Title: GLOBAL REACH 2018: Iron infusion at high altitude reduces hypoxic pulmonary vasoconstriction equally in both lowlanders and healthy Andean highlanders

Alexander Patrician MSc[1], Tony Dawkins PhD[2], Geoff B Coombs PhD[1], Benjamin Stacey PhD[3], Christopher Gasho MD[4], Travis Gibbons MSc[5], Connor A Howe MSc[1], Joshua C Tremblay PhD[1], Rachel Stone BHK[1], Kaitlyn Tymko BKin[1], Courtney Tymko BSN[1], John D Akins PhD[6], Ryan L Hoiland PhD[1], Gustavo A. Vizcardo-Galindo BSc[1,7], Rómulo Figueroa-Mujíca BSc[7], Francisco C. Villafuerte PhD[7], Damian M. Bailey PhD[3], Michael Stembridge PhD[2], James D. Anholm MD[4], Michael M Tymko PhD[1,8], Philip N Ainslie PhD[1].

[1] Centre for Heart, Lung and Vascular Health, School of Health and Exercise Sciences, University of British Columbia – Okanagan, Kelowna, British Columbia, Canada
[2] Cardiff School of Sport and Health Sciences, Cardiff Metropolitan University, Cardiff, UK
[3] Neurovascular Research Laboratory, Faculty of Life Sciences and Education, University of South Wales, Glamorgan, UK
[4] Department of Medicine, Division of Pulmonary and Critical Care, Loma Linda University School of Medicine, Loma Linda, CA, USA
[5] School of Physical Education, Sport & Exercise Science, University of Otago, Dunedin, New Zealand
[6] Department of Kinesiology, University of Texas, Arlington, TX, USA.
[7] Laboratorio de Fisiología Comparada/Fisiología del Transporte de Oxígeno, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Perú
[8] Neurovascular Health Lab, Faculty of Kinesiology, Sport, & Recreation, University of Alberta, Edmonton, Alberta, Canada.

Abstract word count: 285
Text word count: 3071

Corresponding author:
Alexander Patrician, Centre for Heart, Lung and Vascular Health, University of British Columbia, 3333 University Way, Kelowna, BC, V1V 1V7.
Email: alexander.patrician@alumni.ubc.ca

Running title: Iron and the pulmonary vasculature in healthy Andeans

Abstract

Background: Increasing iron bioavailability attenuates hypoxic pulmonary vasoconstriction in both lowlanders and Sherpa at high altitude. In contrast, the pulmonary vasculature of Andeans suffering with chronic mountain sickness is resistant to iron administration. While pulmonary vascular remodeling and hypertension are characteristic features of chronic mountain sickness, the impact of iron administration in healthy Andeans has not been investigated. If the interplay between iron status and pulmonary vascular tone in healthy Andeans remains intact, this could provide valuable clinical insight into the role of iron regulation at high altitude.

Research question: Is the pulmonary vasculature in healthy Andeans responsive to iron infusion?

Study Design and Methods: In a double-blinded, block-randomized design, 24 healthy high-altitude Andeans and 22 partially acclimatized lowlanders at 4300 m (Cerro de Pasco, Peru), received an *i.v.* infusion of either iron [iron (III)-hydroxide sucrose; 200mg] or saline. Markers of iron status were collected at baseline and 4 hours after infusion. Echocardiography was performed during room-air breathing (P_IO_2=~96 mmHg) and during exaggerated hypoxia (P_IO_2=~73 mmHg), at baseline, and at 2 and 4 hours following the infusion.

Results: Iron infusion reduced pulmonary artery systolic pressure (PASP) by ~2.5 mmHg in room air (main effect P<0.001), and by ~7 mmHg during exaggerated hypoxia (main effect P<0.001) in both lowlanders and healthy Andean highlanders. There was no change in PASP following the infusion of saline. Iron metrics were comparable between groups, except for serum ferritin, which was 1.8-fold higher at baseline in the Andeans when compared to lowlanders [95% confidence interval (CI) 74-121 ng/ml vs. 37-70 ng/ml, respectively; P=0.003].

Interpretation: The pulmonary vasculature of healthy Andeans and lowlanders remains sensitive to iron infusion and this response seems to differ from the pathological characteristics of chronic mountain sickness.

Keywords: Andeans, iron, high altitude, pulmonary

Take home points

Study question: Is the pulmonary vasculature in healthy Andeans responsive to iron infusion?

Results: Iron infusion reduced pulmonary artery systolic pressure (PASP) by ~2.5 mmHg in room air (main effect P<0.001), and by ~7 mmHg during exaggerated hypoxia (main effect P<0.001) in both lowlanders and healthy Andean highlanders.

Interpretation: The pulmonary vasculature of healthy Andeans and lowlanders remains sensitive to iron infusion and this response seems to differ from the pathological characteristics of chronic mountain sickness.

Abbreviations:

CI, cardiac index
CMS, chronic mountain sickness
CO, cardiac output
EPO, erythropoietin
Hb, hemoglobin
Hct, hematocrit
HIF, hypoxia inducible factor
F_IO_2, fraction of inspired oxygen
LVOT VTI, left ventricular outflow tract velocity time integral
MAP, mean arterial pressure
NaCl, sodium chloride
PASP, Pulmonary artery systolic pressure
Pb, lead
P_IO_2, partial pressure of inspired oxygen
RAP, Right atrial pressure
SV, stroke volume
SpO_2, peripheral arterial oxygen saturation
TIBC, Total iron binding capacity
TPR, Total pulmonary resistance
TrF Sat, Transferrin saturation

Introduction

With ascent to high altitude, there is a progressive reduction in the partial pressure of oxygen (PO_2) and a progressive decline in iron bioavailability.[1-3] As such, there is a robust inverse relationship between iron status and the pulmonary vasculature in hypoxia (reviewed in [4]). Part of this relationship stems from the integral role of iron on propyl hydroxylase activity[5,6] and contribution to the stabilization of hypoxia inducible factors (HIF). The HIF pathway acts as a cellular oxygen sensor,[7,8] and controls a broad range of transcriptional responses to hypoxic exposure, including a variety of down-stream gene products that can influence hypoxic pulmonary vasoconstriction (e.g. endothelin, vascular endothelial grown factor and transient receptor potential channels).[4,9] Experimentally, iron manipulation has been used as: 1) a tool to attenuate pulmonary vascular responsiveness of lowlanders exposed to acute normobaric hypoxia,[10] and upon ascent and stay at high altitude;[3,11-14] and 2) a method to interrogate HIF-dependent differences associated with clinical pulmonary hypertension[15] and adaptation to high altitude – such as Sherpa of Tibetan descent,[3] and Andeans suffering with chronic mountain sickness (CMS).[14] In both lowlanders and Sherpa, iron infusion attenuates hypoxic pulmonary vasoconstriction at high altitude; however, this does not occur in Andeans with CMS. Andeans with CMS characteristically display marked pulmonary remodeling and hypertension (reviewed in [16]). Therefore, in the study by Smith and colleagues,[14] it is perhaps not surprising that 400 mg of iron(III)-sucrose [200 mg·day^{-1} over 2 days preceded by 4 days of venesection (at 500 ml·day^{-1})], was ineffective at reducing pulmonary artery systolic pressure (PASP), even after 5 days.[14]

While Andeans have had a putatively shorter high-altitude lineage, especially compared to Sherpa/Tibetans,[17] healthy Andeans still demonstrate numerous attributes that enhance their hypoxic tolerance compared to lowlanders including: elevated birth weights, increased exhaled nitric oxide concentrations, larger lungs, improved aerobic capacity and genotypic adaptations[18-22]. With the recently proposed notion that high altitude adaptation may proffer an enhanced state of iron metabolism,[3,23] exploring whether healthy Andeans are receptive to iron would provide insight into the pathological divergence of CMS on the pulmonary vasculature. Therefore, the aim of this study was to examine the role of iron bioavailability on the pulmonary vascular regulation to hypoxia in high-altitude adapted Andeans, with and without, acute reductions in inspired PO_2 (P_IO_2). To address this aim, partially acclimatized lowlanders and healthy Andeans received either an infusion of iron or saline at 4300 m, in a double-blinded and block-randomized design. Our primary hypothesis was that healthy Andeans and lowlanders would demonstrate comparable pulmonary vascular responses to hypoxia, and both groups would be sensitive to iron infusion.

Methods

Ethical Approval

All experimental procedures were approved by the University of British Columbia Research Ethics Board (H17-02687 and H18-01404) and the Universidad Peruana Cayetano Heredia Comité de Ética (no. 101686), and conformed to the Declaration of Helsinki, except for registration in a database. All participants received both written and oral information about the study and provided written informed consent. Forms were translated into Spanish, and Andeans participants spoke with a Peruvian research assistant and provided written informed consent in Spanish before participating.

Participants

A cohort of 24 healthy Andean highlanders and 22 age-matched lowlanders completed the study (Table 1). All were normotensive, and did not report a history of cardiovascular, cerebrovascular, or respiratory diseases during completion of a medical history questionnaire. All Andeans were born at high altitude (n=2 at 1880-2900 m, n=20 at 4300m; as were their parents) and resided at 4300 m, were non-smokers, did not exhibit excessive erythrocytosis (Hb ≤19 g/dl for females and ≤21 g/dl for males) and had a Qinghai CMS questionnaire score of 0.5±0.8 – and thus were not considered to suffer from CMS.[24] Of the few lowlander participants who opted for high altitude prophylaxis (i.e., acetazolamide) upon immediate arrival to 4300 m, all lowlander participants had discontinued use for at least a week, prior to being tested in the current study.

This study was part of the Global Research Expedition on Altitude Related Chronic Health (REACH) to Cerro de Pasco, Peru [4300m; barometric pressure of 457 mmHg; P_IO_2 of ~96 mmHg].[25] Upon arrival in Lima and finalizing logistics, lowlander participants (of which were members of the expedition and sea-level residents of European descent) were driven up to Cerro de Pasco in 8 hours. Lowlanders were tested after 8±4 days at 4300 m – lowlander infusion groups were equally weighted, for number of days at 4300 m – see *Infusion* section.

Experimental Design

Participants arrived at the laboratory having only eaten a small meal ~2 hours prior, and abstained from caffeine and exercise for >6 and >12 hours, respectively. Following 10 min of supine rest an indwelling venous catheter (21G needle, BD Vacutainer eclipse) was inserted into the median antecubital vein for repeated blood sampling and the infusion. Echocardiography (see *Experimental Measures* section) was performed at baseline, and at 2 and 4 hours after receiving an infusion of either iron or saline (see *Infusion* section). Between time-points, participants were allowed to sit up, but all participants resumed supine rest for at least 10 minutes before any measurement. At baseline and at 4 hours, echocardiography was also performed during a hypoxic stage (15 min of F_IO_2=0.16; P_IO_2=73 mmHg; simulating ~1700 m gain in elevation). At 2.5 minutes into the hypoxic stage, automated blood pressure was collected in duplicate using brachial oscillometry. After 10 min of reduced P_IO_2, echocardiographic imaging was repeated, and at the end of the hypoxic stage, peripheral oxygen saturation was recorded.

Infusion: In a double-blinded, block-randomized design, Andean and lowlander participants received an *i.v.* infusion of either iron (iron (III)-hydroxide sucrose; 200 mg in 250 ml 0.9% NaCl saline) or saline (250 ml of 0.9% NaCl saline) over 30 min. Both participants and investigators were blinded during collection (via opaque bags to cover the infusion and lines), and maintained throughout analysis. Due to the >40 day longevity of iron infusion[10] and the logistic constraints of an expedition,[25] a cross-over design was not feasible. Therefore, the purpose of block-randomizing participants was two-fold: 1) enabled us to optimize logistics and coordination with other ongoing studies, and 2) ensured that lowlanders allocated to iron or saline conditions, were appropriately weighted, in terms of the number of days at 4300 m, to limit any potentially confounding influence of hypobaric exposure on iron stores [lowlanders receiving saline and iron were tested after 9±5 and 6±3 days, respectively (unpaired t-test P=0.158)].

Experimental Measures

Echocardiography: Stroke volume (SV) was estimated using the following equation:

$$SV = \left(\pi \cdot \left[\text{aortic diameter}/2\right]^2\right) \cdot \text{LVOT VTI}$$

where the left ventricular outflow tract (LVOT) velocity time integral (VTI) was measured at the point of aortic leaflet insertion during systole using a pulsed wave Doppler signal. Cardiac output (CO) was calculated as the product of stroke volume and heart rate (via electrocardiography). Cardiac index (CI) is the quotient of CO and body surface area, calculated as[26]:

$$\text{Body surface area} = \sqrt{(\text{height} \times \text{weight})/3600}$$

PASP was measured by Doppler echocardiography based upon the measurement of the maximum velocity of the tricuspid regurgitation jet (TR velocity).[27] The peak systolic pressure gradient of the right ventricle (ΔP_{max}) to the right atrium was calculated according to the simplified Bernoulli equation ($4 \cdot V^2$), where V is the peak systolic velocity of the tricuspid regurgitate. PASP was then determined by adding the right atrial pressure (RAP), which was estimated by evaluating inferior vena cava diameter and collapsibility index – in line with the standard American Society of Echocardiography guidelines.[28]

$$\text{PASP} = (4 \cdot V^2) + \text{RAP}$$

PASP was normalized to a hematocrit (Hct) of 45% in all individuals using the following equation:[29]

$$\text{PASP}_{[\text{Hct}]} = \text{PASP}/\exp[2(\varphi - 0.45)]$$

Where φ represents the measured hematocrit level. Total pulmonary resistance (TPR) was calculated as an index of PASP against CO (i.e., PASP/CO), as previously described.[30]

Biochemical analysis: Venous blood samples were separated by microcentrifugation, with serum samples frozen in liquid nitrogen at -196 °C for subsequent analysis. Serum iron, ferritin, transferrin, and erythropoietin (EPO) were analysed according to clinical laboratory standards (Medlab clinical laboratories, Lima, Peru). Total iron binding capacity (TIBC) was calculated as[31]:

$$\text{TIBC} = \text{transferrin} \cdot 1.389$$

Transferrin saturation (TrF Sat) was calculated as:

$$\text{Transferrin saturation} = (\text{serum iron}/\text{TIBC}) \cdot 100$$

Hb and Hct were obtained from whole venous blood sample using microcentrifugation, and analyzed immediately. The Hct at 2 hours, was estimated as the median value between the baseline and 4-hour timepoints.

Statistical analysis

Data were analyzed using a linear mixed effects model with a compound symmetry repeated measure co-variance structure (SPSS v24, IBM Statistics). The fixed factors for the model were ancestry and P_IO_2 (i.e., 96 to 73 mmHg) or time (i.e. pre to post infusion) – with the latter, P_IO_2 or time, being a repeated factor with a compound symmetry repeated covariance structure. Subjects were included as a random effect. When a significant interaction effect (e.g., altitude×ancestry) was detected, Bonferroni adjusted post-hoc tests were utilized to test pairwise comparisons. Comparison of hematological measures at baseline, of pooled lowlanders and Andeans, were assessed with independent samples t-test. The absolute difference between normoxia and hypoxia (Δ), is also presented. A linear regression was used to evaluate the prevalence of decreasing serum iron, across days at altitude. All results are reported as mean±SD and significance was set at $P<0.05$.

Results

Comparison of lowlanders and healthy Andeans at baseline

Iron metrics: At baseline, when all participants were pooled, ferritin was 82% higher in Andeans at baseline, compared to lowlanders (97.3±54.6 ng/ml vs. 53.5±37.1 ng/ml, respectively; P=0.003; Table 1). There were no other differences in markers of iron status (e.g., serum iron, transferrin, TIBC, TrF Sat, or EPO) at baseline between Andeans and lowlanders.

Hypoxic pulmonary vasoconstriction: At baseline (prior to infusion), there was no difference in pulmonary vascular reactivity to hypoxia (P_IO_2=73 mmHg) between lowlanders and Andeans (for both uncorrected and corrected to 45% Hct; Table 2). LV SV slightly decreased during hypoxia in Andeans (75±17 ml at baseline to 72±18 ml during hypoxia; P=0.047); however, both lowlanders and Andeans demonstrated comparable increases in CO, CI, TPR, and MAP during hypoxia.

