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Title: Global Reach 2018: The adaptive phenotype to life with chronic mountain sickness and polycythaemia

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List of Abbreviations:

$a\text{-}vO_{2Diff}$	arterial venous oxygen difference
CaO_2	arterial oxygen content
CMS	chronic mountain sickness
CMS-	Andeans without chronic mountain sickness
CMS+	Andeans with chronic mountain sickness
CO	cardiac output
CvO_2	venous oxygen content
DO_2	oxygen delivery
FBF	forearm blood flow
FVC	forearm vascular conductance
FVR	forearm vascular resistance
Hb	haemoglobin
HGE	handgrip exercise
HR	heart rate
MAP	mean arterial pressure
MBV	mean blood velocity
MSNA	muscle sympathetic nervous activity
MSS	mean shear stress
MVC	maximum voluntary contraction
PaO_2	partial pressure of oxygen
SaO_2	arterial oxygen saturation
SpO_2	oxygen saturation pulse oximetry
$\dot{V}O_2$	oxygen consumption

Key Point Summary

- Humans suffering from polycythaemia undergo multiple circulatory adaptations including changes in blood rheology and structural and functional vascular adaptations to maintain normal blood pressure and vascular shear stresses, despite high blood viscosity.

- During exercise, several circulatory adaptations are observed, especially involving adrenergic and non-adrenergic mechanisms within non-active and active skeletal muscle to maintain exercise capacity, which is not observed in animal models.

- Despite profound circulatory stress, i.e., polycythaemia, several adaptations can occur to maintain exercise capacity, therefore making early identification of the disease difficult without overt symptomology.

- Pharmacological treatment of the background heightened sympathetic activity may impair the adaptive sympathetic response needed to match local oxygen delivery to active skeletal muscle oxygen demand and therefore inadvertently impair exercise capacity.

Abstract

Excessive haematocrit and blood viscosity can increase blood pressure, cardiac work and reduce aerobic capacity. However, past clinical investigations have demonstrated that certain human high-altitude populations suffering from excessive erythrocytosis, Andeans with chronic mountain sickness, appear to have phenotypically adapted to life with polycythaemia, as their exercise capacity is comparable to healthy Andeans and even with sea level inhabitants residing at high altitude. By studying this unique population, which has adapted thru natural selection, this study aimed to describe how humans can adapt to life with polycythaemia. Experimental studies included Andeans with (n=19) and without (n=17) chronic mountain sickness, documenting exercise capacity, and characterizing the transport of oxygen thru blood rheology, including haemoglobin mass, blood and plasma volume & blood viscosity, cardiac output, blood pressure and changes in total and local vascular resistances thru pharmacological dissected of α-adrenergic signalling pathways within non-active and active skeletal muscle. At rest, Andeans with chronic mountain sickness had a substantial plasma volume contraction, which alongside a higher red blood cell volume, caused an increase in blood viscosity yet similar total blood volume. Moreover, both morphological and functional alterations in the periphery normalized vascular shear stress and blood pressure despite high sympathetic nerve activity. During exercise, blood pressure, cardiac work and global oxygen delivery increased similar to healthy Andeans but were sustained by modifications in both non-active and active skeletal muscle vascular function. These findings highlight widespread physiological adaptations that can occur in response to polycythaemia, which allow the maintenance of exercise capacity.

Introduction

Exercise fatigue and reduced exercise capacity is common in several disorders associated with polycythaemia, including polycythaemia vera (Scherber *et al.*, 2016), Chuvash polycythaemia (Formenti *et al.*, 2010), and chronic mountain sickness (Monge *et al.*, 1989). These observations of reductions in exercise capacity are in line with an optimal haematocrit hypothesis (Schuler *et al.*, 2010), whereby exercise capacity is improved as haematocrit increases, until a tipping point (~57-68%), where excessive haematocrit and blood viscosity impairs convective and diffusive oxygen transport while simultaneously augmenting vascular resistance, systemic blood pressure and cardiac work (Letcher *et al.*, 1981). Moreover, elevations in haematocrit and blood viscosity may alter capillary haemodynamics and oxygen offloading due to a reduction in oxygen diffusivity according to Fick's first law of diffusion (Østergaard, 2020). While an attractive hypothesis, several studies indirectly suggest that adaptive mechanisms exist to maintain oxygen transport in the face of a high haematocrit (Lindenfeld *et al.*, 1985; Juvonen *et al.*, 1991). Indeed, a clinical investigation has challenged the conventional wisdom by documenting normal maximal aerobic capacities (~32 $ml \cdot kg \cdot min^{-1}$) in Andeans diagnosed with mild to moderate chronic mountain sickness compared not only to healthy Andean controls, but also to unacclimatized lowlanders to Cerro de Pasco (~4300m), who were on average 15 years younger (Hb: 15 $g \cdot dL^{-1}$) (Groepenhoff *et al.*, 2012). These data provides strong evidence that an adaptive phenotype to polycythaemia exists; whereby exercise capacity (Groepenhoff *et al.*, 2012) and systemic blood pressure (Simpson *et al.*, 2020) can be maintained despite a high haematocrit.

The present study aimed to explore several phenotypical adaptations related to the oxygen transport cascade and blood pressure regulation that might be amenable to adaptation including blood rheology [including haemoglobin mass, plasma and blood volumes & blood viscosity] and systemic haemodynamics that facilitate convective and diffusive oxygen transport. Moreover, recently attention has shifted away from the conventional focus of pulmonary and haematological factors governing acclimatization/adaptation, towards more specific vascular factors that promote elevations in blood flow and reduced vascular resistance, at least among Tibetans (Erzurum *et al.*, 2007). Our emphasis also focused on vascular function including direct

measurements of sympathetic nerve activity, and pharmacological dissection of α-adrenergic signalling pathways that control vascular resistance in non-active and active skeletal muscle. Using a comprehensive and multifaceted array of physiological techniques, we demonstrate phenotypical adaptions, both on a systemic and mechanistic level, that occur in a unique population of polycythaemia that have normal exercise capacities.

Ultimately, we sought to address four questions in high altitude natives with and without polycythaemia, yet normal maximal exercise capacity: Firstly, does polycythaemia associated with chronic hypoxia lead to changes in blood rheology, specifically in terms of total blood volume and its constituents plasma and red cell volume that may impact blood viscosity and its influence on cardiovascular function. Secondly, are there cardiovascular adaptations to polycythaemia that preserve blood pressure regulation, with a focus on the autonomic nervous system, vascular morphology and resistance at rest. Thirdly, during moderate intensity exercise, do adaptations in autonomic control of vascular resistance in non-active skeletal muscle support the distribution of cardiac output and oxygen delivery. Fourthly, a focus on the active skeletal muscle and if α-adrenergic receptor signalling is altered to optimize local muscle blood flow and oxygen delivery during forearm handgrip exercise.

Materials and Methods

The Clinical Research Ethics Board at the University of British Columbia (CREB ID: H18-01404) and the Universidad Peruana Cayetano Heredia Comité de Ética (CIEH-UPCH #101686) approved all experimental procedures and protocols in adherence with the principles of the Declaration of Helsinki (except registration in a database). All participants provided verbal and written informed consent before participation in this study. Andean participants were provided with a translated consent form and a Spanish translator thoroughly explained the experimental protocol prior to consent. This investigation was part of the Global REACH International Expedition to Peru between June and July 2018 (Tymko *et al.*, 2020a). Several participants volunteered for multiple studies conducted at the high altitude laboratory located in Cerro de Pasco, Peru (~4300m). However, the current investigation was performed prior to or at least 24 hours after participation in any other study that would alter resting haemodynamics. All datasets generated during and/or analysed within the current study are available from the corresponding authors upon request.

Participants

Thirty-six Andean (Quechua origin) males born and permanently residing at an altitude ≥4000 m, who had at least two previous known generations of high-altitude Andean ancestry, were recruited for the current investigation. None of the participants had a history of working in the mining industry, had not travelled to an altitude lower than <2500 m in the previous six months and were not taking any prescribed or over-the-counter medication before or during participation. All 36 participants attended the laboratory on at least two occasions for 1) assessment of chronic mountain sickness (CMS) using the Qinghai CMS questionnaire (Leon-Velarde *et al.*, 2005) (described below), and haematological parameters and 2) assessment of maximal cardiopulmonary exercise capacity, which were separated by a minimum of 24 hours. In addition, a subset of seven CMS+ (age: 41±13 yrs.; weight: 77±13 kg; height: 163±3 cm) and nine CMS- (age: 44±15 yrs.; weight: 62±7 kg; height: 159±4 cm) attended the laboratory on a third occasion for pharmacological testing and assessment of blood pressure regulation. Only male Andeans were recruited for the current investigation to avoid the confounding effects of

age, as CMS primarily affects post-menopausal women (Monge *et al.*, 1989; Leon-Velarde *et al.*, 2005; Villafuerte & Corante, 2016). All subject characteristics are presented in Table 1.

Experimental protocol

Experimental Protocol 1: Qinghai CMS assessment questionnaire, haematological parameters and aerobic capacity.

The presence and severity of chronic mountain sickness (CMS) was assessed using the Qinghai CMS assessment questionnaire (Leon-Velarde *et al.*, 2005), which assesses eight signs and symptoms of CMS, as agreed by an international consensus (Leon-Velarde *et al.*, 2005); Breathlessness, Sleep disturbance, Cyanosis, Dilatation of veins, Paraesthesia, Headache, Tinnitus and presence of excessive erythrocytosis (defined as ≥21 $g \cdot dL^{-1}$). Each symptom is rated from 0 (i.e. absent of symptom) to 3 (i.e. severe symptom), and the presence of excessive erythrocytosis adds three to the cumulative score. The sum of scores constitutes the CMS score, and CMS is defined as Absent (0-5) Mild (6-10), Moderate (11-14) or Severe (>15) (Leon-Velarde *et al.*, 2005). The overall objective of the study was to examine potential adaptations to polycythaemia. Thus, persons with mild CMS were sought. That being said, several subjects had what could be described as excessive erythrocytosis (Hb ≥ 21 $g \cdot dL^{-1}$) and polycythaemia, but no or very few symptoms, which could be considered as "healthy" adaptation at ~4300m. Collectivelly, the sample included 17 healthy Andeans without chronic mountain sickness (CMS-; age: 44±15 yrs.; weight: 70±12 kg; height: 161±6 cm). 19 Andeans had a Hb ≥ 21 $g \cdot dL^{-1}$ (chronic mountain sickness (CMS+; age: 42±14 yrs.; weight: 68±11 kg; height: 161±6 cm) and of these n=13 had clinically defined CMS. Of the total sample n=5 Andeans as smokers. Haematological parameters (i.e. haemoglobin mass, total blood and plasma volume) were determined using the carbon monoxide rebreathing method, as previously described elsewhere (Schmidt & Prommer, 2005; Stembridge *et al.*, 2019), and scaled to body weight. Haematocrit was assessed via centrifugation, as previously described elsewhere (Schmidt & Prommer, 2005; Stembridge *et al.*, 2019). Assessment of maximal aerobic power and maximal hand-grip voluntary contraction.

All 36 Andean participants performed a stepwise cardiopulmonary exercise test on a custom-built semi-recumbent cycle ergometer (Corival Pediatric, Lode; Lode B.V., Groningen,

The Netherlands) until volitional exhaustion. Following a two-minute warmup (20 watts), the test began at 40 watts and increased by 20 watts every minute. Additionally, in the seven CMS+ and nine CMS- participants who underwent additional pharmacological testing, maximal voluntary hand-grip contraction (MVC) of the non-dominant arm was determined using a grip force transducer (MLT004/ST, ADInstruments, Sydney, Australia). MVC was taken as the average value of three isometric MVC's and used to calculate 5, 15 and 25% of the participants' MVC for use Visit 3 (described in the Experimental protocol section below).

Sub-study experimental protocol.

Experimental Protocol 2: Factors effecting resting blood pressure and vascular tone.

The aim of protocol 2 was to determine the sympathetic influence on resting blood pressure and tonic vascular tone within CMS- and CMS+. Resting blood flow was measured as a control baseline, whereby vascular resistance and conductance was subsequently calculated. During this period, resting blood samples were extracted from both the arterial and venous catheters for haematological assessments (i.e., assessment of partial pressure of arterial oxygen, blood viscosity, arterial and venous oxygen content, and oxygen delivery and extraction). Following baseline and control measurements, localized propranolol hydrochloride (β-adrenergic receptor antagonist; West-ward Pharmaceutical Corp. Eatontown, NJ, USA) was infused at a rate of 10 $\mu g \cdot dL^{-1} \cdot FAV^{-1} \cdot min^{-1}$ over five minutes (loading dose) and maintained at five $\mu g \cdot dL^{-1} \cdot FAV^{-1} \cdot min^{-1}$ throughout the remainder of the pharmacological trial (Richards *et al.*, 2014) to control for any direct or indirect β-adrenergic vasodilatory effects of the study drugs (Torp *et al.*, 2001). After which selective α_1-adrenergic receptor agonist phenylephrine (PE) was infused at three graded doses (PE; 0.0625 (low), 0.125 (medium), 0.250 (high) $\mu g \cdot dL^{-1} \cdot FAV^{-1} \cdot min^{-1}$; Hospira Healthcare Corp. Montreal, Quebec, Canada) each for a period of three-minutes (Dinenno & Joyner, 2003), to isolate post-junctional α_1-adrenergic signalling. While increases in sympathetic nervous activity releases neuropeptide Y, adenosine triphosphate, along with norepinephrine (Holwerda *et al.*, 2015), norepinephrine is the primary neurotransmitter evoking vasoconstriction during rest and exercise (Buckwalter & Clifford, 1999; Fairfax *et al.*, 2013). Therefore, phenylephrine is the most controlled and reliable way to isolate α_1-adrenergic responsiveness and vasoconstrictor signalling. After a wash out period, non-selective α-adrenergic antagonist

phentolamine (Sandoz Inc, Princeton, NJ, USA) was infused at a rate of 12 $\mu g \cdot dL^{-1} \cdot FAV^{-1} \cdot min^{-1}$ over five minutes (loading dose) and maintained at five $\mu g \cdot dL^{-1} \cdot FAV^{-1} \cdot min^{-1}$ (Torp *et al.*, 2001; Richards *et al.*, 2014) alongside β-adrenergic receptor antagonist, to allow complete α-adrenergic blockade (+α-β blockade). Drug efficacy has been previously described and demonstrated elsewhere (Dinenno *et al.*, 2002b; Richards *et al.*, 2014).

Experimental Protocol 3: Steady-state dynamic lower limb exercise.

The aim of protocol 3 was to determine the contributing mechanism for vasoconstrictor signalling within inactive skeletal muscle blood flow during moderate intensity leg cycling exercise. Participants were instructed to cycle at 60% total peak workload for three minutes, whereby blood flow in the brachial artery was assessed during the final one-minute of exercise. This intensity and duration were chosen to induce a significant increase in sympathetic nervous activity (Saito *et al.*, 1993; Moralez *et al.*, 2018) and vasoconstriction (Taylor *et al.*, 1992; Padilla *et al.*, 2011), whilst avoiding a secondary rise in skin blood flow with prolonged exercise (Simmons *et al.*, 2011). The exercise protocol was then repeated with localized +α-β blockade.

Experimental Protocol 4: Forearm handgrip exercise.

Lastly, the aim of protocol 4 was to investigate the mechanism(s) of vasoconstrictor signalling of active skeletal muscle blood flow during graded hand-grip exercise. Participants performed continuous dynamic handgrip/forearm contractions with a duty cycle of one-second contraction and two-second relaxation (Richards *et al.*, 2014) at 5, 15, and 25% MVC for three-minutes. Both audio and visual cues were provided to ensure correct timing and force output. Handgrip exercise at 5% MVC does not blunt α-adrenergic signalling whereas 15% attenuates, but does not abolish, α-adrenergic signalling (Tschakovsky *et al.*, 2002; Kirby *et al.*, 2005; Hearon *et al.*, 2016; Hearon *et al.*, 2020) whereas using 25% MVC indicates a moderate exercise intensity to elicit elevated blood flow hyperaemia. Blood flow in the brachial artery was measured at the final one-minute of each stage. Arterial blood samples were taken at baseline with venous blood samples taken during the last 30-seconds of each stage. Exercise was then repeated with localized +α-β blockade. All exercise and infusion protocols were separated with an appropriate

amount of time (i.e., 30 minutes) to re-establish resting baseline haemodynamics and drug washout.

