

**The influence of hemoconcentration on hypoxic pulmonary vasoconstriction in acute, prolonged and life-long hypoxemia.**

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American Journal of Physiology - Heart and Circulatory Physiology

DOI:

[10.1152/ajpheart.00357.2021](https://doi.org/10.1152/ajpheart.00357.2021)

Published: 01/10/2021

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):

Stembridge, M., Hoiland, R., Williams, A., Howe, C., Dawkins, T., Drane, A., Tymko, M. M., Gasho, C., Anholm, J., Simpson, L., Bailey, D., Moore, J., MacLeod, D., & Ainslie, P. (2021). The influence of hemoconcentration on hypoxic pulmonary vasoconstriction in acute, prolonged and life-long hypoxemia. *American Journal of Physiology - Heart and Circulatory Physiology*, 321(4), H738-H747. <https://doi.org/10.1152/ajpheart.00357.2021>

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1 The influence of hemoconcentration on hypoxic pulmonary vasoconstriction in acute, prolonged
2 and life-long hypoxemia.

3
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27
28 *Word counts*

29 *Abstract = 242*

30 *Main text = 3681*

31
32 *Key words:*

33 *Hypoxic pulmonary vasoconstriction, haemodilution, pulmonary pressure, hypoxia, viscosity.*

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40 New and Noteworthy

41

42 Red blood cell concentration influences the pulmonary vasculature via direct frictional force and
43 vasoactive signalling, but whether the magnitude of the response is modified with duration of
44 exposure is not known. By assessing the pulmonary vascular response to haemodilution in acute
45 normobaric and prolonged hypobaric hypoxia in lowlanders and life-long hypobaric hypoxemia in
46 Andean natives, we demonstrated that a reduction in red cell concentration augments the
47 vasoconstrictive effects of hypoxia in lowlanders. In high altitude natives, haemodilution lowered
48 pulmonary vascular resistance but a compensatory increase in cardiac output following
49 haemodilution rendered PASP unchanged.

50

51

52 Abstract

53

54 Haemoconcentration can influence hypoxic pulmonary vasoconstriction (HPV) via increased
55 frictional force and vasoactive signalling from erythrocytes, but whether the balance of these
56 mechanism is modified by the duration of hypoxia remains to be determined. We performed three
57 sequential studies: (i) at sea level, in normoxia and isocapnic hypoxia with and without isovolumic
58 haemodilution (n=10, aged 29±7 years); (ii) at altitude (6±2 days acclimatization at 5050 m),
59 before and during hypervolumic haemodilution (n=11, aged 27±5 years) with room air and
60 additional hypoxia (FiO₂ = 0.15), and; (iii) at altitude (4340 m) in Andean high-altitude natives with
61 excessive erythrocytosis (EE; n=6, aged 39±17 years), before and during isovolumic
62 haemodilution with room air and hyperoxia (end-tidal PO₂ = 100 mmHg). Results: At sea level,
63 haemodilution mildly increased pulmonary artery systolic pressure (PASP; +1.6±1.5 mmHg,
64 P=0.01) and pulmonary vascular resistance (PVR; +0.7±0.8 wu, P=0.04). In contrast, after
65 acclimation to 5050 m, haemodilution did not significantly alter PASP (22.7±5.2 vs. 24.5±5.2
66 mmHg, P=0.14) or PVR (2.2±0.9 vs. 2.3±1.2 wu, p=0.77), although both remained sensitive to
67 additional acute hypoxia. In Andeans with EE at 4340 m, haemodilution lowered PVR in room air
68 (2.9±0.9 vs. 2.3±0.8 wu, P=0.03), but PASP remained unchanged (31.3±6.7 vs. 30.9±6.9 mmHg,
69 P=0.80) due to an increase in cardiac output. Collectively, our series of studies reveal that HPV is
70 modified by the duration of exposure and the prevailing haematocrit level. In application, these
71 findings emphasize the importance of accounting for haematocrit and duration of exposure when
72 interpreting the pulmonary vascular responses to hypoxemia.

73

74

75

76 Introduction

77

78 Hypoxic pulmonary vasoconstriction (HPV) of the pulmonary artery is mediated by a range of
79 mechanisms across the neuro-cardiopulmonary axis (for review see Swenson, 2013). Pulmonary
80 vascular smooth muscle cells are intrinsically sensitive to hypoxia, causing the pulmonary
81 arterioles and veins to constrict in response to a decrease in partial pressure of oxygen (PO_2) (2).
82 Extrinsic factors such as vascular endothelium, neurohormonal and erythrocyte-dependent
83 mechanisms can also alter the balance of vasoactive forces during hypoxic exposure.
84 Erythrocytes can both augment (7, 15) and attenuate (5, 10) the HPV response, through nitric
85 oxide (NO) scavenging and release, depending on whether they are in the oxygenated or
86 deoxygenated state. The balance between these regulatory processes has been suggested to
87 vary depending on the duration of hypoxic exposure (46), but different time domains are often
88 explored in separate studies with differing methodologies making comparisons problematic.

89

90 The signal for HPV is predominately derived from alveolar PO_2 (PAO_2) and mixed venous PvO_2 ,
91 accounting for ~62% and ~38% respectively. A lower concentration of red cells will increase HPV
92 by reducing PvO_2 at a constant arterial-venous O_2 difference. The vasodilatory action of
93 deoxygenated haemoglobin will be modified by the concentration of red cells i.e. during
94 polycythaemia, the greater concentration of red cells will lead to a larger signal, and *vice versa*.
95 Therefore, the degree of HPV for a given PO_2 may vary across a range of haematocrit. Indeed,
96 mild haemodilution has recently been demonstrated to exaggerate the pulmonary artery pressure
97 response to acute poikilocapnic hypoxia (8). Haemoconcentration also occurs concomitantly to an
98 increase in pulmonary artery pressure during acclimatisation to high altitude, but it's not known
99 whether the increased concentration of red cells amplifies the HPV response through the action of
100 deoxy-haemoglobin. During this period, the sensitivity of the pulmonary vasculature to changes in
101 PO_2 is also modified, with pulmonary artery pressure remaining responsive to hypoxia after 6-10
102 days of incremental altitude exposure to 5300 m (20), but unresponsive to oxygen reversal after
103 approximately three weeks' simulated ascent of Mt Everest in hypobaric hypoxia (11). With
104 increasing time at altitude, pulmonary vascular remodelling may predominate the vasoactive
105 effects of red blood cells and other mechanisms in the neuro-cardiopulmonary axis (28).
106 Understanding the role of haematocrit on HPV is especially pertinent to high altitude residents of
107 the Andean mountains, where up to one third of the population experiences excessive
108 erythrocytosis (EE) (18), often alongside elevated pulmonary artery pressures (30) and *RV*
109 *enlargement* (21). Current guidelines promote the consideration of EE in the diagnosis of high-
110 altitude pulmonary hypertension (17), due to the potential for right heart failure in this population
111 (25). However, only recently have mathematical models been developed to correct pulmonary
112 vascular resistance (PVR) to haematocrit (26, 50). While such models attempt to account for the
113 mechanical effect of blood viscosity, they overlook the vasoactive processes that occur when

114 erythrocyte concentration is reduced or the proportion of haemoglobin that is bound to oxygen is
115 altered. Therefore, comprehensively understanding the interaction between haematocrit,
116 pulmonary vasoconstriction and duration of exposure will aid the diagnosis and treatment of
117 lifelong high-altitude residents.