Effects of intravenous iron

Iron metrics: The iron infusion increased serum iron (by 382±193% and 288±100% in lowlanders and Andeans, respectively; main effect P<0.001), increased serum ferritin (by 18±16% and 14±10% in lowlanders and Andeans, respectively; main effect P<0.001) and decreased transferrin (by 4±7% and 5±6% in lowlanders and Andeans, respectively; main effect P=0.010; Figure 1). Consistent with previous reports in CMS,[14] EPO remained unaltered following iron or placebo in both groups. There were no significant changes in iron metrics during the saline condition.

Time course over 4 hours at high altitude: In lowlanders and Andeans receiving saline, PASP did not significantly change across the 4 hours (main effect of time P=0.262; Table 3a). Following iron infusion, there was a decrease in PASP by 7% and 4% at 2 hours and 11% and 7% at 4 hours in lowlanders and Andeans, respectively (main effect of time P<0.001; Figure 2 and Table 3b). Following iron, both lowlanders and Andeans demonstrated a tendency for an increased MAP (main effect of time P=0.052; Table 3b). Even though there were no change in TPR across the 4 hours in lowlanders and Andeans with iron (Table 3b), TPR was higher in Andeans compared to lowlanders at 4 hours (P=0.034) – this difference is likely driven by the tendency for TPR to decrease across the 4 hours in lowlanders following iron (P=0.051).

Hypoxic pulmonary vasoconstriction: During acute hypoxia (i.e., P_IO_2=73 mmHg), iron infusion attenuated PASP by 4 mmHg, in both Andeans and lowlanders (main effect P=0.01; Table 4b and Figure 3), which coincided with a decrease in TPR (main effect P=0.001) and an increase in LV SV (main effect P=0.031). The rise in PASP during hypoxia was unaltered following the placebo condition in both groups (main effect P=0.326; Table 4a). While the expected decrease in SpO_2 was apparent in the exaggerated hypoxia trial, this reduction was not altered by iron infusion in lowlanders and Andeans; however, the rise in PASP for a given drop in SpO_2 was attenuated by iron infusion (main effect P=0.011; Table 4b).

Discussion

The pulmonary vasculature of adapted Andeans remained responsive to iron infusion – a feature consistent among lowlanders and high altitude Sherpa.[3] Because the pulmonary vasculature of Andeans suffering with CMS does not exhibit this responsiveness to iron,[14] this may provide valuable insight into the pathological divergence of CMS. The following discussion outlines the implications of these findings, including the physiological significance of elevated ferritin levels in the Andeans.

Pulmonary vascular responsiveness to hypoxia and iron

Aligning with our hypothesis, this is the first study to confirm that the pulmonary vasculature of adapted and otherwise healthy Andeans is responsive to iron infusion in hypoxia – a feature akin to the well adapted Sherpa and lowlanders.[3] Such congruent responses across different populations (i.e. acclimatizing lowlanders and high altitude adapted Andeans and Sherpa), highlight a conserved phenotype reflected in an otherwise healthy pulmonary vasculature. Healthy Andeans have demonstrated either slightly higher PASP (at rest and during exercise)[32], or similar PASP (at rest),[33,34] compared to partially acclimatized lowlanders at the same altitude. Therefore, it is perhaps not surprising in the current study that PASP at rest and during further hypoxia was again similar between partially acclimatized lowlanders and healthy Andeans.

The unremarkable influence of a comparable iron infusion on PASP in Andeans with CMS[14] is intriguing. Certainly, CMS has long been established to coincide with pulmonary vascular remodeling and persistence of pulmonary hypertension (during rest and exercise)[32,35-39]. However, CMS has also been linked to the dysfunction of a myriad of HIF-related factors [including sentrin-specific protease 1[40], prevailing circulatory status of vasoactive peptides (i.e. vascular endothelial growth factor and endothelin-1[41]), methylation of propyl hydroxylase activity[42], and/or mutations to the von Hippel-Lindau protein[43]] – these factors could blunt or counteract the efficacy of iron infusion on pulmonary vascular tone. Ultimately, the notable divergence of healthy adapted Andeans from the CMS pulmonary phenotype, hints at some facet of HIF-dysregulation, that may share mechanistic basis with other clinical pathologies.

Iron metabolism – higher ferritin levels in Andeans

Ascent to high altitude is associated with a progressive decline in iron bioavailability,[1-3] which can exaggerate hypoxia-induced HIF stabilization and contribute to pulmonary hypertension.[44] Therefore, in the context of iron regulation, the observed higher ferritin (a stimulus for iron storage) in Andeans could imply a greater state of iron sufficiency and a lower iron/erythropoietic demand, compared to lowlanders. While this feature might have resonance in the interesting dialogue of enhanced iron metabolism in adapted highlanders,[3,23] using ferritin as an index of iron sufficiency is limited, as elevated ferritin levels (e.g., >200-300 ng/ml) are indicative of a myriad of medical concerns [e.g., liver disease, hepatitis, infection, state of inflammation, and hemochromatosis].[45,46] As such, Sherpa do not present with elevated ferritin levels. For example, in the study by Willie and colleagues, serum ferritin of Sherpa was 46.7±37.8 ng/ml[3] – values which more closely resemble our lowlander data at 4300 m, rather than healthy Andeans (Table 1). Coincidingly, similar to lowlanders, Sherpa also did not exhibit any obvious inflammatory profile.[3] Conversely, previous reports of Andeans with CMS (and/or excessive erythrocytosis), show ferritin levels of 169±90 ng/ml (n=11),[14] 184±28 ng/ml (n=42)[47] and 156±132 ng/ml (n=41).[48] However, in the latter two studies, ferritin in healthy Andeans were comparable to Andeans with CMS.[47,48] While it is unclear why the ferritin in our "healthy" Andeans appear substantially lower than the ferritin values in other "healthy" Andeans[47,48], one possibility may be related to CMS score severity (0.5±0.8 in this study; 2.7±0.38 in[47]; no score reported in[48]). Future studies are needed to consider how and why CMS severity might influence ferritin levels, or why ferritin level can be so variable between otherwise healthy individuals.

The factors potentially mediating these elevations in ferritin, in both healthy and CMS Andeans compared to lowlanders, may have some basis when considering the city of Cerro de Pasco itself. First, it has been demonstrated that more than half of the soil sample sites evaluated in Cerro de Pasco, had lead (Pb) levels exceeding 1200mg/kg,[49] which is ~10-fold higher the health recommendations in Canada [140 mg/kg[50]]. Recently, and not surprisingly, whole blood Pb concentrations in residents of Cerro de Pasco (with Hb 10-25 g/dl) were >2-fold greater than Pb concentrations in sea-level residents of Lima [4.75±1.53 ug/dl, range: 1.5-9.8 ug/dl versus 2.03±0.62 ug/dl, range: 1.0-4.6 ug/dl, respectively[51]]. While Pb toxicity may not be prevalent in our cohort [due to the lack of iron deficiency and anemia (reviewed in: [52])], consequence(s) of Pb exposure on upstream inflammatory factors[53] and ferroportin activity,[54] have been demonstrated in animal and cell-preparation models. Whether Pb exposure could extend beyond iron deficiency/anemia and potentially contribute to the elevated ferritin levels in Andeans, compared lowlanders, remains to be determined. Second, the city of Cerro de Pasco starkly contrasts those of small towns/villages in the Himalayan highlands, in terms of lifestyle (e.g. diet, physical activity) and level of industrialization (e.g. drive-in and mining versus hike-in and agriculture/tourism, respectively). As such, comparisons between populations are certainly multifaceted and future studies are needed to consider the potential detrimental influence of even mildly elevated Pb levels on inflammation[53,55] and explore the mechanistic links to contributing to the elevated ferritin levels in Andeans

Importantly, our findings that iron attenuated the rise in PASP during hypoxia, with a coinciding increase in left ventricular SV and decrease in TPR (Table 4), is broadly consistent with the recent study by Holdsworth and colleagues.[13] Here, the authors reported that increasing iron bioavailability (via infusion of 1g of ferric carboxymaltose) in lowlanders, *prior* to ascent to high altitude, translated into enhanced right ventricular SV and lower pulmonary vascular resistance at 5050 m.

Considerations of iron status during prolonged stay at high altitude

In our lowlander cohort, a pattern of decreasing serum iron levels appeared to develop over the 17 days at 4300 m (r^2=0.204, P=0.035; Figure 4) – a reproducible feature of high-altitude sojourn.[1-3] Even though our cohort of Andeans did not travel and resided at 4300 m, our groups were also appropriately weighted for serum iron (unpaired t-test P=0.522; Figure 4). Our study appropriately weighted lowlanders to saline and iron conditions, so the confounding influence of time at altitude would be less. However, the progressive reduction in iron with stay at altitude, highlights the need to be cognisant of such changes in iron status, if the outcome variable of interest hinges or relies upon downstream iron-regulatory processes.

Interpretation

Unlike Andeans with CMS,[14] yet aligning with Sherpa,[3] the pulmonary vasculature of adapted Andeans remained responsive to iron infusion. Therefore, these findings highlight a feature of healthy pulmonary vascular regulation, and a trait of high-altitude adaptation that remains divergent from the typical pathological characteristics of high-altitude maladaptation (i.e., CMS).

Acknowledgements

This study was conducted within the framework of Global REACH 2018. The authors are grateful to the expedition team members and collaborators in Cerro de Pasco for their support in the weeks

leading up to, and during data collection in Peru. We would also like to thank the Andean residents of Cerro de Pasco for their participation.

Author contributions

All authors were involved in data collection. AP and PNA were involved in data analyses and interpretation. AP drafted the manuscript. All authors provided intellectual feedback, approved the final version of this manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Financial/nonfinancial disclosures

None to declare

Tables

Table 1. Baseline summary of pooled lowlanders and pooled Andeans.

	Lowlander	Andean	p-value
Sex (males/females)	16 / 6	21 / 3	
Age (years)	29 ± 7	29 ± 11	0.898
Height (cm)	175 ± 8	162 ± 5	**<0.001**
Weight (kg)	71 ± 10	63 ± 7	**<0.001**
BMI (kg/m^2)	23.3 ± 2.3	23.8 ± 2.8	0.530
Hb (g/dl)	16.2 ± 1.4 [17]	16.9 ± 1.9 [14]	0.235
Hct (%)	48.4 ± 3.9. [20]	53.2 ± 4.1 [18]	**0.001**
EPO (mIU/ml)	17.3 ± 9.5	14.1 ± 8.9	0.257
Serum Ferritin (ng/ml)	53.5 ± 37.1	97.3 ± 54.6 [23]	**0.003**
Serum Iron (ug/dL)	81.8 ± 40.9	94.2 ± 29.7 [23]	0.250
Serum Transferrin (mg/dl)	275.4 ± 24.4	268 ± 28.3 [23]	0.353
TIBC (ug/dl)	382.5 ± 33.9	372.2 ± 39.3 [23]	0.353
TrF Sat (%)	21.9 ± 11.5	25.3 ± 7.6 [23]	0.240
SpO_2 (%)	89 ± 3	88 ± 3	0.528
PASP (mmHg)	31.0 ± 6.4 [21]	29.5 ± 5.9 [23]	0.403
$PASP_{[Hct]}$ (mmHg)	29.5 ± 7.3 [19]	25.4 ± 5.8 [17]	0.072

Mean ± SD in 22 Lowlanders and 24 Andeans (unless otherwise noted [n]). Independent samples t-test.

Table 2. Cardiopulmonary measures in pooled lowlanders and Andeans (prior to infusion) during room air breathing and reduced P_IO_2.

		Room air	Hypoxia	Ancestry	P_IO_2	Inter
PASP (mmHg)	Lowlander	31.0 ± 6.4 [21]	40.2 ± 8.4 [21]	0.866	<**0.001**	0.343
	Andean	29.5 ± 5.9 [23]	40.9 ± 12.9 [23]			
$PASP_{[hct]}$ (mmHg)	Lowlander	29.5 ± 7.3 [19]	38.4 ± 10.7 [19]	0.242	<**0.001**	0.586
	Andean	25.4 ± 5.8 [17]	35.7 ± 12.3 [17]			
TPR (mmHg/l)	Lowlander	6.4 ± 1.5 [21]	7.0 ± 1.8 [21]	0.401	<**0.001**	0.120
	Andean	6.5 ± 1.6 [23]	7.7 ± 2.3 [23]			
LV SV (ml)	Lowlander	83 ± 18	86 ± 19	**0.041**	0.931	**0.009**
	Andean	75 ± 17	72 ± 18*†			
HR (beats/min)	Lowlander	62 ± 12	71 ± 15	0.320	<**0.001**	0.061
	Andean	63 ± 9	77 ± 12			
CO (l/min)	Lowlander	5.0 ± 1.0	6.0 ± 1.3	0.173	<**0.001**	0.167
	Andean	4.7 ± 0.9	5.4 ± 1.2			
CI (ml/min/m^2)	Lowlander	2.7 ± 0.5	3.2 ± 0.7	0.751	<**0.001**	0.349
	Andean	2.8 ± 0.5	3.2 ± 0.7			
MAP (mmHg)	Lowlander	90 ± 8	94 ± 10	<**0.001**	**0.001**	0.813
	Andean	78 ± 8	82 ± 10			
SpO_2 (%)	Lowlander	89 ± 3	72 ± 7	0.916	<**0.001**	0.506
	Andean	88 ± 3	73 ± 10 [23]			

Mean ± SD in 22 Lowlanders and 24 Andeans (unless otherwise noted [n]). Linear mixed model. * Significant difference from baseline, P<0.05; † Significant difference between Andeans and Lowlanders, P<0.05.

408

Table 3a. Resting cardiopulmonary measures at baseline and following the infusion of saline at 4300 m

		Baseline	2 hours	4 hours	Ancestry	Time	Inter
PASP (mmHg)	Lowlander	32.1 ± 7.5	31.5 ± 6.2	31.1 ± 5.8	0.366	0.262	0.751
	Andean	29.7 ± 6.4 [11]	29.6 ± 7.8 [11]	27.8 ± 8.1 [11]			
$PASP_{[hct]}$ (mmHg)	Lowlander	30.5 ± 9.0	29.7 ± 7.7	29.3 ± 8.4	0.094	0.174	0.549
	Andean	24.8 ± 6.3 [11]	25.5 ± 6.1 [9]	23.1 ± 7 [9]			
TPR (mmHg/l)	Lowlander	6.1 ± 1.4	6 ± 1.2	5.8 ± 1.1	0.739	0.307	0.594
	Andean	6.1 ± 1.5 [11]	6.5 ± 1.7 [11]	5.5 ± 2.4			
HR (beats/min)	Lowlander	60 ± 10	59 ± 13	57 ± 13	0.592	0.067	0.648
	Andean	63 ± 10	59 ± 9	60 ± 9			
LV SV (ml)	Lowlander	90 ± 17	92 ± 11	98 ± 22	**0.021**	0.129	0.099
	Andean	80 ± 16	78 ± 9	79 ± 11			
CO (l/min)	Lowlander	5.3 ± 1.0	5.3 ± 1	5.4 ± 1.3	0.079	0.430	0.278
	Andean	5.0 ± 0.8	4.6 ± 0.7	4.7 ± 0.5			
CI (l/min/m^2)	Lowlander	2.8 ± 0.6	2.8 ± 0.6	2.9 ± 0.7	0.855	0.398	0.227
	Andean	2.9 ± 0.6	2.7 ± 0.5	2.7 ± 0.4			
MAP (mmHg)	Lowlander	92 ± 8	90 ± 7	91 ± 10	**0.027**	0.914	0.305
	Andean	81 ± 8	84 ± 11	83 ± 14			
SpO_2 (%)	Lowlander	89 ± 3	89 ± 3	88 ± 4	0.607	0.557	0.806
	Andean	88 ± 2	88 ± 3	88 ± 2			

Mean ± SD in 11 lowlanders and 12 Andeans (unless otherwise noted [n]). P_IO_2=96mmHg. Significant p-values are denoted in bold. Linear mixed model.

