and/or resting pulmonary haemodynamics in hypoxic environments may have led to differences in that amount of blood that flows through right-to-left shunt pathways between Sherpas and sea-level inhabitants.
- We studied sea-level inhabitants during rest at sea level and acute isocapnic hypoxia and during rest at high altitude following 3 weeks of acclimatization and compared their responses to those of Sherpas during rest at high altitude.
- Contrary to some previous literature, we found similar resting pulmonary pressure and total pulmonary resistance between acclimatized sea-level inhabitants and Sherpas at high altitude.
- We also found a similar number of subjects from each group with intracardiac shunt and intrapulmonary shunt at high altitude.
- These results help us better understand resting cardiopulmonary adaptations to high altitude by comparing life-long high-altitude residents with sea-level inhabitants acclimatized to high altitude.

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Abstract.

Incidence of blood flow through intracardiac shunt and intrapulmonary arteriovenous anastomoses (IPAVA) may differ between Sherpas permanently residing at high altitude (HA) and sea-level (SL) inhabitants as a result of evolutionary pressure to improve gas exchange and/or resting pulmonary hemodynamics. To test this hypothesis we compared sea-level inhabitants at SL (SL-SL; n=17), during acute isocapnic hypoxia (SL-HX; n=7) and following 3 weeks at 5,050m (SL-HA; n=8 non-PFO subjects) to Sherpas at 5,050m (n=14). SpO_2, heart rate, pulmonary artery systolic pressure (PASP) and cardiac index (Q_i) were measured during 5min of room air breathing at SL and HA, during 20min of isocapnic hypoxia (SL-HX; $P_{ET}O_2$ = 47mmHg) and during 5min of hyperoxia (FiO_2=1.0; Sherpas only). Intracardiac shunt and IPAVA blood flow was evaluated by agitated saline contrast echocardiography. Although PASP was similar between groups at HA (Sherpas: 30.0±6.0 mmHg; SL-HA: 32.7±4.2 mmHg; P=0.27), it was greater than SL-SL (19.4±2.1 mmHg; P<0.001). The percent of subjects with intracardiac shunt was similar between groups (SL-SL: 41%; Sherpas: 50%). In the remaining subjects, IPAVA blood flow was found in 100% of subjects during acute isocapnic hypoxia at SL, but in only 4 of 7 Sherpas and 1 of 8 SL-HA subjects at rest. In conclusion, differences in resting pulmonary vascular regulation, intracardiac shunt and IPAVA blood flow do not appear to account for any adaptation to high altitude in Sherpas. Despite elevated pulmonary pressures and profound hypoxaemia, IPAVA blood flow in all subjects at HA was lower than expected compared with acute normobaric hypoxia.

Abbreviations list: FiO_2: *fraction of inspired oxygen;* HA: *High altitude;* HPV: *hypoxic pulmonary vasoconstriction;* IPAVA*: intrapulmonary arteriovenous anastomoses;* HR: *heart rate;* IVC: *inferior vena cava;* SL: *sea level;* SL-HA: *sea level group at high altitude;* SL-HX: *sea level group during acute hypoxia;* SL-SL: *sea level group at sea-level;* SpO_2: *oxyhaemoglobin saturation;* SV: *stroke volume;* PASP: *pulmonary artery systolic pressure;* PAVM: *pulmonary*

arteriovenous malformation; PFO: *patent foramen ovale;* Q: *cardiac output;* Qi: *cardiac index;*

Introduction.

The resting pulmonary haemodynamic response when individuals from lowland areas are exposed to high altitude (HA) is reasonably well characterized (Reeves, 1973; Swenson, 2013). For example, hypoxic pulmonary vasoconstriction (HPV) is stimulated largely by alveolar partial pressure of oxygen (P_AO_2) and ventilation-perfusion relationships leading to increases in pulmonary artery pressure (Marshall & Marshall, 1983). At sea level (SL), this response to regional alveolar hypoxia might be considered adaptive, as its teleological role is to decrease perfusion of poorly ventilated alveoli and assist in the matching of ventilation and perfusion throughout the lung (Marshall *et al.*, 1981; Orchard *et al.*, 1983). However, when the whole lung is hypoxic, such as with chronic exposure to high altitude, HPV may in fact be a maladaptive response leading to high perfusion pressure, capillary damage, and an increased risk of high altitude pulmonary oedema. High altitude residents from the Andes are prone to chronic mountain sickness and greater hypoxaemia, pulmonary vasoconstriction and increased haematocrit contribute to their increased pulmonary arterial vascular pressure (Penaloza & Arias-Stella, 2007; Stuber & Scherrer, 2010). However, not all high altitude ethnic groups are prone to elevated pulmonary artery pressures and chronic mountain sickness, which suggests that some individuals and populations of high-altitude residents have different phenotypes for life in environments with low PO_2. Tibetans and Sherpas, for example, are thought to be well adapted to high altitude owing partly to their relatively low pulmonary artery pressures, minimal polycythemia, elevated resting ventilation, and a high lung diffusing capacity (Groves *et al.*, 1993; Hoit, 2005; Hoit *et al.*, 2011; Faoro *et al.*, 2013). Although we do not contest that Tibetans and Sherpas are well-adapted to their high altitude environment, the direct evidence for low resting pulmonary artery pressures with alveolar hypoxia in this high-altitude population is based on investigations with small sample sizes and lack adequate control groups. To shed light on resting pulmonary haemodynamics in Sherpas, it would be most appropriate to study resting pulmonary haemodynamics in high altitude Sherpas and in sea-level inhabitants

at sea level and following acclimatization to high altitude.

The circulatory system has two pathways that could help to alleviate pulmonary pressure by providing a low resistance pathway for blood. However, these pathways also prevent some blood from passing through the blood gas interface within the lung, which could impair pulmonary gas exchange efficiency. These pathways include intracardiac shunt, of which the most notable is the patent foramen ovale (PFO), and intrapulmonary arteriovenous anastomoses (IPAVA). A PFO is detected in 35-38% of the general population (Woods, 2010; Marriott *et al.*, 2013; Elliott *et al.*, 2013). Whereas, blood flow through IPAVA is detected at rest in approximately 30% of healthy humans at SL (Woods, 2010; Elliott *et al.*, 2013) and increases to near 100% in healthy male and female humans during exercise (Eldridge *et al.*, 2004; Stickland *et al.*, 2007; Kennedy *et al.*, 2012) and with exposure to hypoxia (Imray *et al.*, 2008; Lovering *et al.*, 2008*a*; Laurie *et al.*, 2010). At lower altitudes, e.g. <3000m, the presence of a right-to-left shunt pathway, such as a PFO or IPAVA, could potentially worsen systemic arterial hypoxaemia and over-time lead to greater pulmonary hypertension, suggesting blood flow through these pathways could be detrimental (Levine *et al.*, 1991; Swenson & Bärtsch, 2012). However, with increasing altitude, the impact of blood flow through IPAVA and/or PFO on pulmonary gas exchange efficiency will decrease as PaO_2 approaches PvO_2. Accordingly, at altitudes > 3000m, blood flow through these pathways may provide a beneficial effect by allowing for lower pulmonary vascular pressure without having a significant impact on gas exchange. Based on this rationale, there is reasonable ground to investigate whether or not Sherpas, a group of well-adapted high altitude residents living in the Himalayas, may have adapted to utilize large diameter right-to-left shunt pathways when hypoxaemic to divert blood flow away from the conventional pulmonary circulation in an attempt to reduce pulmonary vascular pressure for a given level of HPV.