Arterial and venous catheterization, haemodynamic and haematological measurements

Under local anaesthesia (2% lidocaine) and using ultrasound guidance a 20-gauge, 7.6cm catheter (Arrow, Markham, ON, Canada) was inserted under sterile conditions into the brachial artery of the non-dominant arm for administration of pharmacological agents (PhD Ultra Syringe Pumps, Harvard Apparatus, Holliston, MA, USA). The catheter was connected to an arterial blood sampling kit (VAMP adult, Edwards Lifescience, Irvine, CA) for repeated arterial blood sampling and flushing (0.9% saline). The blood sampling kit was also connected to a blood pressure amplifier (ADInstruments, FE117, Sydney, Australia) that was calibrated (i.e. zeroed) at the level of the right atrium and fourth intercostal space, for continuous measurements of mean arterial blood pressure (MAP), from which systolic (SAP) and diastolic (DAP) arterial pressure were derived (Richards *et al.*, 2014; Hearon *et al.*, 2016). Additionally, a 20-gauge venous catheter was inserted retrograde into the antecubital vein for deep venous blood sampling. Arterial and venous blood samples were extracted using 10ml safePICO syringes and immediately analysed via ABL90 FLEX blood gas analyser (Radiometer Medical ApS, Brønshøi, Denmark), to determine arterial (CaO_2) and venous (CvO_2) oxygen content, using previously described methods (Nyberg *et al.*, 2018). Heart rate was continuously measured via three-lead electrocardiogram (ADInstruments, Sydney, Australia). Stroke volume (SV) and cardiac output ($\dot{Q}_C$) were estimated from the arterial waveform, via the Model-flow technique (LabChart NICO extension version 8.1, ADInstruments, Sydney, Australia) (Hill *et al.*, 2013). Using $\dot{Q}_C$ and SAP, we calculated cardiac work.

Muscle sympathetic nerve activity

Resting multiunit muscle sympathetic nerve activity (MSNA) was recorded from the radial nerve via microneurography (GM), using the standardized ultrasound-guided technique (Curry & Charkoudian, 2011; Moralez *et al.*, 2018). Raw nerve signals were amplified (100x pre-amplifier and variable gain isolated amplifier, Neuroamp Ex, ADInstruments, Sydney, Australia) band pass filtered (300-2000 Hz), rectified and integrated (time decay constant 0.1s) (LabChart Pro V8.3.1, ADInstruments, Sydney, Australia). Multiunit bursts of MSNA were

identified using standardized guidelines (i.e. 3:1 signal to noise ratio) by a single trained observer (GM) (Macefield, 2013; White *et al.*, 2015). Resting MSNA was quantified as burst frequency (bursts·min^{-1}) and burst incidence (bursts·100heartbeats^{-1}).

Forearm haemodynamics

Mean blood velocity (MBV) and diameter of the brachial artery were measured via a 12 MHz pulsed Doppler probe (Vivid 7, General Electric, Milwaukee, WI, USA) and used to calculate forearm blood flow (FBF). All ultrasound measurements were performed by a single qualified sonographer (C.M.H). Subsequently, forearm vascular resistance (FVR) and forearm vascular conductance (FVC) were calculated, as described previously (Crecelius *et al.*, 2010). To limit the influence of skin blood flow to the estimation of skeletal muscle blood flow through the brachial artery, skin temperature and skin conductance were continuously monitored using a laser Doppler monitor (moorVMS-LDF), and the forearm skin was cooled using a fan (Richards *et al.*, 2014; Limberg *et al.*, 2020), in accordance with current guidelines for the assessment of resistance vessel function during exercise and pharmacological infusions (Limberg *et al.*, 2020). Forearm skeletal muscle DO_2 and $\dot{V}O_2$ were calculated (DO_2 = [FBF x CaO_2]/100 & $\dot{V}O_2$ = FBF x [CaO_2 – CvO_2]/100) and expressed as ml·min^{-1}, and arterial-venous oxygen difference was calculated as a-vO_{2Diff} = CaO_2 - CvO_2 and expressed as a ml·dL^{-1}. Venous whole blood viscosity was measured in duplicate at a shear rate of 225 s^{-1} at 37ºC using a cone and plate viscometer (DV2T Viscometer, Brookfield Amtek, USA) and circulating heating water bath (TC-150, Brookfield Amtek, USA) and used to calculate mean shear stress [MSS = (4 x (viscosity/100) x (MBV/diameter) along with antegrade and retrograde shear stress and expressed as dyne·cm^{-2} (Tremblay *et al.*, 2019b). Neurovascular transduction was calculated as a quotient between FVR and MSNA burst frequency (FVR/burst frequency) and expressed as mmHg·ml^{-1}·min^{-1}/burst^{-1}·min^{-1}.

Data acquisition.

All cardiovascular variables were sampled at 1-KHz via an analogue-to-digital converter (Powerlab, 16/30: ADInstruments, Sydney, Australia), displayed on LabChart (version 8.1; ADInstruments, Sydney, Australia) and analysed offline.

Experimental protocol 1

Breath-by-breath respiratory measures were collected throughout the exercise protocol (Oxycon Mobile, Carefusion, San Diego, CA, USA). Peak oxygen consumption ($\dot{V}O_2$ max) was calculated as the highest oxygen uptake over a 30-sec average and expressed as both absolute (i.e. $ml \cdot min^{-1}$) and relative (i.e. $ml \cdot kg^{-1} \cdot min^{-1}$) values. Total peak workload was used to calculate 60% total peak workload for Visit 3.

Experimental protocol 2

The vasoconstrictor response to graded phenylephrine was quantified as both absolute and relative changes in FBF and FVC, because MAP did not change during infusion of the α-agonist (Padilla *et al.*, 2011). Resting vascular tone was quantified as absolute changes in FBF, FVC, and MSS. Due to α+β-adrenergic blockade causing a localized vasodilatory response, it was most reliable to quantify this change as FBF and FVC. Absolute changes in shear stress quantifies the interaction relationship of blood viscosity and FBF. Therefore, the absolute and relative vasoconstrictor responsiveness to graded phenylephrine quantification was termed as:

$$\Delta FBF = (FBF_{\text{Phenylephrine dose1, 2, 3}} - FBF_{\text{Baseline}})$$

$$\%\Delta FBF = ((FBF_{\text{Phenylephrine dose1, 2, 3}} - FBF_{\text{Baseline}})/FBF_{\text{Baseline}})*100$$

$$\Delta FVC = (FVC_{\text{Phenylephrine dose1, 2, 3}} - FVC_{\text{Baseline}})$$

$$\%\Delta FVC = ((FVC_{\text{Phenylephrine dose1, 2, 3}} - FVC_{\text{Baseline}})/FVC_{\text{Baseline}})*100$$

Moreover, the absolute vasodilatory response to α+β-adrenergic blockade quantification was termed as:

$$\Delta FBF = (FBF_{\alpha+\beta \text{ blockade}} - FBF_{\text{control}})$$

$$\Delta FVC = (FVC_{\alpha+\beta \text{ blockade}} - FVC_{\text{control}})$$

Experimental Protocol 3

The vasoconstrictor response to cycle exercise-induced vasoconstriction in the inactive forearm was quantified as changes in both FVC and FVR. The change in FVR was the primary variable of interest because MAP was elevated during cycle exercise compared to rest. Absolute

and relative change in FVR were calculated to account for baseline differences in FVR after α+β blockade. Therefore, the increase in FVR during cycling exercise was calculated in accordance with our groups previous work (Hansen *et al.*, 2020).

Experimental Protocol 4

Forearm vasodilation was the primary variable changing during HGE with a minor impact on MAP; therefore, forearm vasodilation is most reliably quantified as a change in FVC (Richards *et al.*, 2014). The vasodilatory response to HGE was quantified as a change in FVC from rest to each MVC intensity in both control and after α+β blockade. The vasodilatory response was quantified in absolute terms as:

$$\Delta FVC = (FVC_{5,\ 15,\ 25\%\ HGE} - FVC_{Baseline\ HGE})$$

Statistical analysis

Experimental protocol 1:

Haematological, CMS scores and exercise parameters we analysed using un-paired *t*-tests (n=17 CMS-vs. n=19 CMS+). Moreover, to confirm these results were not biased by including some individuals with high erythrocytes but minimal symptomology, a second analysis including only those with clinically diagnosed CMS was performed (n=17 CMS-vs. n=13 CMS+). As the findings remained similar (see results), figure 2 was developed form the entire data set. Relationships between haematological indices and $\dot{V}O_2$ max were examined by Pearson correlation coefficient.

Experimental protocol 2: All hemodynamic, haematological, resting and exercising vascular tone data were analysed using a combination of two-tailed matched paired (CMS-/CMS+ x control vs +α-β blockade) and un-paired (Control/+α-β blockade x CMS- vs. CMS+) *t*-tests. To assess the effect of graded PE infusions on both FBF and FVC we used a one-way repeated measures analyses of variance (ANOVA) (CMS-/CMS+ vs. three PE doses) with multiple comparisons using Fisher's least significant difference (LSD) test (BL vs PE1/PE2/PE3). Absolute and percent changes for both FBF and FVC during graded PE infusion were analysed using a two-way mixed-effects repeated measures ANOVA with multiple

comparisons using Fisher's LSD test (CMS- vs. CMS+ x three PE doses). To assess the effect of +α-β blockade on resting FBF, FVC, and MSS we used a two-way repeated measures ANOVA with multiple comparisons using Fisher's LSD test (Control vs. +α-β blockade x CMS-/CMS+). Absolute changes in FBF, FVC, and MSS were analysed using a two-tailed un-paired *t*-tests (CMS- vs. CMS+).

Experimental protocol 3: To assess the absolute and percent changes during cycling induced sympathetic restraint within inactive skeletal muscle with and without +α-β blockade, we used a two-way repeated measures ANOVA with multiple comparisons using Fisher's LSD test (Control vs. +α-β blockade x CMS-/CMS+). Haemodynamic comparisons between groups during cycling exercise was analysed using a two-tailed un-paired *t*-tests (CMS- vs CMS+).

Experimental protocol 4: To assess the vasoconstrictor signalling and haematological differences with and without +α-β blockade in active skeletal muscle during HGE we used a two-way repeated measures ANOVA with multiple comparisons using Fisher's LSD test (CMS-/CMS+ x control vs. +α-β blockade x 5%/15%/25% MVC). Absolute change in vasoconstrictor signalling and haematological differences between groups during HGE was analysed using a two-way repeated measures ANOVA with multiple comparisons using Fisher's LSD test (Control/+α-β blockade x CMS- vs. CMS+ x 5%/15%/25%).

All statistical analyses were performed using Prism GraphPad (Version 8.4.2, GraphPad Software, San Diego, USA) and were reported as mean ± standard deviation (SD) unless stated otherwise. Statistical significance was set at $P<0.05$.

Results

Polycythaemia and the diagnosis of chronic mountain sickness.

Haemoglobin concentration and haematocrit were higher (P<0.001) in CMS+ than CMS- (Figure 2A). As the measurement of circulating haemoglobin concentration is affected by plasma volume, we also measured total haemoglobin mass to derive red blood cell volume, plasma volume and total blood volume. Andeans with CMS+ had a higher haemoglobin mass (P=0.022), and red blood cell volume (P=0.030), but substantially reduced plasma volume (P=0.022) compared to Andeans without CMS, which resulted in a normalization of total blood volume (P=0.434) between groups (Figure 2B & C, Table 1). When only individuals with the clinical definition of CMS were used, the pattern of these results remained consistent for Hb (P<0.0001), Hct (P<0.0001), plasma volume (P=0.061) and Hb mass (P=0.048) with total blood volume remaining similar (P=0.635). In summary, these results quantified a mild to moderate severity of CMS symptoms within our volunteers, along with an exaggerated erythrocytosis, degree of hypoxemia, and confirming trends from previous research (Claydon *et al.*, 2004; Simpson *et al.*, 2020), a statistically lower plasma volume in CMS+ versus healthy Andeans.

Assessment of maximal aerobic capacity.

To confirm our model of polycythaemia but preserved aerobic exercise capacity, healthy Andeans and CMS+ performed a stepwise cardiopulmonary exercise test on a semi-recumbent cycle ergometer. Our results indicate that healthy Andeans and CMS+ have a similar level of aerobic fitness as indicated by power output (P=0.105), and absolute (P=0.213) and relative maximal oxygen uptakes (P=0.592) (Figures 2H, I & J). The heart rate response (P=0.591) and oxygen saturation (P=0.850) at peak exercise did not differ between healthy Andeans and CMS+ (Figures 2k & L). While previous data indicates an “optimal haematocrit” exists for aerobic capacity in animal models (Schuler *et al.*, 2010) we found no such relationship despite a wide range of haematocrits (R^2=0.061; P=0.148), *in vivo* blood viscosities (R^2=0.052; P=0.180), total haemoglobin mass (R^2=0.070; P=0.124), or haemoglobin (R^2=0.024; P=0.364, Figures 2M-P) in humans. As previously mentioned, the CMS group included 5 Andeans with high haemoglobin concentration and haematocrit, but a borderline CMS score. Nevertheless, when these individuals were removed, power output (P=0.172), absolute (P=0.320) and relative (P=0.704) aerobic

capacity remained similar with blood viscosity significantly higher in CMS+ ($P<0.0001$). These data confirm that while individuals with CMS+ have lower plasma volumes, higher haematocrits and increased blood viscosity (Figures 2A & 3E),their cardiorespiratory fitness is well maintained.

Assessment of resting blood pressure and vascular shear stress.

Hypoxemia and polycythaemia may effect arterial blood pressure through sympathetic hyperactivity (Tymko *et al.*, 2020b), increased peripheral vascular resistance, decreased endothelial function via altered vascular shear stress (de Simone *et al.*, 1990) or increased adrenergic signalling, all of which increase the risk of hypertensive disease (Corante *et al.*, 2018). As reported previously by our group using the microneurographic technique (Tymko *et al.*, 2020b), MSNA burst frequency ($P=0.035$) and burst incidence ($P=0.013$) were higher in the semi-recumbent position in CMS+ compared to healthy Andeans (Figures 3A-C). Yet despite this background of sympathetic hyperactivity, resting arterial blood pressure (measured directly through an intra-arterial catheter (Figure 3D), cardiac work, and total peripheral resistance were similar between groups (Table 4). Moreover, local FVR, calculated from arterial blood pressure and forearm blood flow (O'Leary, 1991), was slightly reduced in CMS+ (Figure 3L, Table 2) as was forearm vascular conductance (Figure 3S, FVC; $P=0.040$). Alterations in the relationship between blood viscosity, pressure, flow and resistance may influence shear stress imposed on conduit arteries, with the expectation that greater viscosity and peripheral vascular resistance, would lead to increased mean, antegrade or retrograde shear profiles associated with vascular dysfunction (Casey *et al.*, 2012). However, despite higher *ex vivo* blood viscosities in CMS+ [Figure 3E, $P<0.0001$ (Tremblay *et al.*, 2019b)], in vivo mean shear stress (MSS), calculated by combining *ex vivo* blood viscosity with ultrasound-based measurements of blood velocity and artery diameter, was equal between groups (Figures 3E, G & H, Table 2). This response was also apparent when separating antegrade and retrograde shear stress (Antegrade: $P=0.236$; Retrograde: $P=0.241$; Figure 3F, Table 2). The normalization of shear is likely due to the larger diameter of the brachial arteries in Andeans with CMS+ ($P=0.027$; Figure 3H) which likely represents a chronic adaptation of conduit arteries to high blood viscosity.

Mechanistic assessment of resting sympathetic transduction, α-adrenergic responsiveness.