409

410

411

Table 3b. Resting cardiopulmonary measures at baseline and following the infusion of iron at 4300 m

		Baseline	2 hours	4 hours	Ancestry	Time	Inter
PASP (mmHg)	Lowlander	29.8 ± 5.1 [10]	27.7 ± 5.6 [10]	26.6 ± 6.3 [10]	0.967	**<0.001**	0.587
	Andean	29.3 ± 5.6	28.1 ± 7.2	27.2 ± 6.9			
$PASP_{[hct]}$ (mmHg)	Lowlander	28.2 ± 4.3 [8]	26.5 ± 4.8 [8]	25.1 ± 5.5 [10]	0.183	**0.001**	0.485
	Andean	26.5 ± 5.2 [6]	26.6 ± 6.9 [4]	21.3 ± 4.3 [8]			
TPR (mmHg/l)	Lowlander	6.7 ± 1.6 [10]	6.4 ± 1.8 [10]	5.3 ± 2.6†	0.190	0.867	0.008
	Andean	6.8 ± 1.7	7 ± 2	7.5 ± 2.9			
HR (beats/min)	Lowlander	63 ± 14	63 ± 20	64 ± 16	0.483	0.098	0.125
	Andean	63 ± 7	58 ± 8	58 ± 8			
LV SV (ml)	Lowlander	76 ± 17	75 ± 15	77 ± 19	0.439	0.764	0.181
	Andean	71 ± 18	73 ± 18	68 ± 19			
CO (l/min)	Lowlander	4.7 ± 0.9	4.5 ± 1	4.7 ± 0.9	0.262	0.277	0.135
	Andean	4.4 ± 0.9	4.2 ± 1.2	3.9 ± 1.3			
CI (l/min/m^2)	Lowlander	2.6 ± 0.4	2.5 ± 0.5	2.6 ± 0.5	0.854	0.256	0.096
	Andean	2.7 ± 0.5	2.5 ± 0.6	2.4 ± 0.7			
MAP (mmHg)	Lowlander	89 ± 8	89 ± 10	91 ± 7	**0.001**	0.052	0.647
	Andean	75 ± 6	76 ± 8	79 ± 11			
SpO_2 (%)	Lowlander	88 ± 3	88 ± 2	88 ± 3 [10]	0.444	0.160	0.315
	Andean	88 ± 4	87 ± 3	87 ± 3			

Mean ± SD in 11 lowlanders and 12 Andeans (unless otherwise noted [n]). P_IO_2=96mmHg. Significant p-values are denoted in bold. Linear mixed model.

412
413
414

Table 4a. Cardiopulmonary reactivities to hypoxia, prior-to and following the infusion of saline.

		Pre-saline	Post-saline	Ancestry	Time	Inter
ΔSpO_2 (%)	Lowlander	-14.9 ± 6.9	-15.5 ± 6.7	0.949	0.899	0.610
	Andean	-15.8 ± 9.1	-14.9 ± 8			
ΔPASP (mmHg)	Lowlander	9 ± 5.3	10.6 ± 5.6	0.995	0.326	0.989
	Andean	9.1 ± 5.7	10.6 ± 8.5			
$\Delta PASP/\Delta SpO_2$ (mmHg/%)	Lowlander	-0.6 ± 0.4	-0.9 ± 0.9	0.565	0.070	0.691
	Andean	-0.5 ± 0.7 [10]	-0.9 ± 0.5			
ΔPASP[hct] (mmHg)	Lowlander	8.7 ± 5.8	10.1 ± 5.9	0.654	0.258	0.854
	Andean	7.5 ± 4.9	9.8 ± 6.4 [9]			
$\Delta PASP[hct]/\Delta SpO_2$ (mmHg/%)	Lowlander	-0.6 ± 0.4	-0.9 ± 0.8	0.427	**0.045**	0.477
	Andean	-0.3 ± 0.6 [9]	-0.8 ± 0.3 [9]			
ΔTPR (mmHg/l)	Lowlander	0.4 ± 0.8	1.0 ± 1.9	0.420	0.137	0.922
	Andean	0.9 ± 1.3	1.4 ± 2			
ΔHR (beats/min)	Lowlander	10.0 ± 9.5	9.6 ± 7.2	0.091	0.702	0.849
	Andean	15.0 ± 4.8	13.9 ± 8.6			
ΔSV (ml)	Lowlander	3.6 ± 8.1	1.5 ± 7.5	**0.004**	0.433	0.651
	Andean	-6.2 ± 8.8	-6.8 ± 6.9			
ΔCO (ml/min)	Lowlander	1.1 ± 0.6	1.1 ± 0.9	0.055	0.665	0.591
	Andean	0.7 ± 0.6	0.6 ± 0.4			
ΔCI (ml/min/m^2)	Lowlander	0.6 ± 0.4	0.6 ± 0.5	0.123	0.624	0.657
	Andean	0.4 ± 0.3	0.3 ± 0.3			
ΔMAP (mmHg)	Lowlander	5.1 ± 6.6	5.9 ± 5.5	0.495	0.760	0.888
	Andean	3.9 ± 8.1	4.2 ± 5.1			

Mean ± SD. N=10-11 and n=11-12 for lowlanders and Andeans respectively, unless otherwise noted [n]. Δ, the absolute change between normoxia and hypoxia.

Table 4b. Cardiopulmonary reactivities to hypoxia, prior-to and following the infusion of iron.

		Pre-iron	Post-iron	Ancestry	Time	Inter.
ΔSpO_2 (%)	Lowlander	-18.4 ± 3.3	-18 ± 4.2	0.238	0.966	0.749
	Andean	-14.8 ± 7.7	-15.2 ± 9.8			
ΔPASP (mmHg)	Lowlander	9.2 ± 3.9	6.8 ± 3.1	0.451	**0.010**	0.219
	Andean	13.6 ± 12.9	7.4 ± 8.4			
$\Delta PASP/\Delta SpO_2$ (mmHg/%)	Lowlander	-0.5 ± 0.2	-0.4 ± 0.2 [9]	0.979	**0.011**	0.051
	Andean	-0.9 ± 0.7	-0.2 ± 0.9			
$\Delta PASP_{[Hct]}$ (mmHg)	Lowlander	9 ± 4.4 [8]	6.6 ± 3.1 [10]	0.556	0.075	0.300
	Andean	15.5 ± 16.6 [5]	7.7 ± 10 [6]			
$\Delta PASP_{[Hct]}/\Delta SpO_2$ (mmHg/%)	Lowlander	-0.51 ± 0.25 [8]	-0.4 ± 0.23 [9]	0.509	0.057	0.176
	Andean	-0.85 ± 0.7 [5]	-0.001 ± 1.04 [6]			
ΔTPR (mmHg/l)	Lowlander	0.7 ± 1.3	0.02 ± 1.4	0.62	**0.001**	0.16
	Andean	1.5 ± 1.9	-0.1 ± 2.1			
ΔHR (beats/min)	Lowlander	9.1 ± 5.9	6.9 ± 10.9	0.256	0.303	0.671
	Andean	11.9 ± 6.8	11 ± 7.2			
ΔSV (ml)	Lowlander	2.8 ± 5.6	7.3 ± 8.3	0.234	**0.031**	0.729
	Andean	-0.6 ± 9.3	2.7 ± 11			
ΔCO (l/min)	Lowlander	0.9 ± 0.5	1.1 ± 0.6	0.505	0.264	0.929
	Andean	0.8 ± 0.7	0.9 ± 0.7			
ΔCI (ml/min/m^2)	Lowlander	0.5 ± 0.3	0.6 ± 0.3	0.809	0.244	0.997
	Andean	0.5 ± 0.4	0.6 ± 0.4			
ΔMAP (mmHg)	Lowlander	2.6 ± 9.3	1.1 ± 5.9	0.718	0.145	0.468
	Andean	4.9 ± 6.9	0.5 ± 6.1			

Mean ± SD. N=10-11 and n=11-12 for lowlanders and Andeans respectively, unless otherwise noted [n]. Δ, the absolute change between normoxia and hypoxia.

Figures

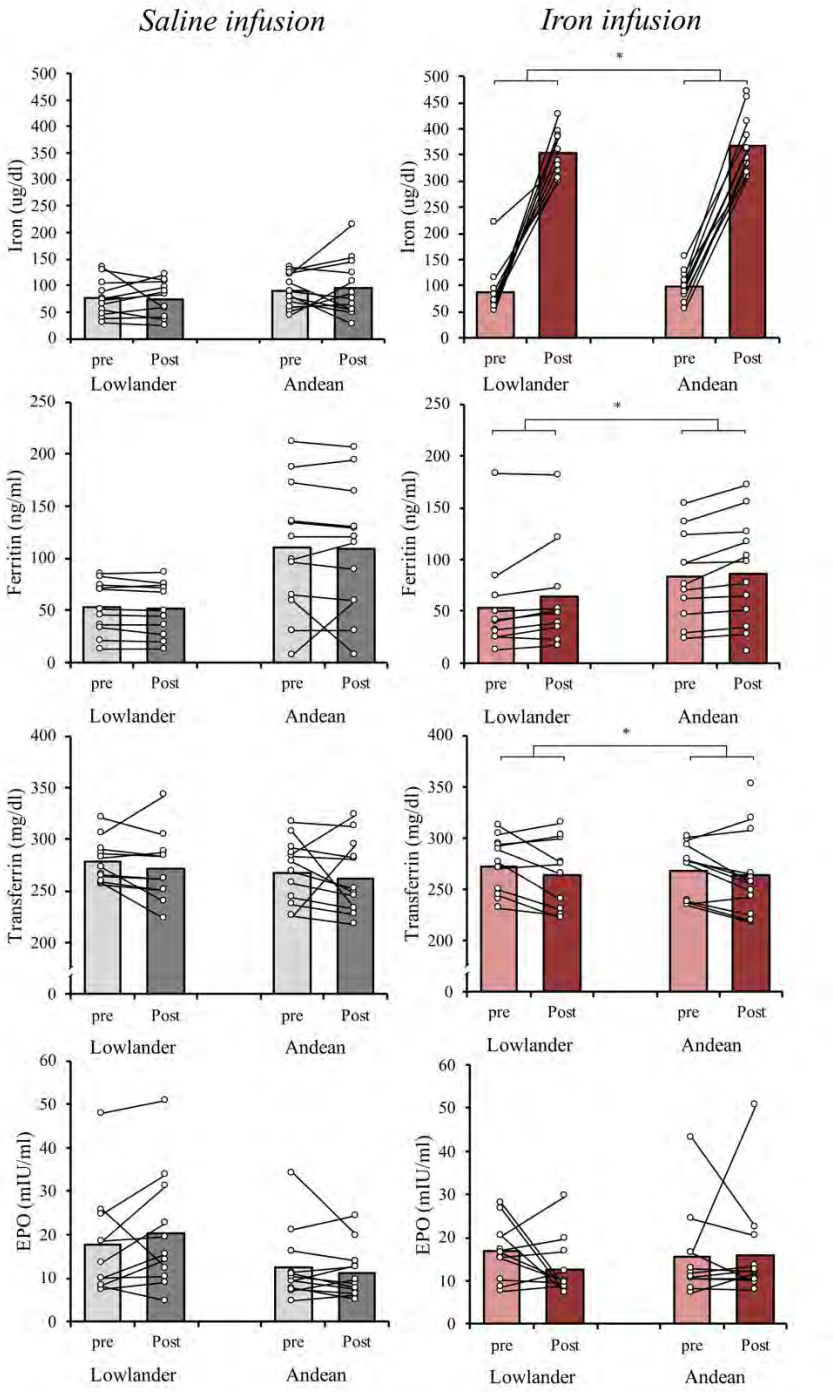

Figure 1. Impact of saline (grey bars) or iron infusion (red bars) on iron metrics in lowlanders and healthy Andeans. *P<0.05 main effect of time.

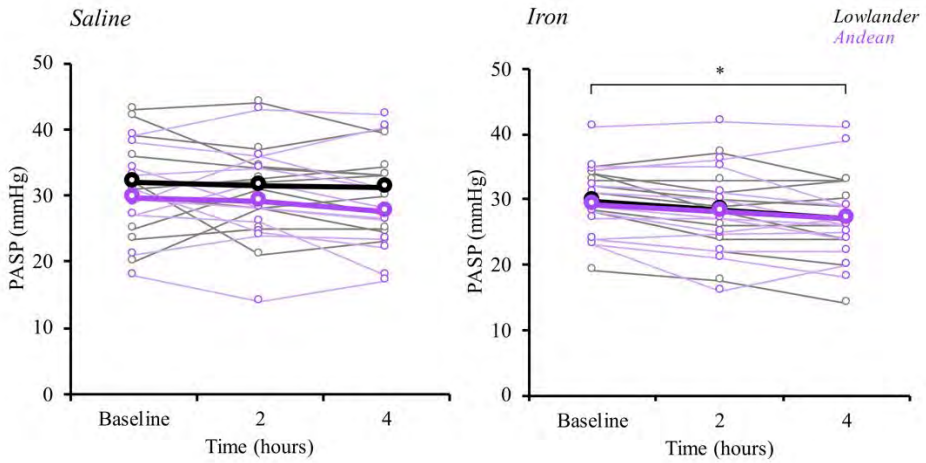

Figure 2. Impact of saline and iron infusion on the PASP in lowlanders and healthy Andeans. Horizonal bar with asterisk denotes a main effect significance of time (*P<0.05).

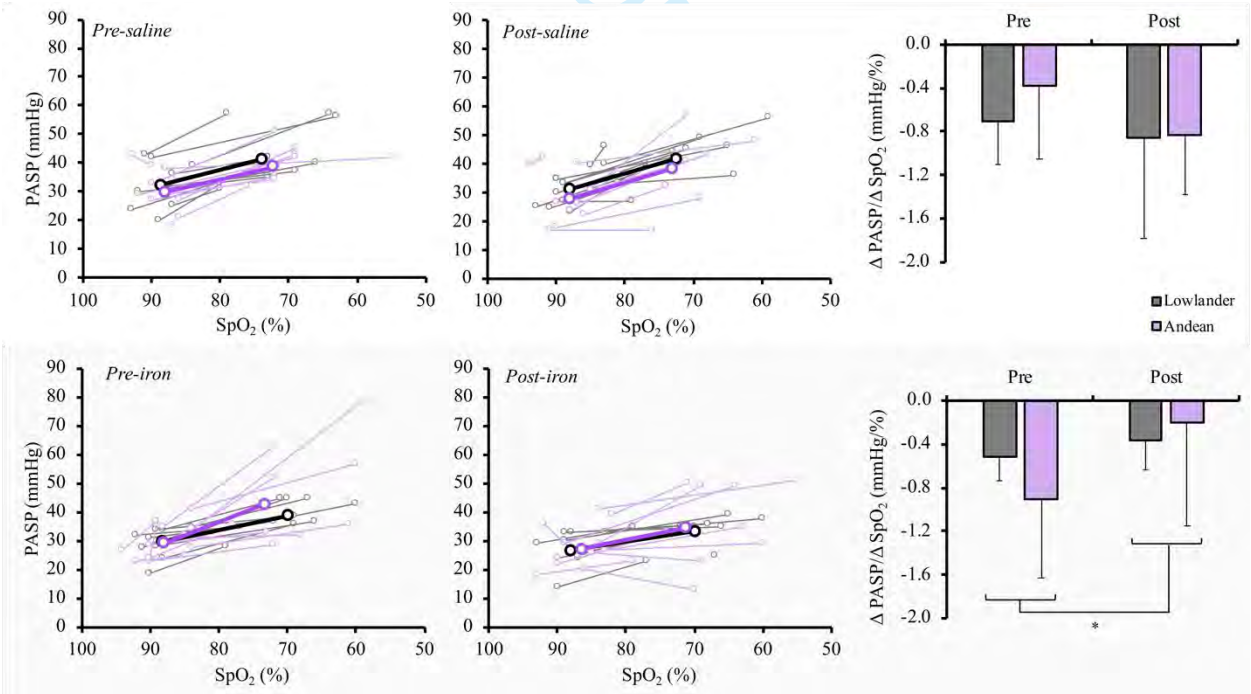

Figure 3. Pulmonary vascular reactivity to hypoxia in lowlanders and Andeans receiving either saline or iron infusion. * main effect P<0.05.