With this rationale in mind, we aimed to measure pulmonary haemodynamics, SpO_2 and to characterize the occurrence of intracardiac shunt and blood flow through IPAVA in Sherpas who permanently reside at high

altitude compared to sea-level inhabitants at sea level and following ~3 weeks acclimatization to 5,050m. To provide a reasonable comparison to acute hypoxia studies investigating pulmonary haemodynamics and right-to-left shunt (Laurie *et al.*, 2010), we studied the pulmonary haemodynamic response in a subset of sea-level subjects during exposure to acute isocapnic hypoxia. Based on the results of previous investigations (Groves *et al.*, 1993; Hoit, 2005; Schwab *et al.*, 2008; Stuber & Scherrer, 2010; Hoit *et al.*, 2011; Groepenhoff *et al.*, 2012; Faoro *et al.*, 2013) we hypothesized that Sherpas at high altitude would have low pulmonary vascular pressure similar to sea-level inhabitants at sea level and would have decreased pulmonary vascular pressure compared to sea-level inhabitants following acclimatization to high altitude. Secondly, we hypothesized that the lower pulmonary artery vascular pressure in Sherpas at altitude would be explained by more blood flow through right-to-left shunt pathways compared with sea-level inhabitants. Such differences might help to explain the previously reported reduced pulmonary pressure and total pulmonary resistance in Sherpas compared with sea-level inhabitants.

Methods.

Ethical approval. 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 Medical Research Council, and conformed to the latest revision of the Declaration of Helsinki. All participants provided written informed consent prior to participation in this study.

Subjects. We studied a group of sea-level subjects in Kelowna, British Columbia, Canada near sea level (SL-SL; n= 17; elevation = 344m). From this group, a subset of subjects were identified as PFO negative and were subsequently studied for pulmonary haemodynamics and blood flow through IPAVA at sea level during acute isocapnic hypoxia (n=7) and at high altitude following 3 weeks of acclimatization at the EV-K2-CNR Laboratory (SL-HA; n=8; elevation = 5,050m). Three weeks of exposure to high altitude was selected to ensure a reasonable length of time for acclimatization. Previous work by Lundy *et al.* (2004) have demonstrated that there is little difference in arterial oxyhaemoglobin saturation (SpO_2) and haematocrit between 2 and 8 weeks of acclimatization to 4,100m. Due to logistical barriers one subject in the SL-HA group was not studied during acute isocapnic hypoxia at sea level. Sea-level subjects were excluded if they were born above 1,200m in elevation or if they had travelled to high altitude (>2,500m) in the past 6 months. Finally, a group of high-altitude residents of Sherpa ancestry were recruited from high-altitude regions of the Khumbu Valley and were studied at high altitude (n=14; elevation = 5,050m). Sherpas were excluded if they were not permanent residents above 2,800m, if they were born below an altitude of 2,800m, and if they did not self-identify as a Sherpa. No Sherpas had travelled below 2,800m within 2 months of this study.

Experimental Protocol. At both sea level and high altitude, all subjects were first measured for height and weight and then instrumented with an intravenous catheter at the antecubital fossa. Following 5 minutes of supine rest, three

consecutive blood pressure measurements were obtained from the right upper arm by using a manual sphygmomanometer and stethoscope for auscultation of Korotkoff sounds. Also, at this time, SpO_2 (Model 3100, WristOx, Nonin Medical Inc., MN, USA) was measured by pulse oximetry of the right index finger. While in the supine position, collapse of the inferior vena cava (IVC) during a rapid inspiration was imaged by ultrasound to estimate right atrial pressure (Yildirimturk *et al.*, 2011). The subject was then positioned laterally on their left side and echocardiographic images were recorded to determine stroke volume (SV) and pulmonary artery systolic pressure (PASP). Heart rate (HR) was measured by standard three-lead electrocardiograph. Next, an apical 4-chamber view of the heart was acquired and the presence or absence of intracardiac shunt or blood flow through IPAVA was determined by the technique of agitated saline contrast echocardiography (Kennedy *et al.*, 2012; Elliott *et al.*, 2013). This was repeated during the release of a Valsalva maneuver as a provocative stimulus to ensure the patency of the foramen ovale was identified appropriately. At sea level, subjects identified as PFO negative were subsequently exposed to 20 minutes of isocapnic hypoxia to determine pulmonary haemodynamics and blood flow through IPAVA during an acute hypoxic exposure. Pulmonary haemodynamics and blood flow through IPAVA were measured during the last 5 minutes of hypoxia. In Sherpas only, at high altitude the above measurements were repeated after breathing hyperoxia (FiO_2 = 1.0) for 5 minutes to determine the impact of hypoxia relief. Agitated contrast studies were repeated if there was any doubt in the classification of the shunt to intracardiac or intrapulmonary.

Pulmonary Hemodynamics. All echocardiography measurements were performed using the same commercially available ultrasound system (Vivid Q, 3.5 MHz transducer, GE Healthcare) by the same trained sonographer (M.S.). 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 (VTI) 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 VTI and aortic area, and Q was obtained by multiplication with HR. These methods have been previously described and validated against thermodilution and direct Fick (Christie *et al.*, 1987). Cardiac index (Q_i) was determined by indexing Q with body surface area to facilitate comparisons between groups. 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 could then be estimated from the simplified Bernoulli equation and PASP could be estimated by addition of right atrial pressure. The IVC diameter was measured from subcostal longitudinal images approximately 2 cm distal to the right atrial junction. The collapsibility index was calculated as the percentage of difference between maximal and minimal size of IVC. Right atrial pressure was predicted using the collapsibility index as recommended by the American Society of Echocardiography (Lang *et al.*, 2005). 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. Total pulmonary resistance was estimated by indexing PASP against Q (i.e. PASP/Q; mmHg l^{-1}). This noninvasive estimate of total pulmonary resistance correlates well with invasive measurements (Abbas *et al.*, 2003; Roule *et al.*, 2010).

Agitated Saline Contrast Echocardiography. The presence of intracardiac shunt (i.e. PFO) was determined under resting conditions and during a provocative stimulus (i.e. release from Valsalva) using the technique of agitated saline contrast echocardiography (Marriott *et al.*, 2013). Two, 5-ml syringes were connected by three-way stopcocks and connected to the 22-guage 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 forcefully to agitate the mixture prior to rapid injection. The agitated contrast was

visualized travelling through the heart from an apical 4-chamber view of the heart. If a negative test was identified, a provocative maneuver was used to further assess the patency of the foramen ovale. In this case, subjects were instructed to valsalva at the end of a normal expiration. Agitated contrast was subsequently injected and once visualized in the right atrium, the subjects were asked to relax and breathe quietly. A PFO was identified if contrast appeared in the left ventricle within <5 cardiac cycles after the contrast cloud filled the right atrium (Marriott *et al.*, 2013). After all contrast injections, a minimum of 20 cardiac cycles was recorded. In the event that a subject was identified as being PFO negative they were then studied for resting blood flow through IPAVA. An IPAVA was defined when contrast appeared in the left ventricle 6 or more cardiac cycles after the contrast appeared in the right atrium. Therefore, all subjects were identified as PFO positive or negative and IPAVA positive and negative. A PFO positive subject could also have blood flow through an IPAVA, but this technique cannot distinguish between the two. As a result, blood flow through IPAVA can only be studied in subjects identified as PFO negative. This technique has been used to investigate blood flow through IPAVA in subjects during rest and exercise at SL breathing room air and during acute normobaric hypoxia (Stickland *et al.*, 2004; Imray *et al.*, 2008; Kennedy *et al.*, 2012; Laurie *et al.*, 2012; Elliott *et al.*, 2013). A scoring system was used to determine the severity of blood flow through 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 (Lovering *et al.*, 2008*b*). 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. All echocardiograms were conducted and analyzed by the same un-blinded investigator (M.S.). Previous work by members of our group has shown good agreement between scorers who have been blinded to the experimental conditions (Laurie *et al.*, 2010).