Chronic hypoxia causes a substantial sympathetic hyperactivity in humans (Hansen & Sander, 2003; Simpson *et al.*, 2020; Tymko *et al.*, 2020b), which appears to be exaggerated in CMS+ (Figures 3B & C). However, animal studies have shown decreased α-adrenergic receptor responsiveness with chronic hypoxia (Doyle & Walker, 1991). Alterations in sympathetic transduction, thru a desensitization of α-adrenergic receptors may represent an adaptive response to sympathetic hyperactivity and account for the observed lower FVR/FVC (Figures 3L & S). Indeed, sympathetic vascular transduction, calculated as a quotient of resting FVR and MSNA burst frequency, was lower in CMS+ compared to healthy Andeans (P=0.013; Figure 3M). To examine the role of α_1-adrenergic mediated vasoconstriction, we infused three graded doses of phenylephrine into the forearm. Because adrenergic agonists cause β-adrenergic mediated vasodilation, the β-receptor agonist propranolol was co-infused to isolate α_1-adrenergic responsiveness (Figures 3I-K). As expected, graded phenylephrine infusion caused vasoconstriction in both CMS- and CMS+; however, while there was a very slightly attenuated of the decrease in FVC at the lowest dose of phenylephrine in CMS+ compared to healthy Andeans (P=0.039; Figure 3N, Table 3), no differences were observed at higher phenylephrine doses (Figures 3N-P, Table 3). To further pharmacologically dissect the contribution of tonic α-adrenergic constriction to resting vascular tone, we infused phentolamine, a non-selective α-adrenergic antagonist and propranolol (β-adrenergic blockade; Figures 3Q & R, Table 2) to remove local sympathoadrenal vascular signalling. When α-adrenergic tone was removed, FVC increased similarly in both groups (Figures 3S & T), indicating no difference in basal adrenergic restraint. Localized +α-β blockade increased MSS in both groups (CMS-: P=0.005; CMS+: P=0.001) with no difference between groups (P=0.490; Figures 3U & V, Table 2). In summary, Andeans with mild-to-moderate polycythaemia have a substantial reduction in the peripheral vascular resistance given the prevailing sympathetic activity, which is likely related to the lower basal vascular tone noted above. However, this observation is unlikely to be fully explained by the modest reduction in vasoconstriction to low dose α_1-adrenergic receptor activation.

Haemodyamics and convective oxygen transport during steady-state cycling.

During exercise, blood lactate (P=0.250) alongside MAP (P=0.372), heart rate (P=0.352), cardiac work (P=0.975) and oxygen delivery (P=0.657) increased similarly in both groups (Figures 4B-E, Table 4); such metabolic and hemodynamic similarities are interpreted to rule out

positive adaptations in convective oxygen transport. Conversely, the fall in total peripheral resistance was slightly less during exercise in CMS+ (P=0.010, Table 4), suggesting an alteration(s) in peripheral vascular tone.

α-adrenergic vasoconstrictor signalling in non-active forearm skeletal muscle during cycling.

Redistribution of peripheral blood flow/volume is vital during exercise to maintain cardiac filling pressure and optimize oxygen delivery to active skeletal muscles from non-active tissues (Saltin & Mortensen, 2012), with recent evidence highlighting α-adrenergic receptor signalling as the primary mechanism at sea level (Hansen *et al.*, 2020). To identify if polycythaemia causes adaptations to physiological α-adrenergic vasoconstriction in non-active skeletal muscle during exercise, we determined the forearm vasoconstrictor response to moderate intensity (i.e., 60% total peak workload) leg cycling, before and after +α-β blockade. During cycle exercise, CMS- showed a modest increase in vasoconstriction that was not impacted by +α-β blockade, whereas CMS+ had a greater vasoconstrictor response in non-active skeletal muscle (Figures 4F-I, Table 4), which likely explains the attenuated fall in total peripheral resistance (Table 4). In contrast to what has been observed in people exercising at sea-level (Hansen *et al.*, 2020), the vasoconstrictor response in non-active tissue was due in large part to non-adrenergic mediated vasoconstrictor signalling, as local +α-β blockade only accounted for ~60% of the vasoconstrictor response in CMS+ (Figures 4F-I, Table 4). During exercise in CMS+, we found that while greater, the retrograde shear stress, did not reach the threshold for statistical significance (Figure 4J, Table 4). After +α-β blockade, retrograde shear stress was reduced in both groups (CMS-: P=0.023; CMS+: P=0.001; Figure 4J) whereas antegrade shear stress was unaffected (CMS-: P=0.892; CMS+: P=0.813; Figure 4J). Collectively, these data highlight that CMS+ have a greater degree of vasoconstriction within non-active skeletal muscle during moderate intensity exercise. Moreover, in both CMS+ and healthy Andeans, vasoconstriction within non-active skeletal muscle relies heavily on non-α-adrenergic mediated pathways.

α-adrenergic vasoconstrictor signalling in forearm active skeletal muscle.

The preservation of aerobic capacity in Andeans with polycythaemia may also be due to vascular and/or metabolic adaptations in active skeletal muscle (Tremblay *et al.*, 2019a). In

particular modulation of α-adrenergic signalling may regulate the vasodilatory response to exercise in order to match oxygen delivery with metabolic demand. To examine whether α-adrenergic vasoconstrictor signalling contributes to metabolic flow matching, we determined components of the Fick equation across the forearm during graded handgrip exercise (HGE; 5, 15, 25% MVC, Figure 5A) before and after +α-β blockade. The vasodilatory response to graded HGE was quantified as the absolute change in FBF and FVC. During progressive HGE, FBF ($P<0.0001$), FVC ($P<0.0001$), oxygen delivery ($P<0.0001$) and a-VO_{2diff} ($P<0.0001$) increased in proportion to oxygen uptake in the Healthy Andeans (Figures 5B-F, Table 5). After local +α-β blockade, the physiological responses to HGE were identical in healthy Andeans (Figures 5B-G). In contrast, +α-β blockade in Andeans with polycythaemia revealed a greater vasodilator response to 25% MVC (Figures 5H-K), showing α-adrenergic restraint of exercise hyperaemia. Moreover, +α-β blockade caused an increase in oxygen uptake at rest and during all three exercise intensities, which elicited a rightward shift in absolute oxygen uptake and an exaggerated requirement for oxygen delivery, as indicted by a steeper slope between oxygen delivery and utilization (Figure 5M). Interestingly, a similar effect of +α-β blockade has been observed previously in healthy individuals exercising at sea level (Wilkins *et al.*, 2008). Moreover, as the hypermetabolic response to +α-β blockade was not observed in healthy Andeans without CMS, these data suggest that the sympathetic nervous system adapts to polycythaemia by restraining blood flow to regulate oxygen delivery and utilization. Finally, in contrast to observations in the autosomal disorder Chuvash polycythaemia (Formenti *et al.*, 2010), lactate production and pH concentrations to small muscle mass exercise were within the normal range in CMS+ and were consistent with healthy Andeans (Table 5). In summary, these data indicate that polycythaemia is associated with α-adrenergic vasoconstrictor signalling that restrains the vasodilatory response to exercise in order to precisely match oxygen delivery to active skeletal muscle oxygen uptake during moderate intensity exercise. Moreover, while changes in lactate and pH were normal, infusion of +α-β blockade unmasked a potential adaptation in skeletal muscle metabolic regulation or muscle regional oxidative capacity in Andeans who have adapted to life at high altitude.

Discussion

To identify potential phenotypical adaptations to polycythaemia we employed a unique human model of elevated haematocrit, blood viscosity and haemoglobin mass, but preserved exercise capacity. In doing so, we observed that the main findings were: 1) in CMS+ with a relative polycythaemia, blood volume was similar to healthy Andeans due to a significant reduction in plasma volume; 2) despite high haematocrit and blood viscosity, moderate intensity cycling exercise did not increase cardiac work and oxygen delivery was maintained despite a smaller fall in total peripheral resistance, 3) with a background of high resting sympathetic activity, a lower basal vascular tone is unlikely explained by the modest reduction in low dose α-adrenergic receptor mediated vasoconstriction under resting conditions. Ultimately, vascular adaptations in CMS+, in both conduit artery and downstream arterioles, allows the maintenance of local and total vascular resistances, resting blood pressure and vascular shear patterns; 4) both adrenergic and non-adrenergic vasoconstrictor signalling contributes to a greater vasoconstriction of non-active skeletal muscle in individuals with CMS+, which likely aids central redistribution of blood volume during exercise; 5) heightened α-adrenergic signalling also restrains vasodilation to better match oxygen delivery within active skeletal muscle despite higher oxygen carrying capacity of blood.

Plasma volume contraction and normalized total blood volume in CMS.

High altitude residents with CMS have elevated haemoglobin, haematocrit and total haemoglobin mass; however, due to a substantial plasma volume contraction, blood volume is similar to their healthy counterparts. While two previous studies did not observe a similar "statistically significant" plasma volume contraction in CMS+ (Claydon *et al.*, 2004; Simpson *et al.*, 2020), this is likely due to a smaller sample sizes in contrast to our larger cohort (e.g. Current study: n=36 vs. Claydon et al: n=22). Plasma volume contraction in CMS+ is also in contrast to Sherpa, where an expanded plasma volume allows for a total blood volume similar to Andeans with and without CMS but maintains a haemoglobin concentration and haematocrit similar to acclimatized lowlanders (Stembridge *et al.*, 2019). The underlying mechanism(s) and physiological significance of these haematological differences are poorly understood, but may involve genetic (e.g., EGLN1 or I/I and I/D ACE genotypes (Rupert *et al.*, 1999)) or physiological (e.g., EPO or renin-angiotensin aldosterone system or chronic vasoconstriction

(Fekete *et al.*, 2011)) factors regulating the production of red blood cells and fluid balance that contracts plasma volume to "normalize" total blood volume. Indeed, the kidneys are ideally placed as a "critmeter" to haematocrit and plasma volume through erythropoiesis and extra-cellular water retention and secretion thru alterations in renal tissue oxygen partial pressure stimulation (Donnelly, 2003). however renal metabolic adaptions to hypoxemia are unclear and should also be taken into consideration. Indeed, while classically CMS is conceptualized as a hypoventilation syndrome, differences in SaO2 (~4%) and PaO2 (~9%) were relatively minor between our CMS+ and healthy Andeans, especially compared to changes in haematocrit (20%), plasma volume (16%), haemoglobin mass (~28%) and blood viscosity (44%). Our data suggests an alternative idea in that the normalization of blood volume is prioritized, with the likely benefits of maintaining cardiac function, blood pressure and global oxygen delivery, yet the disadvantage of haemoconcentration. Although it must be highlighted that this speculation regarding the lifelong maladaptation to chronic hypoxia is in complete contrast to the physiological response observed during the acclimation of lowlanders to high altitude, whereby a rapid plasma volume contraction is followed by a slow increase in Hb-mass/total erythrocyte-volume. Why healthy Andeans and those with CMS adapt differently to Sherpa and initially "tolerate" higher blood viscosity remains to be elucidated but is likely pivotal to the pathology that develops with lifelong CMS.

Vascular adaptations in CMS maintain normal resting blood pressure.

Elevated MSNA can coincide with arterial stiffness and vascular dysfunction (Hijmering *et al.*, 2002; Swierblewska *et al.*, 2010) which, alongside high blood viscosity, should result in an increase in vascular resistance and arterial hypertension at rest. However, despite CMS+ presenting with a greater resting sympathetic nerve activity in the semi-recumbent position, vascular resistance was lower (conductance higher), and blood pressure and vascular shear stress comparable to healthy Andeans. This observation suggests an uncoupling between sympathetic bursts and neurotransmitter release and/or binding to downstream receptors, or elevated vasodilator influences. While sympathetic hyperactivity has been shown to cause desensitization of post-junctional α_1-adrenergic receptors (Dinenno *et al.*, 2002a), α_1-adrenergic vasoconstriction was largely preserved between healthy Andeans and CMS+, except a marginal reduction to low dose phenylephrine infusion. Moreover, it is unlikely that protection is conferred via elevated

downstream vasodilator signalling at rest, as the increase in forearm vascular conductance was similar after +α-β blockade. This observation seems in direct contrast to Sherpa who have high circulating concentrations of bioactive nitric oxide (NO) products and high resting forearm blood flow (Erzurum *et al.*, 2007). While limited data exist concerning chronic hypoxia, an elegant series of studies in the rat iliac artery has identified a switch between desensitization of α_1-adrenergic receptors in acute hypoxia to a nitric oxide facilitated desensitization of α_2-adrenergic receptors with chronic hypoxia (Bartlett & Marshall, 2002, 2003). Future work in humans will be required to elucidate these time dependent adaptations. Indeed, epidemiological studies have seen evidence of systolic and masked hypertension in Andean's with CMS at Cerro de Pasco (Corante *et al.*, 2018; Bilo *et al.*, 2020), suggesting that time maybe key and as CMS pathology progress the functional adaptations we observed herein are lost and hypertension develops. In addition to these physiological changes, vascular remodelling in terms of greater conduit artery diameter was a prominent feature in CMS+ (Tremblay *et al.*, 2019a; Tremblay *et al.*, 2019b), which contributed to the normalization of in vivo conduit artery anterograde and retrograde shear stress despite elevated blood viscosity.

Maintained cardiac work and convective oxygen transport during exercise despite polycythaemia and elevated blood viscosity.

A positive relationship between Hb mass and maximal aerobic capacity is observed in humans at sea level (Schmidt & Prommer, 2010). Moreover, a study using animal models showed that, as haematocrit increases (~57-68%), so does exercise capacity up until a turning point (>68%) where the rate pressure product, indicative of myocardial oxygen consumption, also increases, and thereafter exercise capacity diminishes. These thresholds define what has been termed the "optimal haematocrit theory" (Schuler *et al.*, 2010). However, these data imply that no compensatory adaptations occur to chronic polycythaemia. On the contrary, our data alongside those of Groepenhoff et al., (Groepenhoff *et al.*, 2012) have clearly documented that Andeans with "excessive" polycythaemia can have normal exercise capacities, despite high haematocrits and blood viscosity and concomitant high pulmonary artery pressures. A major difference between animal models and humans with CMS seems to be that vascular adaptations permit similar increase in blood pressure and cardiac work in healthy Andeans and CMS+, implying normal changes in myocardial oxygen consumption, which likely explains the normal increases

convective oxygen transport, at least during 60% cycling exercise. Moreover, in contrast to exercise training where an increase in haemoglobin mass coincides with plasma volume expansion (increased total blood volume) (Montero *et al.*, 2017), Andeans with CMS had a relative plasma volume contraction, which likely limits any improvements in aerobic capacity. Thus, overall, no relationship was observed between $\dot{V}O_2$ max despite a large range of Hb mass (700 – 2000 g) & blood viscosities (4 to 10 cP). One caveat to this general interpretation is the slight negative relationship between viscosity and V̇O2 max in the healthy Andeans. This correlation could be spurious, as similar relationships were not observed for other haematological variables. Yet, if true, the data still supports the idea that adaptations have occurred with chronic polycythaemia in CMS as their V̇O2 max was maintained despite further increases in viscosity.

Moderate intensity cycling exercise elicits greater vasoconstrictor signalling in non-active forearm skeletal muscle in CMS.

During moderate intensity cycling, the CMS+ group had comparable hemodynamic responses to moderate exercise (in terms of heart rate, cardiac work, lactate etc.,), but greater vasoconstriction within non-active skeletal muscle (i.e., the forearm). While the measurement of forearm vascular resistance is isolated to non-active skeletal muscle, when coupled with the attenuated fall in total peripheral resistance (greater constriction) and greater restraint of active skeletal muscle vasodilation, it is likely that greater vasoconstriction also occurs in other vascular beds/organs with substantially higher perfusion. These data imply a greater requirement for redistribution of blood volume during moderate intensity exercise. Importantly, cardiac output during exercise was comparable to healthy Andeans, suggesting the heightened vasoconstrictor response is not to improve cardiac function, but a compensatory mechanism to maintain normal blood pressures and distribution of cardiac output to active skeletal muscle. Recently, our group demonstrated that at sea-level, moderate intensity leg cycling elicited vasoconstriction within the forearm primarily via α-adrenergic signalling (Hansen *et al.*, 2020). Conversely, in CMS+, forearm vasoconstriction remained prominent even after +α-β blockade. Animal studies have shown that during severe acute hypoxia, neuropeptide-Y mediated vasoconstrictor signalling remains intact and contributes ~50% to vasoconstriction during high frequency bursts of sympathetic activity (Coney & Marshall, 2007). Moreover, humans with chronic heart failure

have high SNA and elevated circulating catecholamines (Cohn *et al.*, 1984); whereby recent investigations have pointed towards the contribution of neuropeptide-Y in controlling vascular tone due to the elevated neuronal traffic (Ajijola *et al.*, 2020). Therefore, it seems that during moderate exercise in chronic hypoxia when sympathetic burst frequency is high, non-adrenergic vasoconstrictor mechanisms continue to mediate blood flow to non-active skeletal muscle.