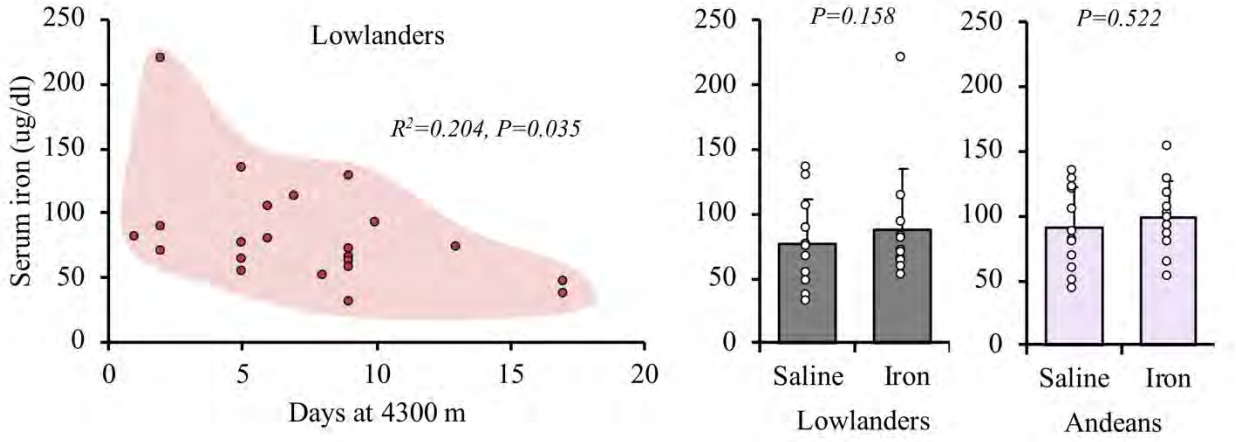

Figure 4. Baseline serum iron measurements in lowlanders and Andeans. Lowlander data is presented as 1) to correspond to the number of days at 4300 m, before being tested (with linear regression; *left panel*) and 2) highlight the equal weighting of iron status between the saline and iron groups (with unpaired t-test; *middle panel*). Andean data demonstrates iron levels in the saline and iron groups at baseline were comparable (with unpaired t-test; *right panel*).

References

1. Robach P, Cairo G, Gelfi C, et al. Strong iron demand during hypoxia-induced erythropoiesis is associated with down-regulation of iron-related proteins and myoglobin in human skeletal muscle. *Blood.* 2007;109(11):4724-4731.
2. Gassmann M, Muckenthaler MU. Adaptation of iron requirement to hypoxic conditions at high altitude. *J Appl Physiol (1985).* 2015;119(12):1432-1440.
3. Willie CK, Patrician A, Hoiland RL, et al. Influence of iron manipulation on hypoxic pulmonary vasoconstriction and pulmonary reactivity during ascent and acclimatization to 5050 m. *J Physiol.* 2021;599(5):1685-1708.
4. Frise MC, Robbins PA. Iron, oxygen, and the pulmonary circulation. *J Appl Physiol (1985).* 2015;119(12):1421-1431.
5. Kaelin WG, Ratcliffe PJ. Oxygen Sensing by Metazoans: The Central Role of the HIF Hydroxylase Pathway. *Molecular Cell.* 2008;30(4):393-402.
6. Knowles HJ, Raval RR, Harris AL, Ratcliffe PJ. Effect of ascorbate on the activity of hypoxia-inducible factor in cancer cells. *Cancer Res.* 2003;63(8):1764-1768.
7. Semenza GL, Agani F, Iyer N, et al. Hypoxia-inducible factor 1: from molecular biology to cardiopulmonary physiology. *Chest.* 1998;114(1 Suppl):40s-45s.
8. Prabhakar NR, Semenza GL. Oxygen Sensing and Homeostasis. *Physiology (Bethesda).* 2015;30(5):340-348.
9. Sylvester JT, Shimoda LA, Aaronson PI, Ward JP. Hypoxic pulmonary vasoconstriction. *Physiol Rev.* 2012;92(1):367-520.

10. Bart NK, Curtis MK, Cheng HY, et al. Elevation of iron storage in humans attenuates the pulmonary vascular response to hypoxia. *J Appl Physiol (1985).* 2016;121(2):537-544.
11. Smith TG, Balanos GM, Croft QP, et al. The increase in pulmonary arterial pressure caused by hypoxia depends on iron status. *J Physiol.* 2008;586(24):5999-6005.
12. Talbot NP, Smith TG, Privat C, et al. Intravenous Iron Supplementation May Protect Against Acute Mountain Sickness: A Randomized, Double-Blinded, Placebo-Controlled Trial. *High Alt. Med. Biol.* 2011;12(3):265-269.
13. Holdsworth DA, Frise MC, Bakker-Dyos J, et al. Iron bioavailability and cardiopulmonary function during ascent to very high altitude. *Eur Respir J.* 2020;56(3).
14. Smith TG, Talbot NP, Privat C, et al. Effects of iron supplementation and depletion on hypoxic pulmonary hypertension: two randomized controlled trials. *Jama.* 2009;302(13):1444-1450.
15. Balanos GM, Dorrington KL, Robbins PA. Desferrioxamine elevates pulmonary vascular resistance in humans: potential for involvement of HIF-1. *J Appl Physiol (1985).* 2002;92(6):2501-2507.
16. Penaloza D, Arias-Stella J. The heart and pulmonary circulation at high altitudes: healthy highlanders and chronic mountain sickness. *Circulation.* 2007;115(9):1132-1146.
17. Aldenderfer MS. Moving Up in the World: Archaeologists seek to understand how and when people came to occupy the Andean and Tibetan plateaus. *Am Sci.* 2003;91(6):542-549.
18. Moore LG, Shriver M, Bemis L, et al. Maternal adaptation to high-altitude pregnancy: an experiment of nature--a review. *Placenta.* 2004;25 Suppl A:S60-71.
19. Beall CM, Laskowski D, Strohl KP, et al. Pulmonary nitric oxide in mountain dwellers. *Nature.* 2001;414(6862):411-412.
20. Lenfant C, Sullivan K. Adaptation to high altitude. *N Engl J Med.* 1971;284(23):1298-1309.
21. Frisancho AR. Developmental functional adaptation to high altitude: review. *Am J Hum Biol.* 2013;25(2):151-168.
22. Storz JF. High-Altitude Adaptation: Mechanistic Insights from Integrated Genomics and Physiology. *Mol Biol Evol.* 2021;msab064.
23. Muckenthaler MU, Mairbäurl H, Gassmann M. Iron metabolism in high altitude residents. *J Appl Physiol (1985).* 2020;129(4):920-925.
24. León-Velarde F, Maggiorini M, Reeves JT, et al. Consensus statement on chronic and subacute high altitude diseases. *High Alt Med Biol.* 2005;6(2):147-157.
25. Tymko MM, Hoiland RL, Tremblay JC, et al. The 2018 Global Research Expedition on Altitude Related Chronic Health (Global REACH) to Cerro de Pasco, Peru: an Experimental Overview. *Exp Physiol.* 2020.
26. Mosteller RD. Simplified calculation of body-surface area. *N Engl J Med.* 1987;317(17):1098.
27. Bertini M, Sengupta PP, Nucifora G, et al. Role of left ventricular twist mechanics in the assessment of cardiac dyssynchrony in heart failure. *JACC Cardiovasc Imaging.* 2009;2(12):1425-1435.
28. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of

Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging.* 2015;16(3):233-270.

29. Vanderpool RR, Naeije R. Hematocrit-corrected Pulmonary Vascular Resistance. *Am J Respir Crit Care Med.* 2018;198(3):305-309.
30. Foster GE, Ainslie PN, Stembridge M, et al. Resting pulmonary haemodynamics and shunting: a comparison of sea-level inhabitants to high altitude Sherpas. *J Physiol.* 2014;592(6):1397-1409.
31. Auerbach M, Adamson JW. How we diagnose and treat iron deficiency anemia. *Am J Hematol.* 2016;91(1):31-38.
32. Groepenhoff H, Overbeek MJ, Mulè M, et al. Exercise pathophysiology in patients with chronic mountain sickness exercise in chronic mountain sickness. *Chest.* 2012;142(4):877-884.
33. de Bisschop C, Kiger L, Marden MC, et al. Pulmonary capillary blood volume and membrane conductance in Andeans and lowlanders at high altitude: a cross-sectional study. *Nitric Oxide.* 2010;23(3):187-193.
34. Schwab M, Jayet PY, Stuber T, et al. Pulmonary-artery pressure and exhaled nitric oxide in Bolivian and Caucasian high altitude dwellers. *High Alt Med Biol.* 2008;9(4):295-299.
35. Peñaloza D, Sime F, Banchero N, Gamboa R, Cruz J, Marticorena E. Pulmonary hypertension in healthy men born and living at high altitudes. *Am J Cardiol.* 1963;11(2):150-157.
36. Rotta A, Cánepa A, Hurtado A, Velásquez T, Chávez R. Pulmonary circulation at sea level and at high altitudes. *Journal of Applied Physiology.* 1956;9(3):328-336.
37. Arias-Stella J, Saldana M. The terminal portion of the pulmonary arterial tree in people native to high altitudes. *Circulation.* 1963;28:915-925.
38. Lockhart A, Zelter M, Mensch-Dechene J, et al. Pressure-flow-volume relationships in pulmonary circulation of normal highlanders. *J Appl Physiol.* 1976;41(4):449-456.
39. Grover R. Chronic hypoxic pulmonary hypertension. In: Fishman AP, ed. *The pulmonary circulation: normal and abnormal*. Philadelphia: University of Pennsylvania Press; 1990:283-299.
40. Villafuerte FC. New genetic and physiological factors for excessive erythrocytosis and Chronic Mountain Sickness. *J Appl Physiol (1985).* 2015;119(12):1481-1486.
41. Ge RL, Mo VY, Januzzi JL, et al. B-type natriuretic peptide, vascular endothelial growth factor, endothelin-1, and nitric oxide synthase in chronic mountain sickness. *Am J Physiol Heart Circ Physiol.* 2011;300(4):H1427-1433.
42. Julian CG. Epigenomics and human adaptation to high altitude. *J Appl Physiol (1985).* 2017;123(5):1362-1370.
43. Ang SO, Chen H, Hirota K, et al. Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. *Nat Genet.* 2002;32(4):614-621.
44. Frise MC, Cheng HY, Nickol AH, et al. Clinical iron deficiency disturbs normal human responses to hypoxia. *J Clin Invest.* 2016;126(6):2139-2150.
45. Wallace DF, Subramaniam VN. Co-factors in liver disease: the role of HFE-related hereditary hemochromatosis and iron. *Biochim Biophys Acta Gen Subj.* 2009;1790(7):663-670.

46. Wessling-Resnick M. Iron homeostasis and the inflammatory response. *Annu Rev Nutr.* 2010;30:105-122.
47. Villafuerte FC, Macarlupú JL, Anza-Ramírez C, et al. Decreased plasma soluble erythropoietin receptor in high-altitude excessive erythrocytosis and Chronic Mountain Sickness. *J Appl Physiol (1985).* 2014;117(11):1356-1362.
48. De Ferrari A, Miranda JJ, Gilman RH, et al. Prevalence, clinical profile, iron status, and subject-specific traits for excessive erythrocytosis in Andean adults living permanently at 3,825 meters above sea level. *Chest.* 2014;146(5):1327-1336.
49. Van Geen A, Bravo C, Gil V, Sherpa S, Jack D. Lead exposure from soil in Peruvian mining towns: a national assessment supported by two contrasting examples. *Bull World Health Organ.* 2012;90:878-886.
50. Health Canada. Final Human Health State of the Science Report on Lead. Ottawa: Minister of Health; 2013.
51. Childebayeva A, Goodrich JM, Chesterman N, et al. Blood lead levels in Peruvian adults are associated with proximity to mining and DNA methylation. *Environ Int.* 2021;155:106587.
52. Kwong WT, Friello P, Semba RD. Interactions between iron deficiency and lead poisoning: epidemiology and pathogenesis. *Sci Total Environ.* 2004;330(1-3):21-37.
53. Hosseinirad H, Shahrestanaki JK, Moosazadeh Moghaddam M, Mousazadeh A, Yadegari P, Afsharzadeh N. Protective Effect of Vitamin D3 Against Pb-Induced Neurotoxicity by Regulating the Nrf2 and NF-κB Pathways. *Neurotox Res.* 2021;39(3):687-696.
54. Harada N, Kanayama M, Maruyama A, et al. Nrf2 regulates ferroportin 1-mediated iron efflux and counteracts lipopolysaccharide-induced ferroportin 1 mRNA suppression in macrophages. *Arch Biochem Biophys.* 2011;508(1):101-109.
55. Brewster LM, Bain AR, Garcia VP, et al. Global REACH 2018: dysfunctional extracellular microvesicles in Andean highlander males with excessive erythrocytosis. *Am J Physiol Heart Circ Physiol.* 2021;320(5):H1851-h1861.

Title: GLOBAL REACH 2018: Iron infusion at high altitude reduces hypoxic pulmonary vasoconstriction equally in both lowlanders and healthy Andean highlanders

Alexander Patrician MSc[1], Tony Dawkins PhD[2], Geoff B Coombs PhD[1], Benjamin Stacey PhD[3], Christopher Gasho MD[4], Travis Gibbons MSc[5], Connor A Howe MSc[1], Joshua C Tremblay PhD[1], Rachel Stone BHK[1], Kaitlyn Tymko BKin[1], Courtney Tymko BSN[1], John D Akins PhD[6], Ryan L Hoiland PhD[1], Gustavo A. Vizcardo-Galindo BSc[1,7], Rómulo Figueroa-Mujíca BSc[7], Francisco C. Villafuerte PhD[7], Damian M. Bailey PhD[3], Michael Stembridge PhD[2], James D. Anholm MD[4], Michael M Tymko PhD[1,8], Philip N Ainslie PhD[1].

[1] Centre for Heart, Lung and Vascular Health, School of Health and Exercise Sciences, University of British Columbia – Okanagan, Kelowna, British Columbia, Canada
[2] Cardiff School of Sport and Health Sciences, Cardiff Metropolitan University, Cardiff, UK
[3] Neurovascular Research Laboratory, Faculty of Life Sciences and Education, University of South Wales, Glamorgan, UK
[4] Department of Medicine, Division of Pulmonary and Critical Care, Loma Linda University School of Medicine, Loma Linda, CA, USA
[5] School of Physical Education, Sport & Exercise Science, University of Otago, Dunedin, New Zealand
[6] Department of Kinesiology, University of Texas, Arlington, TX, USA.
[7] Laboratorio de Fisiología Comparada/Fisiología del Transporte de Oxígeno, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Perú
[8] Neurovascular Health Lab, Faculty of Kinesiology, Sport, & Recreation, University of Alberta, Edmonton, Alberta, Canada.

Abstract word count: 285
Text word count: 3071

Corresponding author:
Alexander Patrician, Centre for Heart, Lung and Vascular Health, University of British Columbia, 3333 University Way, Kelowna, BC, V1V 1V7.
Email: alexander.patrician@alumni.ubc.ca

Running title: Iron and the pulmonary vasculature in healthy Andeans

Abstract

Background: Increasing iron bioavailability attenuates hypoxic pulmonary vasoconstriction in both lowlanders and Sherpa at high altitude. In contrast, the pulmonary vasculature of Andeans suffering with chronic mountain sickness is resistant to iron administration. While pulmonary vascular remodeling and hypertension are characteristic features of chronic mountain sickness, the impact of iron administration in healthy Andeans has not been investigated. If the interplay between iron status and pulmonary vascular tone in healthy Andeans remains intact, this could provide valuable clinical insight into the role of iron regulation at high altitude.

Research question: Is the pulmonary vasculature in healthy Andeans responsive to iron infusion?

Study Design and Methods: In a double-blinded, block-randomized design, 24 healthy high-altitude Andeans and 22 partially acclimatized lowlanders at 4300 m (Cerro de Pasco, Peru), received an *i.v.* infusion of either iron [iron (III)-hydroxide sucrose; 200mg] or saline. Markers of iron status were collected at baseline and 4 hours after infusion. Echocardiography was performed during room-air breathing (P_IO_2=~96 mmHg) and during exaggerated hypoxia (P_IO_2=~73 mmHg), at baseline, and at 2 and 4 hours following the infusion.

Results: Iron infusion reduced pulmonary artery systolic pressure (PASP) by ~2.5 mmHg in room air (main effect P<0.001), and by ~7 mmHg during exaggerated hypoxia (main effect P<0.001) in both lowlanders and healthy Andean highlanders. There was no change in PASP following the infusion of saline. Iron metrics were comparable between groups, except for serum ferritin, which was 1.8-fold higher at baseline in the Andeans when compared to lowlanders [95% confidence interval (CI) 74-121 ng/ml vs. 37-70 ng/ml, respectively; P=0.003].

Interpretation: The pulmonary vasculature of healthy Andeans and lowlanders remains sensitive to iron infusion and this response seems to differ from the pathological characteristics of chronic mountain sickness.

