Title: Global REACH: Andean Highlanders, Chronic Mountain Sickness and the Integrative Regulation of Resting Blood Pressure.

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Running Title: Blood pressure regulation in chronic mountain sickness

Abstract: High-altitude maladaptation syndrome chronic mountain sickness (CMS) is characterised by excessive erythrocytosis and frequently accompanied by accentuated arterial hypoxaemia. Whether altered autonomic cardiovascular regulation is apparent in CMS is unclear. Therefore, we assessed integrative control of blood pressure (BP) and determined basal sympathetic vasomotor outflow and arterial baroreflex function in 8 Andean natives with CMS ([Hb] 22.6{plus minus}0.9g/dL) and 7 healthy highlanders ([Hb] 19.3{plus minus}0.8g/dL) at their resident altitude (Cerro de Pasco, Peru; 4383m). R-R interval (RRI, electrocardiogram), beat-by-beat BP (photoplethysmography) and muscle sympathetic nerve activity (MSNA; microneurography) were recorded at rest and during pharmacologically-induced changes in BP (modified Oxford test). Although [Hb] and blood viscosity (7.8{plus minus}0.7 vs 6.6{plus minus}0.7cP; d=1.7, P=0.01) were elevated in CMS compared to healthy highlanders, cardiac output, total peripheral resistance and mean BP were similar between groups. The vascular sympathetic baroreflex MSNA set-point (i.e. MSNA burst incidence) and reflex gain (i.e. responsiveness) were also similar between groups (MSNA set-point; d=0.75, P=0.16, gain; d=0.2, P=0.69). In contrast, in CMS the cardiovagal baroreflex operated around a longer RRI (960{plus minus}159 vs 817{plus minus}50msec; d=1.4, P=0.04) with a greater reflex gain (17.2{plus minus}6.8 vs 8.8{plus minus}2.6msec·mmHg-1; d=1.8, P=0.01) versus healthy highlanders. Basal sympathetic vasomotor activity was also lower compared to healthy highlanders (33{plus minus}11 vs 45{plus minus}13bursts·min-1; d=1.0, P=0.08). In conclusion, our findings indicate adaptive differences in basal sympathetic vasomotor activity and heart rate compensate for the haemodynamic consequences of excessive erythrocyte volume and contribute to integrative blood pressure regulation in Andean highlanders with mild CMS.

New Findings: What is the central question of this study? Does chronic mountain sickness (CMS) alter sympathetic neural control and arterial baroreflex regulation of blood pressure in Andean (Quechua) highlanders? What is the main finding and its importance? Compared to healthy Andean highlanders, basal sympathetic vasomotor outflow is lower, baroreflex control of MSNA is similar, supine heart rate is lower, and cardiovagal baroreflex gain is greater in mild CMS. Taken together, these findings reflect flexibility in integrative regulation of blood pressure that may be important when blood viscosity and blood volume are elevated in CMS.

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2 **Regulation of Resting Blood Pressure.**

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24 **New findings**
25

26 *What is the central question of this study?*

27 Does chronic mountain sickness (CMS) alter sympathetic neural control and arterial
28 baroreflex regulation of blood pressure in Andean (Quechua) highlanders?

29 *What is the main finding and its importance?*

30 Compared to healthy Andean highlanders, basal sympathetic vasomotor outflow is lower,
31 baroreflex control of MSNA is similar, supine heart rate is lower, and cardiovagal baroreflex
32 gain is greater in mild CMS. Taken together, these findings reflect flexibility in integrative

regulation of blood pressure that may be important when blood viscosity and blood volume are elevated in CMS.

Key words: Chronic mountain sickness, excessive erythrocytosis, arterial baroreflex, muscle sympathetic nerve activity, blood pressure control

Abbreviations:

CMS*: Chronic mountain sickness*

BP*: Blood pressure*

Hb*: Haemoglobin concentration*

RRI*: R-R interval*

MSNA*: Muscle sympathetic nerve activity*

EE*: Excessive erythrocytosis*

HR*: Heart rate*

SBP*: Systolic blood pressure*

DBP*: Diastolic blood pressure*

MAP*: Mean arterial pressure*

SV*: Stroke volume*

Qc*: Cardiac output*

TPR*: Total peripheral resistance*

PASP*: Pulmonary artery systolic pressure*

PE*: Phenylephrine*

SNP*: Sodium nitroprusside*

SaO$_2$*: Arterial oxygen saturation*

PaO$_2$*: Arterial oxygen partial pressure*

PaCO$_2$*: Arterial oxygen partial pressure*

Abstract

High-altitude maladaptation syndrome chronic mountain sickness (CMS) is characterised by excessive erythrocytosis and frequently accompanied by accentuated arterial hypoxaemia. Whether altered autonomic cardiovascular regulation is apparent in CMS is unclear. Therefore, we assessed integrative control of blood pressure (BP) and determined basal sympathetic vasomotor outflow and arterial baroreflex function in 8 Andean natives with CMS ([Hb] 22.6±0.9g/dL) and 7 healthy highlanders ([Hb] 19.3±0.8g/dL) at their resident altitude (Cerro de Pasco, Peru; 4383m). R-R interval (RRI, electrocardiogram), beat-by-beat BP (photoplethysmography) and muscle sympathetic nerve activity (MSNA; microneurography) were recorded at rest and during pharmacologically-induced changes in BP (modified Oxford test). Although [Hb] and blood viscosity (7.8±0.7 vs 6.6±0.7cP; d=1.7, P=0.01) were elevated in CMS compared to healthy highlanders, cardiac output, total peripheral resistance and mean BP were similar between groups. The vascular sympathetic baroreflex MSNA set-point (i.e. MSNA burst incidence) and reflex gain (i.e. responsiveness) were also similar between groups (MSNA set-point; d=0.75, P=0.16, gain; d=0.2, P=0.69). In contrast, in CMS the cardiovagal baroreflex operated around a longer RRI (960±159 vs 817±50msec; d=1.4, P=0.04) with a greater reflex gain (17.2±6.8 vs 8.8±2.6msec·mmHg^{-1}; d=1.8, P=0.01) versus healthy highlanders. Basal sympathetic vasomotor activity was also lower compared to healthy highlanders (33±11 vs 45±13bursts·min^{-1}; d=1.0, P=0.08). In conclusion, our findings indicate adaptive differences in basal sympathetic vasomotor activity and heart rate compensate for the haemodynamic consequences of excessive erythrocyte volume and contribute to integrative blood pressure regulation in Andean highlanders with mild CMS.

Introduction

Globally, between 5–10% of the ~140 million people living at high-altitude (>2500m) lack the ability to cope with chronic hypoxia and develop a progressively incapacitating maladaptation syndrome termed chronic mountain sickness (León-Velarde *et al.*, 2005). Chronic mountain sickness (CMS), which is most prevalent in natives of the Andean plateau, is characterised by excessive erythrocytosis (EE, haemoglobin concentration [Hb] ≥21g/dL for men, ≥19g/dL for women) and is frequently accompanied by accentuated arterial hypoxaemia for the resident altitude; and, in more severe stages of the disease, pulmonary hypertension (León-Velarde *et al.*, 2005). In addition, CMS individuals may present with a number of clinical symptoms including headache, breathlessness, sleep disturbances, and cognitive impairment (León-Velarde *et al.*, 2005; Villafuerte & Corante, 2016). Importantly, CMS is also associated with an increased cardiovascular disease risk (Corante *et al.*, 2018), which increases with disease severity. Specifically, an increased prevalence of thrombotic events, stroke, coronary heart disease and systemic and pulmonary hypertension, which can give rise to cardiac hypertrophy and congestive heart failure, have all been reported in CMS (Monge, 1942; Peñaloza *et al.,* 1971; Leon-Velarde and Arregui, 1994; Leon-Velarde *et al.*, 2014). Excessive erythrocyte volume and the resulting elevations in haemoglobin and hematocrit are known to contribute to this increased risk (Corante *et al.*, 2018; Tremblay *et al.,* 2019). However, several other clinical conditions characterised by sustained hypoxaemia (i.e. Chronic Obstructive Pulmonary Disease) are often accompanied by arterial baroreflex dysfunction and elevated sympathetic vasomotor outflow (van Gestel & Steier, 2010; Andreas *et al.*, 2013). Such changes, which can facilitate increased blood pressure variability, elevated blood pressure, increased arterial stiffness and vascular dysfunction (Smit *et al.*, 2002; Hijmering *et al.,* 2002; Swierblewska *et al.*, 2010), can all contribute to the development of cardiovascular disease. Whether arterial baroreflex dysfunction and elevated sympathetic vasomotor outflow are also apparent in CMS is unclear.

109   The arterial baroreflex plays a fundamental role in the control of blood pressure

110   through its regulation of cardiac pacemaker activity and sympathetic vasomotor outflow.