Heightened α-adrenergic signalling during handgrip exercise in active forearm skeletal muscle.

At the final stages of the oxygen cascade, there is a complex interplay of vasoconstrictor and metabolic vasodilator signalling pathways that act to precisely distribute and match muscle blood flow, substrate, and oxygen delivery to oxygen consumption (Saltin *et al.*, 1998). Our findings indicate that during moderate hand grip exercise, there is a greater vasodilatory signal in Andeans with CMS+, which is appropriately restrained by adrenergic signalling mechanisms. While the list of potential factors effecting the vascular smooth muscle is extensive, attractive hypotheses include enhanced contribution of NO mediated vasodilation or greater release of adenosine triphosphate from erythrocytes (Ellsworth *et al.*, 1995) due to the elevated red cell volume in CMS+ (Table 1). In contrast to CMS+, α-adrenergic signalling did not alter the relationship between oxygen delivery and utilization in healthy Andeans, which is similar to healthy individuals living at sea level (Richards *et al.*, 2014). An important insight from these data is that while prescribing alpha blockers to CMS patients may reduce microvascular endothelial dysfunction (Swenson, 2020; Tymko *et al.*, 2020b), system adrenergic inhibition may also limit exercise capacity, as elevated α-adrenergic signalling plays an important role in active skeletal muscle.

Hypermetabolic response to handgrip exercise.

After forearm +α-β blockade, we observed an elevation in muscle oxygen consumption at rest, with a hypermetabolic response to HGE in CMS+, but not in healthy Andeans. Interestingly, Wilkins *et al* (2008) (Wilkins *et al.*, 2008) observed similar results at sea level during exposure to acute hypoxaemia (SpO_2: 80%), which they isolated the effect of local β_2-adrenergic receptors. The mechanistic reasoning behind this result is unclear; but increased blood flow heterogeneity within individual skeletal muscle capillaries can lead to elevated tissue

oxygen consumption (Goldman *et al.*, 2004). Thus, +α-β blockade may increase perfusion to fibre types that are not typically perfused at rest and provide a simple explanation for the increase oxygen consumption. Importantly, healthy Andeans, who by definition are better adapted to life at high altitude compared to CMS, did not display this hypermetabolic response, implying a beneficial muscle metabolic and/or regional adaptation to chronic hypoxemia.

Limitations

We acknowledge that the current study was not without limitations. Firstly, albeit not a limitation *per se*, we acknowledge that the Andean participants within the current study were only of Quechua origin. Thus, our data cannot be extrapolated to other Andean populations such as the Aymara where potential haematological differences exist (Hb: Aymara: 18.2±1.1 vs. Quechua: 15.8±1.5 $g \cdot dL^{-1}$) (Arnaud *et al.*, 1981). Although the inclusion of both males and females in this previous study makes a direct comparison difficult (Gassmann *et al.*, 2019). Secondly, CMS+ subjects within the current study only had mild-to-moderate polycythaemia and CMS symptoms. While this modest degree of polycythaemia was sought to examine potential positive adaptations to high blood viscosity, the observations found here maybe very different when the disease becomes severe, and maladaptation becomes the predominant phenotype. Along the same lines, what is considered excessive erythrocytosis is not clear and an argument could be made that any Hb concentration without symptomology is healthy, especially at ~4300m. We used a cut-off from the CMS literature as CMS positive and/or excessive erythrocytosis (Hb $\geq$ 21 $g \cdot dL^{-1}$) (Leon-Velarde *et al.*, 2005), but Gassmann and colleagues (2019) have re-evaluated haemoglobin concentrations based on varying altitudes and shown that ~21 $g \cdot dL^{-1}$ is not uncommon in healthy males at ~4000m (Gassmann *et al.*, 2019). If these individuals remain "healthy" by definition of low clinical symptoms despite high haemoglobin concentrations due to the adaptations observed in the current investigation should be the study of future research.

Conclusion.

Collectively, the present study identified several mechanistic insights into how humans can adapt to life with excessive polycythaemia. These include important physiological adaptions such as changes in red cell volume relative to plasma volume, variations in α-adrenergic and

non-adrenergic vasoconstrictor signalling within skeletal muscle and morphological adaption with larger conduit arteries. Ultimately these adaptations subvert the expected increase in resting blood pressure, vascular shear stress, myocardial oxygen consumption and decreased exercise capacity due to polycythaemia.

Author Contributions

G.M., S.A.R., C.M.H., and J.S.L. designed research; A.B.H., G.M., S.B.A., F.H., J.D.A., C.G., T.G.D., M.M.T., P.N.A., F.C.V., C.M.H., and J.S.L. performed research; A.B.H., L.L.S., G.M., C.M.H., and J.S.L analyzed data; and A.B.H., C.M.H., and J.S.L. wrote the paper; All authors revised the paper.

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Disclosures

None

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Tables

Table 1. Subject characteristics.

Initial screening			
Subjects	**n=36**		
	CMS- (n=17)	**CMS+ (n=19)**	**P value**
Age (yrs)	44±15	42±14	0.666
Weight (kg)	70±12	68±11	0.695
Height (cm)	161±6	161±6	0.950
BMI ($kg \cdot m^2$)	27±5	26±3	0.660
Qinghai CMS score	1±1	6±3	<0.0001
Total peak workload (W)	166±33	150±25	0.105
$\dot{V}O_2$ absolute ($ml \cdot min^{-1}$)	2287.6±574.7	2073.1±437.7	0.213
$\dot{V}O_2$ relative ($ml \cdot kg^{-1} \cdot min^{-1}$)	33.4±9.3	31.8±7.6	0.592
Hb ($g \cdot dL^{-1}$)	19.2±1.7	23.0±1.3	<0.0001
Hct (%)	56.2±4.7	67.2±6.0	<0.0001
Total haemoglobin mass (g)	1121.2±270.2	1431.2±461.7	0.022
Red blood cell volume (l)	3.3±0.9	4.2±1.4	0.030
Blood volume ($ml \cdot kg^{-1}$)	93.6±22.7	99.6±21.7	0.434
Plasma volume ($ml \cdot kg^{-1}$)	45.6±10.8	38.2±7.3	0.022
Blood Viscosity (cP)	5.9±1.0	8.5±1.2	<0.0001
SaO_2 (%)	86.9±5.0	83.6±2.9	0.019
Experimental testing			
Subjects	**n=16**		
	CMS- (n=9)	**CMS+ (n=7)**	**P value**
Age (yrs)	44±15	43±13	0.931
Weight (kg)	62±7	76±11	0.007
Height (cm)	159±4	164±3	0.028
BMI ($kg \cdot m^2$)	25±3	28±4	0.044
Qinghai CMS score	2±1	7±3	0.0001
Total peak workload (W)	162±24	149±28	0.336
60% total peak workload (W)	97±14	89±17	0.336
MVC (kg)	31.4±6.3	29.0±7.0	0.482
Hb ($g \cdot dL^{-1}$)	19.4±1.2	22.9±1.2	<0.0001
Hct (%)	56.0±4.3	67.1±7.1	0.002
Blood Viscosity (cP)	6.1±0.8	8.0±0.6	0.0001
SaO_2 (%)	87.8±3.7	83.0±2.3	0.010
PaO_2 (mmHg)	50.0±3.9	45.4±3.0	0.025

List of Abbreviations: CMS-, Andeans without chronic mountain sickness; CMS+, Andeans with chronic mountain sickness; yrs, years; BMI, body mass index; W, watts; VO_2, maximal oxygen consumption; MVC, maximum voluntary contraction; Hb, haemoglobin; Hct, haematocrit; cP, centipoise; SaO_2, arterial oxygen saturation; PaO_2, partial pressure of arterial oxygen. Resting PaO_2 was taken only in protocol 2 via intra-arterial catheter. All values are presented as mean ± standard deviation. Statistical comparisons performed using two-tailed unpaired *t*-tests (CMS- vs. CMS+). Subject characteristics for both total n=36 Andean participants and n=16 Andean participants.

Table 2. CMS- and CMS+ resting haemodynamics between control and +α-β blockade.

Subjects (n=16)	Conditon	CMS- (n=9)	Control vs. +α-β Blockade P Value	CMS+ (n=7)	Control vs. +α-β Blockade P value	CMS- vs. CMS+ P value
HR (bpm)	*Control*	72±11	0.256	69±16	0.965	0.654
	+α-β Blockade	69±9		67±13		0.793
MAP (mmHg)	*Control*	99±13	0.834	91±6	0.546	0.163
	+α-β Blockade	100±11		89±7		0.059
Systolic (mmHg)	*Control*	135±16	0.293	130±7	0.012	0.456
	+α-β Blockade	129±17		118±9		0.178
Diastolic (mmHg)	*Control*	76±9	0.606	70±6	0.593	0.133
	+α-β Blockade	78±8		71±7		0.076
MBV ($cm \cdot s^{-1}$)	*Control*	4.7±2.5	0.004	4.9±2.1	0.0004	0.855
	+α-β Blockade	24.0±14.5		18.0±5.9		0.328
FBF ($ml \cdot min^{-1)}$	*Control*	28.2±11.7	0.008	37.9±12.8	0.0003	0.135
	+α-β Blockade	155.3±108.1		154.1±45.8		0.979
FVC ($ml \cdot min^{-1} \cdot 100mmHg^{-1}$)	*Control*	28.2±10.9	0.004	41.1±12.0	<0.0001	0.040
	+α-β Blockade	150.5±90.6		180.9±37.4		0.453
FVR ($mmHg \cdot ml^{-1} \cdot min^{-1}$)	*Control*	4.2±2.0	0.001	2.7±1.0	0.002	0.093
	+α-β Blockade	0.9±0.5		0.6±0.1		0.148
MSS ($dyne \cdot cm^{-2}$)	*Control*	3.3±2.0	0.005	3.8±1.7	0.001	0.563
	+α-β Blockade	15.9±10.2		13.6±4.9		0.599
Antegrade SS ($dyne \cdot cm^{-2}$)	*Control*	4.2±1.7	0.006	5.2±1.5	0.002	0.236
	+α-β Blockade	15.9±10.2		13.8±4.8		0.613
Retrograde SS ($dyne \cdot cm^{-2}$)	*Control*	-0.9±0.6	0.002	-1.3±0.7	0.001	0.241
	+α-β Blockade	-0.05±0.1		-0.1±0.2		0.359
Skin conductance (a.u.)	*Control*	14.4±9.2	0.248	15.9±8.3	0.133	0.742
	+α-β Blockade	23.4±25.6		21.6±12.3		0.876
CaO_2 ($ml \cdot dL^{-1}$)	*Control*	23.0±0.9	0.392	25.6±2.1	0.118	0.001
	+α-β Blockade	22.8±0.6		26.2±1.9		<0.0001
CvO_2 ($ml \cdot dL^{-1}$)	*Control*	17.6±1.8	0.002	20.9±4.1	0.006	0.047
	+α-β Blockade	21.3±1.6		23.9±2.7		0.028
DO_2 ($ml \cdot min^{-1}$)	*Control*	6.5±2.8	0.008	9.7±3.3	0.0005	0.054
	+α-β Blockade	35.5±24.6		40.9±14.1		0.594
a-vO_{2Diff} ($ml \cdot dL^{-1}$)	*Control*	5.5±1.7	0.004	4.6±2.8	0.023	0.549
	+α-β Blockade	1.5±1.2		2.4±1.4		0.221

***List of Abbreviations*:** CMS-, Andeans without chronic mountain sickness; CMS+, Andeans with chronic mountain sickness; HR, heart rate; MAP, mean arterial pressure; Systolic, systolic blood pressure; Diastolic, diastolic blood pressure; MBV, mean blood velocity; FBF, forearm blood flow; FVC, forearm vascular conductance; FVR, forearm vascular resistance; MSS, mean shear stress; Antegrade SS, antegrade shear stress; Retrograde SS, retrograde shear stress; a.u., arbitrary units; CaO_2, arterial oxygen content; CvO_2, venous oxygen content; DO_2, Oxygen delivery; a-vO_{2Diff}, arterial venous oxygen difference; +α-β blockade, combination of phentolamine and propranolol. All values are presented as mean ± standard deviation. Statistical comparisons performed using two-tailed paired *t*-test (control vs. +α-β blockade) and two-tailed unpaired *t*-test (CMS- vs. CMS+). Total Andean subjects n=16 (CMS-: n=9; CMS+: n=7).

Table 3. Haemodynamics during graded phenylephrine doses between CMS- and CMS+

Subjects (n=16)	CMS- (n=9)					CMS+ (n=7)					CMS- vs. CMS+
Phenylephrine ($\mu g \cdot dL^{-1} \cdot FAV^{-1} \cdot min^{-1}$)	**Baseline**	**PE 1 (0.0625)**	**PE 2 (0.125)**	**PE 3 (0.250)**	**One-way P value**	**Baseline**	**PE 1 (0.0625)**	**PE 2 (0.125)**	**PE 3 (0.250)**	**One-way P value**	**Two-way P value**
HR (bpm)	68±10	70±11	69±10	71±10	0.958	69±11	70±10	67±11	69±10	0.960	0.873
MAP (mmHg)	97±12	98±13	99±13†	97±12	0.981	92±7	92±7	93±7	91±8	0.956	0.284
Systolic (mmHg)	124±21	125±22	124±24	125±21	0.9838	125±8	124±7	125±8	126±8	0.969	0.964
Diastolic (mmHg)	78±11	80±9	81±9	77±10	0.813	73±5	74±4	74±5	74±5	0.975	0.221
MBV ($cm \cdot s^{-1}$)	4.7±2.5	4.1±2.0	2.5±1.5	1.9±1.1	0.011	4.8±2.1	4.4±2.0	2.3±1.2	1.6±1.0	0.003	0.976
FBF ($ml \cdot min^{-1}$)	29.1±13.4	24.3±9.8	14.7±7.1	11.2±5.3	0.001	36.9±12.4	35.0±14.5	19.6±11.7	13.7±10.0	0.003	0.202
FVC ($ml \cdot min^{-1} \cdot 100mmHg^{-1}$)	29.6±12.4	24.3±8.7	14.4±6.3	11.2±4.8	0.0002	39.9±12.2	38.4±15.9	21.5±13.4	15.4±12.1	0.004	0.090
FVR ($mmHg \cdot ml^{-1} \cdot min^{-1}$)	4.2±2.4	7.0±9.7	11.3±13.9	11.1±6.6	0.295	2.8±1.3	3.6±3.1	12.6±21.2	19.2±29.1	0.296	0.835
MSS ($dyne \cdot cm^{-2}$)	3.2±1.9	2.9±1.6	1.8±1.2	1.4±0.9	0.036	3.9±1.8	3.5±1.8	1.8±1.0	1.2±0.8	0.004	0.670
Skin Conductance (a.u.)	34.7±49.4	29.2±38.9	23.7±24.9	19.7±9.9	0.804	21.6±20.4	23.7±26.1	18.6±13.0	14.1±7.5	0.774	0.575

***List of Abbreviations*:** CMS-, Andeans without chronic mountain sickness; CMS+, Andeans with chronic mountain sickness; PE, phenylephrine; $\mu g \cdot dL^{-1} \cdot FAV^{-1} \cdot min^{-1}$, microgram per deciliter per forearm volume per minute; HR, heart rate; MAP, mean arterial pressure; Systolic, systolic blood pressure; Diastolic, diastolic blood pressure; MBV, mean blood velocity; FBF, forearm blood flow; FVC, forearm vascular conductance; FVR, forearm vascular resistance; MSS, mean shear stress; a.u., arbitrary units. All values are presented as mean ± standard deviation. Statistical comparisons performed using one-way repeated measures analysis of variance (ANOVA) (baseline vs. PE1/PE2/PE3) and two-way repeated measures ANOVA (CMS- vs. CMS+). Total Andean subjects n=16 (CMS-: n=9; CMS+: n=7).