Isocapnic Hypoxia. Respiratory parameters were acquired at 200Hz using an analog-to-digital converter (PL3504, ADInstruments, Colorado Springs, USA) interfaced with a personal computer and analyzed using commercially available software (LabChart, ADInstruments, Colorado Springs, USA). During the isocapnic hypoxia protocol subject's breathed through a mouthpiece and two-way non-rebreathing valve, and a nose clip was applied to obstruct the nasal passage. Respired gas pressures were sampled at the mouth and analyzed for PO_2 and PCO_2 (ML206, ADInstruments, Colorado Springs, USA). Respiratory flow was measured at the mouth using a pneumotachograph (HR 800L, HansRudolph, Shawnee, USA). $P_{ET}O_2$, $P_{ET}CO_2$, inspiratory and expiratory tidal volume was determine for each breath online using custom designed software (LabView, National Instruments, Austin, USA). SpO_2 was measured continuously by pulse oximetry (ML320/F, ADInstruments, Colorado Springs, USA). $P_{ET}O_2$ was maintained at 45 mmHg and $P_{ET}CO_2$ was maintained at resting levels by using a portable end-tidal forcing system (AirForce, G.E.Foster, Vancouver, Canada)(Querido *et al.*, 2013; Bain *et al.*, 2013). This system uses independent gas solenoid valves for O_2, CO_2, and N_2 and controls the volume of each gas being delivered to an inspiratory reservoir through a mixing and humidification chamber. Using feedback information regarding $P_{ET}O_2$, $P_{ET}CO_2$, inspired and expired tidal volumes, and estimates of O_2 consumption and CO_2 production the system prospectively targets the inspirate using the alveolar gas equation to bring end-tidal gas levels to the desired level. Gas control fine-tuning is accomplished using a complex feedback control and error reduction algorithm. Pulmonary haemodynamics and blood flow through IPAVA were determined during 5 min of baseline breathing and during the last 5 minutes of isocapnic hypoxia.

Statistical Analysis. Statistical comparisons and calculations were made using statistical software (Statistica v.7.0, Statsoft Inc., Tulsa, OK, USA). Subject resting characteristics were compared between the three groups by one-way analysis of variance. Pulmonary haemodynamics were compared between

groups by a one-way ANOVA. For all analyses, when significant F-ratios were detected, Tukey's HSD was applied and corrected for unequal sample sizes where appropriate to determine where the differences lay. Differences between haemodynamic parameters were compared within each group for PFO+ and PFO- subjects by un-paired T-test. Fisher's exact test was used to compare the frequency of intracardiac shunt and IPAVA between groups. Shunt score was compared between groups by Friedman's test, with Dunn's posttest if appropriate. All data are presented as mean ± SD (unless otherwise noted) and statistical significance was set at $P<0.05$ for all comparisons.

Results.

Subject characteristics and resting haemodynamic data are presented in Table 1. Groups were similar in age, but only SL-SL was taller than Sherpas ($P<0.05$). Body mass for all three SL groups was similar, but only SL-HX and SL-HA were significantly heavier than Sherpas ($P<0.05$). Body mass index (BMI) was similar between groups but body surface area was greater in SL-SL, SL-HX and SL-HA compared with Sherpas. SpO_2 was lowered similarly during acute hypoxia and HA ($P<0.001$), and was similar between SL-HA and Sherpas. Likewise, HR was elevated during acute hypoxia and HA ($P<0.001$) but was similar between groups exposed to hypoxia. Both MAP and Q_i were not appreciably different between the four groups. Table 2 displays the geographical locations and altitude of where Sherpas were born.

Figure 1A displays PASP for all groups, including sea-level subjects during acute hypoxia and Sherpas during hyperoxia breathing. Acute hypoxia and high altitude increased PASP in SL-HX and SL-HA to a level similar to that of Sherpas. Hyperoxia decreased PASP in Sherpas ($P<0.01$); however, PASP was still greater than that observed in the SL-SL group ($P<0.01$). Figure 1B displays total pulmonary resistance as estimated by PASP/Q. Total pulmonary resistance was elevated at high altitude in the SL-HA group, but was not significantly different from Sherpas. Hyperoxia had no effect on total pulmonary resistance in Sherpas. Total pulmonary resistance was not significantly elevated during acute hypoxia owing to a small (albeit statistically insignificant) increase in Q_i.

Figure 2 shows a contrast echocardiogram for one Sherpa at 5,050m who was identified as having a resting intracardiac shunt. Figure 3A displays the percent of subjects positive for intracardiac shunt in Sherpas and SL-SL. In Sherpas, 7 out of 14 subjects were positive for intracardiac shunt, which was not statistically different from SL-SL where 7 out of 17 subjects were positive for intracardiac shunt. There were no significant differences between PFO positive and PFO negative subjects in either sea level subjects or Sherpas (Table 1 and Figure 1).

In both SL-SL and Sherpas, 5 out of 7 intracardiac shunts were identified in the resting state without valsalva. Eight subjects studied at sea level were negative for both intracardiac shunt and resting blood flow through IPAVA and were subsequently studied during acute isocapnic hypoxia (n=7 only) and at 5,050m (i.e. SL-HA) to determine the presence of hypoxia-induced blood flow through IPAVA. Similarly, seven Sherpas were negative for intracardiac shunt and were subsequently studied for resting blood flow through IPAVA at 5,050m. Figure 3B shows the percent of subjects identified for resting blood flow through IPAVA during acute hypoxia and at high altitude. During acute isocapnic hypoxia exposure, 100% of subjects displayed blood flow through IPAVA. At high altitude the proportions were much less and not statistically different between Sherpas and SL-HA. Nonetheless, 4 out of 7 Sherpas and 1 out of 8 SL-HA were identified as having a resting IPAVA at high altitude. In addition, blood flow through IPAVA remained in 3 out of 4 Sherpas breathing 100% O_2. Figure 4 shows two contrast echocardiograms in one Sherpa who had a resting blood flow through IPAVA at 5,050m (Figure 4A) and while breathing 100% O_2 (Figure 4B).

The magnitude of blood flow through IPAVA for all subjects at high altitude was low. At 5,050m all subjects who were positive for blood flow through IPAVA at rest had a microbubble score of '1' while breathing ambient air. While breathing hyperoxia, one Sherpa had a microbubble score of '2' with the remaining two Sherpas having a score of '1' (i.e., 1 subject increased their score with hyperoxia while 1 subject normalized their response). We did not observe a microbubble score for blood flow through IPAVA greater than '2' in any subject during any experimental conditions.

Discussion.