111   Previous research has found impaired baroreflex control of R-R interval (RRI) in Andean

112   highlanders with CMS compared to healthy highlanders (Keyl *et al.*, 2003); however, this is

113   not a consistent finding (Gulli *et al.*, 2007). Baroreflex control of arterial pressure also occurs

114   via alterations in sympathetic vasomotor outflow. Previously, no difference in maximum gain

115   (i.e. responsiveness) of carotid baroreflex control of forearm vascular resistance (index of

116   sympathetic vasomotor activity) was reported for CMS compared to healthy Andean

117   highlanders (Moore *et al.*, 2006). Nevertheless, to the best of our knowledge, no direct

118   measurement of sympathetic vasomotor activity exists for CMS individuals. Whilst plasma

119   catecholamine concentrations may not accurately represent sympathetic nervous system

120   activity (Esler, 1988), they are reported to be either elevated (Gamboa *et al.,* 2006) or

121   unchanged (Antezana *et al.*, 1995) in CMS, indicating either an increased or comparable

122   global sympathetic activation compared to their healthy Andean counterparts. On one hand,

123   elevated sympathetic activity might be predicted in CMS if exaggerated arterial hypoxaemia

124   is present; thus augmenting tonic peripheral chemoreflex activation. On the other hand, a

125   larger blood volume in CMS (Claydon *et al.*, 2004) might have a sympathoinhibitory effect on

126   basal MSNA, as shown in healthy individuals at sea-level (Charkoudian *et al.*, 2004; Best *et*

127   *al.*, 2014).

128   In light of the equivocal findings, and absence of microneurographic data for CMS, it

129   is unclear what effect CMS has on sympathetic neural control and arterial baroreflex

130   regulation of blood pressure in Andean highlanders. The present study, therefore, aimed to

131   comprehensively assess integrative regulation of resting blood pressure in Andean

132   highlanders with CMS, and to compare this with healthy highlanders. To achieve this we

133   assessed blood volume, basal sympathetic vasomotor outflow, and arterial baroreflex control

134   of the heart and sympathetic vasomotor outflow. Based upon limited previous reports, we

135   hypothesised that 1) the vascular sympathetic baroreflex would operate around a higher

136   MSNA burst incidence for CMS, with no difference in reflex gain (i.e. responsiveness),

137   compared to healthy highlanders; 2) the cardiovagal baroreflex would operate around a

138   shorter RRI (higher HR) in CMS with a concurrent reduction in reflex gain, therefore, 3) basal

139   sympathetic vasomotor outflow and arterial pressure would be elevated for CMS. A

140   secondary aim was to determine the contribution of the peripheral chemoreflex to basal

141   MSNA and arterial baroreflex function in CMS.

142

143 **Methodology**

144 **Ethical approval**

145 This study was part of the Global REACH high-altitude research expedition to the
146 Universidad Peruana Cayetano Heredia's Instituto de Investigacions de Altura (4380 m;
147 Cerro de Pasco, Peru) in July 2018. All experimental procedures had Institutional Review
148 Board approval from Universidad Peruana Cayetano Heredia (#101686, date of approval
149 20.02.2018) and conformed to the latest revision of the *Declaration of Helsinki*, except for
150 registration in a database. Prior to participation, all experimental procedures were explained
151 to subjects in writing, and verbally, in their native language, and written informed consent
152 was provided. Participants took part in a number of other studies; however, care was taken
153 to ensure adequate recovery between protocols to prevent any potential for confounding
154 results. Furthermore, the present study addressed a distinct *a priori* research question.

155 **Participants**

156 Twenty Andean men born at an altitude above 3250 m, permanently residing in the Cerro de
157 Pasco area and who had at least two previous known generations of high-altitude Andean
158 ancestry were recruited for the study. None of the subjects had travelled to an altitude lower
159 than 3000m in the previous six months and did not have a history of working in the mining
160 industry. None of the participants were taking prescribed medication and had no prior history
161 of cardiovascular, pulmonary, metabolic, neurological or renal disease. Participants attended
162 the laboratory on two occasions, with a minimum of 24 hours between visits 1) preliminary
163 screening visit, and 2) experimental visit.

164 *Preliminary screening visit,*

165 On arrival to the laboratory, participants provided a detailed clinical history and history of
166 high-altitude residence and ancestral background. A venous blood sample was drawn from
167 the antecubital vein to measure [Hb], hematocrit and blood viscosity. An arterial blood
168 sample was drawn from the radial artery (CG), following local anaesthesia (2% lidocaine), to

169   determine arterial blood gases (PaO_2 and $PaCO_2$) and arterial oxygen saturation (SaO_2).

170   Total blood volume (packed cell volume and plasma volume) was determined via the

171   modified carbon monoxide (CO) rebreathing method as previously described in detail

172   (Schmidt & Prommer 2005) and used previously by our group in lowland and highland

173   natives at high-altitude (Stembridge *et al.*, 2018, 2019). Participants also performed an

174   incremental exercise test (20 W/min) to exhaustion, in the semi recumbent position, on an

175   electronically braked cycle ergometer (Lode Angio; Lode). Breath-by-breath respiratory data

176   were collected throughout (Oxycon Mobile; Carefusion) to determine peak oxygen

177   consumption (VO_{2peak}).