Keywords: Andeans, iron, high altitude, pulmonary

Take home points

Study question: Is the pulmonary vasculature in healthy Andeans responsive to iron infusion?

Results: Iron infusion reduced pulmonary artery systolic pressure (PASP) by ~2.5 mmHg in room air (main effect P<0.001), and by ~7 mmHg during exaggerated hypoxia (main effect P<0.001) in both lowlanders and healthy Andean highlanders.

Interpretation: The pulmonary vasculature of healthy Andeans and lowlanders remains sensitive to iron infusion and this response seems to differ from the pathological characteristics of chronic mountain sickness.

Abbreviations:
CI, cardiac index
CMS, chronic mountain sickness
CO, cardiac output
EPO, erythropoietin
Hb, hemoglobin
Hct, hematocrit
HIF, hypoxia inducible factor
F_IO_2, fraction of inspired oxygen
LVOT VTI, left ventricular outflow tract velocity time integral
MAP, mean arterial pressure
NaCl, sodium chloride
PASP, Pulmonary artery systolic pressure
Pb, lead
P_IO_2, partial pressure of inspired oxygen
RAP, Right atrial pressure
SV, stroke volume
SpO_2, peripheral arterial oxygen saturation
TIBC, Total iron binding capacity
TPR, Total pulmonary resistance
TrF Sat, Transferrin saturation

Introduction

With ascent to high altitude, there is a progressive reduction in the partial pressure of oxygen (PO_2) and a progressive decline in iron bioavailability.[1-3] As such, there is a robust inverse relationship between iron status and the pulmonary vasculature in hypoxia (reviewed in [4]). Part of this relationship stems from the integral role of iron on propyl hydroxylase activity[5,6] and contribution to the stabilization of hypoxia inducible factors (HIF). The HIF pathway acts as a cellular oxygen sensor,[7,8] and controls a broad range of transcriptional responses to hypoxic exposure, including a variety of down-stream gene products that can influence hypoxic pulmonary vasoconstriction (e.g. endothelin, vascular endothelial grown factor and transient receptor potential channels).[4,9] Experimentally, iron manipulation has been used as: 1) a tool to attenuate pulmonary vascular responsiveness of lowlanders exposed to acute normobaric hypoxia,[10] and upon ascent and stay at high altitude;[3,11-14] and 2) a method to interrogate HIF-dependent differences associated with clinical pulmonary hypertension[15] and adaptation to high altitude – such as Sherpa of Tibetan descent,[3] and Andeans suffering with chronic mountain sickness (CMS).[14] In both lowlanders and Sherpa, iron infusion attenuates hypoxic pulmonary vasoconstriction at high altitude; however, this does not occur in Andeans with CMS. Andeans with CMS characteristically display marked pulmonary remodeling and hypertension (reviewed in [16]). Therefore, in the study by Smith and colleagues,[14] it is perhaps not surprising that 400 mg of iron(III)-sucrose [200 mg·day^{-1} over 2 days preceded by 4 days of venesection (at 500 ml·day^{-1})], was ineffective at reducing pulmonary artery systolic pressure (PASP), even after 5 days.[14]

While Andeans have had a putatively shorter high-altitude lineage, especially compared to Sherpa/Tibetans,[17] healthy Andeans still demonstrate numerous attributes that enhance their hypoxic tolerance compared to lowlanders including: elevated birth weights, increased exhaled nitric oxide concentrations, larger lungs, improved aerobic capacity and genotypic adaptations[18-22]. With the recently proposed notion that high altitude adaptation may proffer an enhanced state of iron metabolism,[3,23] exploring whether healthy Andeans are receptive to iron would provide insight into the pathological divergence of CMS on the pulmonary vasculature. Therefore, the aim of this study was to examine the role of iron bioavailability on the pulmonary vascular regulation to hypoxia in high-altitude adapted Andeans, with and without, acute reductions in inspired PO_2 (P_IO_2). To address this aim, partially acclimatized lowlanders and healthy Andeans received either an infusion of iron or saline at 4300 m, in a double-blinded and block-randomized design. Our primary hypothesis was that healthy Andeans and lowlanders would demonstrate comparable pulmonary vascular responses to hypoxia, and both groups would be sensitive to iron infusion.

Methods

Ethical Approval

All experimental procedures were approved by the University of British Columbia Research Ethics Board (H17-02687 and H18-01404) and the Universidad Peruana Cayetano Heredia Comité de Ética (no. 101686), and conformed to the Declaration of Helsinki, except for registration in a database. All participants received both written and oral information about the study and provided written informed consent. Forms were translated into Spanish, and Andeans participants spoke with a Peruvian research assistant and provided written informed consent in Spanish before participating.

Participants

A cohort of 24 healthy Andean highlanders and 22 age-matched lowlanders completed the study (Table 1). All were normotensive, and did not report a history of cardiovascular, cerebrovascular, or respiratory diseases during completion of a medical history questionnaire. All Andeans were born at high altitude (n=2 at 1880-2900 m, n=20 at 4300m; as were their parents) and resided at 4300 m, were non-smokers, did not exhibit excessive erythrocytosis (Hb ≤19 g/dl for females and ≤21 g/dl for males) and had a Qinghai CMS questionnaire score of 0.5±0.8 – and thus were not considered to suffer from CMS.[24] Of the few lowlander participants who opted for high altitude prophylaxis (i.e., acetazolamide) upon immediate arrival to 4300 m, all lowlander participants had discontinued use for at least a week, prior to being tested in the current study.

This study was part of the Global Research Expedition on Altitude Related Chronic Health (REACH) to Cerro de Pasco, Peru [4300m; barometric pressure of 457 mmHg; P_IO_2 of ~96 mmHg].[25] Upon arrival in Lima and finalizing logistics, lowlander participants (of which were members of the expedition and sea-level residents of European descent) were driven up to Cerro de Pasco in 8 hours. Lowlanders were tested after 8±4 days at 4300 m – lowlander infusion groups were equally weighted, for number of days at 4300 m – see *Infusion* section.

Experimental Design

Participants arrived at the laboratory having only eaten a small meal ~2 hours prior, and abstained from caffeine and exercise for >6 and >12 hours, respectively. Following 10 min of supine rest an indwelling venous catheter (21G needle, BD Vacutainer eclipse) was inserted into the median antecubital vein for repeated blood sampling and the infusion. Echocardiography (see *Experimental Measures* section) was performed at baseline, and at 2 and 4 hours after receiving an infusion of either iron or saline (see *Infusion* section). Between time-points, participants were allowed to sit up, but all participants resumed supine rest for at least 10 minutes before any measurement. At baseline and at 4 hours, echocardiography was also performed during a hypoxic stage (15 min of F_IO_2=0.16; P_IO_2=73 mmHg; simulating ~1700 m gain in elevation). At 2.5 minutes into the hypoxic stage, automated blood pressure was collected in duplicate using brachial oscillometry. After 10 min of reduced P_IO_2, echocardiographic imaging was repeated, and at the end of the hypoxic stage, peripheral oxygen saturation was recorded.

Infusion: In a double-blinded, block-randomized design, Andean and lowlander participants received an *i.v.* infusion of either iron (iron (III)-hydroxide sucrose; 200 mg in 250 ml 0.9% NaCl saline) or saline (250 ml of 0.9% NaCl saline) over 30 min. Both participants and investigators were blinded during collection (via opaque bags to cover the infusion and lines), and maintained throughout analysis. Due to the >40 day longevity of iron infusion[10] and the logistic constraints of an expedition,[25] a cross-over design was not feasible. Therefore, the purpose of block-randomizing participants was two-fold: 1) enabled us to optimize logistics and coordination with other ongoing studies, and 2) ensured that lowlanders allocated to iron or saline conditions, were appropriately weighted, in terms of the number of days at 4300 m, to limit any potentially confounding influence of hypobaric exposure on iron stores [lowlanders receiving saline and iron were tested after 9±5 and 6±3 days, respectively (unpaired t-test P=0.158)].

Experimental Measures

Echocardiography: Stroke volume (SV) was estimated using the following equation:

$$SV = \left(\pi \cdot \left[\text{aortic diameter}/2\right]^2\right) \cdot \text{LVOT VTI}$$

where the left ventricular outflow tract (LVOT) velocity time integral (VTI) was measured at the point of aortic leaflet insertion during systole using a pulsed wave Doppler signal. Cardiac output (CO) was calculated as the product of stroke volume and heart rate (via electrocardiography). Cardiac index (CI) is the quotient of CO and body surface area, calculated as[26]:

$$\text{Body surface area} = \sqrt{(\text{height} \times \text{weight})/3600}$$

PASP was measured by Doppler echocardiography based upon the measurement of the maximum velocity of the tricuspid regurgitation jet (TR velocity).[27] The peak systolic pressure gradient of the right ventricle (ΔP_{max}) to the right atrium was calculated according to the simplified Bernoulli equation ($4 \cdot V^2$), where V is the peak systolic velocity of the tricuspid regurgitate. PASP was then determined by adding the right atrial pressure (RAP), which was estimated by evaluating inferior vena cava diameter and collapsibility index – in line with the standard American Society of Echocardiography guidelines.[28]

$$\text{PASP} = (4 \cdot V^2) + \text{RAP}$$

PASP was normalized to a hematocrit (Hct) of 45% in all individuals using the following equation:[29]

$$\text{PASP}_{[\text{Hct}]} = \text{PASP}/\exp[2(\varphi - 0.45)]$$

Where φ represents the measured hematocrit level. Total pulmonary resistance (TPR) was calculated as an index of PASP against CO (i.e., PASP/CO), as previously described.[30]

Biochemical analysis: Venous blood samples were separated by microcentrifugation, with serum samples frozen in liquid nitrogen at -196 °C for subsequent analysis. Serum iron, ferritin, transferrin, and erythropoietin (EPO) were analysed according to clinical laboratory standards (Medlab clinical laboratories, Lima, Peru). Total iron binding capacity (TIBC) was calculated as[31]:

$$\text{TIBC} = \text{transferrin} \cdot 1.389$$

Transferrin saturation (TrF Sat) was calculated as:

$$\text{Transferrin saturation} = (\text{serum iron}/\text{TIBC}) \cdot 100$$

Hb and Hct were obtained from whole venous blood sample using microcentrifugation, and analyzed immediately. The Hct at 2 hours, was estimated as the median value between the baseline and 4-hour timepoints.

Statistical analysis

Data were analyzed using a linear mixed effects model with a compound symmetry repeated measure co-variance structure (SPSS v24, IBM Statistics). The fixed factors for the model were ancestry and P_IO_2 (i.e., 96 to 73 mmHg) or time (i.e. pre to post infusion) – with the latter, P_IO_2 or time, being a repeated factor with a compound symmetry repeated covariance structure. Subjects were included as a random effect. When a significant interaction effect (e.g., altitude×ancestry) was detected, Bonferroni adjusted post-hoc tests were utilized to test pairwise comparisons. Comparison of hematological measures at baseline, of pooled lowlanders and Andeans, were assessed with independent samples t-test. The absolute difference between normoxia and hypoxia (Δ), is also presented. A linear regression was used to evaluate the prevalence of decreasing serum iron, across days at altitude. All results are reported as mean±SD and significance was set at $P<0.05$.

Results

Comparison of lowlanders and healthy Andeans at baseline

Iron metrics: At baseline, when all participants were pooled, ferritin was 82% higher in Andeans at baseline, compared to lowlanders (97.3±54.6 ng/ml vs. 53.5±37.1 ng/ml, respectively; P=0.003; Table 1). There were no other differences in markers of iron status (e.g., serum iron, transferrin, TIBC, TrF Sat, or EPO) at baseline between Andeans and lowlanders.

Hypoxic pulmonary vasoconstriction: At baseline (prior to infusion), there was no difference in pulmonary vascular reactivity to hypoxia (P_IO_2=73 mmHg) between lowlanders and Andeans (for both uncorrected and corrected to 45% Hct; Table 2). LV SV slightly decreased during hypoxia in Andeans (75±17 ml at baseline to 72±18 ml during hypoxia; P=0.047); however, both lowlanders and Andeans demonstrated comparable increases in CO, CI, TPR, and MAP during hypoxia.

Effects of intravenous iron

Iron metrics: The iron infusion increased serum iron (by 382±193% and 288±100% in lowlanders and Andeans, respectively; main effect P<0.001), increased serum ferritin (by 18±16% and 14±10% in lowlanders and Andeans, respectively; main effect P<0.001) and decreased transferrin (by 4±7% and 5±6% in lowlanders and Andeans, respectively; main effect P=0.010; Figure 1). Consistent with previous reports in CMS,[14] EPO remained unaltered following iron or placebo in both groups. There were no significant changes in iron metrics during the saline condition.

Time course over 4 hours at high altitude: In lowlanders and Andeans receiving saline, PASP did not significantly change across the 4 hours (main effect of time P=0.262; Table 3a). Following iron infusion, there was a decrease in PASP by 7% and 4% at 2 hours and 11% and 7% at 4 hours in lowlanders and Andeans, respectively (main effect of time P<0.001; Figure 2 and Table 3b). Following iron, both lowlanders and Andeans demonstrated a tendency for an increased MAP (main effect of time P=0.052; Table 3b). Even though there were no change in TPR across the 4 hours in lowlanders and Andeans with iron (Table 3b), TPR was higher in Andeans compared to lowlanders at 4 hours (P=0.034) – this difference is likely driven by the tendency for TPR to decrease across the 4 hours in lowlanders following iron (P=0.051).

Hypoxic pulmonary vasoconstriction: During acute hypoxia (i.e., P_IO_2=73 mmHg), iron infusion attenuated PASP by 4 mmHg, in both Andeans and lowlanders (main effect P=0.01; Table 4b and Figure 3), which coincided with a decrease in TPR (main effect P=0.001) and an increase in LV SV (main effect P=0.031). The rise in PASP during hypoxia was unaltered following the placebo condition in both groups (main effect P=0.326; Table 4a). While the expected decrease in SpO_2 was apparent in the exaggerated hypoxia trial, this reduction was not altered by iron infusion in lowlanders and Andeans; however, the rise in PASP for a given drop in SpO_2 was attenuated by iron infusion (main effect P=0.011; Table 4b).

Discussion

The pulmonary vasculature of adapted Andeans remained responsive to iron infusion – a feature consistent among lowlanders and high altitude Sherpa.[3] Because the pulmonary vasculature of Andeans suffering with CMS does not exhibit this responsiveness to iron,[14] this may provide valuable insight into the pathological divergence of CMS. The following discussion outlines the implications of these findings, including the physiological significance of elevated ferritin levels in the Andeans.

Pulmonary vascular responsiveness to hypoxia and iron

Aligning with our hypothesis, this is the first study to confirm that the pulmonary vasculature of adapted and otherwise healthy Andeans is responsive to iron infusion in hypoxia – a feature akin to the well adapted Sherpa and lowlanders.[3] Such congruent responses across different populations (i.e. acclimatizing lowlanders and high altitude adapted Andeans and Sherpa), highlight a conserved phenotype reflected in an otherwise healthy pulmonary vasculature. Healthy Andeans have demonstrated either slightly higher PASP (at rest and during exercise)[32], or similar PASP (at rest),[33,34] compared to partially acclimatized lowlanders at the same altitude. Therefore, it is perhaps not surprising in the current study that PASP at rest and during further hypoxia was again similar between partially acclimatized lowlanders and healthy Andeans.

The unremarkable influence of a comparable iron infusion on PASP in Andeans with CMS[14] is intriguing. Certainly, CMS has long been established to coincide with pulmonary vascular remodeling and persistence of pulmonary hypertension (during rest and exercise)[32,35-39]. However, CMS has also been linked to the dysfunction of a myriad of HIF-related factors [including sentrin-specific protease 1[40], prevailing circulatory status of vasoactive peptides (i.e. vascular endothelial growth factor and endothelin-1[41]), methylation of propyl hydroxylase activity[42], and/or mutations to the von Hippel-Lindau protein[43]] – these factors could blunt or counteract the efficacy of iron infusion on pulmonary vascular tone. Ultimately, the notable divergence of healthy adapted Andeans from the CMS pulmonary phenotype, hints at some facet of HIF-dysregulation, that may share mechanistic basis with other clinical pathologies.