118

119 We therefore sought to determine the role of erythrocyte-dependent modulation of hypoxic
120 pulmonary vasoconstriction in humans by performing three sequential studies. First, we explored
121 the influence of haematocrit and arterial PO_2 in lowlanders at sea level by performing an
122 isovolumic haemodilution in normoxia and with acute isocapnic hypoxia. Second, we examined
123 how changes in haematocrit and arterial PO_2 affect pulmonary haemodynamics in lowlanders at
124 high altitude by performing a hypervolemic haemodilution (i.e. normalise haematocrit and blood
125 volume to sea level values) following acclimatization to 5050 m. Finally, we assessed the
126 pulmonary vascular responses to altered haematocrit and arterial PO_2 in Andeans chronically
127 living at 4340 m with EE by performing an acute isovolumic haemodilution in normal room air and
128 during oxygen supplementation. In line with these three aims, we hypothesised that haemodilution
129 would (i) increase the pulmonary pressure response to acute isocapnic hypoxia (ii) augment
130 pulmonary pressure at high altitude and increase the sensitivity of the pulmonary vasculature to
131 acute hypoxia and (iii) lower pulmonary pressure in Andean high altitude natives mediated by
132 reduced viscosity.

133

134

135 Materials and Methods

136

137 Ethical approval

138

139 Ethical approval was granted by the Clinical Research Ethics Board at the University of British
140 Columbia (H16-01297 and HS17-02687 for studies 1 and 2, respectively) and the Universidad
141 Peruana Cayetano Heredia, Lima, Peru (#101686 for study 3), and conformed to the *Declaration*
142 *of Helsinki* except for registration in a database. All experimental procedures were explained in
143 writing and verbally in the participants' native language, and written informed consent obtained
144 from all volunteers.

145

146 Experimental Design

147

148 *Study 1- Acute Hypoxia*

149 A total of ten male participants (aged 29 ± 7 , height 176 ± 4 cm, weight 72 ± 2 kg) participated in this
150 laboratory study, all of whom were sea level residents who were normotensive, non-smokers with
151 no previous history of cardiovascular, respiratory or haematological conditions and were taking no
152 prescription medications. The participants visited the laboratory once, and first underwent radial
153 artery and internal jugular vein catheterisation. Pre-haemodilution cardiovascular assessments
154 were taken during normoxia and acute isocapnic hypoxia ($P_{ET}O_2 = 40 \pm 2$ mmHg). Dynamic end-
155 tidal forcing (12) was used to closely match arterial PO_2 to that experienced in Study 2 described
156 below, and carbon dioxide fixed throughout to avoid the additional influence on the pulmonary
157 vasculature (3). Next, isovolumic haemodilution was performed via the removal of whole blood
158 from the internal jugular vein and replaced with infusion of volume-matched human serum albumin
159 to achieve an absolute drop in haematocrit of $\sim 10\%$ (e.g. 45 to 35% haematocrit). Following which,
160 cardiovascular measurements were repeated in normoxia and acute hypoxia.

161

162 *Study 2- Prolonged Exposure*

163 This study was conducted as part of the University of British Columbia Nepal 2016 expedition.
164 Precise details of the adopted ascent profile (52) and experimental design (14) are detailed
165 elsewhere. Although the expedition consisted of numerous studies, care was taken to avoid
166 overlap with drug interventions, and all participants avoided exercise and caffeine >24 hours prior
167 to testing. Following a cautious ascent profile over 10 days, participants were enrolled into the
168 study following 6 ± 2 days of acclimatization to 5050 m. Eleven male sea level residents (aged
169 27 ± 5 , height 177 ± 5 cm, weight 75 ± 8 kg) participated in Study 2. The participants arrived at the
170 laboratory in the fasted state (>4 hrs) and first underwent arterial and peripheral venous
171 catheterisation. Pre-haemodilution measurements were made whilst breathing room air and during
172 poikilocapnic hypoxia ($FiO_2 = 0.15$). All measurements were repeated following successful

173 hypervolemic haemodilution via rapid infusion of saline, a procedure our group has successfully
174 performed previously at high altitude (41). Similar to Study 1, the intervention aimed to reduce
175 haematocrit by ~10%. In contrast to Study 1, hyper- rather than isovolemic haemodilution was
176 chosen. This was to normalise total blood volume and cardiac filling to sea level values (41, 44,
177 45), as a lower blood volume would have lowered stroke volume and, in turn, pulmonary artery
178 pressure.

179

180 *Study 3- Life-long High-Altitude Residence*

181 A total of ten male Andean highlanders were recruited from the town of Cerro de Pasco, Peru
182 (4340 m) (49). Of these, two did not complete the experiments due to clotting of the arterial line,
183 one did not tolerate the catheterization procedure and withdrew and one had unacceptable
184 echocardiographic windows. Analysis of blood samples was not possible in one individual due to
185 equipment failure. Therefore, cardiopulmonary data are reported on six Andeans (aged 39 ± 17
186 years, height 162 ± 7 cm, body mass 69 ± 12 kg) and haematological data in five participants.
187 Following medical screening that included blood pressure, haematocrit and full medical and
188 altitude history, the participants reported to the laboratory on one occasion. Instrumentation began
189 with radial artery catheterization for blood gas sampling and antecubital vein cannulation for
190 volume infusion. Cardiovascular assessments were completed in (hypoxic) room air and simulated
191 isocapnic normoxia (end-tidal forcing with hyperoxia to achieve a PaO_2 of 100 mmHg). Hyperoxia
192 was chosen as it allowed a greater magnitude of change in oxygen saturation compared to what
193 was safe and realistic individuals with a starting saturation of ~75-80%. These measurements
194 were taken before and after blood volume removal via the arterial catheter, and replacement with
195 human serum albumin to achieve an absolute drop in haematocrit of ~10% (48).