178   Chronic mountain sickness scores were calculated using the Qinghai CMS questionnaire

179   based on the presence and severity of eight signs and symptoms of CMS, as agreed by

180   international consensus (León-Velarde *et al.*, 2005); EE, heart palpitations, difficulty

181   sleeping, cyanosis, parathesia, headache, tinnitus and dilated veins. A value of zero was

182   assigned to negative answers. Positive answers were categorised as light, moderate, or

183   severe and assigned values of one, two and three respectively. The sum of assigned values

184   constituted the CMS score. Subjects were diagnosed with CMS by a score ≥5 in the

185   presence of EE ([Hb] ≥21g/dL) and individuals not meeting these criteria were categorised

186   as healthy highlanders. The sum of the score defines CMS severity as absent (0–5), mild (6–

187   10), moderate (11–14) or severe (≥ 15). Two highlanders (both CMS) were current smokers,

188   but refrained from smoking on the day of testing.

189   **Experimental visit**

190   **Experimental protocol**

191   All participants were asked to abstain from caffeine, alcohol and vigorous exercise for at

192   least 24 hours before to the experimental session and arrived at the laboratory a minimum of

193   4 hours after a light meal. Following arrival at the laboratory, subjects rested in the supine

194   position and an antecubital venous cannula was inserted for subsequent drug administration.

195 Following instrumentation, acquisition of an acceptable MSNA signal and a period of

196 stabilisation, 10 minutes of baseline data were recorded to determine resting cardiovascular

197 and pulmonary haemodynamics and sympathetic vasomotor activity. A modified Oxford test

198 was then performed to assess vascular sympathetic and cardiovagal baroreflex function.

199 Following baseline measurements, participants were then transferred to breathing 100% O_2,

200 in an attempt to eliminate peripheral chemoreceptor drive, as used previously (Simpson *et*

201 *al.,* 2019). Subjects breathed hyperoxia, via a mouthpiece, for a period of five minutes.

202 Following five minutes of hyperoxia, a second modified Oxford test was performed, whilst

203 subjects continued to breathe hyperoxia, in order to determine the influence of the peripheral

204 chemoreflex on arterial baroreflex function. Due to the unknown time course of recovery

205 from hyperoxia, the order of conditions was not randomised. A minimum of 20 minutes

206 separated each modified Oxford test.

207 **Experimental measurements**

208 **Haematological analysis**

209 Venous blood samples were collected into lithium heparin-coated vacutainers (Becton,

210 Dickinson and Company, Mississauga, Canada) and tested within 15 minutes of acquisition.

211 Arterial blood samples were collected into pre-heparinized syringes (safePICO syringes,

212 Radiometer, Copenhagen, Denmark) for immediate analyses. Whole blood viscosity was

213 measured in duplicate at a shear rate 225 s-1 at 37 ºC using a cone and plate viscometer

214 (DV2T Viscometer, Brookfield Amtek, USA) and a circulating water heating bath (TC-150,

215 Brookfield Amtek, USA). [Hb] and hematocrit, arterial blood gases and SaO_2 were

216 determined by radiometer ABL90 analyzer (ABL90 Flex, Radiometer, Canada).

217 ***Cardiovascular haemodynamics***

218 Heart rate and blood pressure were continuously recorded using Lead II electrocardiogram

219 and finger photoplethysmography (Finometer Pro; Finapres Medical Systems BV,

220 Amsterdam, The Netherlands). Systolic (SBP), diastolic (DBP) and mean (MAP) pressures

221 were calculated on a beat-by-beat basis from the finger arterial pressure waveform.

222 Finometer values were calibrated against the average of three brachial artery blood pressure

223 measurements taken during baseline. Stroke volume (SV) and cardiac output (Qc) were

224 estimated using the Model Flow algorithm (Wesseling *et al.*, 1993) and used to estimate total

225 peripheral resistance (TPR = MAP/Qc).

226

227 ***Pulmonary Haemodynamics***

228 Echocardiograhy was used to assess pulmonary artery systolic pressure (PASP). Images

229 were obtained using a commercially available system (Vivid Q, GE, Fairfield, CT, USA) and

230 stored for subsequent off-line analysis. Pulmonary arterial systolic pressure (PASP) was

231 quantified as the maximum systolic pressure gradient across the tricuspid valve added to

232 right atrial pressure estimated from the collapsibility of the inferior vena cava, in line with the

233 guidelines of the American Society of Echocardiography (Rudski *et al.*, 2010). To derive

234 pressure, the modified Bernoulli equation $(4 \cdot V^2)$ was applied to the peak systolic

235 regurgitation jet velocity measured via continuous wave Doppler (Rudski *et al.*, 2010)

236

237 ***Muscle sympathetic nerve activity***

238 Multi-unit MSNA was recorded from the peroneal nerve via microneurography as previously

239 described (Hagbarth & Vallbo, 1968; Sundolf and Wallin, 1978). MSNA signal was confirmed

240 by pulse-synchronous activity that responded to end-expiratory apnea but not to startle

241 stimuli or skin stroking. Nerve signals were acquired (Neuroamp EX headstage,

242 ADInstruments, Sydney, Australia), amplified (100,000x), filtered (band pass 700-2,000Hz),

243 rectified and integrated (decay constant 0.1s) (LabChart Pro v8.3.1, ADInstruments, Sydney,

244 Australia). No adverse events or complications occurred during or following the

245 microneurography procedure in any subject.