Iron metabolism – higher ferritin levels in Andeans

Ascent to high altitude is associated with a progressive decline in iron bioavailability,[1-3] which can exaggerate hypoxia-induced HIF stabilization and contribute to pulmonary hypertension.[44] Therefore, in the context of iron regulation, the observed higher ferritin (a stimulus for iron storage) in Andeans could imply a greater state of iron sufficiency and a lower iron/erythropoietic demand, compared to lowlanders. While this feature might have resonance in the interesting dialogue of enhanced iron metabolism in adapted highlanders,[3,23] using ferritin as an index of iron sufficiency is limited, as elevated ferritin levels (e.g., >200-300 ng/ml) are indicative of a myriad of medical concerns [e.g., liver disease, hepatitis, infection, state of inflammation, and hemochromatosis].[45,46] As such, Sherpa do not present with elevated ferritin levels. For example, in the study by Willie and colleagues, serum ferritin of Sherpa was 46.7±37.8 ng/ml[3] – values which more closely resemble our lowlander data at 4300 m, rather than healthy Andeans (Table 1). Coincidingly, similar to lowlanders, Sherpa also did not exhibit any obvious inflammatory profile.[3] Conversely, previous reports of Andeans with CMS (and/or excessive erythrocytosis), show ferritin levels of 169±90 ng/ml (n=11),[14] 184±28 ng/ml (n=42)[47] and 156±132 ng/ml (n=41).[48] However, in the latter two studies, ferritin in healthy Andeans were comparable to Andeans with CMS.[47,48] While it is unclear why the ferritin in our "healthy" Andeans appear substantially lower than the ferritin values in other "healthy" Andeans[47,48], one possibility may be related to CMS score severity (0.5±0.8 in this study; 2.7±0.38 in[47]; no score reported in[48]). Future studies are needed to consider how and why CMS severity might influence ferritin levels, or why ferritin level can be so variable between otherwise healthy individuals.

The factors potentially mediating these elevations in ferritin, in both healthy and CMS Andeans compared to lowlanders, may have some basis when considering the city of Cerro de Pasco itself. First, it has been demonstrated that more than half of the soil sample sites evaluated in Cerro de Pasco, had lead (Pb) levels exceeding 1200mg/kg,[49] which is ~10-fold higher the health recommendations in Canada [140 mg/kg[50]]. Recently, and not surprisingly, whole blood Pb concentrations in residents of Cerro de Pasco (with Hb 10-25 g/dl) were >2-fold greater than Pb concentrations in sea-level residents of Lima [4.75±1.53 ug/dl, range: 1.5-9.8 ug/dl versus 2.03±0.62 ug/dl, range: 1.0-4.6 ug/dl, respectively[51]]. While Pb toxicity may not be prevalent in our cohort [due to the lack of iron deficiency and anemia (reviewed in: [52])], consequence(s) of Pb exposure on upstream inflammatory factors[53] and ferroportin activity,[54] have been demonstrated in animal and cell-preparation models. Whether Pb exposure could extend beyond iron deficiency/anemia and potentially contribute to the elevated ferritin levels in Andeans, compared lowlanders, remains to be determined. Second, the city of Cerro de Pasco starkly contrasts those of small towns/villages in the Himalayan highlands, in terms of lifestyle (e.g. diet, physical activity) and level of industrialization (e.g. drive-in and mining versus hike-in and agriculture/tourism, respectively). As such, comparisons between populations are certainly multifaceted and future studies are needed to consider the potential detrimental influence of even mildly elevated Pb levels on inflammation[53,55] and explore the mechanistic links to contributing to the elevated ferritin levels in Andeans

Importantly, our findings that iron attenuated the rise in PASP during hypoxia, with a coinciding increase in left ventricular SV and decrease in TPR (Table 4), is broadly consistent with the recent study by Holdsworth and colleagues.[13] Here, the authors reported that increasing iron bioavailability (via infusion of 1g of ferric carboxymaltose) in lowlanders, *prior* to ascent to high altitude, translated into enhanced right ventricular SV and lower pulmonary vascular resistance at 5050 m.

Considerations of iron status during prolonged stay at high altitude

In our lowlander cohort, a pattern of decreasing serum iron levels appeared to develop over the 17 days at 4300 m (r^2=0.204, P=0.035; Figure 4) – a reproducible feature of high-altitude sojourn.[1-3] Even though our cohort of Andeans did not travel and resided at 4300 m, our groups were also appropriately weighted for serum iron (unpaired t-test P=0.522; Figure 4). Our study appropriately weighted lowlanders to saline and iron conditions, so the confounding influence of time at altitude would be less. However, the progressive reduction in iron with stay at altitude, highlights the need to be cognisant of such changes in iron status, if the outcome variable of interest hinges or relies upon downstream iron-regulatory processes.

Interpretation

Unlike Andeans with CMS,[14] yet aligning with Sherpa,[3] the pulmonary vasculature of adapted Andeans remained responsive to iron infusion. Therefore, these findings highlight a feature of healthy pulmonary vascular regulation, and a trait of high-altitude adaptation that remains divergent from the typical pathological characteristics of high-altitude maladaptation (i.e., CMS).

Acknowledgements

This study was conducted within the framework of Global REACH 2018. The authors are grateful to the expedition team members and collaborators in Cerro de Pasco for their support in the weeks

leading up to, and during data collection in Peru. We would also like to thank the Andean residents of Cerro de Pasco for their participation.

Author contributions

All authors were involved in data collection. AP and PNA were involved in data analyses and interpretation. AP drafted the manuscript. All authors provided intellectual feedback, approved the final version of this manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

Financial/nonfinancial disclosures

None to declare

Tables

Table 1. Baseline summary of pooled lowlanders and pooled Andeans.

	Lowlander	Andean	p-value
Sex (males/females)	16 / 6	21 / 3	
Age (years)	29 ± 7	29 ± 11	0.898
Height (cm)	175 ± 8	162 ± 5	**<0.001**
Weight (kg)	71 ± 10	63 ± 7	**<0.001**
BMI (kg/m^2)	23.3 ± 2.3	23.8 ± 2.8	0.530
Hb (g/dl)	16.2 ± 1.4 [17]	16.9 ± 1.9 [14]	0.235
Hct (%)	48.4 ± 3.9. [20]	53.2 ± 4.1 [18]	**0.001**
EPO (mIU/ml)	17.3 ± 9.5	14.1 ± 8.9	0.257
Serum Ferritin (ng/ml)	53.5 ± 37.1	97.3 ± 54.6 [23]	**0.003**
Serum Iron (ug/dL)	81.8 ± 40.9	94.2 ± 29.7 [23]	0.250
Serum Transferrin (mg/dl)	275.4 ± 24.4	268 ± 28.3 [23]	0.353
TIBC (ug/dl)	382.5 ± 33.9	372.2 ± 39.3 [23]	0.353
TrF Sat (%)	21.9 ± 11.5	25.3 ± 7.6 [23]	0.240
SpO_2 (%)	89 ± 3	88 ± 3	0.528
PASP (mmHg)	31.0 ± 6.4 [21]	29.5 ± 5.9 [23]	0.403
$PASP_{[Hct]}$ (mmHg)	29.5 ± 7.3 [19]	25.4 ± 5.8 [17]	0.072

Mean ± SD in 22 Lowlanders and 24 Andeans (unless otherwise noted [n]). Independent samples t-test.

Table 2. Cardiopulmonary measures in pooled lowlanders and Andeans (prior to infusion) during room air breathing and reduced P_IO_2.

		Room air	Hypoxia	Ancestry	P_IO_2	Inter
PASP (mmHg)	Lowlander	31.0 ± 6.4 [21]	40.2 ± 8.4 [21]	0.866	<**0.001**	0.343
	Andean	29.5 ± 5.9 [23]	40.9 ± 12.9 [23]			
$PASP_{[hct]}$ (mmHg)	Lowlander	29.5 ± 7.3 [19]	38.4 ± 10.7 [19]	0.242	<**0.001**	0.586
	Andean	25.4 ± 5.8 [17]	35.7 ± 12.3 [17]			
TPR (mmHg/l)	Lowlander	6.4 ± 1.5 [21]	7.0 ± 1.8 [21]	0.401	<**0.001**	0.120
	Andean	6.5 ± 1.6 [23]	7.7 ± 2.3 [23]			
LV SV (ml)	Lowlander	83 ± 18	86 ± 19	**0.041**	0.931	**0.009**
	Andean	75 ± 17	72 ± 18*†			
HR (beats/min)	Lowlander	62 ± 12	71 ± 15	0.320	<**0.001**	0.061
	Andean	63 ± 9	77 ± 12			
CO (l/min)	Lowlander	5.0 ± 1.0	6.0 ± 1.3	0.173	<**0.001**	0.167
	Andean	4.7 ± 0.9	5.4 ± 1.2			
CI (ml/min/m^2)	Lowlander	2.7 ± 0.5	3.2 ± 0.7	0.751	<**0.001**	0.349
	Andean	2.8 ± 0.5	3.2 ± 0.7			
MAP (mmHg)	Lowlander	90 ± 8	94 ± 10	<**0.001**	**0.001**	0.813
	Andean	78 ± 8	82 ± 10			
SpO_2 (%)	Lowlander	89 ± 3	72 ± 7	0.916	<**0.001**	0.506
	Andean	88 ± 3	73 ± 10 [23]			

Mean ± SD in 22 Lowlanders and 24 Andeans (unless otherwise noted [n]). Linear mixed model. * Significant difference from baseline, P<0.05; † Significant difference between Andeans and Lowlanders, P<0.05.

408

Table 3a. Resting cardiopulmonary measures at baseline and following the infusion of saline at 4300 m

		Baseline	2 hours	4 hours	Ancestry	Time	Inter
PASP (mmHg)	Lowlander	32.1 ± 7.5	31.5 ± 6.2	31.1 ± 5.8	0.366	0.262	0.751
	Andean	29.7 ± 6.4 [11]	29.6 ± 7.8 [11]	27.8 ± 8.1 [11]			
$PASP_{[hct]}$ (mmHg)	Lowlander	30.5 ± 9.0	29.7 ± 7.7	29.3 ± 8.4	0.094	0.174	0.549
	Andean	24.8 ± 6.3 [11]	25.5 ± 6.1 [9]	23.1 ± 7 [9]			
TPR (mmHg/l)	Lowlander	6.1 ± 1.4	6 ± 1.2	5.8 ± 1.1	0.739	0.307	0.594
	Andean	6.1 ± 1.5 [11]	6.5 ± 1.7 [11]	5.5 ± 2.4			
HR (beats/min)	Lowlander	60 ± 10	59 ± 13	57 ± 13	0.592	0.067	0.648
	Andean	63 ± 10	59 ± 9	60 ± 9			
LV SV (ml)	Lowlander	90 ± 17	92 ± 11	98 ± 22	**0.021**	0.129	0.099
	Andean	80 ± 16	78 ± 9	79 ± 11			
CO (l/min)	Lowlander	5.3 ± 1.0	5.3 ± 1	5.4 ± 1.3	0.079	0.430	0.278
	Andean	5.0 ± 0.8	4.6 ± 0.7	4.7 ± 0.5			
CI (l/min/m^2)	Lowlander	2.8 ± 0.6	2.8 ± 0.6	2.9 ± 0.7	0.855	0.398	0.227
	Andean	2.9 ± 0.6	2.7 ± 0.5	2.7 ± 0.4			
MAP (mmHg)	Lowlander	92 ± 8	90 ± 7	91 ± 10	**0.027**	0.914	0.305
	Andean	81 ± 8	84 ± 11	83 ± 14			
SpO_2 (%)	Lowlander	89 ± 3	89 ± 3	88 ± 4	0.607	0.557	0.806
	Andean	88 ± 2	88 ± 3	88 ± 2			

Mean ± SD in 11 lowlanders and 12 Andeans (unless otherwise noted [n]). P_IO_2=96mmHg. Significant p-values are denoted in bold. Linear mixed model.

409

410

411

Table 3b. Resting cardiopulmonary measures at baseline and following the infusion of iron at 4300 m

		Baseline	2 hours	4 hours	Ancestry	Time	Inter
PASP (mmHg)	Lowlander	29.8 ± 5.1 [10]	27.7 ± 5.6 [10]	26.6 ± 6.3 [10]	0.967	**<0.001**	0.587
	Andean	29.3 ± 5.6	28.1 ± 7.2	27.2 ± 6.9			
$PASP_{[hct]}$ (mmHg)	Lowlander	28.2 ± 4.3 [8]	26.5 ± 4.8 [8]	25.1 ± 5.5 [10]	0.183	**0.001**	0.485
	Andean	26.5 ± 5.2 [6]	26.6 ± 6.9 [4]	21.3 ± 4.3 [8]			
TPR (mmHg/l)	Lowlander	6.7 ± 1.6 [10]	6.4 ± 1.8 [10]	5.3 ± 2.6†	0.190	0.867	0.008
	Andean	6.8 ± 1.7	7 ± 2	7.5 ± 2.9			
HR (beats/min)	Lowlander	63 ± 14	63 ± 20	64 ± 16	0.483	0.098	0.125
	Andean	63 ± 7	58 ± 8	58 ± 8			
LV SV (ml)	Lowlander	76 ± 17	75 ± 15	77 ± 19	0.439	0.764	0.181
	Andean	71 ± 18	73 ± 18	68 ± 19			
CO (l/min)	Lowlander	4.7 ± 0.9	4.5 ± 1	4.7 ± 0.9	0.262	0.277	0.135
	Andean	4.4 ± 0.9	4.2 ± 1.2	3.9 ± 1.3			
CI ($l/min/m^2$)	Lowlander	2.6 ± 0.4	2.5 ± 0.5	2.6 ± 0.5	0.854	0.256	0.096
	Andean	2.7 ± 0.5	2.5 ± 0.6	2.4 ± 0.7			
MAP (mmHg)	Lowlander	89 ± 8	89 ± 10	91 ± 7	**0.001**	0.052	0.647
	Andean	75 ± 6	76 ± 8	79 ± 11			
SpO_2 (%)	Lowlander	88 ± 3	88 ± 2	88 ± 3 [10]	0.444	0.160	0.315
	Andean	88 ± 4	87 ± 3	87 ± 3			

Mean ± SD in 11 lowlanders and 12 Andeans (unless otherwise noted [n]). P_IO_2=96mmHg. Significant p-values are denoted in bold. Linear mixed model.

412
413
414

Table 4a. Cardiopulmonary reactivities to hypoxia, prior-to and following the infusion of saline.

		Pre-saline	Post-saline	Ancestry	Time	Inter
ΔSpO_2 (%)	Lowlander	-14.9 ± 6.9	-15.5 ± 6.7	0.949	0.899	0.610
	Andean	-15.8 ± 9.1	-14.9 ± 8			
ΔPASP (mmHg)	Lowlander	9 ± 5.3	10.6 ± 5.6	0.995	0.326	0.989
	Andean	9.1 ± 5.7	10.6 ± 8.5			
$\Delta PASP/\Delta SpO_2$ (mmHg/%)	Lowlander	-0.6 ± 0.4	-0.9 ± 0.9	0.565	0.070	0.691
	Andean	-0.5 ± 0.7 [10]	-0.9 ± 0.5			
ΔPASP[hct] (mmHg)	Lowlander	8.7 ± 5.8	10.1 ± 5.9	0.654	0.258	0.854
	Andean	7.5 ± 4.9	9.8 ± 6.4 [9]			
$\Delta PASP[hct]/\Delta SpO_2$ (mmHg/%)	Lowlander	-0.6 ± 0.4	-0.9 ± 0.8	0.427	**0.045**	0.477
	Andean	-0.3 ± 0.6 [9]	-0.8 ± 0.3 [9]			
ΔTPR (mmHg/l)	Lowlander	0.4 ± 0.8	1.0 ± 1.9	0.420	0.137	0.922
	Andean	0.9 ± 1.3	1.4 ± 2			
ΔHR (beats/min)	Lowlander	10.0 ± 9.5	9.6 ± 7.2	0.091	0.702	0.849
	Andean	15.0 ± 4.8	13.9 ± 8.6			
ΔSV (ml)	Lowlander	3.6 ± 8.1	1.5 ± 7.5	**0.004**	0.433	0.651
	Andean	-6.2 ± 8.8	-6.8 ± 6.9			
ΔCO (ml/min)	Lowlander	1.1 ± 0.6	1.1 ± 0.9	0.055	0.665	0.591
	Andean	0.7 ± 0.6	0.6 ± 0.4			
ΔCI (ml/min/m^2)	Lowlander	0.6 ± 0.4	0.6 ± 0.5	0.123	0.624	0.657
	Andean	0.4 ± 0.3	0.3 ± 0.3			
ΔMAP (mmHg)	Lowlander	5.1 ± 6.6	5.9 ± 5.5	0.495	0.760	0.888
	Andean	3.9 ± 8.1	4.2 ± 5.1			

Mean ± SD. N=10-11 and n=11-12 for lowlanders and Andeans respectively, unless otherwise noted [n]. Δ, the absolute change between normoxia and hypoxia.