196

197 *Experimental Measures*

198

199 *Haematological and haemodynamic measures*

200 Arterial (20G, Arrow, Markham, Ontario, Canada) and central venous catheterisation (13G, Cook
201 Medical, Bloomington, IN) were performed with local lidocaine (1%) and under sterile conditions
202 with ultrasound guidance. The arterial catheters were connected to an inline waste-less sampling
203 system containing a pressure transducer located at the height of the right atrium (VAMP System,
204 Edwards Lifesciences) for the monitoring and assessment of systemic blood pressure. Arterial
205 blood gases, haemoglobin concentrations and haematocrit were determined via co-oximetry
206 (ABL90 Flex, Radiometer, Copenhagen, Denmark) and used to calculate arterial oxygen content
207 (CaO_2). Absolute blood and plasma volumes were determined using the modified carbon
208 monoxide rebreathing technique (35). Blood viscosity was determined from venous samples using
209 a cone and plate viscometer (Model DV2T, Brookfield Amtek, USA) at 37°C and a shear rate of
210 225 s^{-1} .

211

212 *Cardiovascular Assessments*

213 Pulmonary vascular haemodynamic and cardiac function were determined via echocardiography
214 following the American Society of Echocardiography recommendations (33), as previously
215 performed by our group at high altitude (42, 43). Participants were supine and tilted into the left-
216 lateral position where images were acquired in the parasternal and apical imaging windows. Five
217 successive cardiac cycles were recorded on a portable ultrasound (Vivid q, GE Healthcare,
218 Piscataway, NJ, USA) for subsequent offline analysis (Echopac, GE Healthcare, Piscataway, NJ,
219 USA). Pulmonary artery systolic pressure (PASP) was estimated as the maximum systolic
220 pressure gradient across the tricuspid valve. The modified Bernoulli equation ($4V^2$) was applied to
221 the peak systolic regurgitation jet velocity measured via continuous wave Doppler, and right atrial
222 pressure was estimated from the collapsibility of the inferior vena cava. Cardiac output was
223 determined from stroke volume obtained from the velocity-time integral of the left ventricular
224 outflow tract in the five-chamber view and heart rate acquired from a 3-lead electrocardiograph.
225 PVR was estimated by calculating mean pulmonary artery pressure from PASP (4), then
226 subtracting left atrial pressure derived from early mitral inflow velocity and early tissue Doppler
227 velocity of the septal and lateral mitral annulus(27) and dividing by cardiac output. This approach
228 was chosen as our volume interventions may have caused changes in left atrial pressure, rather
229 than assuming left atrial pressure to be zero or applying a set value obtained from the literature
230 (47).

231

232 HPV is largely determined by alveolar (PAO_2 ; ~38%) and venous PO_2 (PvO_2 ; 62%), and the
233 interventions we employed will alter the degree of HPV in line with these relative contributions. For
234 example, haemodilution will reduce arterial oxygen content, such that maintained $a-vO_2$
235 differences will result in a lower PvO_2 . Changes in inspired oxygen will reduce both PaO_2 and
236 PvO_2 due to decreased diffusion in the lung, and therefore provide a stronger stimulus for HPV. To
237 compare the effects of both interventions across our three protocols, we calculated the estimated
238 stimulus PO_2 so that our data can be interpreted relative to the stimulus applied. PAO_2 was
239 estimated from the alveolar gas equation, using $PaCO_2$ from our blood gas data and a fixed
240 respiratory quotient of 0.82. PvO_2 was estimated by assuming a fixed arterial-venous oxygen
241 difference of 5ml under resting conditions and subtracting that from CaO_2 measured from arterial
242 blood gas data. The proportional contribution towards HPV was then applied using the following
243 equation from Marshall and Marshall (23):

244

245 • Stimulus $PO_2 = PvO_2^{0.375} \times PAO_2^{0.626}$

246

247 Statistical Analysis

248

249 All statistical analyses were performed in Graphpad Prism (Version 7, San Diego, US). Distribution
250 normality was confirmed with the Shapiro-Wilk test. Two-way repeated measures analysis of
251 variance (factors: haemodilution and oxygen saturation) was conducted for dependent data in
252 each study separately, and when a significant main effect was detected *post hoc* comparisons
253 were performed with Bonferroni correction to account for multiple comparisons and adjusted P
254 values reported. Where appropriate, effect sizes (Cohen's *d*) are reported to help indicate the
255 magnitude of change. The slope of the response for a given change in stimulus PO₂ was calculate
256 using the rise over run method, and tested for differences using a paired-samples t test.
257 Significance was established at P<0.05, and data are presented as mean ± SD. The data that
258 support the findings of this study are available from the corresponding author upon reasonable
259 request.
260
261

262 Results

263

264 *Study 1- Acute Hypoxia*

265

266 Haemodilution was effective in reducing both haematocrit (43.5 ± 2.6 vs. $35.0\pm 1.6\%$, $P<0.001$) and
267 blood viscosity (3.5 ± 0.3 vs. 2.8 ± 0.3 centipoise (cP), $P<0.001$). By design, there was only a small
268 difference in oxygen content between the hypoxia pre-haemodilution and normoxia post-
269 haemodilution, and was lowest in hypoxia following haemodilution (Table 1). Haemodilution did not
270 alter mean arterial pressure, but it was elevated in response to hypoxia.

271

272 As expected, acute hypoxia elevated PASP in both pre- and post-haemodilution conditions (main
273 effect $p<0.001$). Haemodilution resulted in a small increase in PASP in normoxia ($+1.6 \pm 1.5$
274 mmHg, $P=0.008$), and a relatively larger increase during hypoxia compared to pre-haemodilution
275 hypoxia ($+4.5\pm 2.4$ mmHg, $P<0.001$, Figure 1). The changes in PASP in normoxia occurred
276 independent of changes in cardiac output, with no significant main effect for haemodilution
277 ($P=0.237$). Whilst there was a significant main effect for the change in oxygen saturation ($P=0.037$,
278 Figure 1), *post hoc* analysis revealed no significant changes in cardiac output. Therefore,
279 observed changes in PVR are likely attributable to changes in pulmonary vasculature tone.
280 Indeed, PVR was increased in response to acute hypoxia in both pre-haemodilution ($P=0.002$) and
281 post haemodilution ($P=0.030$) states, and increased in normoxia pre-post haemodilution
282 ($P=0.015$).

283

284 *Study 2- Prolonged Exposure to High Altitude*

285

286 At high altitude, haemodilution decreased haematocrit from 49.2 ± 2.9 to 43.2 ± 3.2 ($P<0.001$), so
287 that it was comparable to sea level baseline in Study 1 (43.5%). Concomitant with the decrease in
288 haematocrit was a reduction in blood viscosity (4.5 ± 0.6 vs. 3.7 ± 0.4 cP, $P<0.001$) and CaO_2 (Table
289 1). Acute poikilocapnic hypoxia decreased arterial PO_2 and CaO_2 to similar degrees pre- and post-
290 haemodilution (Table 1). Despite the hypervolemic nature of the haemodilution, mean arterial
291 pressure was not altered by the intervention (main effect $P=0.097$).