This is the first study to simultaneously examine pulmonary haemodynamics, presence of intracardiac shunt, and blood flow through IPAVA between Sherpas and acclimatized sea-level inhabitants at high altitude. The major findings from this study indicate that (1) there are no differences in resting pulmonary haemodynamics between acclimatized sea-level inhabitants and Sherpas, and (2) blood flow through shunt pathways do not appear to contribute to high altitude adaptation in Sherpas. Principally, we found that pulmonary arterial pressure and total pulmonary resistance were similar between Sherpas at high altitude and sea-level inhabitants following 3 weeks of acclimatization. In addition, we found a similar number of subjects with intracardiac shunt and blood flow through IPAVA between Sherpas and sea-level inhabitants. Despite elevated pulmonary pressures and profound hypoxaemia at high altitude, the number of subjects with blood flow through IPAVA and the magnitude of blood flow through IPAVA for a given level of hypoxaemia in all subjects was lower than expected based on: (1) acute isocapnic hypoxia testing at sea-level in a subset of our SL subjects, and (2) from the results of previous investigations involving acute normobaric hypoxia (Laurie *et al.*, 2010; Elliott *et al.*, 2011). The lack of difference in cardiac output between high-altitude and sea level measurements may be one explanation for the lower than expected blood flow through IPAVA. Another explanation may relate to pulmonary vascular remodeling to high altitude in both sea-level inhabitants and Sherpas. Relevant methodological considerations and evidence to support these conclusions will now be discussed.

Pulmonary haemodynamics and adaptation to high altitude. In humans, there are several populations of life-long high-altitude residents including the residents of Leadville, Colorado in North America (Reeves & Grover, 1975), the Andean natives of South America (Banchero *et al.*, 1966; Schwab *et al.*, 2008; Groepenhoff *et al.*, 2012), the Amhara highlanders in Ethiopia (Hoit *et al.*, 2011) and the Tibetans from the Himalayas (Groves *et al.*, 1993; Hoit, 2005) who have been studied for pulmonary adaptations to high-

altitude environments. Of this group of high-altitude residents, the Tibetans have been reported to have the lowest mean pulmonary pressure at rest and display no rise in pulmonary vascular resistance (Groves *et al.*, 1993; Hoit, 2005). Tibetans have been reported to exhibit little hypoxic pulmonary vasoconstriction as evidenced by no change in pulmonary vascular resistance with hyperoxia breathing (Groves *et al.*, 1993). However, the operation Everest II studies also demonstrated in healthy lowlanders that elevated pulmonary resistance with high-altitude exposure was not immediately reversed by hyperoxia (Groves *et al.*, 1987).

Sherpas are another group of high-altitude natives from the Himalayan plateau who share recent ancestry with the Tibetan highlanders (Hochachka, 1998). Pulmonary haemodynamics have previously been assessed directly in five Tibetan highlanders by right heart catheterization and studied during rest at 3,658m and with further hypoxic stress (14% O_2) (Groves *et al.*, 1993). Mean pulmonary pressure, cardiac output, and pulmonary vascular resistance were reported to be unchanged with exposure to hypoxia and not different from sea-level norms. A closer assessment of individual subject data indicates that a limitation in statistical power may have prevented the detection of hypoxia-induced pulmonary vasoconstriction. Specifically, three out of five subjects increased pulmonary vascular resistance markedly (range of the difference = -0.2 – +2.2 woods units). However, it is difficult to compare our data with this study (Groves *et al.*, 1993) due to differences in techniques (i.e. right heart catheterization vs. echocardiography). Instead, the direct comparison of our group of Sherpas to the group of Tibetans studied by Hoit and colleagues (Hoit, 2005) is more valid since similar techniques and methodology were used (albeit at slightly different altitudes). Specifically, we found similar PASP (mean ± SE; 30.0 ± 1.6 vs 31.5 ± 1.0 mmHg), Q_i (2.2 ± 0.1 vs 2.7 ± 0.8 l/min/cm^2), and total pulmonary resistance (8.2 ± 0.6 vs 8.5 ± 0.4) between our Sherpas and the Tibetans of Hoit *et al.* (2005), respectively. Recently, Faoro *et al.* (2013) assessed pulmonary haemodynamics and gas exchange at rest and during exercise in Sherpas. They estimated mean pulmonary artery pressure using a

prediction equation from the measurement of PASP and subsequently calculated PVR. In that study, the authors compared their measurements made in Sherpas to measurements made in a control group of sea-level inhabitants within two days of arrival at 5,050m. In our experience, 40-50% of subjects will have symptoms of AMS during this time-point and be in the very early stages of acclimatization (i.e., more hypoxaemic with little renal compensation for the respiratory alkalosis) (Fan *et al.*, 2010; Lucas *et al.*, 2011; Willie *et al.*, 2013); therefore, comparison with Sherpas at this time-point seems problematic. Here, we compared our findings in Sherpas to a group of sea-level inhabitants both at sea level and following ~3 weeks of acclimatization to high altitude and our subjects were free of AMS symptoms and therefore we believe this to be a more appropriate comparison. Our data showed that while PASP and total pulmonary resistance in the Sherpas were significantly elevated compared to sea-level inhabitants at sea level, following acclimatization sea-level inhabitants and Sherpas did not differ in terms of PASP and total pulmonary resistance (*see figure 1A and 1B*). Based on these results and direct comparisons, we suggest that Sherpas and Tibetans have similar pulmonary haemodynamics at high altitude and they do not differ substantially from sea-level inhabitants following acclimatization to high altitude.

Intracardiac shunt. Agitated saline contrast echocardiography is a highly sensitive technique useful in identifying intracardiac shunts (Marriott *et al.*, 2013). The prevalence of intracardiac shunting in the general population (sample sizes ranging from 104 – 1162 subjects) has been estimated to be 35-38% (Woods, 2010; Marriott *et al.*, 2013; Elliott *et al.*, 2013). In the current investigation, we characterized the occurrence of intracardiac shunt in a relatively small sample of subjects at sea-level and Sherpas at high altitude. The observed occurrence of intracardiac shunt in our groups of subjects is consistent with previous reports (Woods, 2010; Marriott *et al.*, 2013; Elliott *et al.*, 2013). We detected intracardiac shunt in 41% of sea-level inhabitants; a finding not statistically different from our Sherpa population, where intracardiac shunt was detected in 50% of subjects

(*see figure 3*). Although Valsalva was used as a provocative maneuver to verify the patency of the foramen ovale, intracardiac shunts were detected in the majority of subjects (~83%) at rest without requiring a provocative maneuver. This indicates that the majority of PFO positive subjects had an intracardiac shunt in the resting state while at high altitude. Despite this, the amount of blood flow through the PFO did not lead to a measureable effect on SpO_2 and pulmonary vascular pressure between PFO positive and negative subjects at altitude. Therefore, at least in this population, the prevalence of intracardiac shunt is similar between Sherpas and sea-level inhabitants and the amount of right-to-left shunt was small enough such that there was no appreciable impact on saturation or pulmonary vascular pressure at 5,050m. Of note, in subjects breathing an FiO_2 between 0.10 and 0.12, which is equivalent to an altitude of 5,050m, we have previously calculated that the total venous admixture would need to be >25% in order to account for all of the pulmonary gas exchange efficiency that occurs with this level of hypoxia (Laurie *et al.*, 2010). Thus, at 5,050m, a significantly large amount of shunt would be required to have an impact on saturation and/or pulmonary gas exchange efficiency. However, this does not preclude that the presence of a PFO would have a larger impact on saturation and gas exchange at lower altitudes where shunt fractions on the order of 5-8% would have a significant effect on pulmonary gas exchange efficiency (Laurie *et al.*, 2010).