246

247 ***Assessment of Sympathetic and Cardiac Baroreflex function***

248   Baroreflex function was assessed from the MSNA and RRI (and HR) responses during

249   arterial blood pressure perturbations induced by the modified Oxford test (Rudas *et al.*,

250   1999). Briefly, this involved bolus injection of sodium nitroprusside (SNP), followed 90

251   seconds later by phenylephrine (PE). Prior to experimental testing, bolus doses of SNP and

252   PE that evoked ~15mmHg perturbations above and below resting arterial blood pressure

253   were determined for each individual. Briefly, individualized doses of vasoactive drugs were

254   calculated based on total blood volume (20µg·L^{-1} SNP; 30 µg·L^{-1} PE), which were adjusted if

255   insufficient BP perturbations were achieved. Identical doses were administered during all

256   trials in the same individual. Doses of vasoactive drugs injected were similar in CMS (SNP,

257   1.66 ± 0.35 µg·kg^{-1}; PE, 2.48 ± 0.25 µg·kg^{-1}) and healthy highlanders (SNP, 1.48 ± 0.30

258   µg·kg^{-1}, *P* = 0.30 *d* = 0.5; PE, 2.34 ± 0.42 µg·kg^{-1}, *P* = 0.49 *d* = 0.42) and induced similar

259   total blood pressure perturbations in both groups (CMS, 26 ± 5 mmHg; Healthy highlanders,

260   25 ± 9 mmHg; *P = 0.72*, *d =* 0.11).

261   **Data analyses**

262   All haemodynamic data were sampled at 1KHz using a commercial data acquisition software

263   (LabChart Pro v8.3.1, ADInstruments, Sydney, Australia) and stored on a laboratory

264   computer for offline analysis. The raw MSNA signal was sampled at 10 KHz. Multi-unit

265   bursts of MSNA were identified using an automated detection algorithm (Chart Pro 8.3.1)

266   and confirmed by a trained observer (SAB/LLS), using established criteria (White *et al.*,

267   2015). To account for sympathetic baroreflex latency, MSNA data were shifted backwards

268   (Average shift: CMS -1.23 ± 0.05s, healthy highlanders -1.17 ± 0.05s) so that the peak of

269   each sympathetic burst coincided with the diastolic period which initiated it (Simpson *et al.*,

270   2019). To account for differences in microelectrode positioning, burst amplitude data were

271   normalised by assigning a value of 100 to the largest burst observed during baseline. All

272   other bursts were calibrated against this value. Resting sympathetic vasomotor activity was

273   quantified as MSNA burst frequency (burst·min^{-1}) and total activity (mean burst amplitude x

274   burst frequency [au·min^{-1}]) as it reflects the amount of neurotransmitter release and thus

275 vasoconstrictor drive to the vasculature over a given time period (Charkoudian & Wallin,

276 2014).

277 Baroreflex control of MSNA was assessed from the relationship between i) DBP and MSNA

278 burst probability. DBP was used because MSNA correlates more closely with DBP than SBP

279 (Sundlof & Wallin, 1978). All DBP values during the modified Oxford test were assigned to a

280 3 mmHg bin to reduce the statistical impact of respiratory related oscillations (Eckberg &

281 Eckberg, 1982). The percentage of cardiac cycles associated with a burst of MSNA (ranging

282 from 0-100%) was calculated for each DBP bin to give values of burst probability. Non-linear

283 saturation and threshold regions, if present, were excluded through visual inspection of data

284 points by agreement of two observations. The slope of the linear relationship was

285 determined by weighted linear regression analysis, and this value provided an index of

286 vascular sympathetic vascular baroreflex gain. Only slopes with (i) at least five data points

287 and (ii) R≥ 0.5 were included in the group mean data (Hart *et al.*, 2011). Vascular

288 sympathetic baroreflex gain for rising and falling pressures were not determined

289 independently. The operating point of the vascular sympathetic baroreflex was taken as the

290 average value for MSNA burst incidence (burst·100HB^{-1}) and DBP during the baseline

291 period before to the modified Oxford test. In contrast to burst frequency, burst incidence,

292 which is temporally independent, is an index of reflex control and baroreflex 'gating' of