292

293 PASP was not significantly altered ($P=0.14$) following haemodilution (Figure 2) whilst breathing
294 room air, but it was increased pre-post haemodilution during the acute poikilocapnic hypoxia
295 condition ($P<0.001$) suggesting haemodilution may only increase PASP under acute hypoxic
296 stress and not in the acclimatised state. Consistent with Study 1, acute poikilocapnic hypoxia
297 increased PASP pre-haemodilution ($P=0.004$) and this response remained post-haemodilution
298 ($P=0.002$) (Figure 2). Cardiac output and PVR were unchanged by haemodilution in room air or
299 acute poikilocapnic hypoxia (main effects $P=0.395$ and $P=0.116$, respectively).

300

301

302 *Study 3- Life-long high-altitude residence*

303

304 Haemodilution reduced haematocrit from 68.2 ± 5.4 to 58.4 ± 4.9 % ($P < 0.001$) and lowered viscosity
305 from 8.1 ± 1.4 to 5.4 ± 0.7 cP ($P = 0.002$). While these represent substantial reductions, Andean
306 natives still remained polycythaemic even compared to lowlanders at high altitude in Study 2.
307 Mean arterial pressure was not altered by haemodilution or acute hyperoxia (Table 2). PASP
308 remained unchanged following haemodilution ($P = 0.201$) and during hyperoxia ($P = 0.504$). The
309 consistent PASP during both interventions are likely underpinned by an increase in cardiac output
310 and a decrease in PVR in response to haemodilution (Figure 3). For example, in room air,
311 haemodilution increased cardiac output ($P = 0.027$) but decreased PVR ($P = 0.019$). A similar effect
312 of haemodilution was observed under hyperoxia.

313

314 *Influence of Stimulus PO_2 on pulmonary haemodynamics*

315

316 In lowlanders, haemodilution increased the slope of the PASP response to an increased stimulus
317 PO_2 at both sea level and high altitude (Figure 4), but the slope of the PVR response remained
318 unchanged suggesting that the increase in pressure is largely related to the higher cardiac output
319 needed to sustain oxygen delivery following haemodilution. While the slope of the PVR response
320 was unchanged, as discussed above, PVR for a given stimulus PO_2 was elevated by hemodilution
321 at sea level (main effect $P = 0.045$) but not at high altitude. In contrast, neither the slope of the PVR
322 nor PASP response was altered by haemodilution when they stimulus PO_2 was decreased during
323 hyperoxia in Andeans. Moreover, PVR was reduced (main effect $P = 0.029$) by hemodilution in
324 Andean natives, highlighting differences between the temporal domains of hypoxic exposure.

325

326 Discussion

327

328 In relation to our three hypotheses, the primary novel findings of this series of studies are: (i)
329 haemodilution augments the pulmonary pressure response to acute isocapnic hypoxia; (ii) in
330 lowlanders at high altitude, haemodilution had no effect on pulmonary pressure but the pulmonary
331 vasculature was more responsive to changes in the stimulus PO_2 ; and (iii) pulmonary pressure
332 remained unchanged in Andeans following acute haemodilution because of an increase in cardiac
333 output but reciprocal reduction in PVR. Collectively, our data indicate that the influence of arterial
334 PO_2 and haematocrit on the pulmonary pressure response to hypoxia is modified by the duration of
335 hypoxic exposure and starting haematocrit.

336

337 *Haemodilution and acute hypoxia*

338

339 As hypothesised, the reduction in haematocrit increased the magnitude of pulmonary pressure
340 response to isocapnic hypoxia i.e. there was a greater increase in pulmonary artery pressure for a
341 given change in stimulus PO_2 following haemodilution. A similar response has recently been
342 reported whereby greater increases in pulmonary pressure and cardiac output were observed
343 during graded poikilocapnic hypoxia (8). We confirm and extend these findings by controlling for
344 changes in end-tidal PCO_2 , as the ventilation-induced hypocapnia in the study of Duke et al.
345 (2016) would likely have elicited vasodilatation that would have effectively masked the
346 haemodilution-induced elevation in pulmonary pressure (3). The increased sensitivity to hypoxia
347 following haemodilution may be mediated by the vasodilatory action of red blood cells. The lower
348 concentration of red cells would attenuate the generation of S-nitrosothiol (10), NO (5) and ATP
349 (40) by deoxyhaemoglobin, and low haemoglobin levels can increase free radical signalling (9,
350 29). Together, these mechanisms would shift the vasoactive balance towards constriction. In
351 normoxia, haemodilution also induced a mild increase in PASP and PVR, suggesting the
352 erythrocyte-associated vasodilatory signalling mechanisms outlined above also influence
353 pulmonary vascular tone under baseline normoxic conditions. Interestingly, haemodilution did not
354 alter the slope of the PVR response to hypoxia for a given stimulus PO_2 (i.e. alveolar and venous
355 PO_2). Given that PVR increased, but was not directly related to the change in stimulus PO_2 ,
356 reduced vasodilatory action from the lower concentration of red blood cells could be responsible
357 for the drop in PVR for a given PO_2 .

358

359 *Pulmonary vascular resistance at altitude*

360

361 In the landmark study by Groves, Reeves, Sutton, Wagner, Cymerman, Malconian, Rock, Young
362 and Houston (11), the pulmonary vasculature was shown to be unresponsive to acute restoration
363 of arterial oxygen saturation during simulated ascent to the summit of Mt. Everest over 40 days.

364 Recently, this premise has been somewhat challenged (20) by data showing no alteration to the
365 slope of relationship between pulmonary artery pressure and oxygen saturation during a shorter
366 period of acclimatisation (14 days) up to 5300 m. This is despite the haemoconcentration that
367 occurs during acclimatisation, a process that serves to normalise arterial oxygen content and has
368 the potential to augment erythrocyte-dependent mechanisms of pulmonary vascular control. In line
369 with previous observations (20), we report an increase in pulmonary pressure in response to an
370 additional hypoxic stimulus at high altitude, but no change in absolute PVR or the slope of the
371 response to a change in stimulus PO_2 . Further work is required to explore the time-dependency of
372 the responsiveness to acute changes in PO_2 , as it appears this is lost somewhere between 2-4
373 weeks of high-altitude exposure. Despite the known effects of viscosity, haemodilution did not alter
374 PVR while breathing room air at 5050 m. The absence of change in lowlanders may be due to
375 poor signal-to-noise ratio in our experiment, as models for the PVR-haematocrit predict only a ~0.3
376 mmHg/L/min change in PVR with a haematocrit shift from 49% to 43% lying on the flat portion of
377 the exponential relationship (50). One may have also expected to observe an increase in cardiac
378 output following hypervolemic haemodilution, as the normalisation of cardiac output with
379 acclimatisation has traditionally thought to be due to the restoration of oxygen content via
380 haemoconcentration. However, haemoconcentration occurs progressively over the first seven
381 days at high altitude via plasma volume constriction (34), but cardiac output remains elevated
382 throughout this period (24). Therefore, cardiac output and oxygen content (i.e. delivery) may not
383 be the regulated physiological variable, with blood pressure control a more likely candidate (38).