418

Table 4b. Cardiopulmonary reactivities to hypoxia, prior-to and following the infusion of iron.

		Pre-iron	Post-iron	Ancestry	Time	Inter.
ΔSpO_2 (%)	Lowlander	-18.4 ± 3.3	-18 ± 4.2	0.238	0.966	0.749
	Andean	-14.8 ± 7.7	-15.2 ± 9.8			
ΔPASP (mmHg)	Lowlander	9.2 ± 3.9	6.8 ± 3.1	0.451	**0.010**	0.219
	Andean	13.6 ± 12.9	7.4 ± 8.4			
$\Delta PASP/\Delta SpO_2$ (mmHg/%)	Lowlander	-0.5 ± 0.2	-0.4 ± 0.2 [9]	0.979	**0.011**	0.051
	Andean	-0.9 ± 0.7	-0.2 ± 0.9			
$\Delta PASP_{[Hct]}$ (mmHg)	Lowlander	9 ± 4.4 [8]	6.6 ± 3.1 [10]	0.556	0.075	0.300
	Andean	15.5 ± 16.6 [5]	7.7 ± 10 [6]			
$\Delta PASP_{[Hct]}/\Delta SpO_2$ (mmHg/%)	Lowlander	-0.51 ± 0.25 [8]	-0.4 ± 0.23 [9]	0.509	0.057	0.176
	Andean	-0.85 ± 0.7 [5]	-0.001 ± 1.04 [6]			
ΔTPR (mmHg/l)	Lowlander	0.7 ± 1.3	0.02 ± 1.4	0.62	**0.001**	0.16
	Andean	1.5 ± 1.9	-0.1 ± 2.1			
ΔHR (beats/min)	Lowlander	9.1 ± 5.9	6.9 ± 10.9	0.256	0.303	0.671
	Andean	11.9 ± 6.8	11 ± 7.2			
ΔSV (ml)	Lowlander	2.8 ± 5.6	7.3 ± 8.3	0.234	**0.031**	0.729
	Andean	-0.6 ± 9.3	2.7 ± 11			
ΔCO (l/min)	Lowlander	0.9 ± 0.5	1.1 ± 0.6	0.505	0.264	0.929
	Andean	0.8 ± 0.7	0.9 ± 0.7			
ΔCI (ml/min/m^2)	Lowlander	0.5 ± 0.3	0.6 ± 0.3	0.809	0.244	0.997
	Andean	0.5 ± 0.4	0.6 ± 0.4			
ΔMAP (mmHg)	Lowlander	2.6 ± 9.3	1.1 ± 5.9	0.718	0.145	0.468
	Andean	4.9 ± 6.9	0.5 ± 6.1			

Mean ± SD. N=10-11 and n=11-12 for lowlanders and Andeans respectively, unless otherwise noted [n]. Δ, the absolute change between normoxia and hypoxia.

419
420
421
422

Figures

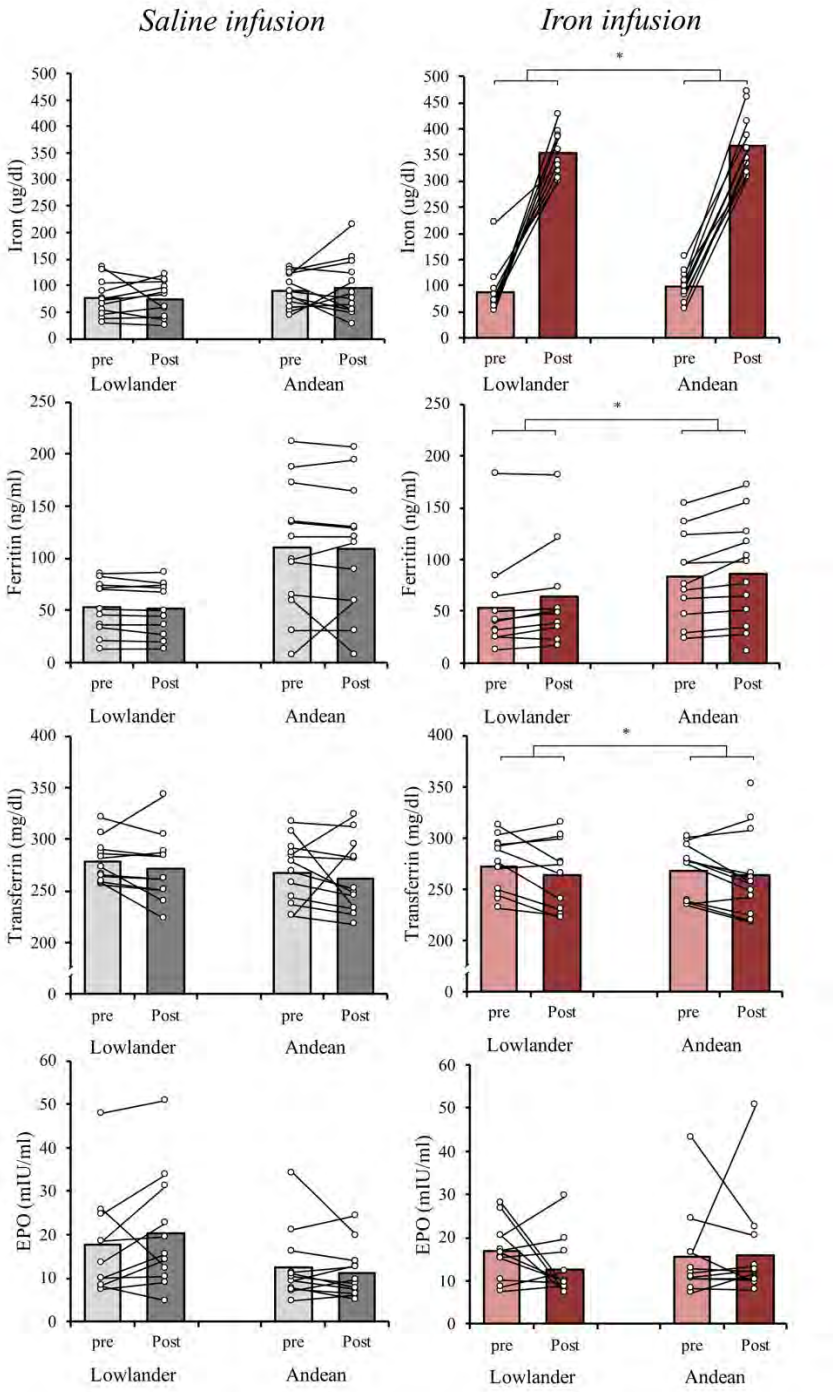

Figure 1. Impact of saline (grey bars) or iron infusion (red bars) on iron metrics in lowlanders and healthy Andeans. *P<0.05 main effect of time.

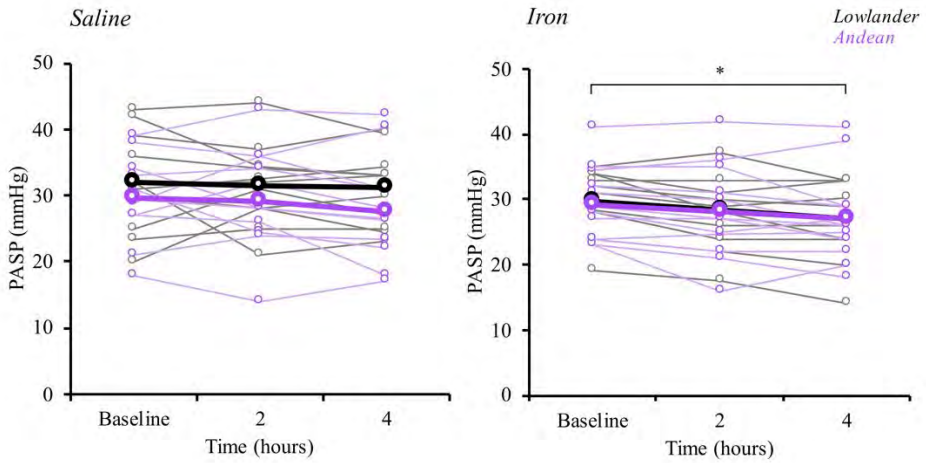

Figure 2. Impact of saline and iron infusion on the PASP in lowlanders and healthy Andeans. Horizonal bar with asterisk denotes a main effect significance of time (*P<0.05).

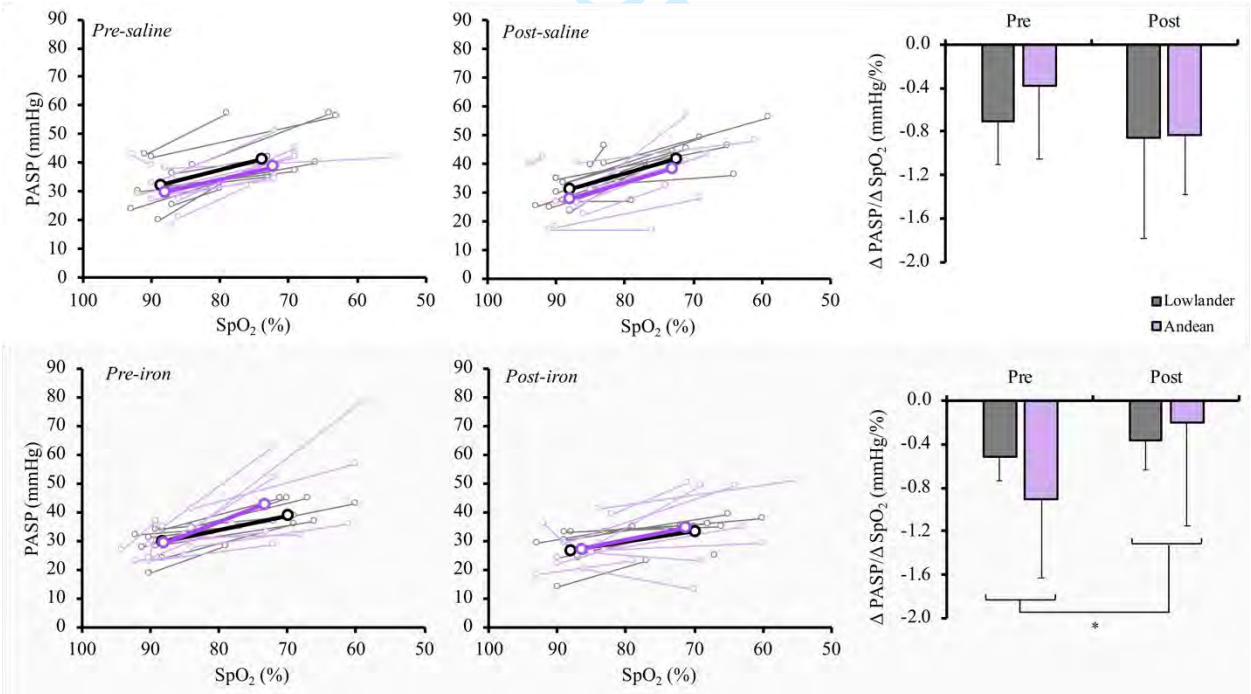

Figure 3. Pulmonary vascular reactivity to hypoxia in lowlanders and Andeans receiving either saline or iron infusion. * main effect P<0.05.

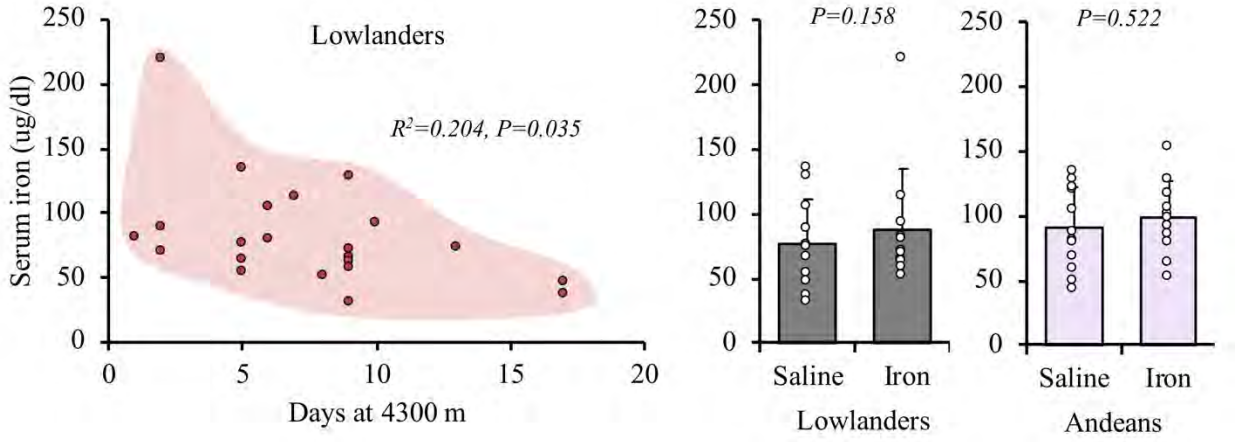

Figure 4. Baseline serum iron measurements in lowlanders and Andeans. Lowlander data is presented as 1) to correspond to the number of days at 4300 m, before being tested (with linear regression; *left panel*) and 2) highlight the equal weighting of iron status between the saline and iron groups (with unpaired t-test; *middle panel*). Andean data demonstrates iron levels in the saline and iron groups at baseline were comparable (with unpaired t-test; *right panel*).

References

1. Robach P, Cairo G, Gelfi C, et al. Strong iron demand during hypoxia-induced erythropoiesis is associated with down-regulation of iron-related proteins and myoglobin in human skeletal muscle. *Blood.* 2007;109(11):4724-4731.
2. Gassmann M, Muckenthaler MU. Adaptation of iron requirement to hypoxic conditions at high altitude. *J Appl Physiol (1985).* 2015;119(12):1432-1440.
3. Willie CK, Patrician A, Hoiland RL, et al. Influence of iron manipulation on hypoxic pulmonary vasoconstriction and pulmonary reactivity during ascent and acclimatization to 5050 m. *J Physiol.* 2021;599(5):1685-1708.
4. Frise MC, Robbins PA. Iron, oxygen, and the pulmonary circulation. *J Appl Physiol (1985).* 2015;119(12):1421-1431.
5. Kaelin WG, Ratcliffe PJ. Oxygen Sensing by Metazoans: The Central Role of the HIF Hydroxylase Pathway. *Molecular Cell.* 2008;30(4):393-402.
6. Knowles HJ, Raval RR, Harris AL, Ratcliffe PJ. Effect of ascorbate on the activity of hypoxia-inducible factor in cancer cells. *Cancer Res.* 2003;63(8):1764-1768.
7. Semenza GL, Agani F, Iyer N, et al. Hypoxia-inducible factor 1: from molecular biology to cardiopulmonary physiology. *Chest.* 1998;114(1 Suppl):40s-45s.
8. Prabhakar NR, Semenza GL. Oxygen Sensing and Homeostasis. *Physiology (Bethesda).* 2015;30(5):340-348.
9. Sylvester JT, Shimoda LA, Aaronson PI, Ward JP. Hypoxic pulmonary vasoconstriction. *Physiol Rev.* 2012;92(1):367-520.