Blood flow through IPAVA. Agitated saline contrast echocardiography is the only non-invasive means of assessing blood flow through IPAVA in PFO negative subjects. Using this technique, blood flow through IPAVA at rest is detectable in 30% of healthy humans who are PFO negative (Elliott *et al.*, 2013), but the prevalence increases to nearly 100% in healthy humans during exercise (Eldridge *et al.*, 2004; Stickland *et al.*, 2007; Kennedy *et al.*, 2012) and with exposure to acute normobaric hypoxia (Lovering *et al.*, 2008*a*; Laurie *et al.*, 2010). In the current study, we also found that 100% of subjects exposed to 20 minutes of isocapnic hypoxia had blood flow through IPAVA (*see Figure 2B)*.

Several mechanisms could be responsible for the opening of IPAVA including: (1) the hypoxic stimulus; (2) increases in pulmonary vascular pressure, or (3) increases in cardiac output. Laurie *et al.* (2010) found that the number of subjects with blood flow through IPAVA increased with reductions in FiO_2. For example, it was noted that 100% of subjects had blood flow through IPAVA while breathing a 10% oxygen mixture for 30 minutes. In addition, the magnitude of blood flow through IPAVA (i.e., microbubble score) increased with reductions in FiO_2 and was related to changes in SpO_2 and PASP. The result from their study provides correlational evidence to indicate that blood flow through IPAVA contributes to the total venous admixture. In direct contrast to these results, we found very little evidence of blood flow through IPAVA in sea-level inhabitants after living at high altitude for 3 weeks, nor in Sherpas permanently living at high altitude (*see figure 3B*). In those subjects who demonstrated blood flow through IPAVA the magnitude estimated by microbubble score was small (i.e., <2) and therefore seems unlikely to have contributed appreciably to the total venous admixture. The discrepancies between studies are potentially explained by differences in normobaric versus hypobaric hypoxia, the impact of acclimatization on cardiac output and/or vascular remodeling. Interestingly, 100% of subjects exposed to acute isocapnic hypoxia at sea level were found to have blood flow through IPAVA and they had a larger rise in cardiac output compared to subjects at high altitude. This indicates that increases in cardiac output may be a stimulus for blood flow through IPAVA.

An increase in right heart cardiac output and the associated pulmonary vascular shear stress has been suggested as a stimulus capable of increasing blood flow through IPAVA; since when cardiac output is increased by dobutamine, dopamine, or epinephrine there is a predictable increase in the microbubble score (Laurie *et al.*, 2012; Bryan *et al.*, 2012). In our investigation, the lack of detectable blood flow through IPAVA in Sherpas and sea-level inhabitants at high altitude could be explained by the fact that cardiac output did not differ from our sea-level inhabitants measured at sea level. Bryan *et al* (2012) intravenously delivered dobutamine in increasing doses to increase

cardiac output. They found that the microbubble score, PASP, and the physiological shunt fraction (Q_s/Q_t) increased in relation to cardiac output. Therefore, blood flow through IPAVA with both acute hypoxia and exercise could potentially be explained by an increase in cardiac output.

An alternative explanation for the lack of blood flow through IPAVA with chronic hypoxia could relate to the remodeling of the pulmonary vasculature. While it is not clear to what extent vascular remodeling will affect IPAVA, small pulmonary arteries, which normally have very little smooth muscle, increase the expression of α-smooth muscle actin, and larger pulmonary arteries develop thickened media and adventitia (Stenmark *et al.*, 2006). Interestingly, the time course of pulmonary vascular remodeling is surprisingly quick upon exposure to high altitude with adventitial fibroblasts and medial smooth muscle cells increasing DNA synthesis after about three to six days of high-altitude exposure (Stenmark *et al.*, 2006). In addition, mRNA levels for procollagens and fibronectin are significantly elevated in lung parenchyma tissue of rats following exposure to as little as three days of hypoxia (Berg *et al.*, 1998). Whether or not IPAVA increase the number of smooth muscle cells or the thickness of their adventitia or media with chronic hypoxia is unknown. One intriguing hypothesis suggests that IPAVA act as pressure relief valves to protect the delicate pulmonary capillaries. In line with this hypothesis, IPAVA may provide a low resistance pathway for blood to travel and thus prevent capillary damage caused by high pulmonary vascular pressure. With chronic hypoxia, as vascular remodeling ensues and the pulmonary vasculature's ability to handle high pulmonary pressure is improved, the need for blood flow through IPAVA may be substantially reduced to augment the amount of blood flow through the lung's gas exchange surfaces.

Limitations. We found that 3 out of 4 Sherpas had resting blood flow through IPAVA at high altitude breathing 100% O_2. It has been previously shown that breathing 100% O_2 prevents blood flow through IPAVA during exercise (Lovering *et al.*, 2008*b*; Elliott *et al.*, 2011) and in 80% of resting subjects who demonstrate

blood flow through IPAVA (Elliott *et al.*, 2013). In the study by Elliott *et al*, we also reported that a subset of healthy young asymptomatic subjects (6/31 or 20%) with blood flow through IPAVA at rest (near sea level) continue to have blood flow through IPAVA despite breathing 100% O_2. Although one explanation may be that these subjects had pulmonary arteriovenous malformations (PAVMs) that allowed blood to flow through them despite the fact that these subjects were breathing 100% O_2, it is currently unknown whether or not hyperoxia only acts to prevent blood through IPAVA and not PAVMs. Because PAVMs are considered to be rare and associated with diseases such as hereditary hemorrhagic telangiectasia, it is unlikely that these healthy asymptomatic young subjects had PAVMs but may simply have blood flow through IPAVA despite breathing 100% O_2, for reasons that are currently unexplainable. Of note, at 5,050m, alveolar PO_2 would be approximately half of that in subjects breathing 100% O_2 at sea level, so it is possible that the alveolar PO_2 may not be sufficient to close the IPAVA in Sherpas at this altitude. We acknowledge the possibility that some of our Sherpas may have pulmonary arteriovenous malformations (PAVMs) that are non-reactive to changes in FiO_2. Therefore, it is possible that we measured blood flow through PAVMs rather than IPAVA and Sherpas could potentially be free from IPAVA. However, based on our work in young asymptomatic subjects, it is also entirely plausible that our Sherpas may have IPAVA that are unresponsive to hyperoxia. This is supported by the fact that Sherpas and sea-level inhabitants during acclimatization to high altitude had similar microbubble scores during rest at high altitude. Future research could study Sherpas during exercise to determine if such a provocative stimulus could open up IPAVA to a greater degree than during rest at high altitude.

Statistical power can be a problem in many high altitude field studies and this increases the risk of a type-II error. While we are confident that the proportion of our subjects who were identified as having a PFO (41% of sea-level subjects and 50% of Sherpas) is a good reflection of the prevalence in the general population, we are less confident in the proportions of subjects with resting blood flow through IPAVA. This is because after removal of the subjects

with PFOs, our sample size is decreased by a half. Although we conclude that the number of subjects with resting blood flow through IPAVA at high altitude (1 of 8 SL-HA subjects and 4 of 7 Sherpas) is similar we recommend that future research is required to reconcile these similarities in a larger sample. The strength of our study lies in determining the differences between PASP in Sherpas and low-landers. Since we are able to compare measurements in Sherpas with measurements made in sea-level inhabitants at sea-level and after acclimatization to high altitude, it is unlikely that a type-I error was made.