384

385 *Haemodilution lowers PVR in Andeans*

386

387 In contrast to our observations in lowlanders at sea level and high altitude, we observed no
388 change in PASP and a reduction in PVR in individuals with EE following haemodilution. These
389 findings are consistent with early invasive studies in chronic mountain sickness patients directly
390 measuring PVR following a comparable 10% reduction in haematocrit (22). The greater influence
391 of haemodilution on PVR is due to the higher starting haematocrit being on the steep portion of the
392 PVR-haematocrit curve (50). However, in contrast to our data and predictive models, when a
393 group of chronic mountain sickness patients were gradually haemodiluted across a four day period
394 (39), pulmonary artery pressure and cardiac output both increased, suggesting there was no
395 change in PVR. This incongruity may be related to differences in the temporal response of the
396 pulmonary vasculature, where the effects of a gradual reduction in blood viscosity on PVR are
397 offset by the vasoconstrictive effects of lowering red blood cell concentration. As in lowlanders in
398 Study 1, fewer red blood cells per unit volume of blood would lead to decreased vasodilatory
399 signalling via S-nitrosothiol (10), NO (5) and ATP (40), as well as amplifying free radical signalling
400 (9, 29). Therefore, the pulmonary vasculature may retain responsiveness to changes in red blood
401 cells in Andeans native to high altitude. However, PVR was unresponsive to acute hyperoxia in our

402 Andean population, which is in direct contrast to lowlanders acclimatised to high altitude (20).
403 Therefore, consistent with the blunted ventilatory response in high altitude natives (36), the
404 pulmonary vasculature of Andeans with EE appears to be unresponsive to acute changes in
405 arterial PO_2 .

406

407 *Influence of Stimulus PO_2*

408

409 The degree of HPV is largely driven by a combination of mixed venous PO_2 (~37%) and alveolar
410 PO_2 (~63%) (23). By estimating the “stimulus PO_2 ” from our data, we are able to observe that
411 PASP is greater in lowlanders following haemodilution for a given stimulus PO_2 . The greater HPV
412 response for the same stimulus PO_2 suggests an alternative vasoactive pathway is acting on the
413 pulmonary vasculature in lowlanders. From our experiments, we speculate that this is erythrocyte-
414 mediated vasodilation. This, however, was not the case in Andeans. The absence of this response
415 in Andeans could be due to the higher starting haematocrit (e.g. ~68%), with a 10% reduction
416 being a smaller relative stimulus compared to the change in lowlanders. Even at 58% haematocrit,
417 the vasoactive effects of erythrocytes may be maximally effective, and a more substantial
418 reduction needed to alter the PASP response for a given change in stimulus PO_2 . Future studies,
419 preferably with long-term relocation to lower altitudes as has been employed previously (37),
420 should look to quantify the pulmonary vascular response across a wider range of stimulus PO_2 .

421

422 *Translational perspective*

423

424 Venesection (i.e. the removal of blood) has historically been used to treat the clinical
425 manifestations of EE and reduce the cardiovascular burden of high viscosity in those diagnosed
426 with chronic mountain sickness (51). However, the benefits are short lived, as red cell production
427 will eventually restore blood volume to pre-removal levels. This approach has been associated
428 iron deficiency resulting in further elevation of PASP (39). Herein, we demonstrate that despite a
429 haemodilution resulting in a reduced PVR, PASP remained elevated due to a compensatory
430 increase in cardiac output, adding further doubt to the effectiveness of this therapeutic approach.
431 Anaemia and iron deficiency are also receiving increasing attention for their role in idiopathic and
432 heritable forms of pulmonary arterial hypertension (32), given the relationship with exercise
433 capacity, symptoms and survival (31). We report that, even in healthy individuals, changes in
434 haematocrit can alter pulmonary vascular haemodynamics at rest and the response to acute
435 changes in PaO_2 , highlighting the importance of haematology in pulmonary vascular regulation in
436 health and disease.

437

438 *Limitations*

439

440 There are several limitations to our study that require acknowledgement. We used indirect
441 measures of pulmonary vascular haemodynamics. However, these measurements have been
442 shown to correlate well with invasive methodologies (6, 53), and invasive techniques were not
443 practicable in such remote mountainous locations. Each of our three studies had a relatively small
444 sample size, especially our study of high-altitude natives. Given the invasiveness associated with
445 haemodilution, and the complex nature of high altitude field work (1), we were unable to recruit a
446 larger sample. Our sample sizes are, however, comparable to previous work in the field (13, 20,
447 39), and we have reported individual data in our figures to be as transparent with as possible. The
448 average age of our Andean high altitude native group was also greater by 10 years, and
449 pulmonary vascular tone is known to increase with age (16, 19). However, when the change in
450 PVR to altered PO_2 was plotted as a function of age during both pre- and post-haemodilution
451 states, there was no significant relationship.

452

453 *Conclusion*

454

455 In summary, our series of studies demonstrate that the influence of haemodilution is modified by
456 the duration of hypoxic exposure in lowlanders, exerting a more profound effect under acute vs.
457 prolonged hypoxia. Rapid haemodilution in life-long high-altitude natives results in an acute drop in
458 pulmonary vascular resistance due to the higher starting haematocrit, but any benefits in
459 unloading the right ventricle are largely negated by an acute increase in cardiac output.
460 Collectively, our data suggest haemodilution to dampen the vasodilatory action of deoxygenated
461 red blood cells, and highlight the need to consider haematology and stimulus PO_2 when
462 investigating pulmonary vascular pathophysiology.

463

464

465 *Acknowledgments*

466 We would like to thank the participants and residents of Cerro de Pasco for their hospitality and
467 help with our field research, and to the Nepalis who facilitated our expedition in the Khumbu valley.

468

469 *Conflicts of interest: None*

470

471 *Funding:* National Science and Engineering Research Council (Canada; PNA) and The
472 Physiological Society (UK; Stembridge).