Table 4. Haemodynamics at rest and during 60% exercise between control and +α-β blockade in CMS- and CMS+.

Subjects (n=16)		CMS- (n=9)			CMS+ (n=7)				
	Condition	**Rest**	**60% Exercise**	**P value**	**Rest**	**60% Exercise**	**P value**	**CMS- vs. CMS+ control**	**CMS- vs. CMS+ +α-β Blockade**
HR (bpm)	*Control*	68±11	124±13	0.013	79±19	120±16	0.215	0.632	0.648
	+α-β Blockade	64±10	118±14	0.148	68±8	110±10	0.094		
Cardiac Work ($L \cdot mmHg^{-1} \cdot min^{-1}$)	*Control*	440.0±145.5	1250.6±455.2	0.0001	469.4±112.3	1262.2±453.3	0.004	0.869	x
	+α-β Blockade	x	x	x	x	x	x		
TPR ($mmHg \cdot L^{-1} \cdot min^{-1}$)	*Control*	29.0±9.3	17.7±6.5	<0.0001	25.3±8.0	18.6±5.8	0.019	0.762	x
	+α-β Blockade	x	x	x	x	x	x		
DO_2 ($ml \cdot min^{-1}$)	*Control*	8.1±2.2	16.7±5.7	0.0002	9.8±2.5	18.0±5.9	0.003	0.525	x
	+α-β Blockade	x	x	x	x	x	x		
MAP (mmHg)	*Control*	99±15	123±15	0.056	93±7	119±8	0.558	0.454	0.411
	+α-β Blockade	105±14	124±17	0.478	95±11	124±15	0.194		
MBV ($cm \cdot s^{-1}$)	*Control*	6.0±2.8	7.9±4.8	<0.0001	5.9±2.9	6.0±3.4	<0.0001	0.577	0.866
	+α-β Blockade	17.6±5.4	21.5±9.3	0.001	18.6±4.6	21.6±5.4	<0.0001		
FBF ($ml \cdot min^{-1}$)	*Control*	41.2±21.7	54.3±30.5	0.002	46.0±123.1	40.8±23.0	<0.0001	0.736	0.204
	+α-β Blockade	119.9±54.2	53.7±72.7	0.006	162.3±23.6	179.5±40.7	<0.0001		
FVC ($ml \cdot min^{-1} \cdot 100mmHg^{-1}$)	*Control*	42.0±21.0	45.5±26.3	0.001	48.8±22.8	33.4±17.7	<0.0001	0.817	0.056
	+α-β Blockade	114.9±45.0	124.3±49.4	0.004	174.5±36.8	144.0±32.2	<0.0001		
FVR ($mmHg \cdot ml^{-1} \cdot min^{-1}$)	*Control*	3.5±3.1	4.5±6.4	0.049	2.4±1.0	3.9±2.1	0.004	0.658	0.117
	+α-β Blockade	1.0±0.5	1.0±0.6	0.160	0.6±0.1	0.7±0.2	0.008		
MSS ($dyne \cdot cm^{-2}$)	*Control*	4.0±2.0	5.9±3.2	<0.0001	4.7±2.4	5.1±2.9	0.001	0.965	0.305
	+α-β Blockade	10.7±3.6	14.3±7.0	0.004	13.9±4.3	16.5±4.8	<0.0001		
Antegrade ($dyne \cdot cm^{-2}$)	*Control*	4.9±1.4	9.6±3.0	0.001	5.6±2.7	9.6±4.0	0.001	0.799	0.313
	+α-β Blockade	10.8±3.5	15.6±6.7	0.011	13.9±4.3	17.6±4.5	0.001		
Retrograde ($dyne \cdot cm^{-2}$)	*Control*	-1.0±0.8	-3.8±1.1	0.019	-1.3±0.7	-5.2±2.4	0.003	0.181	0.620
	+α-β Blockade	-0.1±0.1	-1.5±0.8	<0.0001	-0.03±0.1	-1.3±0.6	0.004		
Skin conductance (a.u.)	*Control*	19.1±12.1	53.6±25.2	0.496	17.7±9.4	50.6±14.8	0.025	0.777	0.035
	+α-β Blockade	21.3±11.8	56.8±30.2	0.724	70.3±47.0	85.4±43.3	0.043		
Lactate ($mmol \cdot L^{-1}$)	*Control*	1.0±0.5	4.2±1.5	0.0001	1.1±0.5	5.2±1.7	0.002	0.219	x
	+α-β Blockade	x	x	x	x	x	x		

***List of Abbreviations*:** CMS-, Andeans without chronic mountain sickness; CMS+, Andeans with chronic mountain sickness; HR, heart rate; TPR, total peripheral resistance; DO_2, oxygen delivery; MAP, mean arterial pressure; MBV, mean blood velocity; FBF, forearm blood flow; FVC, forearm vascular conductance; FVR, forearm vascular resistance; MSS, mean shear stress; Antegrade, antegrade shear stress; Retrograde, retrograde shear stress; +α-β Blockade, combination of phentolamine and propranolol. All values are presented as mean ± standard deviation. Statistical comparison performed using two-tailed paired *t*-test (control vs. +α-β Blockade), two-way repeated measures analysis of variance (ANOVA) (CMS- vs. CMS+ x control/+α-β Blockade). Total Andean subjects n=16 (CMS-: n=9; CMS+: n=7).

Table 5. Haemodynamics during graded handgrip exercise between control and +α-β blockade in both CMS+ and CMS-.

Subjects (n=16)		CMS- (n=9)				Two-way ANOVA	CMS+ (n=7)				Two-way ANOVA
MVC (%)	**Condition**	**Rest**	**5%**	**15%**	**25%**	**P value**	**Rest**	**5%**	**15%**	**25%**	**P value**
MAP (mmHg)	*Control*	99±12	103±14	105±15	106±16	0.816	91±7	95±12	97±11	97±11	0.671
	+α-β blockade	100±13	105±15	106±14	108±18		91±12	93±15	93±15	91±14	
HR (bpm)	*Control*	74±13	76±12	76±13	74±13	0.318	73±15	75±16	75±16	76±16	0.313
	+α-β blockade	69±12	70±11	69±9	69±11		64±13	66±13	66±14	67±13	
Hb_a ($g \cdot dL^{-1}$)	*Control*	18.7±1.7	x	x	x	0.201*	21.7±1.3	x	x	x	0.100*
	+α-β blockade	18.4±1.9	x	x	x		21.3±1.2	x	x	x	
FBF ($ml \cdot min^{-1}$)	*Control*	34.1±14.3	72.4±23.6	105.1±32.0	152.6±47.9	0.005	39.8±14.5	71.6±31.3	85.6±33.2	129.5±30.9	<0.0001
	+α-β blockade	125.8±63.5	158.0±73.1	186.3±75.3	236.8±101.0		146.7±32.3	180.5±28.1	203.6±33.9	266.6±59.5	
FVC ($ml \cdot min^{-1} \cdot 100mmHg^{-1}$)	*Control*	34.0±13.2	70.9±24.2	101.2±32.2	143.0±37.5	0.002	41.7±15.4	69.7±26.7	82.4±26.9	132.9±38.7	0.0003
	+α-β blockade	121.3±54.2	146.5±58.0	173.3±59.9	216.8±77.9		170.5±53.5	200.1±61.0	223.2±74.1	299.1±119.0	
DO_2 ($ml \cdot min^{-1}$)	*Control*	7.6±3.2	16.0±5.0	23.1±6.6	33.7±10.5	0.006	9.8±3.7	17.4±7.2	20.7±7.5	31.6±7.5	<0.0001
	+α-β blockade	27.4±14.0	34.4±16.0	40.3±16.2	51.3±21.8		36.3±9.0	44.6±7.9	50.3±9.5	66.0±16.5	
$\dot{V}O_2$ ($ml \cdot min^{-1}$)	*Control*	1.7±1.1	6.2±2.1	12.3±3.5	17.9±6.9	0.988	1.5±1.5	6.4±2.6	9.8±3.3	17.4±4.8	0.010
	+α-β blockade	1.4±1.8	6.7±7.5	11.8±7.6	18.3±13.0		3.1±1.5	18.0±11.4	22.0±12.5	27.2±11.5	
SaO_2 (%)	*Control*	84.9±4.4	x	x	x	0.393*	80.6±4.1	x	x	x	0.117*
	+α-β blockade	84.3±4.2	x	x	x		82.7±1.8	x	x	x	
SvO_2 (%)	*Control*	64.9±4.8	50.8±7.9	40.7±8.3	37.4±7.0	0.013	67.4±10.5	49.1±11.0	40.8±12.4	34.3±12.9	0.204
	+α-β blockade	79.1±5.5	65.8±14.7	56.5±15.2	50.8±16.7		75.2±5.4	50.0±16.4	46.9±17.6	46.9±17.5	
PaO_2 (mmHg)	*Control*	49.7±6.2	x	x	x	0.137*	47.0±0.8	x	x	x	0.828*
	+α-β blockade	51.0±7.3	x	x	x		47.1±1.4	x	x	x	
PvO_2 (mmHg)	*Control*	33.8±2.4	27.5±3.5	23.9±3.3	22.8±2.3	0.016	37.2±6.1	27.1±4.6	23.3±6.3	21.8±6.5	0.143
	+α-β blockade	42.5±4.4	34.2±7.4	30.6±7.5	28.5±7.5		41.5±4.2	28.6±7.6	27.9±7.1	28.0±7.1	
CaO_2 ($ml \cdot dL^{-1}$)	*Control*	22.1±1.6	x	x	x	0.203*	24.4±1.6	x	x	x	0.634*
	+α-β blockade	21.7±1.6	x	x	x		24.7±1.5	x	x	x	
CvO_2 ($ml \cdot dL^{-1}$)	*Control*	16.5±2.1	13.0±2.8	10.4±2.6	9.5±1.9	0.034	20.5±4.1	15.0±3.9	12.5±4.1	10.3±3.7	0.314
	+α-β blockade	19.7±1.6	16.4±3.8	14.1±3.8	12.7±4.2		22.5±2.5	15.0±5.3	14.1±5.6	14.2±5.8	
a-vO_2 ($ml \cdot dL^{-1}$)	*Control*	5.1±1.6	8.7±2.1	11.2±1.9	12.2±1.9	0.012	3.9±3.3	9.4±3.1	12.0±3.1	14.1±4.3	0.277
	+α-β blockade	1.4±1.1	4.7±3.9	7.0±4.0	8.4±4.4		2.2±1.3	9.7±5.0	10.6±5.3	10.5±4.9	
$PvCO_2$ (mmHg)	*Control*	30.0±4.0	34.9±1.9	40.4±5.4	39.2±12.4	0.055	35.2±6.7	37.7±2.7	41.2±5.1	46.6±5.3	0.123
	+α-β blockade	30.4±2.5	28.6±5.2	33.1±3.4	36.5±3.6		30.7±1.9	35.1±6.1	37.4±6.7	42.6±7.4	
pH	*Control*	7.40±0.00	7.41±0.02	7.39±0.04	7.29±0.13	0.163	7.40±0.00	7.36±0.11	7.36±0.11	7.36±0.11	0.728
	+α-β blockade	7.43±0.05	7.44±0.05	7.36±0.11	7.36±0.11		7.33±0.11	7.40±0.00	7.31±0.15	7.39±0.04	
Lactate ($mmol \cdot L^{-1}$)	*Control*	1.7±0.6	1.6±0.6	1.9±0.7	2.2±0.9	0.003	2.1±0.7	1.7±0.4	1.5±0.4	2.0±0.6	0.002
	+α-β blockade	1.0±0.4	0.9±0.3	1.0±0.3	1.4±0.5		1.2±0.3	0.9±0.3	1.1±0.4	1.7±0.9	

***List of Abbreviations*:** CMS-, Andeans without chronic mountain sickness; CMS+, Andeans with chronic mountain sickness; ANOVA, analysis of variance; MVC, maximal voluntary contraction; MAP, mean arterial blood pressure; HR, heart rate; Hb_a, arterial hemoglobin; FBF, forearm blood flow; FVC, forearm vascular conductance; DO_2, oxygen delivery; $\dot{V}O_2$, oxygen uptake; SaO_2, arterial oxygen saturation; SvO_2, venous oxygen saturation; PaO_2, arterial partial pressure of oxygen; PvO_2, venous partial pressure of oxygen; CaO_2, arterial oxygen content; CvO_2, venous oxygen content; a-vO_2; arterial venous oxygen difference; $PvCO_2$, venous partial pressure of carbon dioxide; +α-β blockade, combination of phentolamine and propranolol. All values are presented as mean ± standard deviation. Statistical comparison performed using two-way repeated measures ANOVA (control vs. +α-β blockade). *Two-tailed unpaired *t*-test (control vs. +α-β blockade). Total Andean subjects n=16 (CMS-: n=9; CMS+: n=7).

Figures

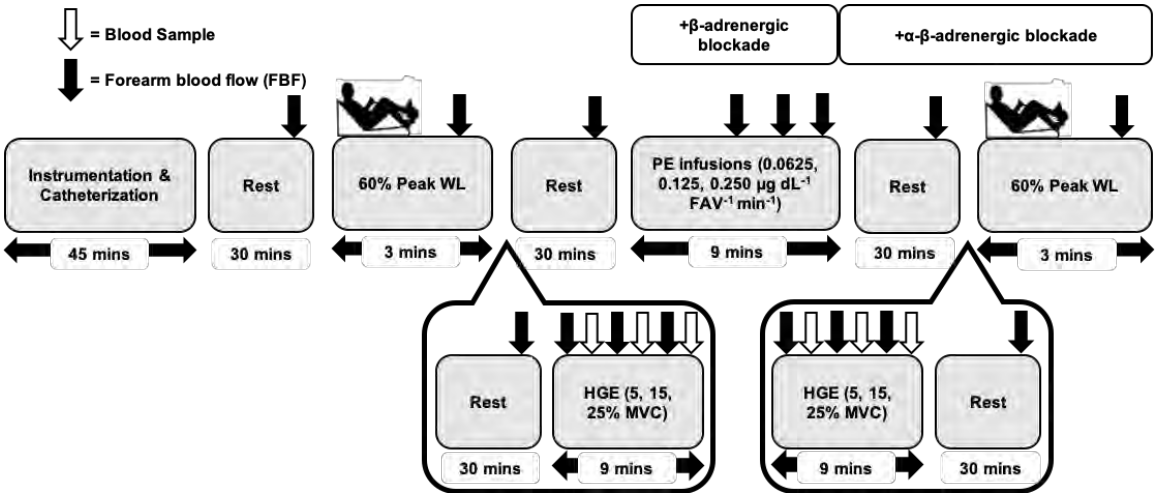


Figure 1. Schematic outline of experimental protocol. Forearm blood flow assessed via Doppler ultrasound represented by black arrows; blood samples represented by white arrows. FBF, forearm blood flow; Peak WL, total peak workload; HGE, handgrip exercise; MVC, maximum voluntary contraction; PE, phenylephrine; +β-adrenergic blockade, localized forearm propranolol infusion; +α-β-adrenergic blockade, localized forearm phentolamine and propranolol infusion; $\mu g \cdot dL^{-1} \cdot FAV^{-1} \cdot min^{-1}$, microgram per deciliter per forearm volume per minute. Time is displayed in minutes. Subjects sample size: n=16; CMS-: n=9; CMS+: n=7.