Acute hypoxia at sea-level was performed under isocapnic conditions. Although a poikilocapnic response test may have been a more appropriate comparison we do not anticipate that this difference in protocol would have a negative impact on our results. Microbubble scores obtained with isocapnic hypoxia are not significantly different from microbubble scores obtained previously using a poikilocapnic hypoxia protocol (Laurie *et al.*, 2010). Rather the purpose of this acute hypoxia protocol provides confidence that the data obtained from our sea-level subjects is similar to other published reports in sea-level subjects and helps to improve interpretation of the lack of blood flow through IPAVA detected while at high altitude.

Finally, total pulmonary resistance could be affected by the relationship of haematocrit and blood viscosity. For example, a 10% increase in haematocrit (45% - 55%) would lead to approximately a 20% increase in relative viscosity (Hoffman, 2011). To determine the impact of changes in haematocrit on our total pulmonary resistance measurements we compared resting haematocrit values from our SL-HX (46.7 ± 0.6%; mean ± SEM) and SL-HA groups (49.4 ± 1.1%). Unfortunately, direct comparisons to our Sherpa group cannot be made as blood samples were not taken from this group. Instead, we have selected haematocrit values from Sherpas from previously published literature (Winslow *et al.*, 1989) and from an unpublished data set from a previous expedition to the EV-K2-CNR laboratory in 2008. During this expedition, arterial blood samples were taken from 8 Sherpas (including 4 who participated in the current study) and analyzed for haematocrit. Mean haematocrit from Winslow *et al* was 48.4 ± 0.8% (at an

altitude of 3,700m) and from our unpublished data set was 53.9 ± 2.0% (at an altitude of 5,050m). Based on these data, the relative error in our measurement of total pulmonary resistance is less than ~10% between high altitude and sea-level measurements. This value is based on the curvilinear relationship between haematocrit and relative blood viscosity (and standardized to a blood haematocrit of 45%) as described previously (Hoffman, 2011). As a result, it is unlikely that differences in haematocrit between Sherpas and sea-level inhabitants can account for the similarity in total pulmonary resistance at high altitude and can only account for less than ~10% of the rise in total pulmonary resistance between sea-level measurements. The effect haematocrit has on blood flow through IPAVA is presently unknown.

Conclusion

In conclusion, we report that pulmonary vascular pressure in Sherpas is elevated by comparison to sea-level inhabitants at sea level but not different from sea-level inhabitants following 3 weeks at high altitude. We found a similar proportion of subjects with intracardiac shunt and blood flow through IPAVA in Sherpa compared to sea-level inhabitants. At high altitude the effect of right-to-left shunting on PaO_2 may be negligible and therefore is not a strong stimulus for selective adaptation for success in the high altitude environment. Alternatively, chronic hypoxaemia (i.e. weeks to life-long), unlike acute hypoxaemia (i.e., minutes to hours), may lead to remodeling of the pulmonary vasculature such that blood flow through IPAVA is reduced compared to acute hypoxia. Or perhaps more simply, the lack of a significant difference in cardiac output after acclimatization while at high altitude could be responsible for the lack of blood flow through IPAVA. These hypotheses are supported by the fact that both sea-level inhabitants and Sherpas had significantly less blood flow through IPAVA by comparison to either our acute hypoxia exposure or based on published studies of acute normobaric hypoxia where cardiac output often increases and time for pulmonary vascular remodeling would not be sufficient. Taken together, blood

flow through PFO and IPAVA do not appear to contribute to resting cardiopulmonary haemodynamics in well-adapted Sherpas at high altitude.

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Tables.

Table 1. Subject characteristics and resting haemodynamics between sea-level inhabitants at sea level (SL-SL), during acute isocapnic hypoxia (SL-HX), following 3 weeks of high-altitude acclimatization (SL-HA), and in Sherpas at high altitude (5,050 m). Subjects are further separated into groups based on their intracardiac shunt status (i.e. PFO+/PFO-).

	SL-SL			SL-HX	SL-HA	Sherpa		
	PFO-	PFO+	Both	PFO- only	PFO- only	PFO-	PFO+	Both
N	10	7	17	7	8	7	7	14
Age (years)	32.6 ± 7.4	27.3 ± 6.0	30.4 ± 7.2	30.9 ± 5.3	30.0 ± 5.5	41.6 ± 17.3	25.6 ± 5.3*	33.7 ± 15.0
Sex (M:F)	9:1	4:3	13:4	6:1	7:1	7:0	7:0	14:0
Height (cm)	176 ± 6	177 ± 7	178 ± 6*	176 ± 7	177 ± 7	167 ± 7	168 ± 7	168 ± 7
Body mass (kg)	80.8 ± 12.4	70.7 ± 13.0	76.6 ± 13.3	84.8 ± 13*	80.7 ± 13.4*	62.7 ± 7.5	68.6 ± 12.4	65.7 ± 10.3
BMI (kg m^{-2})	26.1 ± 3.6	22.6 ± 3.1	24.6 ± 3.7	27.3 ± 3.4	26.4 ± 4.0	22.3 ± 1.2	24.3 ± 4.0	23.3 ± 3.0
BSA (m^2)	1.98 ± 0.17	1.86 ± 0.19	1.93 ± 0.18*	2.03 ± 0.18*	2.01 ± 0.18*	1.71 ± 0.14	1.78 ± 0.07	1.75 ± 0.16
Birth Altitude (m)	275 ± 387	122 ± 162	185 ± 277†	141.7 ± 185†	125 ± 178†	3,979 ± 247	3,884 ± 571	3,931 ± 425
SpO_2 (%)	97.7 ± 1.3	97.9 ± 1.4	97.8 ± 1.3†	77.6 ± 3.9	81.2 ± 1.7	80.9 ± 3.8	83.9 ± 2.0	82.4 ± 3.3
HR (bpm)	56.7 ± 7.1	58.7 ± 11.9	57.5 ± 9.1†	66.3 ± 13.2	70.3 ± 11.3	73.9 ± 14.3	77.9 ± 13.5	75.9 ± 13.5
MAP (mmHg)	89.8 ± 7.9	92.8 ± 8.0	91.1 ± 7.8	93.6 ± 7.0	99.3 ± 9.7	89.9 ± 7.0	94.4 ± 7.9	92.1 ± 7.5
Q_i (l min^{-1} m^{-2})	2.05 ± 0.27	2.41 ± 0.47	2.21 ± 0.40	2.70 ± 0.73	1.91 ± 0.17	2.40 ± 0.53	2.06 ± 0.41	2.24 ± 0.49

Values are mean ± SD. *Significantly different from Sherpa or different than PFO- within each group (P<0.05); †Significantly different from Sherpa (P<0.001). Definition of Abbreviations: BMI = body mass index; BSA = body surface area; SpO_2 = oxyhaemoglobin saturation; HR = heart rate; MAP = mean arterial pressure; Q_i = cardiac index.

Table 2. Distribution of geographical locations and altitudes where Sherpas were born.

Location	Elevation (m)	Frequency (n)
Dingboche	4410	2
Khumjung	3780	1
Khunde	3840	3
Lukla	2840	1
Namche Bazaar	3440	1
Pangboche	3930	1
Pheriche	4240	4
Thame	3750	1

Figures and legends.