293 sympathetic bursts. Baroreflex control of the heart was assessed from the relationship

294 between SBP and RRI/ HR during the modified Oxford test. SBP was used as it correlates

295 more closely with RRI and HR than DBP (Sundlof & Wallin, 1978). Values were averaged

296 over 3mmHg SBP bins. Baroreflex delays were accounted for by associating SBP values

297 with either the concurrent heartbeat (resting RRI <800msec, HR <75 bpm,) or subsequent

298 heartbeat (resting RRI >800msec HR >75bpm) (Eckberg & Eckberg, 1982). Saturation and

299 threshold regions were excluded by visual inspection, slopes were determined by weighted

300 linear regression analysis and only slopes with at least five data points and R ≥0.8 were

301 included in the group mean data (Taylor *et al.*, 2015). To minimize the potential effects of

302 hysteresis, we restricted data analysis to the rising arm of SBP and used values from the

303　nadir to the peak SBP response (Hunt & Farquhar, 2005). The operating point was taken as

304　the average values for RRI/HR and SBP during the resting period prior to the modified

305　Oxford test.

306

307　**Statistical analyses**

308　Differences between groups (CMS vs. healthy highlanders) and between conditions

309　(baseline vs. hyperoxia) were assessed using pre-planned contrasts. To address hypothesis

310　1, 2 and 3, differences in arterial baroreflex function, basal sympathetic vasomotor activity

311　and arterial pressure, between CMS and healthy highlanders, were assessed using

312　independent T-tests. To address our secondary aim and examine the contribution of the

313　peripheral chemoreflex mechanism, differences in arterial baroreflex function, sympathetic

314　vasomotor activity and arterial pressure in CMS and healthy highlanders between baseline

315　and hyperoxia were assessed using dependent T-tests. Significant cardiovagal baroreflex

316　slopes ($R \leq 0.8$) were not obtained in one CMS participant and one healthy highlander;

317　therefore cardiovagal baroreflex gain analyses at baseline were based on seven CMS

318　participants and six healthy highlanders. As a result of MSNA signal losses, repeated

319　measures comparisons for cardiovascular haemodynamics and sympathetic neural activity

320　during hyperoxia were performed on six CMS participants and six healthy highlanders.

321　Furthermore, during hyperoxia, cardiovagal baroreflex slopes did not meet the inclusion

322　criteria ($R \leq 0.8$) in one out of six healthy highlanders; therefore, repeated measures

323　comparisons for cardiovagal baroreflex gain are limited to five healthy highlanders and six

324　CMS individuals. Multiple t-tests were chosen to maximize the number of subjects included

325　in statistical analyses. To correct for multiple comparisons, *a priori* alpha was adjusted, using

326　the experiment-wise error rate (Hinkle *et al*., 2003) as used previously (Busch *et al*., 2017;

327　Simpson *et al*., 2019). Statistical significance was set at $P < 0.05$. Furthermore, due to a

328　small sample size, Cohen's *d* effect sizes are also reported with $d \geq 0.8$ indicative of large

329　effects (Cohen., 1988). Normality was assessed using Shapiro-Wilk test, and data that was

330　not normally distributed underwent log_{10} transformation prior to analysis. All statistical

331 analyses were performed using Prism 7.03 (GraphPad software, USA). Data are presented

332 as means $\pm$ SD. Differences between groups and conditions are also reported as mean

333 difference and (95% confidence interval).

334

335

336 **Results**

337 **Participant characteristics**

338 Although twenty participants were recruited for the study; an MSNA signal could not be

339 obtained in five of them; therefore data is presented for fifteen participants. We tested eight

340 CMS individuals with a mean ± SD CMS score of 8 ± 2 (range 5–11) and seven healthy

341 highlanders with a CMS score of 1 ±1 (range 0–3). Seven CMS participants were classified

342 as having mild CMS and one was classified as having moderate CMS. CMS participants

343 were similar in age (40 ± 12 yrs), weight (69 ± 12 kg), height (1.61 ± 0.06 m) and body mass

344 index (BMI; 26.4 ± 4.9 $kg \cdot m^{-2}$) to healthy highlanders age (45 ± 12 yrs; $d = 0.42$, $P = 0.39$),

345 weight (71 ± 11 kg; $d = 0.18$, $P = 0.79$), height (1.61 ± 0.03 m; $d = 0$, $P = 0.97$), BMI (26.5 ±

346 3.8 $kg \cdot m^{-2}$; $d = 0.21$, $P = 0.74$). VO_{2peak} values were also similar in CMS and healthy

347 highlanders (32.9 ± 10.5 vs 28.7 ± 8.8 $mL \cdot kg^{-1} \cdot min^{-1}$; $d = 0.43$, $P = 0.49$)