473

474

475 *Contributions*

476 MS had full access to all of the data in the study and takes responsibility for the integrity of the
477 data and the accuracy of the data collection, data analysis, including and especially any adverse
478 effects. MS, RLH and PNA contributed substantially to the study design, data analysis and
479 interpretation, and the writing of the manuscript. AMW, CAH, JD, TGD, AD, MMT, CG, JA, LLS,

480 JPM, DMB and DBM contributed substantially to data collection, data analysis and interpretation,
481 and the writing of the manuscript. All authors approved the final version of this manuscript.
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597 Figure Legends

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Figure 1- Pulmonary vascular response to acute hypoxia before and after haemodilution at sea level. Pulmonary artery systolic pressure (PASP) and pulmonary vascular resistance (PVR) were increased in response to both haemodilution and acute hypoxia. PaO_2 = arterial partial pressure of oxygen; CaO_2 arterial oxygen content. Significant p values in **bold**.

Figure 2- Pulmonary vascular response to haemodilution following acclimatisation to 5050 m in room air and acute hypoxia. Pulmonary artery systolic pressure (PASP) was unaltered by haemodilution, but remained responsive to acute hypoxia. FiO_2 = Fraction of inspired oxygen. Significant p values in **bold**.

Figure 3- Pulmonary vascular response to haemodilution in six Andeans with excessive erythrocytosis in room air and after restoration of oxygen saturation to sea level values. Pulmonary artery systolic pressure (PASP) was unresponsive to both acute hypoxia and haemodilution, but pulmonary vascular resistance was reduced following haemodilution despite an increase in cardiac output. PaO_2 = arterial partial pressure of oxygen. Significant p values in **bold**.

Figure 4- Pulmonary vascular changes for a given stimulus PO_2 across all three time domains of hypoxic exposure. The slope of the PASP response was greater in lowlanders following haemodilution, but PVR remained unchanged. The slope of the PASP and PVR response to a change in stimulus PO_2 via hyperoxia was not altered by haemodilution. The slope of the response for a given change in stimulus PO_2 was calculate using the rise over run method, and tested for differences using a paired-samples t test. PaO_2 = arterial partial pressure of oxygen. Significant p values in **bold**.

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Table 1- Haematological and haemodynamic effects of haemodilution in acute and prolonged hypoxia.

	Pre-Haemodilution		Post-Haemodilution		ANOVA P Value		
	Normoxia	Hypoxia	Normoxia	Hypoxia	Haemodilution	O ₂ Saturation	Interaction
<i>Study 1- Acute Hypoxia</i>							
Haematocrit (%)	43.5 ± 2.6	44.5 ± 2.4*	35.0 ± 1.6*#	35.7 ± 1.4*†	<0.001	<0.001	0.09
Arterial Oxygen Saturation (%)	98 ± 0	75 ± 2*#	98 ± 1	74 ± 4*#	0.6	<0.001	0.6
Arterial PO ₂ (mmHg)	94 ± 4	40 ± 2*#	96 ± 7	40 ± 2*#	0.26	<0.001	0.3
Arterial PCO ₂ (mmHg)	42 ± 2	42 ± 2	41 ± 2	42 ± 1#	0.87	0.02	0.02
Arterial Oxygen Content (ml dl ⁻¹)	19.3 ± 1.1	15.7 ± 0.7*#	15.1 ± 1*#	11.9 ± 0.8*†	<0.001	<0.001	0.002
Mean arterial pressure (mmHg)	99 ± 6	107 ± 14*#	98 ± 5	107 ± 8*#	0.18	0.01	0.07
Heart Rate (bpm)	61 ± 12	74 ± 13	61 ± 10	75 ± 11*#	0.885	<0.001	0.839
Stroke Volume (ml)	61.5 ± 10.6	60.5 ± 17.6	63.1 ± 14.7	66.7 ± 16.6	0.095	0.738	0.365
<i>Study 2- Prolonged Exposure</i>							
Haematocrit (%)	49.2 ± 2.9	49.6 ± 2.7*#	43.2 ± 3.2*	45.7 ± 2.4*†	<0.001	<0.001	0.002
Arterial Oxygen Saturation (%)	85 ± 3	70 ± 7*#	86 ± 3	73 ± 7*#	0.397	<0.001	0.98
Arterial PO ₂ (mmHg)	49 ± 3	35 ± 4*#	52 ± 4*	37 ± 4*#†	<0.001	0.001	0.149
Arterial PCO ₂ (mmHg)	25 ± 2	23 ± 3	23 ± 4	20 ± 1*#†	0.001	<0.001	0.244
Arterial Oxygen Content (ml dl ⁻¹)	18.8 ± 1.4	15.7 ± 1.9*#	16.7 ± 1.3*	15.0 ± 1.6*#	<0.001	<0.001	0.003
Mean arterial pressure (mmHg)	107 ± 11	96 ± 10*	111 ± 8	100 ± 13*#	0.097	0.005	0.802
Heart Rate (bpm)	59 ± 13	65 ± 16*#	56 ± 15	65 ± 15*#	0.576	0.002	0.145
Stroke Volume (ml)	62.5 ± 13.6	63.9 ± 13.5	70.3 ± 14.2	63.7 ± 10.9	0.137	0.316	0.2

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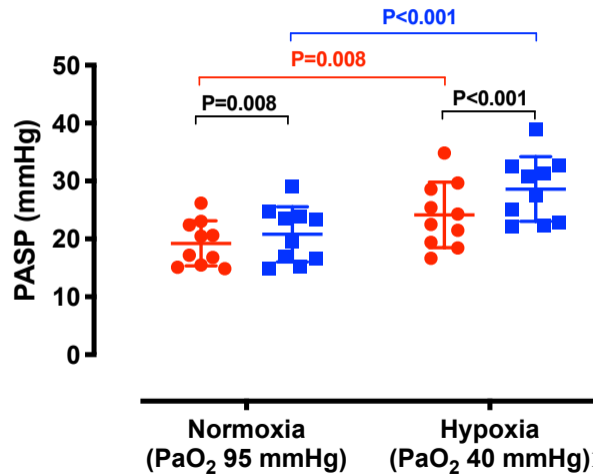
*= vs. normoxia pre-haemodilution, #= vs. normoxia post haemodilution and †= vs. hypoxia pre-haemodilution.