Figure 1. (A) Pulmonary artery systolic pressure (PASP) and (B) total pulmonary resistance (PASP/Q) in sea-level inhabitants at sea level during rest (SL-SL; n=17), during acute hypoxia (SL-HX; n=7) and at high altitude (SL-HA; n=8), and in Sherpas at high altitude (Sherpa; n=14) and during 5 minutes of breathing 100% oxygen (Sherpa O_2; n=14). Where appropriate groups are divided into their status of intracardiac shunt (i.e. PFO-/PFO+). Values are mean ± SD. *P<0.01 compared to SL-SL and †P<0.01 compared to SL-HA and Sherpa. Within each group there are no significant differences between PFO- or PFO+ status.

Figure 2. Contrast echocardiograms (apical four-chamber view; left ventricle focus) of one Sherpa at 5,050m demonstrating an intracardiac shunt. The sequence of images shows (A) pre-injection where no microbubbles are visible followed by (B) the appearance of the microbubble cloud in the right ventricle and the appearance of microbubbles in the left ventricle within 3 cardiac cycles (labeled with circles).

Figure 3. (A) Percent of subjects positive for intracardiac shunt in sea-level inhabitants at sea level (SL-SL) and in Sherpas at high altitude. (B) Percent of subjects positive for IPAVA in sea-level inhabitants during acute hypoxia (SL-HX), at high altitude (SL-HA), and in Sherpas at high altitude who were negative for intracardiac shunt.

Figure 4. Contrast echocardiograms (apical four-chamber view; left ventricle focus) of one Sherpa showing (A) IPAVA at rest and (B) during 5 minutes of breathing hyperoxia (FiO_2 = 1.0). Note that microbubbles are present in the left ventricle in each image (labeled with circles). Both panels A and B were assigned microbubble scores of 1.

Figure 1.

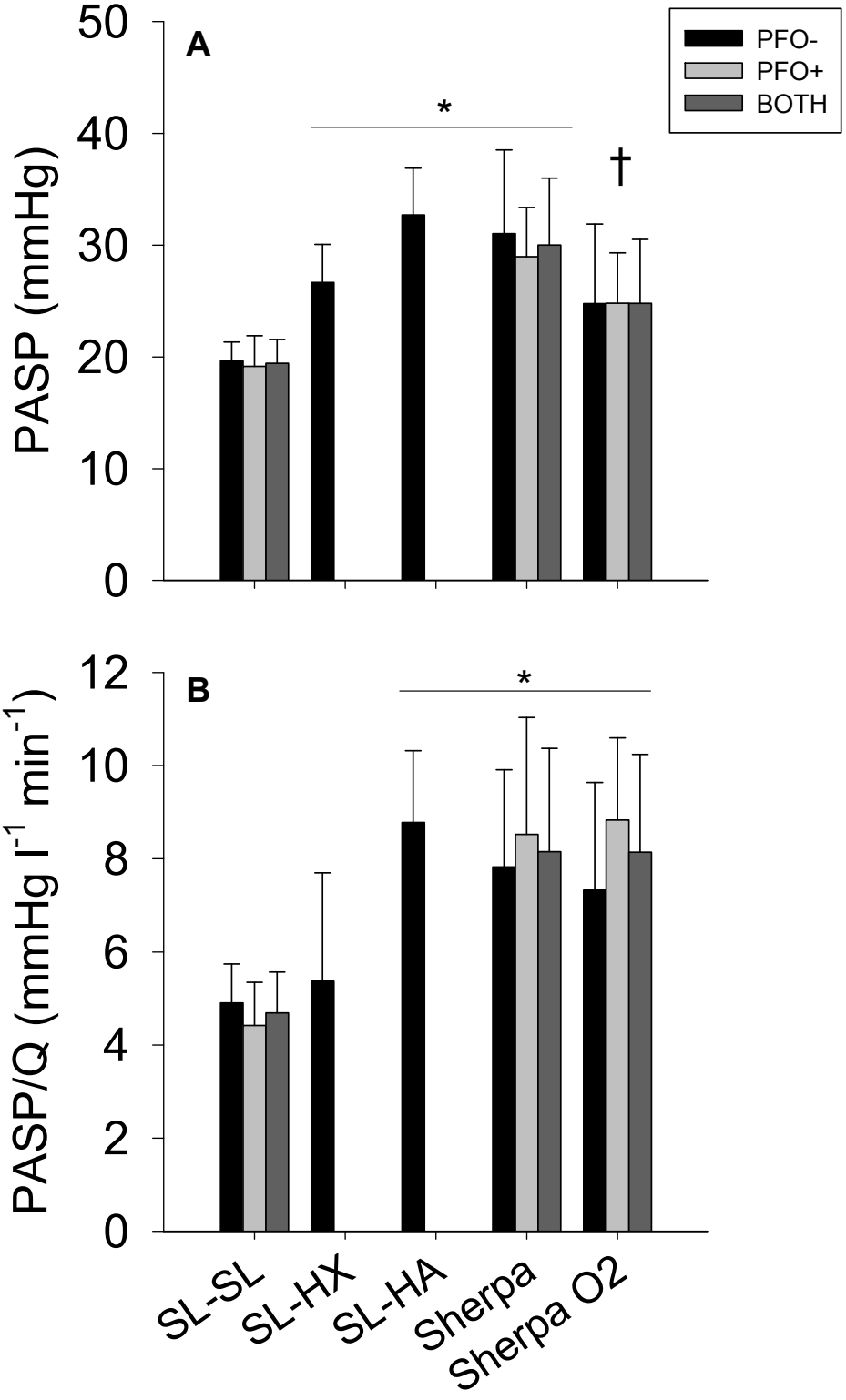

A
PFO-
PFO+
BOTH
*
†
PASP (mmHg)
50
40
30
20
10
0
B
*
PASP/Q (mmHg l-1 min-1)
12
10
8
6
4
2
0
SL-SL
SL-HX
SL-HA
Sherpa
Sherpa O2

Figure 2.