348 **Resting cardiovascular haemodynamics, basal sympathetic neural activity**

349 SaO_2 and PaO_2 were lower and $PaCO_2$ was higher in CMS compared to healthy highlanders

350 (Table 1). As expected, haemoglobin concentration, haematocrit and blood viscosity (7.8 ±

351 0.7 vs 6.6 ± 0.7 cP; $d = 1.7$, $P = 0.01$) were all higher in CMS (Table 1). Although not

352 statistically significant, total blood volume tended to be larger in CMS compared to healthy

353 highlanders (101 ± 25 vs 85 ± 16 $mL \cdot kg^{-1}$; $d = 0.8$, $P = 0.2$), which was due to a larger total

354 red blood cell volume, with a similar plasma volume between groups (Table 1, Figure 1).

355 CMS also tended to exhibit a greater SV (76 ± 13 vs 64 ± 19 mL; $d = 0.8$, $P = 0.32$,) and had

356 a lower HR (64 ± 10 vs 74 ± 4 bpm; $d = 1.4$, $P = 0.03$) compared to healthy highlanders, with

357 a similar Qc in both groups (4.8 ± 0.7 vs 4.7 ± 1.3 $L \cdot min^{-1}$; $d = 0.1$, $P = 0.83$). TPR was also

358 similar between CMS and healthy highlanders (19.2 ± 6.2 vs 19.9 ± 5.5); however CMS

359 exhibited a lower MSNA burst frequency (33 ± 11 vs 45 ± 13 $burst \cdot min^{-1}$; $d = 1.0$, $P = 0.08$)

360 compared to healthy highlanders (Figure 1). Because Qc and TPR were comparable, MAP

361 was also similar in CMS compared to healthy highlanders (Figure 1). Unfortunately, due to

362   the difficulty in identifying the tricuspid value regurgitant jet in four participants we could only

363   obtain pulmonary artery systolic pressure (PASP) measurements in seven CMS and four

364   healthy highlanders. PASP values were not significantly different between groups (Table 1).

365   **Arterial baroreflex function**

366   Vascular sympathetic baroreflex gain (i.e. slope of the DBP-MSNA burst probability

367   relationship) was comparable in CMS and healthy highlanders (-2.5 ± 0.9 vs -2.7 ± 1.1

368   %·mmHg^{-1}; d = 0.2, P = 0.69, mean diff 0.2 [-0.9 to 1.3]). The operating DBP was also

369   similar in both groups (71 ± 4 vs 74 ± 9; d = 0.5, P = 0.41, mean diff -3 [-11 to 4]).The MSNA

370   set-point appeared lower in CMS compared to healthy highlanders (51 ± 12 vs 62 ± 17

371   bursts·100Hb^{-1}; d = 0.75, P = 0.16, mean diff -11 [-28 to 8]), although this was not

372   statistically significant (Figure 2A).

373   Cardiovagal baroreflex gain (i.e. slope of the relationship between RRI and SBP) was

374   greater in CMS compared to healthy highlanders (17.2 ± 6.8 vs 8.8 ± 2.6 msec·mmHg^{-1}; d =

375   1.8, P < 0.01, mean diff 8.4 [2.7 to 15.0]; Figure 2B). These findings were similar regardless

376   of whether RRI or HR was used. Operating SBP was similar in both groups (CMS, 109 ± 8

377   vs healthy highlanders, 113 ± 15 mmHg; d = 0.4, P = 0.5, mean diff – 4 [-17 to 8]); however

378   CMS participants operated around a longer RRI (960 ± 159 vs 817 ± 50 msec; d = 1.4, P =

379   0.04, mean diff -143 [-7 to -279]; Figure 2).

380

|	CMS	Healthy highlanders	P value	Cohen's d	Mean difference (95% CI)
Haematological variable					
Haemoglobin (g/dL)	22.6 ± 0.9	19.3 ± 0.8	**<0.01**	3.9	3.3 (2.2 to 4.2)
Haematocrit (%)	65 ± 5	57 ± 3	**<0.01**	2.0	8 (3 to 13)
SaO_2 (%)†	82 ± 2	87 ± 3	**0.01**	2.0	-5 (-9 to -2)
PaO_2 (mmHg)†	47 ± 2	51 ± 4	**0.05**	1.4	-4 (-8 to 0)
$PaCO_2$ (mmHg)†	34 ± 1	29 ± 4	**0.02**	1.9	5 (1 to 9)
RBC volume (mL·kg^{-1})	57 ± 14	48 ± 8	0.19	0.8	9 (-5 to 21)
Plasma volume (mL·kg^{-1})	41 ± 9	42 ± 9	0.87	0.1	-1 (-11 to 9)
Pulmonary haemodynamics					
PASP (mmHg) ♦	29 ± 7	33 ± 7	0.4	0.6	-4 (-14 to 6)

Table 1.