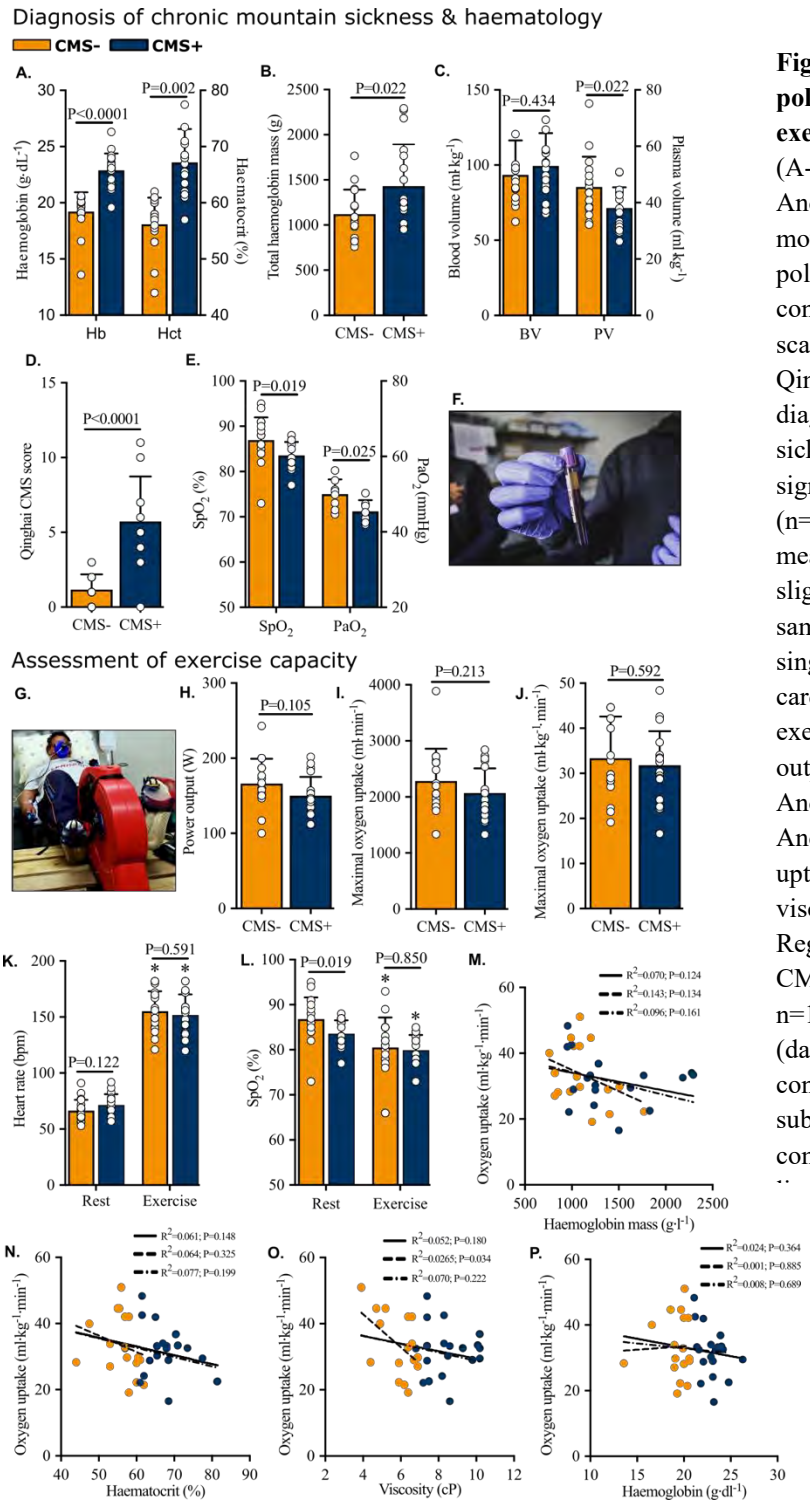


Figure 2. Assessment and determination of polycythaemia, chronic mountain sickness, and exercise capacity in Andean high-altitude residents. (A-C) Haematological measurements in healthy Andeans (n=17, CMS-) and Andeans with chronic mountain sickness (n=19, CMS+), indicating polycythaemia and substantial plasma volume contraction. Note that plasma and blood volume are scaled to subject body weight. (D) Differences in the Qing-Hai questionnaire total score, a common diagnostic tool for diagnosing chronic mountain sickness. (E) Pulse oximetry in Andeans indicating significant hypoxemia in CMS. In a subset of Andeans (n=16), arterial partial pressure of oxygen was measured via arterial blood samples confirming a slightly greater degree of hypoxemia. (F) Blood sample highlighting significant polycythaemia in a single CMS+ subject. (G) Experimental setup for both cardiopulmonary exercise testing and pharmacological exercise testing. (H-L) Results for exercise power output and cardiopulmonary exercise capacity in Andeans indicating a similar exercise capacity between Andean groups. (M-P) Regression analysis for oxygen uptake and haemoglobin mass (M), haematocrit (N), viscosity (O) and haemoglobin concentration (P). Regression analyses were separated into CMS- and CMS+ (solid line, n=36), CMS- only (dashed line, n=17), and all Andeans with a CMS score of <6 (dashed/dotted line, n=23). Photograph (F) was contributed by Alexandra M. Williams. Total Andean subjects n=36 (CMS-: n=17; CMS+: n=19). Statistical comparisons were performed using unpaired *t*-tests and

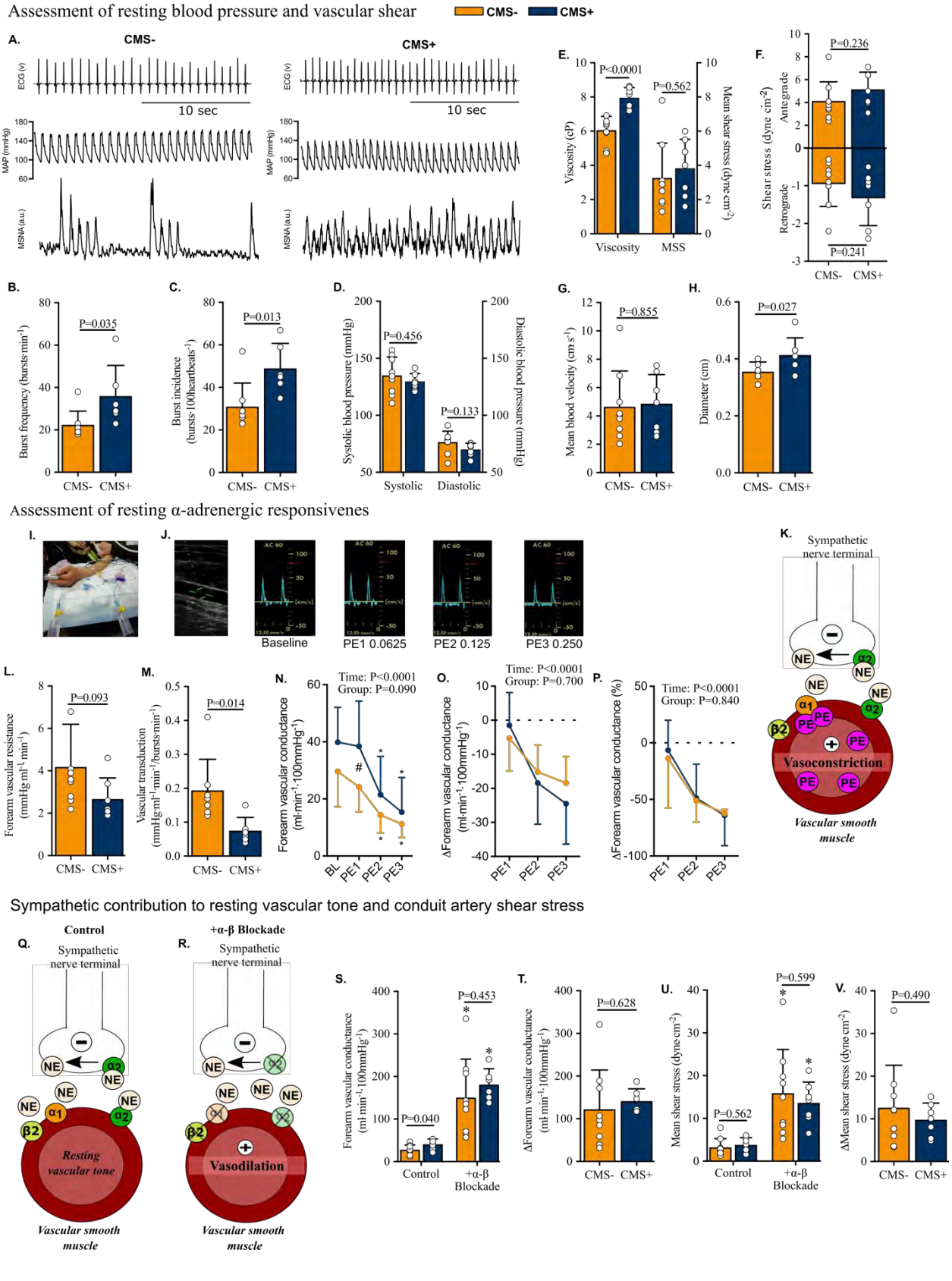

Assessment of resting blood pressure and vascular shear
CMS-
CMS+
A.
ECG (v)
10 sec
MAP (mmHg)
MSNA (a.u.)
B.
Burst frequency (bursts·min-1)
P=0.035
C.
Burst incidence (bursts·100heartbeats-1)
P=0.013
D.
Systolic blood pressure (mmHg)
Diastolic blood pressure (mmHg)
P=0.456
P=0.133
Systolic
Diastolic
E.
Viscosity (cP)
Mean shear stress (dyne cm-2)
P<0.0001
P=0.562
Viscosity
MSS
F.
Shear stress (dyne cm-2)
Antegrade
Retrograde
P=0.236
P=0.241
G.
Mean blood velocity (cm·s-1)
P=0.855
H.
Diameter (cm)
P=0.027
Assessment of resting α-adrenergic responsivenes
I.
J.
Baseline
PE1 0.0625
PE2 0.125
PE3 0.250
K.
Sympathetic nerve terminal
NE
α2
α1
β2
PE
Vasoconstriction
Vascular smooth muscle
L.
Forearm vascular resistance (mmHg·ml-1·min-1)
P=0.093
M.
Vascular transduction (mmHg·ml-1·min-1/bursts·min-1)
P=0.014
N.
Forearm vascular conductance (ml·min-1·100mmHg-1)
Time: P<0.0001
Group: P=0.090
BL
PE1
PE2
PE3
O.
ΔForearm vascular conductance (ml·min-1·100mmHg-1)
Time: P<0.0001
Group: P=0.700
P.
ΔForearm vascular conductance (%)
Time: P<0.0001
Group: P=0.840
Sympathetic contribution to resting vascular tone and conduit artery shear stress
Q.
Control
Sympathetic nerve terminal
Resting vascular tone
Vascular smooth muscle
R.
+α-β Blockade
Sympathetic nerve terminal
Vasodilation
Vascular smooth muscle
S.
Forearm vascular conductance (ml·min-1·100mmHg-1)
P=0.040
P=0.453
Control
+α-β Blockade
T.
ΔForearm vascular conductance (ml·min-1·100mmHg-1)
P=0.628
CMS-
CMS+
U.
Mean shear stress (dyne cm-2)
P=0.562
P=0.599
Control
+α-β Blockade
V.
ΔMean shear stress (dyne cm-2)
P=0.490
CMS-
CMS+
968
969

Figure 3. Assessment of resting blood pressure and α-adrenergic receptors. (A) Raw tracings of intra-arterial measurement of resting systolic and diastolic blood pressure in healthy Andeans (CMS-, n=9) and Andeans with chronic mountain sickness (CMS+, n=7) combined with resting recordings of muscle sympathetic nervous activity (MSNA). (B-D) At rest, MSNA burst frequency and incidence was elevated in CMS+ compared to CMS-, however, resting systolic and diastolic blood pressure was the same. (E-H) Blood viscosity was elevated in CMS+, with no difference in mean shear stress between groups. Mean blood velocity was the same between both groups; however, artery diameter was larger in CMS+, allowing for the normalization of conduit artery mean shear stress. (I-J) Experimental setup of phenylephrine (PE) infusions. Localized Phenylephrine (PE) infusions elicit localized graded vasoconstriction as indicated by (+), thru binding and activating α_1-adrenergic receptors. (K) Figure schematic demonstrating resting α-adrenergic pathway, whereby the sympathetic nerve terminal releases norepinephrine (NE) that binds to both pre-junctional α_2-adrenergic receptors attenuating further NE release as indicated by arrow and (-), and post-junctional α_1 & α_2-adrenergic receptors. (L-M) Resting forearm vascular resistance (FVR), measured via Duplex ultrasound, and vascular transduction, calculated via MSNA burst frequency and forearm vascular resistance, was attenuated in CMS+, despite higher MSNA in CMS+. (N-P) The absolute response in forearm vascular conductance (FVC) to low dose PE was slightly attenuated in CMS+. However, the delta and percent change in FVC were similar between groups. (Q-R) Figure schematic demonstrating resting α-adrenergic pathway on vascular tone and subsequent to α-adrenergic receptors blockade using localized phentolamine, a non-selective α-adrenergic antagonist, eliciting vasodilation indicated by (+). (S-V) After blockade of α-adrenergic receptors, FVC increased similarly in both groups. #signifies difference $P<0.05$ CMS- versus CMS+. *signifies $P<0.05$ control versus +α-β blockade. Statistical comparisons were performed using unpaired *t*-tests, one- and two-way repeated measures of analysis of variance. Total Andean subjects n=16 (CMS-: n=9; CMS+: n=7).

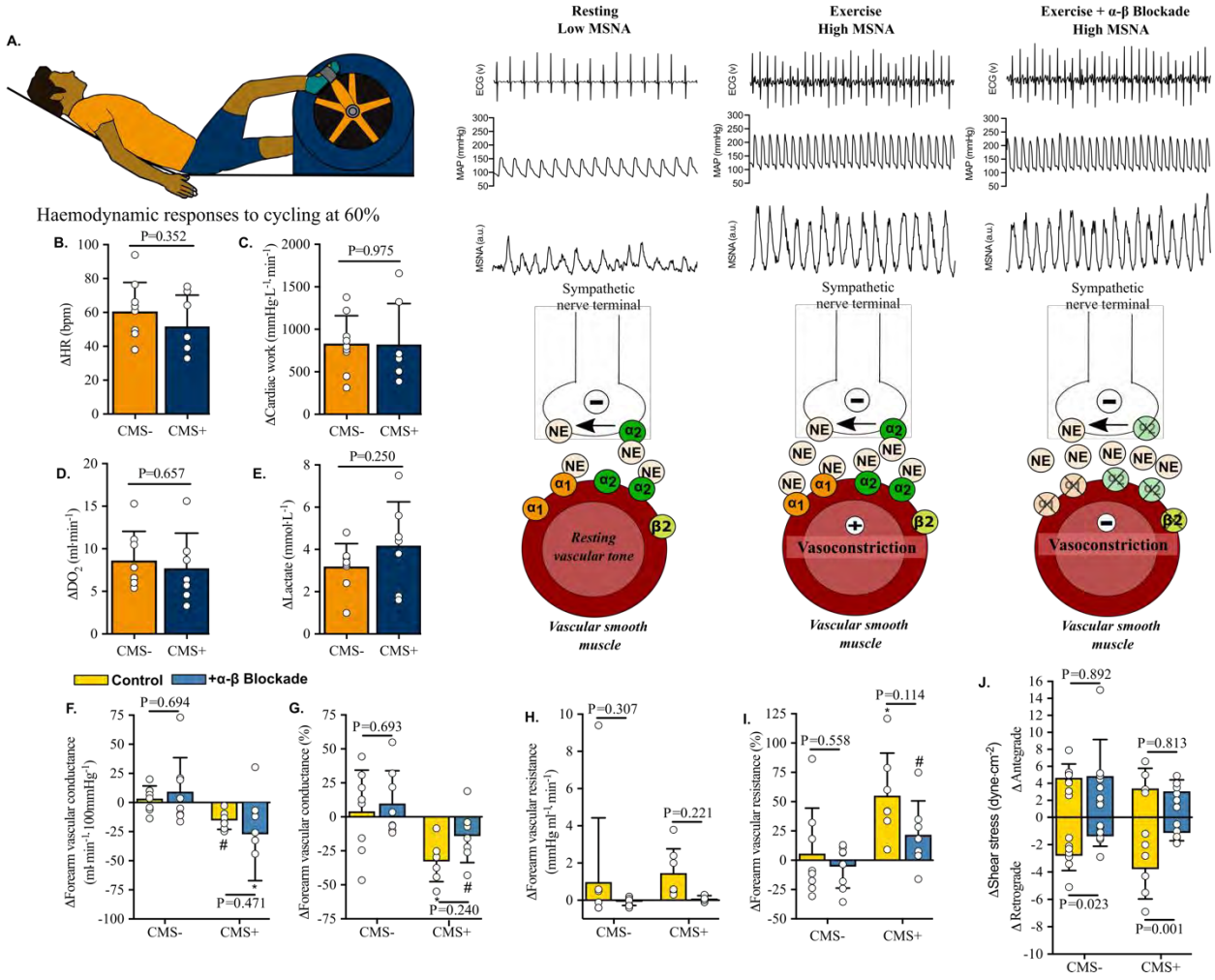


Figure 4. α-adrenergic vasoconstrictor signalling in non-active forearm skeletal muscle during leg cycling. (A) Experimental setup, all participants were positioned on a semi-recumbent cycle ergometer and cycled at 60% total peak workload (TPW) which elicits increases in muscle sympathetic nerve activity (MSNA). Schematic showing the proposed changes in blood pressure and MSNA alongside local pathways whereby increases in SNA releases norepinephrine (NE) from sympathetic nerve terminals which binds to both pre-junctional α_2-adrenergic receptors and attenuates further NE release, and post-junctional α_1 & α_2-adrenergic receptors increasing vasoconstriction in non-active skeletal muscle as indicated by (+). Hemodynamic responses in the form of heart rate (HR), cardiac work, global oxygen delivery (DO_2), and lactate to cycling at 60% TPW in CMS- (yellow) and CMS+ (blue). Using localized phentolamine, a non-selective α-adrenergic antagonist, NE was blocked from binding to α-adrenergic receptors eliciting vasodilation as indicated by (-). Absolute and percent change in forearm vascular conductance (F-G) and resistance (H-I) at rest and during cycling at 60% total peak workload. (J) Absolute change in shear stress patterns (i.e., antegrade and retrograde). *signifies difference P<0.05 and #signifies trending P<0.1 between healthy Andeans (CMS-, n=9) versus chronic mountain sickness (CMS+, n=7). Statistical comparisons were performed using paired and unpaired *t*-tests and two-way repeated measures analysis of variance. Total Andean subjects n=16.