634 Table 2- Haematological and haemodynamic effects of haemodilution in Andean natives during room air and hyperoxia
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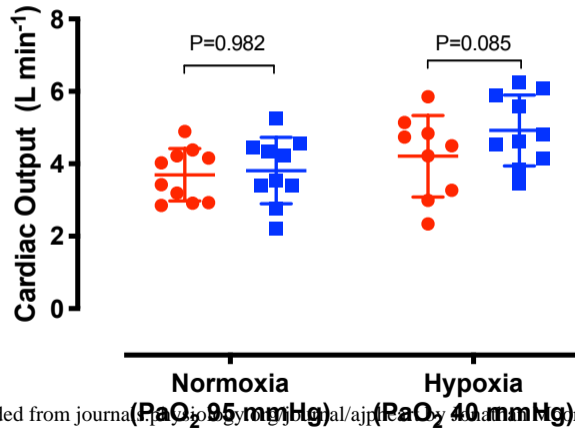
	Pre-Haemodilution		Post-Haemodilution		ANOVA P Value		
	Normoxia	Hyperoxia	Normoxia	Hyperoxia	<i>Haemodilution</i>	<i>O₂ Saturation</i>	<i>Interaction</i>
<i>Study 3- Life-long High Altitude Residence</i>							
Haematocrit (%)	67.7 ± 5.9	69.0 ± 5.7#	57.1 ± 4.1*†	56.2 ± 4.5*#†	<0.001	0.673	0.148
Arterial Oxygen Saturation (%)	78 ± 5	96 ± 1*#	76 ± 5	96 ± 1*#	0.098	<0.001	0.092
Arterial PO ₂ (mmHg)	44 ± 4	101 ± 7*#	41 ± 3	98 ± 5*#	0.255	<0.001	0.098
Arterial PCO ₂ (mmHg)	36 ± 2	36 ± 3	36 ± 2	36 ± 2	0.503	<0.001	0.265
Arterial Oxygen Content (ml dl ⁻¹)	24.3 ± 1.8	30.5 ± 2.4*#	19.8 ± 1.8*	25.0 ± 2.0#†	0.003	<0.001	0.491
Mean arterial pressure (mmHg)	106 ± 18	103 ± 17	96 ± 22	95 ± 21	0.400	0.179	0.214
Heart Rate (bpm)	62 ± 9	61 ± 10	68 ± 11	62 ± 19	0.180	0.058	0.955
Stroke Volume (ml)	60.9 ± 7.4	60.8 ± 10.5	67.8 ± 14.1	65.5 ± 11.3	0.271	0.694	0.305

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 637 *= vs. normoxia pre-haemodilution, #= vs. normoxia post haemodilution and †= vs. hyperoxia pre-haemodilution.
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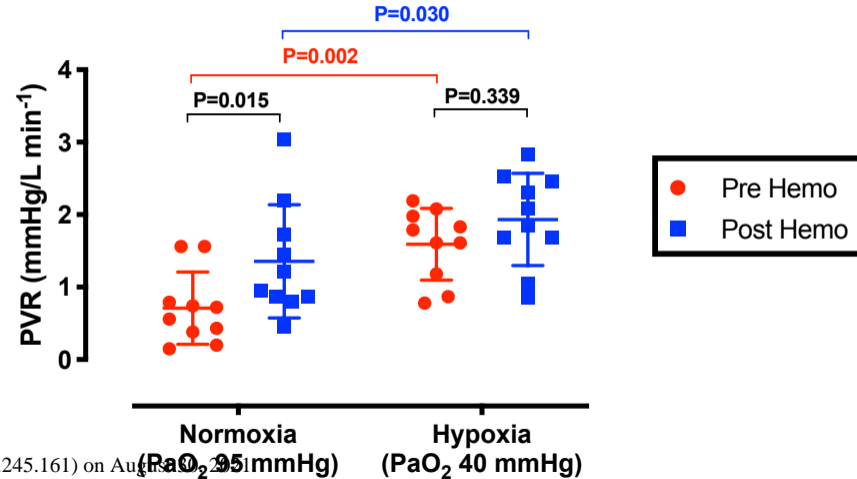
Main effect hemodilution ($P=0.004$), oxygen saturation ($P<0.001$) and interaction ($P=0.005$)



Main effect hemodilution ($P=0.237$), oxygen saturation ($P=0.037$) and interaction ($P=0.156$)

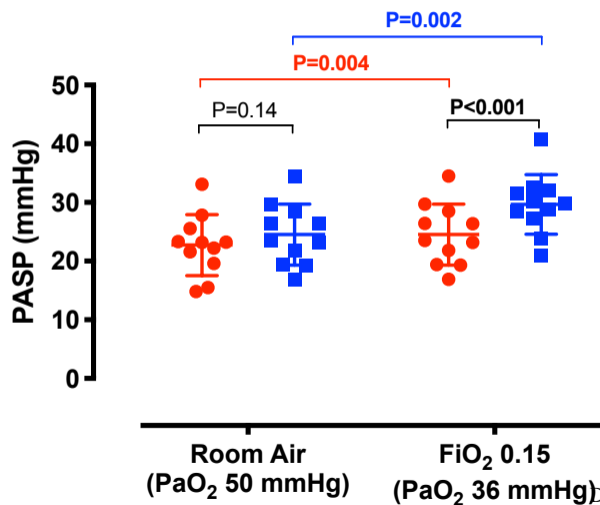


Main effect hemodilution ($P=0.045$), oxygen saturation ($P=0.006$) and interaction ($P=0.200$)

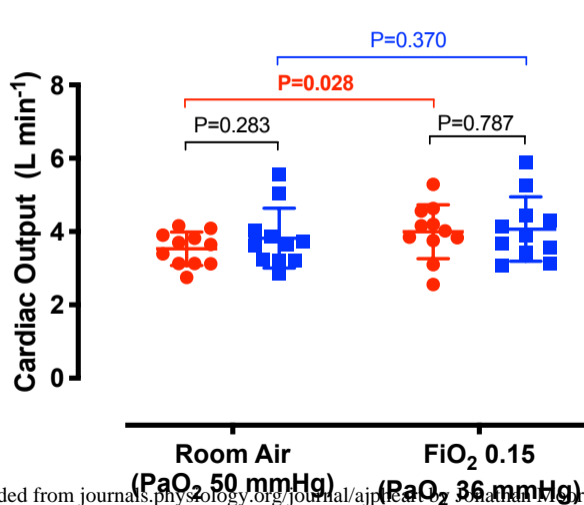


● Pre Hemo
■ Post Hemo

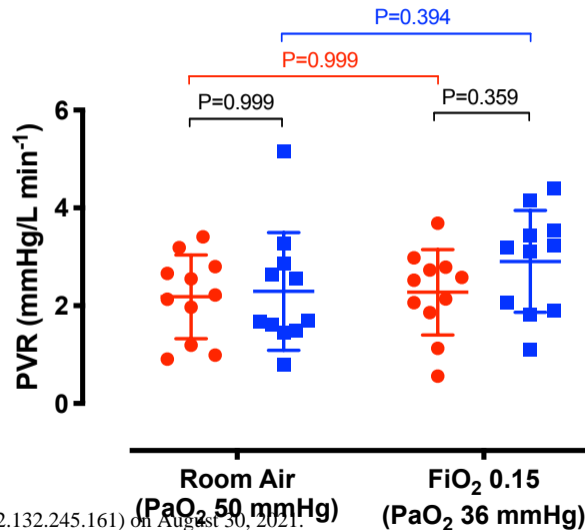
Main effect hemodilution (**P=0.004**), oxygen saturation (**P=0.001**) and interaction (**P=0.034**)