10. Bart NK, Curtis MK, Cheng HY, et al. Elevation of iron storage in humans attenuates the pulmonary vascular response to hypoxia. *J Appl Physiol (1985).* 2016;121(2):537-544.
11. Smith TG, Balanos GM, Croft QP, et al. The increase in pulmonary arterial pressure caused by hypoxia depends on iron status. *J Physiol.* 2008;586(24):5999-6005.
12. Talbot NP, Smith TG, Privat C, et al. Intravenous Iron Supplementation May Protect Against Acute Mountain Sickness: A Randomized, Double-Blinded, Placebo-Controlled Trial. *High Alt. Med. Biol.* 2011;12(3):265-269.
13. Holdsworth DA, Frise MC, Bakker-Dyos J, et al. Iron bioavailability and cardiopulmonary function during ascent to very high altitude. *Eur Respir J.* 2020;56(3).
14. Smith TG, Talbot NP, Privat C, et al. Effects of iron supplementation and depletion on hypoxic pulmonary hypertension: two randomized controlled trials. *Jama.* 2009;302(13):1444-1450.
15. Balanos GM, Dorrington KL, Robbins PA. Desferrioxamine elevates pulmonary vascular resistance in humans: potential for involvement of HIF-1. *J Appl Physiol (1985).* 2002;92(6):2501-2507.
16. Penaloza D, Arias-Stella J. The heart and pulmonary circulation at high altitudes: healthy highlanders and chronic mountain sickness. *Circulation.* 2007;115(9):1132-1146.
17. Aldenderfer MS. Moving Up in the World: Archaeologists seek to understand how and when people came to occupy the Andean and Tibetan plateaus. *Am Sci.* 2003;91(6):542-549.
18. Moore LG, Shriver M, Bemis L, et al. Maternal adaptation to high-altitude pregnancy: an experiment of nature--a review. *Placenta.* 2004;25 Suppl A:S60-71.
19. Beall CM, Laskowski D, Strohl KP, et al. Pulmonary nitric oxide in mountain dwellers. *Nature.* 2001;414(6862):411-412.
20. Lenfant C, Sullivan K. Adaptation to high altitude. *N Engl J Med.* 1971;284(23):1298-1309.
21. Frisancho AR. Developmental functional adaptation to high altitude: review. *Am J Hum Biol.* 2013;25(2):151-168.
22. Storz JF. High-Altitude Adaptation: Mechanistic Insights from Integrated Genomics and Physiology. *Mol Biol Evol.* 2021;msab064.
23. Muckenthaler MU, Mairbäurl H, Gassmann M. Iron metabolism in high altitude residents. *J Appl Physiol (1985).* 2020;129(4):920-925.
24. León-Velarde F, Maggiorini M, Reeves JT, et al. Consensus statement on chronic and subacute high altitude diseases. *High Alt Med Biol.* 2005;6(2):147-157.
25. Tymko MM, Hoiland RL, Tremblay JC, et al. The 2018 Global Research Expedition on Altitude Related Chronic Health (Global REACH) to Cerro de Pasco, Peru: an Experimental Overview. *Exp Physiol.* 2020.
26. Mosteller RD. Simplified calculation of body-surface area. *N Engl J Med.* 1987;317(17):1098.
27. Bertini M, Sengupta PP, Nucifora G, et al. Role of left ventricular twist mechanics in the assessment of cardiac dyssynchrony in heart failure. *JACC Cardiovasc Imaging.* 2009;2(12):1425-1435.
28. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of

Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging.* 2015;16(3):233-270.

29. Vanderpool RR, Naeije R. Hematocrit-corrected Pulmonary Vascular Resistance. *Am J Respir Crit Care Med.* 2018;198(3):305-309.
30. Foster GE, Ainslie PN, Stembridge M, et al. Resting pulmonary haemodynamics and shunting: a comparison of sea-level inhabitants to high altitude Sherpas. *J Physiol.* 2014;592(6):1397-1409.
31. Auerbach M, Adamson JW. How we diagnose and treat iron deficiency anemia. *Am J Hematol.* 2016;91(1):31-38.
32. Groepenhoff H, Overbeek MJ, Mulè M, et al. Exercise pathophysiology in patients with chronic mountain sickness exercise in chronic mountain sickness. *Chest.* 2012;142(4):877-884.
33. de Bisschop C, Kiger L, Marden MC, et al. Pulmonary capillary blood volume and membrane conductance in Andeans and lowlanders at high altitude: a cross-sectional study. *Nitric Oxide.* 2010;23(3):187-193.
34. Schwab M, Jayet PY, Stuber T, et al. Pulmonary-artery pressure and exhaled nitric oxide in Bolivian and Caucasian high altitude dwellers. *High Alt Med Biol.* 2008;9(4):295-299.
35. Peñaloza D, Sime F, Banchero N, Gamboa R, Cruz J, Marticorena E. Pulmonary hypertension in healthy men born and living at high altitudes. *Am J Cardiol.* 1963;11(2):150-157.
36. Rotta A, Cánepa A, Hurtado A, Velásquez T, Chávez R. Pulmonary circulation at sea level and at high altitudes. *Journal of Applied Physiology.* 1956;9(3):328-336.
37. Arias-Stella J, Saldana M. The terminal portion of the pulmonary arterial tree in people native to high altitudes. *Circulation.* 1963;28:915-925.
38. Lockhart A, Zelter M, Mensch-Dechene J, et al. Pressure-flow-volume relationships in pulmonary circulation of normal highlanders. *J Appl Physiol.* 1976;41(4):449-456.
39. Grover R. Chronic hypoxic pulmonary hypertension. In: Fishman AP, ed. *The pulmonary circulation: normal and abnormal*. Philadelphia: University of Pennsylvania Press; 1990:283-299.
40. Villafuerte FC. New genetic and physiological factors for excessive erythrocytosis and Chronic Mountain Sickness. *J Appl Physiol (1985).* 2015;119(12):1481-1486.
41. Ge RL, Mo VY, Januzzi JL, et al. B-type natriuretic peptide, vascular endothelial growth factor, endothelin-1, and nitric oxide synthase in chronic mountain sickness. *Am J Physiol Heart Circ Physiol.* 2011;300(4):H1427-1433.
42. Julian CG. Epigenomics and human adaptation to high altitude. *J Appl Physiol (1985).* 2017;123(5):1362-1370.
43. Ang SO, Chen H, Hirota K, et al. Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. *Nat Genet.* 2002;32(4):614-621.
44. Frise MC, Cheng HY, Nickol AH, et al. Clinical iron deficiency disturbs normal human responses to hypoxia. *J Clin Invest.* 2016;126(6):2139-2150.
45. Wallace DF, Subramaniam VN. Co-factors in liver disease: the role of HFE-related hereditary hemochromatosis and iron. *Biochim Biophys Acta Gen Subj.* 2009;1790(7):663-670.

46. Wessling-Resnick M. Iron homeostasis and the inflammatory response. *Annu Rev Nutr.* 2010;30:105-122.
47. Villafuerte FC, Macarlupú JL, Anza-Ramírez C, et al. Decreased plasma soluble erythropoietin receptor in high-altitude excessive erythrocytosis and Chronic Mountain Sickness. *J Appl Physiol (1985).* 2014;117(11):1356-1362.
48. De Ferrari A, Miranda JJ, Gilman RH, et al. Prevalence, clinical profile, iron status, and subject-specific traits for excessive erythrocytosis in Andean adults living permanently at 3,825 meters above sea level. *Chest.* 2014;146(5):1327-1336.
49. Van Geen A, Bravo C, Gil V, Sherpa S, Jack D. Lead exposure from soil in Peruvian mining towns: a national assessment supported by two contrasting examples. *Bull World Health Organ.* 2012;90:878-886.
50. Health Canada. Final Human Health State of the Science Report on Lead. Ottawa: Minister of Health; 2013.
51. Childebayeva A, Goodrich JM, Chesterman N, et al. Blood lead levels in Peruvian adults are associated with proximity to mining and DNA methylation. *Environ Int.* 2021;155:106587.
52. Kwong WT, Friello P, Semba RD. Interactions between iron deficiency and lead poisoning: epidemiology and pathogenesis. *Sci Total Environ.* 2004;330(1-3):21-37.
53. Hosseinirad H, Shahrestanaki JK, Moosazadeh Moghaddam M, Mousazadeh A, Yadegari P, Afsharzadeh N. Protective Effect of Vitamin D3 Against Pb-Induced Neurotoxicity by Regulating the Nrf2 and NF-κB Pathways. *Neurotox Res.* 2021;39(3):687-696.
54. Harada N, Kanayama M, Maruyama A, et al. Nrf2 regulates ferroportin 1-mediated iron efflux and counteracts lipopolysaccharide-induced ferroportin 1 mRNA suppression in macrophages. *Arch Biochem Biophys.* 2011;508(1):101-109.
55. Brewster LM, Bain AR, Garcia VP, et al. Global REACH 2018: dysfunctional extracellular microvesicles in Andean highlander males with excessive erythrocytosis. *Am J Physiol Heart Circ Physiol.* 2021;320(5):H1851-h1861.

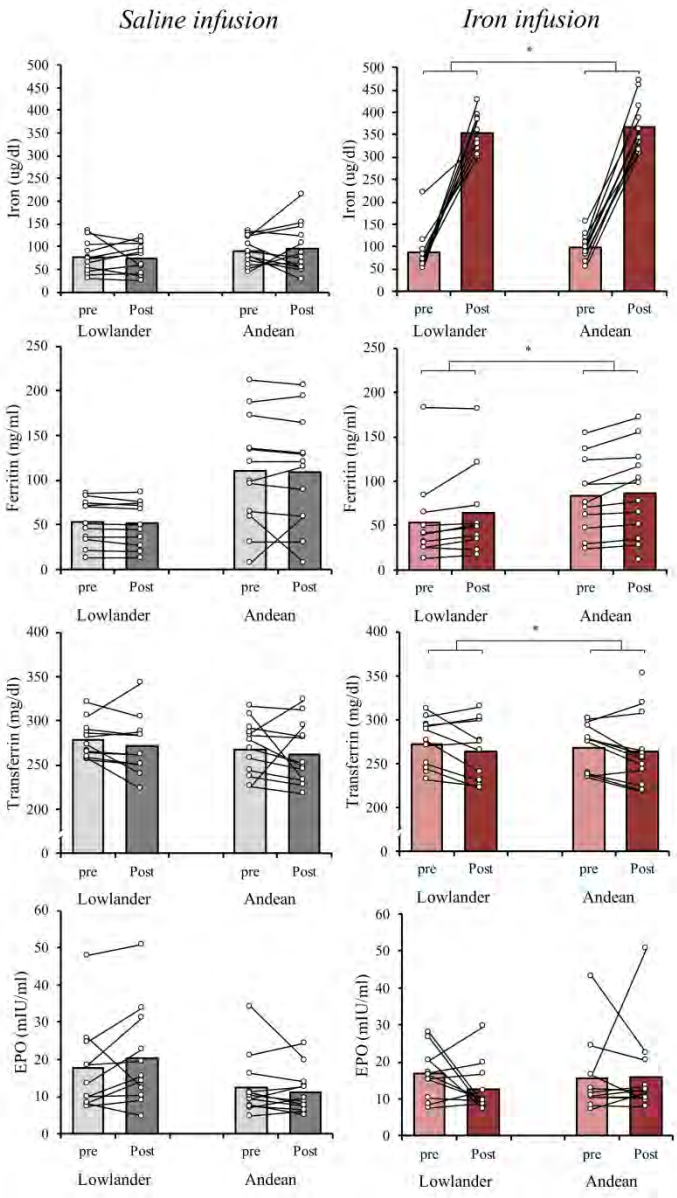

Figure 1. Impact of saline (grey bars) or iron infusion (red bars) on iron metrics in lowlanders and healthy Andeans. *P<0.05 main effect of time.

535x884mm (197 x 197 DPI)

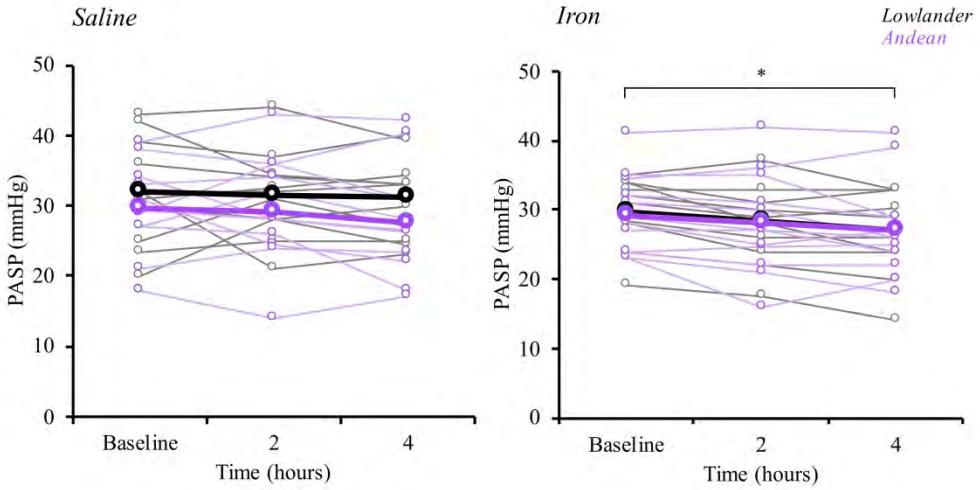

Figure 2. Impact of saline and iron infusion on the PASP in lowlanders and healthy Andeans. Horizonal bar with asterisk denotes a main effect significance of time (*P<0.05).

488x248mm (197 x 197 DPI)

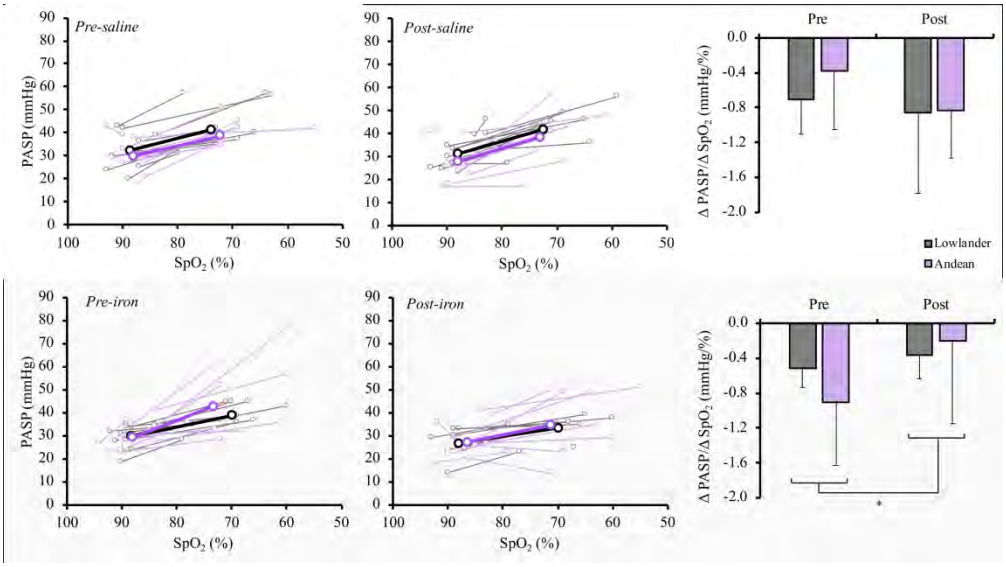

Figure 3. Pulmonary vascular reactivity to hypoxia in lowlanders and Andeans receiving either saline or iron infusion. * main effect P<0.05.

818x456mm (121 x 121 DPI)

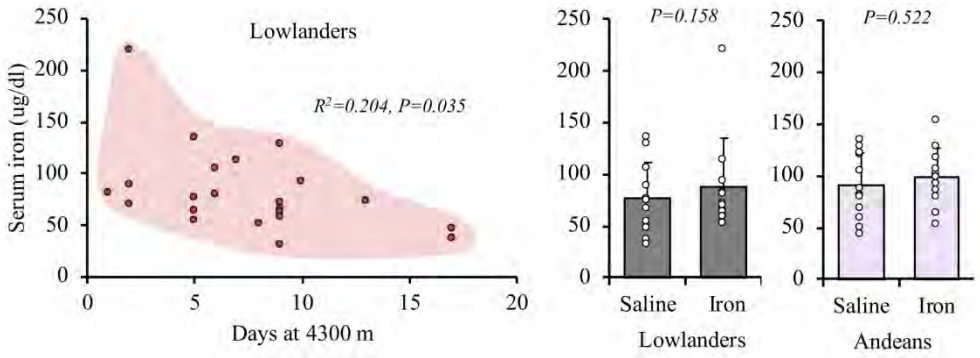

Figure 4. Baseline serum iron measurements in lowlanders and Andeans. Lowlander data is presented as 1) to correspond to the number of days at 4300 m, before being tested (with linear regression; left panel) and 2) highlight the equal weighting of iron status between the saline and iron groups (with unpaired t-test; middle panel). Andean data demonstrates iron levels in the saline and iron groups at baseline were comparable (with unpaired t-test; right panel).

553x208mm (118 x 118 DPI)