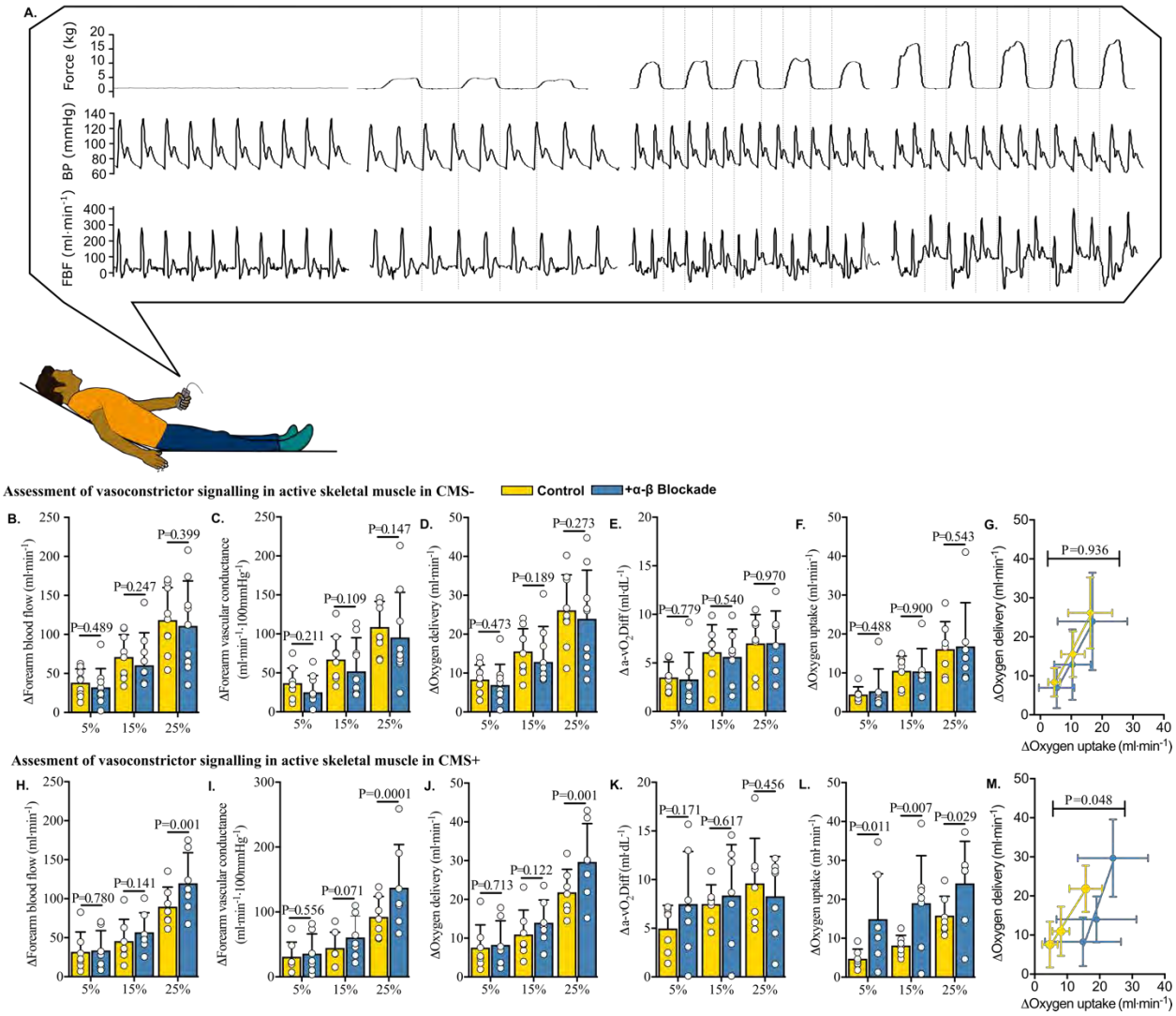
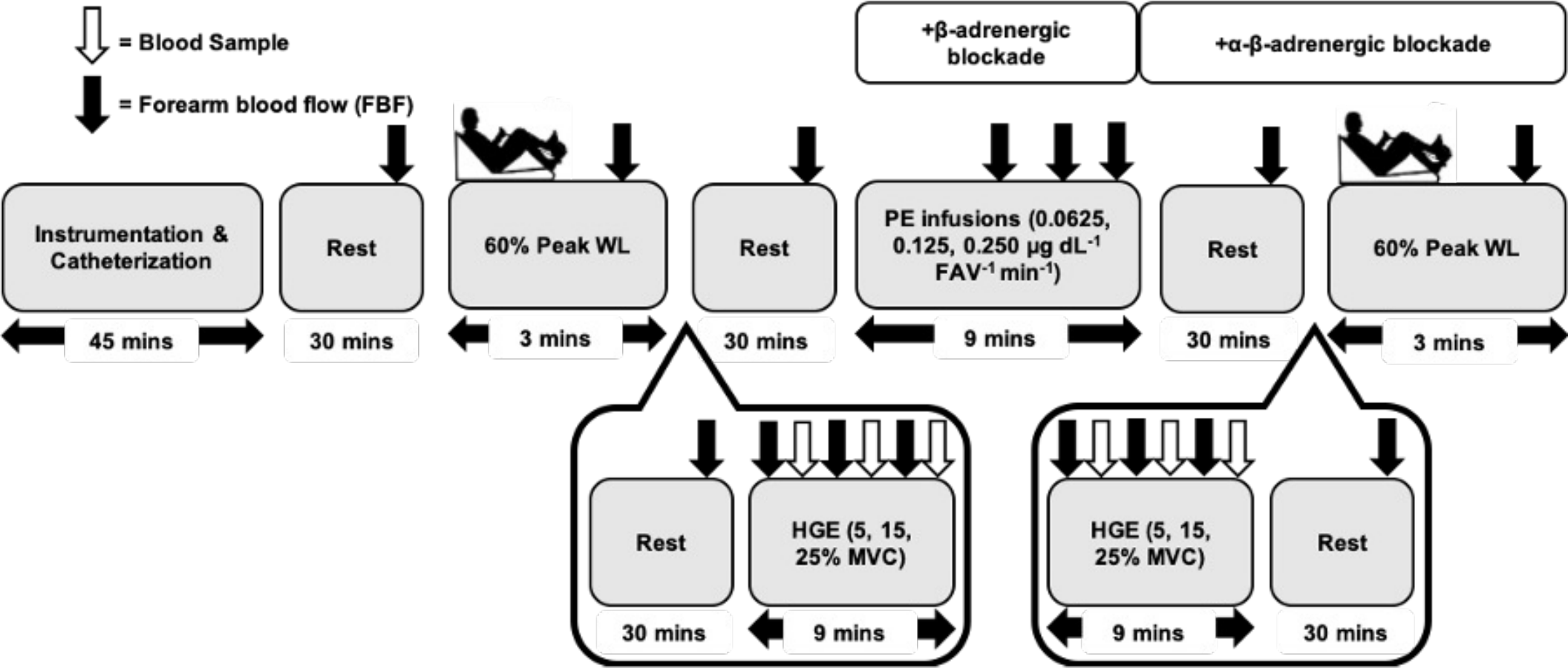
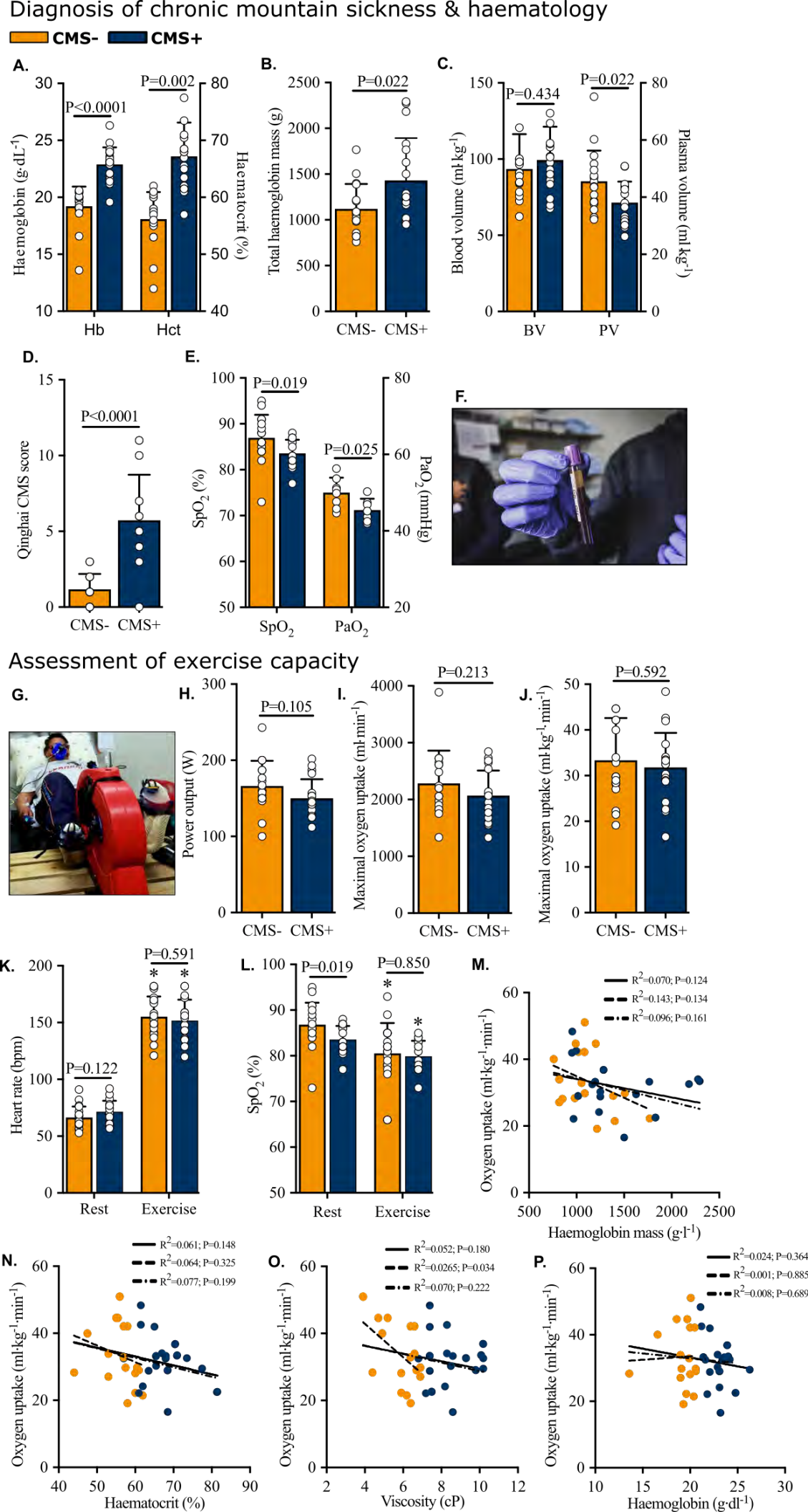
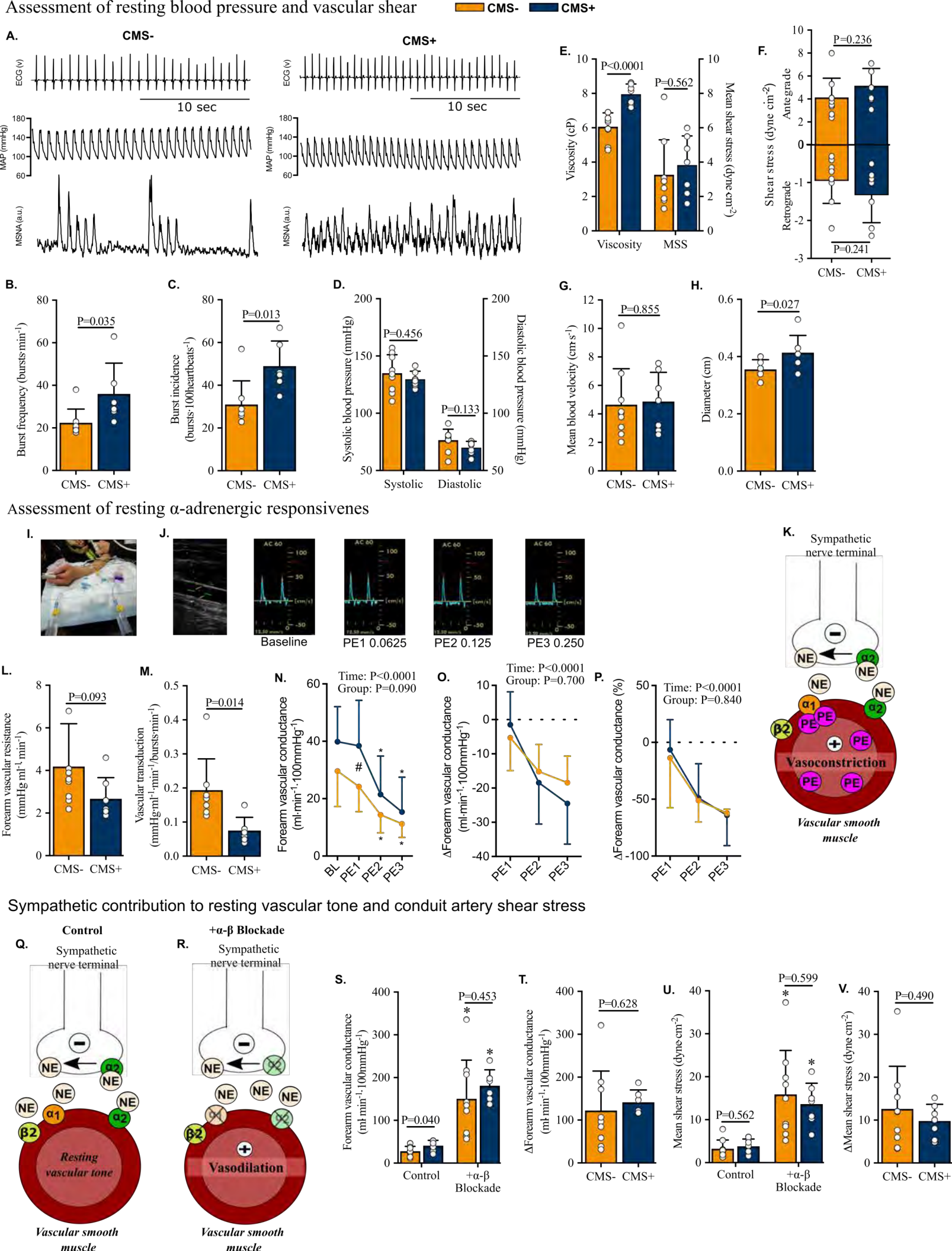