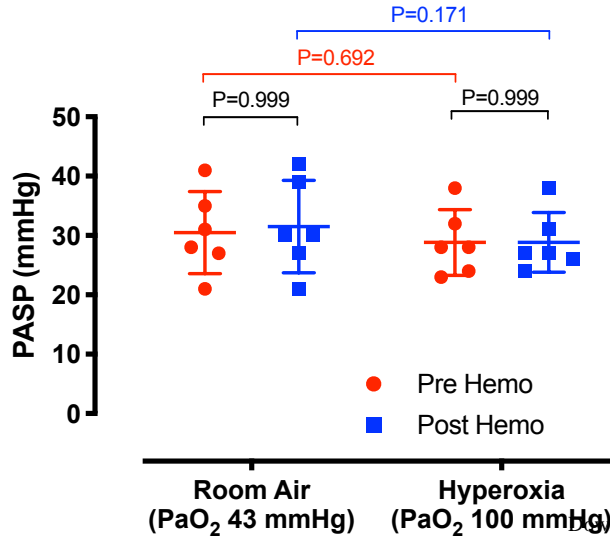
Main effect hemodilution (P=0.395), oxygen saturation (P=0.055) and interaction (P=0.500)



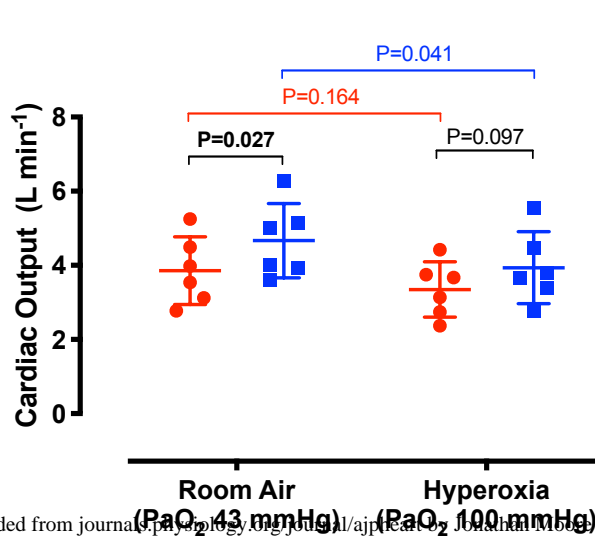
Main effect hemodilution (P=0.116), oxygen saturation (P=0.144) and interaction (P=0.243)



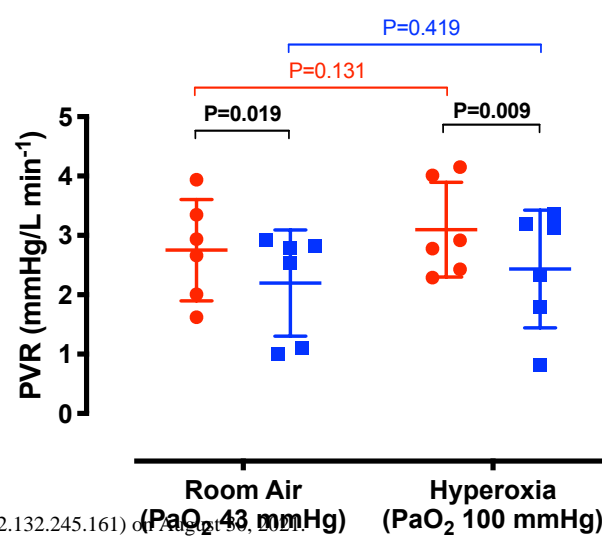
Main effect hemodilution ($P=0.201$), oxygen saturation ($P=0.504$) and interaction ($P=0.456$)



Main effect hemodilution ($P=0.083$), oxygen saturation ($P=0.062$) and interaction ($P=0.386$)



Main effect hemodilution ($P=0.029$), oxygen saturation ($P=0.073$) and interaction ($P=0.515$)

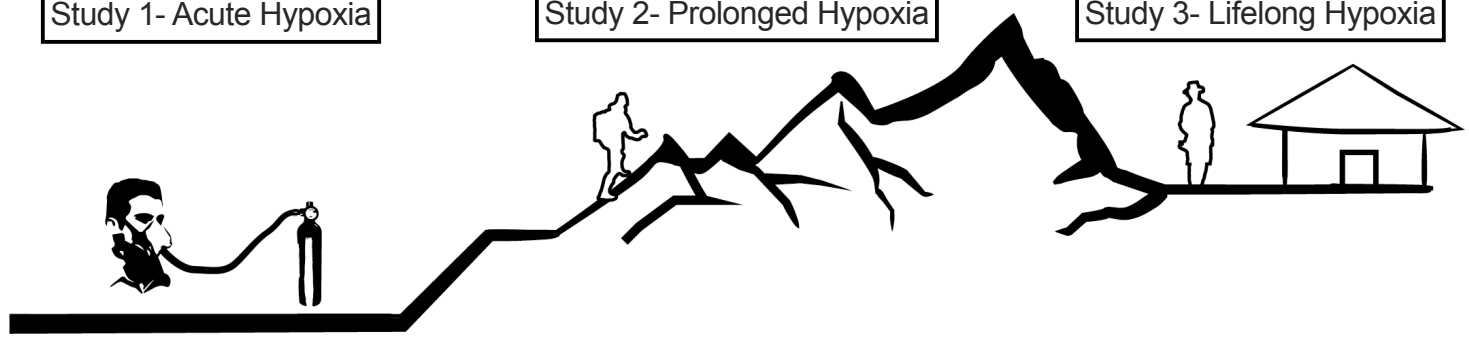


● Pre Hemo
■ Post Hemo

Study 1- Acute Hypoxia

Study 2- Prolonged Hypoxia

Study 3- Lifelong Hypoxia



Response to change in stimulus PO_2 following haemodilution

↑ PASP Response
 -0.14 ± 0.13 vs. -0.234 ± 0.10 mmHg/mmHg -0.19 ± 0.16 vs. -0.39 ± 0.32 mmHg/mmHg