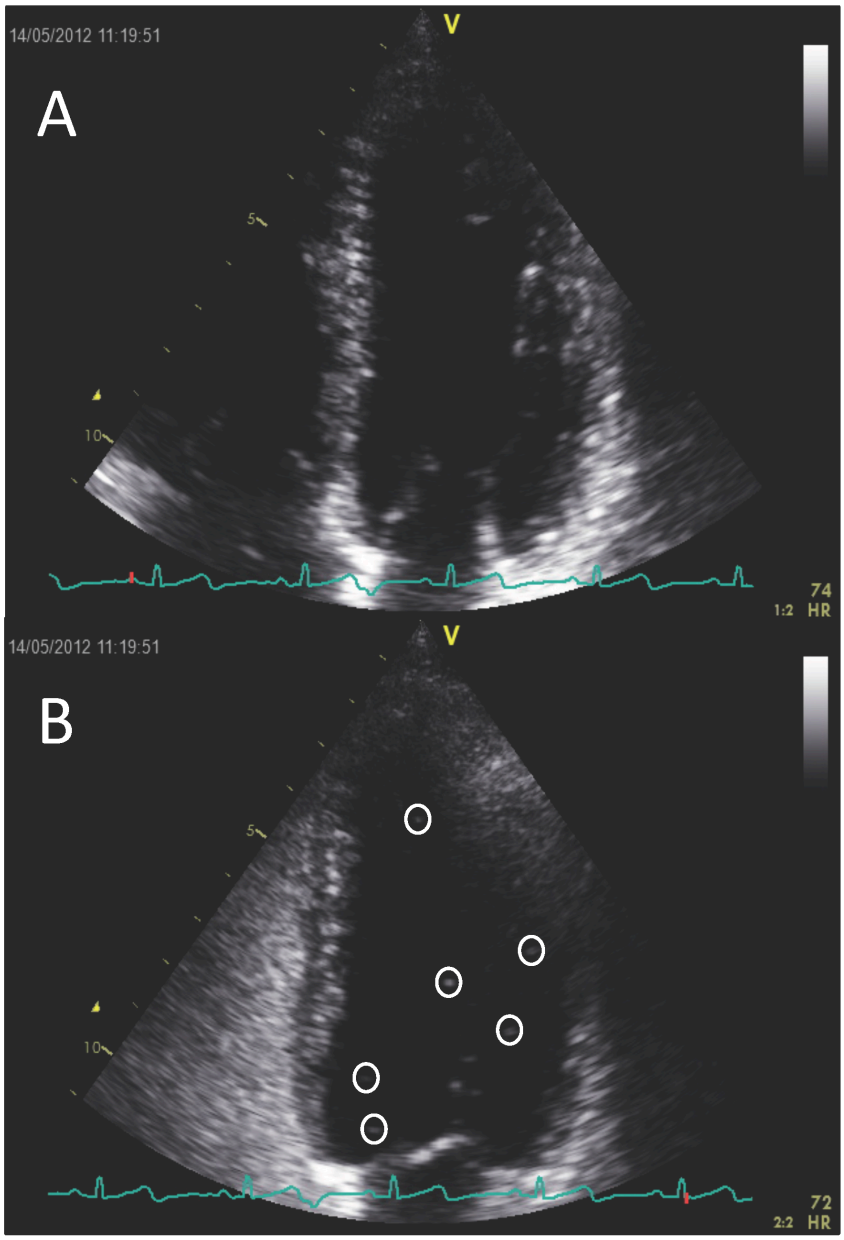

14/05/2012 11:19:51
V
A
5
10
74
1:2 HR
14/05/2012 11:19:51
V
B
5
10
72
2:2 HR

Figure 3.

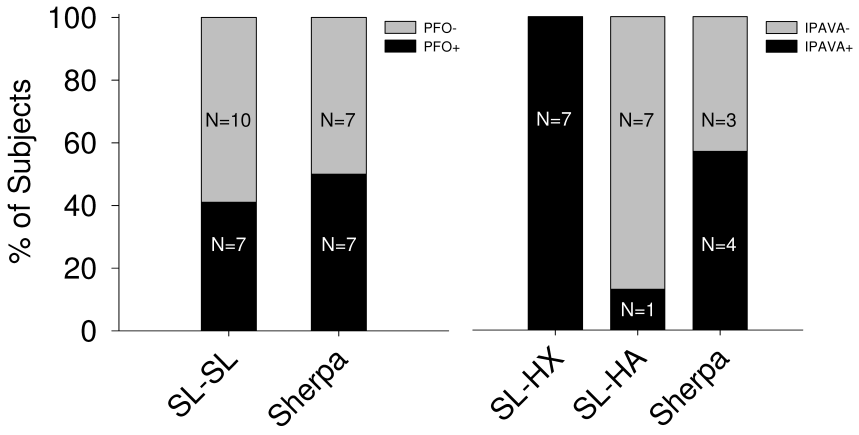
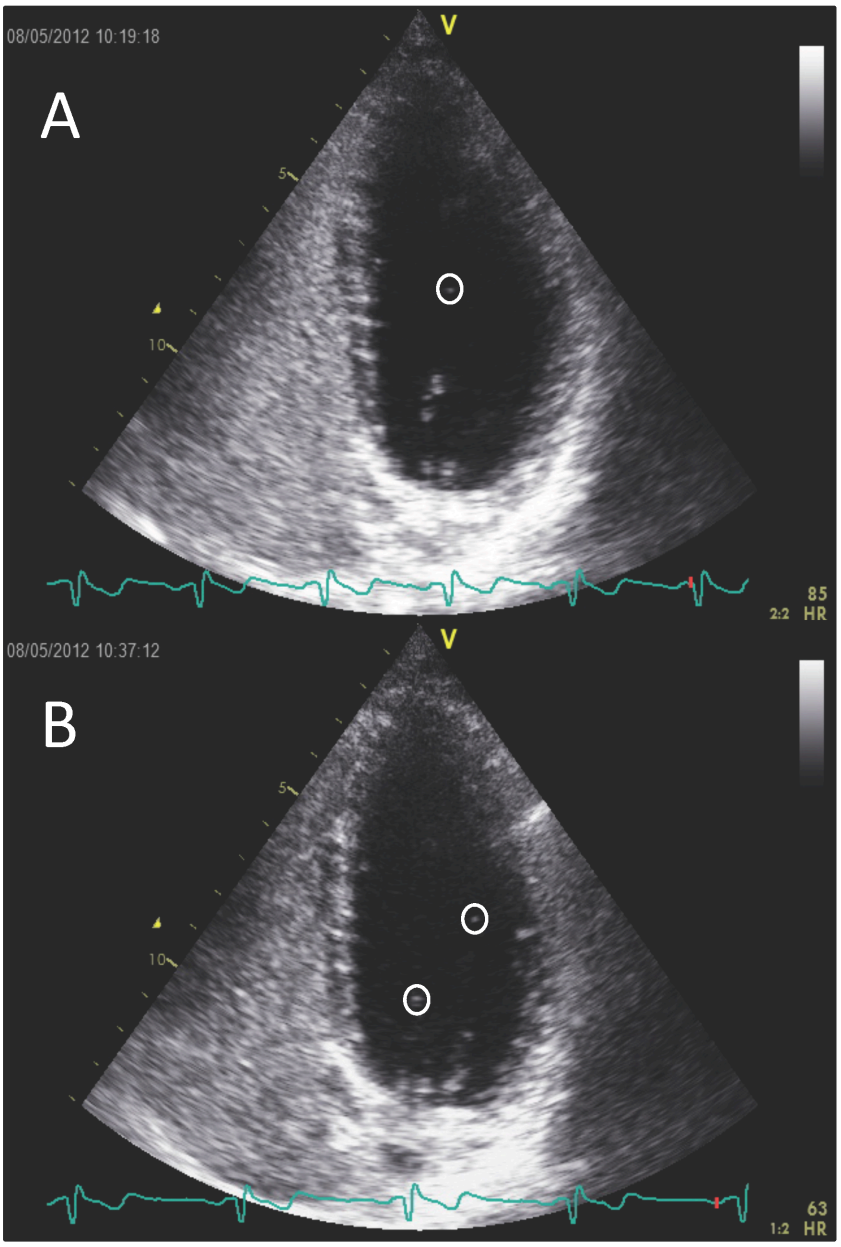

100
80
60
40
20
0
% of Subjects
PFO-
PFO+
N=10
N=7
N=7
N=7
IPAVA-
IPAVA+
N=7
N=7
N=3
N=4
N=1
SL-SL
Sherpa
SL-HX
SL-HA
Sherpa

Figure 4.


08/05/2012 10:19:18
A
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10
85
2:2 HR
08/05/2012 10:37:12
B
V
5
10
63
1:2 HR