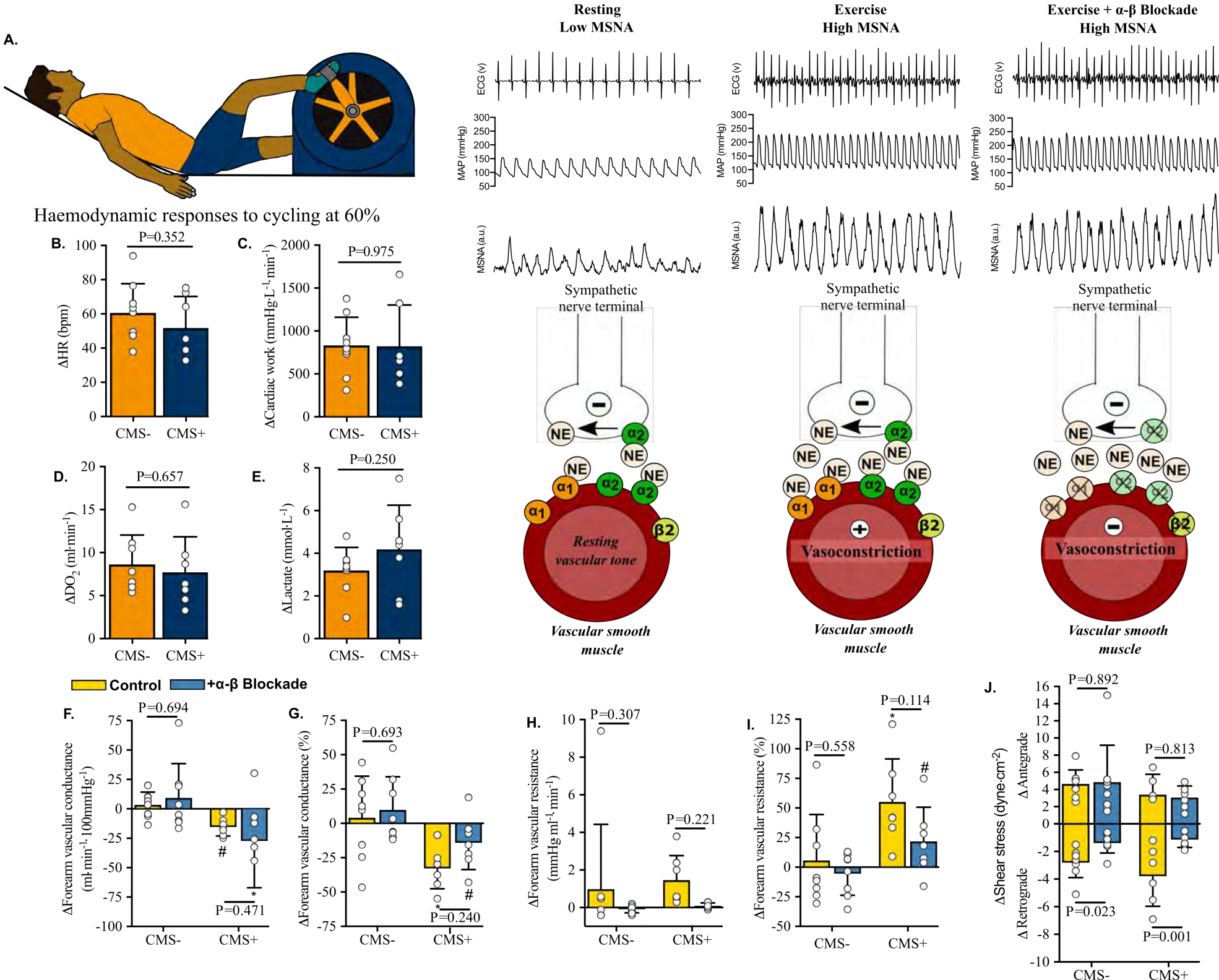
Figure 5. Oxygen flux and the contribution of α-adrenergic vasoconstrictor signalling in active forearm skeletal muscle during handgrip exercise. (A) Experimental setup for graded handgrip exercise using 5, 15, and 25% of maximum voluntary contractions (MVC), indicating blood pressure (BP) and forearm blood flow (FBF) responses to graded handgrip exercise. (B-F) Blood flow, vascular conductance, oxygen delivery, arterial-venous oxygen difference (a-vO_{2diff}), and oxygen uptake responses to handgrip exercise presented as absolute change in healthy Andeans (CMS-, n=9). Localized +α-β blockade did not affect any of the responses to handgrip exercise in CMS-. (G-K) Forearm haemodynamics as above in persons with chronic mountain sickness (CMS+, n=7). Localized +α-β blockade significantly increased blood flow, vascular conductance and oxygen delivery at 25% MVC; however, oxygen uptake was increased at all three exercise intensities. (L-M) Linear regression analysis between oxygen uptake and oxygen delivery revealed and no change in CMS- (control: R^2=0.566; y=1.513x+0.910; P<0.0001; +α-β blockade: R^2=0.382; y=1.476x-1.414; P=0.001) and a rightward shift in CMS+ (control R^2=0.538; y=1.318x+0.954; P=0.0002; +α-β blockade R^2=0.602; y=2.356x-28.23; P<0.0001) with a significant change in slope (P=0.04). Statistical comparisons were performed using paired *t*-tests, two-way repeated measures analysis of variance and simple linear regression analysis. Total Andean subjects n=16 (CMS-: n=9; CMS+: n=7).

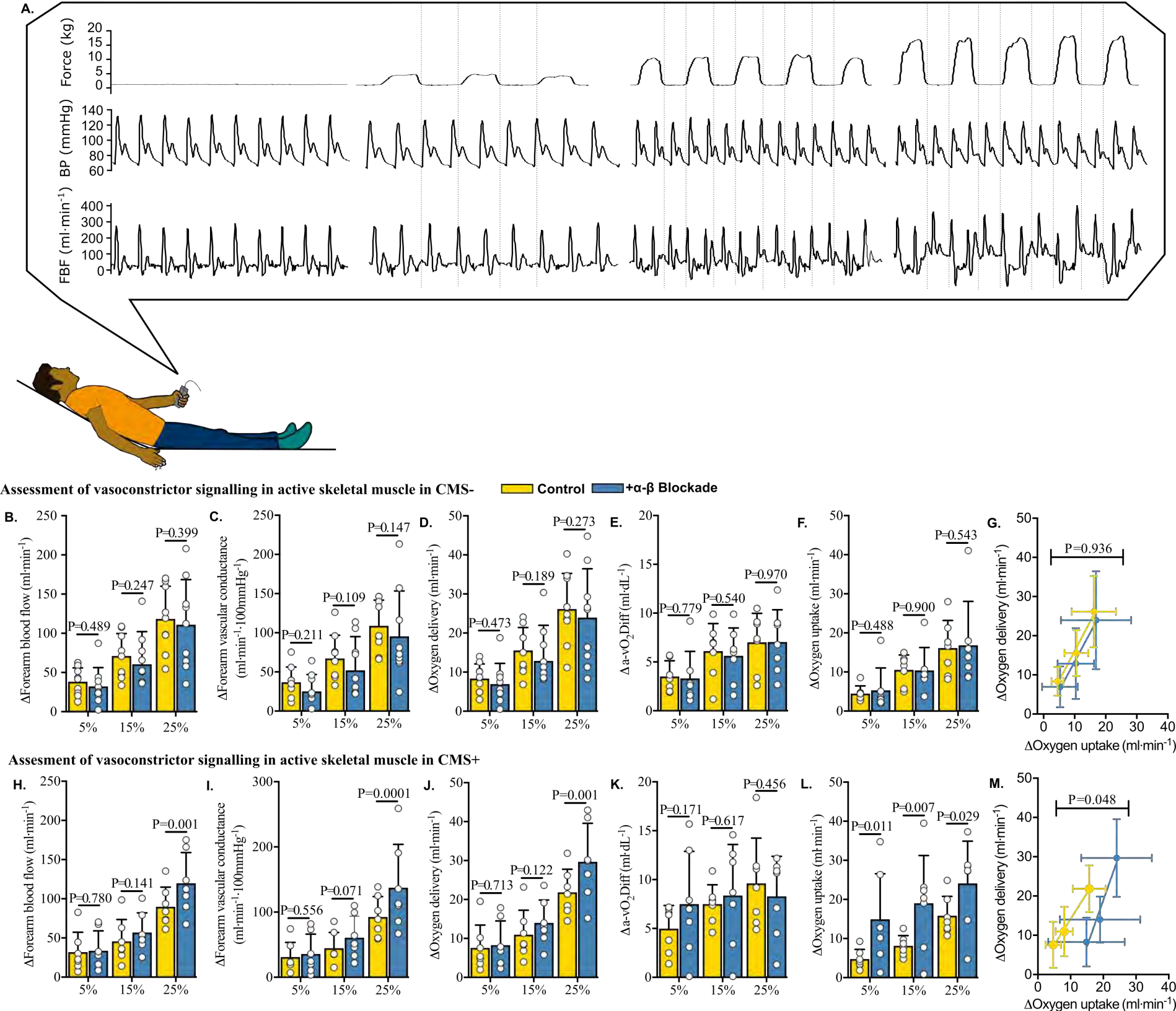

= Blood Sample
= Forearm blood flow (FBF)
+β-adrenergic blockade
+α-β-adrenergic blockade
Instrumentation & Catheterization
45 mins
Rest
30 mins
60% Peak WL
3 mins
Rest
30 mins
PE infusions (0.0625, 0.125, 0.250 μg dL-1 FAV-1 min-1)
9 mins
Rest
30 mins
60% Peak WL
3 mins
Rest
30 mins
HGE (5, 15, 25% MVC)
9 mins
HGE (5, 15, 25% MVC)
9 mins
Rest
30 mins


Diagnosis of chronic mountain sickness & haematology
CMS-
CMS+
A.
Haemoglobin (g·dL-1)
Haematocrit (%)
P<0.0001
P=0.002
Hb
Hct
B.
Total haemoglobin mass (g)
P=0.022
CMS-
CMS+
C.
Blood volume (ml·kg-1)
Plasma volume (ml·kg-1)
P=0.434
P=0.022
BV
PV
D.
Qinghai CMS score
P<0.0001
E.
SpO2 (%)
PaO2 (mmHg)
P=0.019
P=0.025
F.
Assessment of exercise capacity
G.
H.
Power output (W)
P=0.105
I.
Maximal oxygen uptake (ml·min-1)
P=0.213
J.
Maximal oxygen uptake (ml·kg-1·min-1)
P=0.592
K.
Heart rate (bpm)
P=0.122
P=0.591
Rest
Exercise
L.
SpO2 (%)
P=0.019
P=0.850
M.
Oxygen uptake (ml·kg-1·min-1)
R2=0.070; P=0.124
R2=0.143; P=0.134
R2=0.096; P=0.161
Haemoglobin mass (g·l-1)
N.
R2=0.061; P=0.148
R2=0.064; P=0.325
R2=0.077; P=0.199
Haematocrit (%)
O.
R2=0.052; P=0.180
R2=0.0265; P=0.034
R2=0.070; P=0.222
Viscosity (cP)
P.
R2=0.024; P=0.364
R2=0.001; P=0.885
R2=0.008; P=0.689
Haemoglobin (g·dl-1)


Assessment of resting blood pressure and vascular shear
CMS-
CMS+
A.
ECG (v)
10 sec
MAP (mmHg)
MSNA (a.u.)
B.
Burst frequency (bursts·min-1)
P=0.035
C.
Burst incidence (bursts·100heartbeats-1)
P=0.013
D.
Systolic blood pressure (mmHg)
Diastolic blood pressure (mmHg)
P=0.456
P=0.133
Systolic
Diastolic
E.
Viscosity (cP)
Mean shear stress (dyne·cm-2)
P<0.0001
P=0.562
Viscosity
MSS
F.
Shear stress (dyne·cm-2)
Antegrade
Retrograde
P=0.236
P=0.241
G.
Mean blood velocity (cm·s-1)
P=0.855
H.
Diameter (cm)
P=0.027
Assessment of resting α-adrenergic responsivenes
I.
J.
Baseline
PE1 0.0625
PE2 0.125
PE3 0.250
K.
Sympathetic nerve terminal
NE
α2
α1
β2
PE
Vasoconstriction
Vascular smooth muscle
L.
Forearm vascular resistance (mmHg·ml-1·min-1)
P=0.093
M.
Vascular transduction (mmHg·ml-1·min-1/bursts·min-1)
P=0.014
N.
Forearm vascular conductance (ml·min-1·100mmHg-1)
Time: P<0.0001
Group: P=0.090
BL
PE1
PE2
PE3
O.
ΔForearm vascular conductance (ml·min-1·100mmHg-1)
Time: P<0.0001
Group: P=0.700
P.
ΔForearm vascular conductance (%)
Time: P<0.0001
Group: P=0.840
Sympathetic contribution to resting vascular tone and conduit artery shear stress
Control
+α-β Blockade
Q.
R.
Resting vascular tone
Vasodilation
Vascular smooth muscle
S.
Forearm vascular conductance (ml·min-1·100mmHg-1)
P=0.040
P=0.453
Control
+α-β Blockade
T.
ΔForearm vascular conductance (ml·min-1·100mmHg-1)
P=0.628
U.
Mean shear stress (dyne·cm-2)
P=0.562
P=0.599
V.
ΔMean shear stress (dyne·cm-2)
P=0.490
CMS-
CMS+

Contribution of α_1-adrenergic receptors to vasoconstriction in non-active skeletal muscle during exercise




A.
Force (kg)
BP (mmHg)
FBF (ml·min⁻¹)
Assessment of vasoconstrictor signalling in active skeletal muscle in CMS-
Control
+α-β Blockade
B.
ΔForearm blood flow (ml·min⁻¹)
P=0.489
P=0.247
P=0.399
C.
ΔForearm vascular conductance (ml·min⁻¹·100mmHg⁻¹)
P=0.211
P=0.109
P=0.147
D.
ΔOxygen delivery (ml·min⁻¹)
P=0.473
P=0.189
P=0.273
E.
Δa-vO2Diff (ml·dL⁻¹)
P=0.779
P=0.540
P=0.970
F.
ΔOxygen uptake (ml·min⁻¹)
P=0.488
P=0.900
P=0.543
G.
ΔOxygen delivery (ml·min⁻¹)
P=0.936
ΔOxygen uptake (ml·min⁻¹)
Assesment of vasoconstrictor signalling in active skeletal muscle in CMS+
H.
ΔForearm blood flow (ml·min⁻¹)
P=0.780
P=0.141
P=0.001
I.
ΔForearm vascular conductance (ml·min⁻¹·100mmHg⁻¹)
P=0.556
P=0.071
P=0.0001
J.
ΔOxygen delivery (ml·min⁻¹)
P=0.713
P=0.122
P=0.001
K.
Δa-vO2Diff (ml·dL⁻¹)
P=0.171
P=0.617
P=0.456
L.
ΔOxygen uptake (ml·min⁻¹)
P=0.011
P=0.007
P=0.029
M.
ΔOxygen delivery (ml·min⁻¹)
P=0.048
ΔOxygen uptake (ml·min⁻¹)
5%
15%
25%