381

382   Table 1. Haematological variables and resting pulmonary haemodynamics in CMS (n=8) and

383   healthy highlanders (n=7). RBC = red blood cell. Data presented as mean ± SD. † Values

384   based on six CMS and five healthy highlanders ♦ Values based on seven CMS and four

385   healthy highlanders. Statistical comparisons performed using independent t-tests

386

387
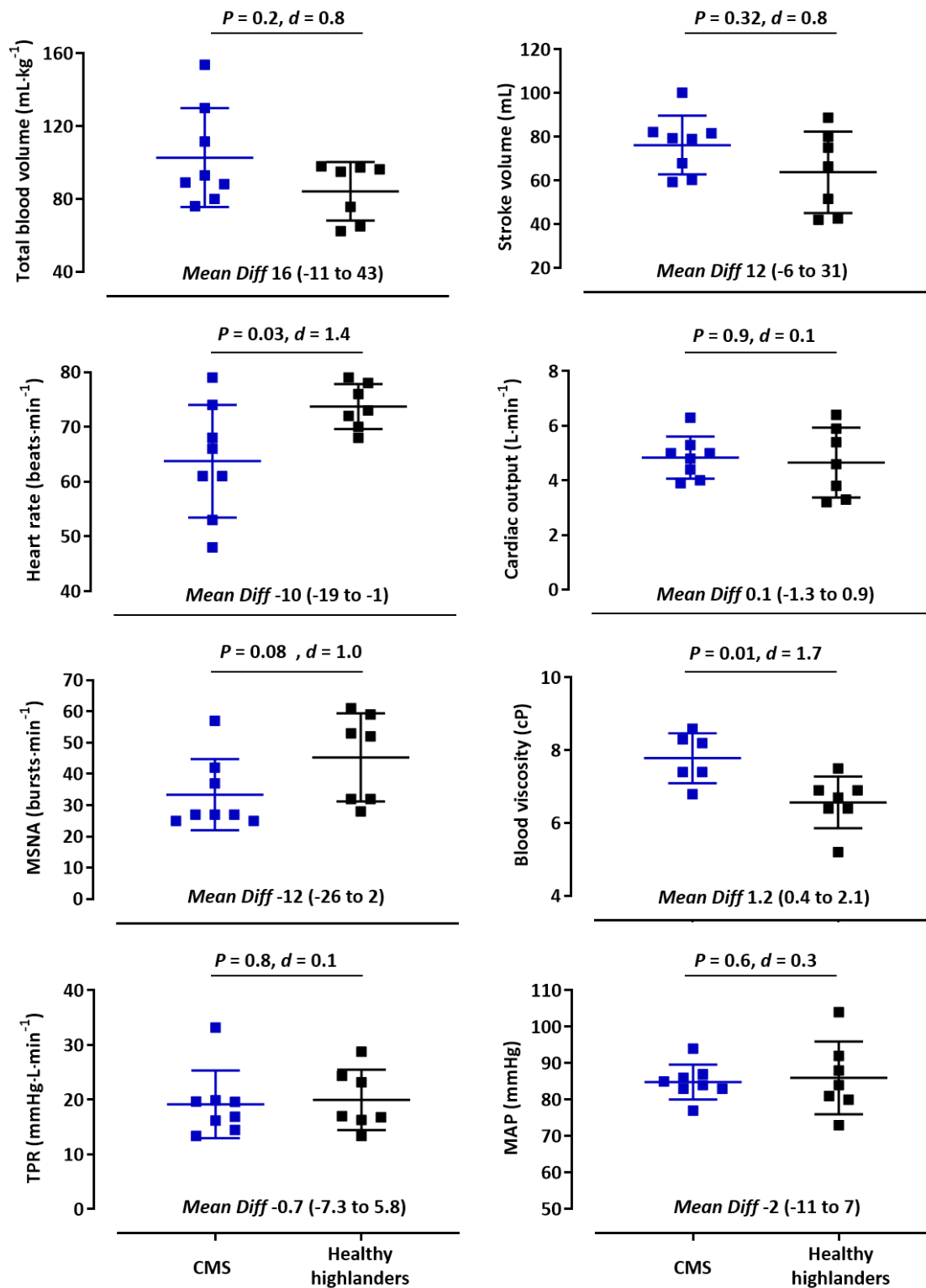
Figure 1. Group mean (±SD) and individual data for haematological and cardiovascular haemodynamic variables in CMS (n=8) and healthy highlanders (n=7). Blood viscosity values based on six CMS and five healthy highlanders. Statistical comparisons performed using independent t-tests. *P* values are reported with Cohen's *d* effect sizes (*d*) and mean differences (95% confidence intervals).
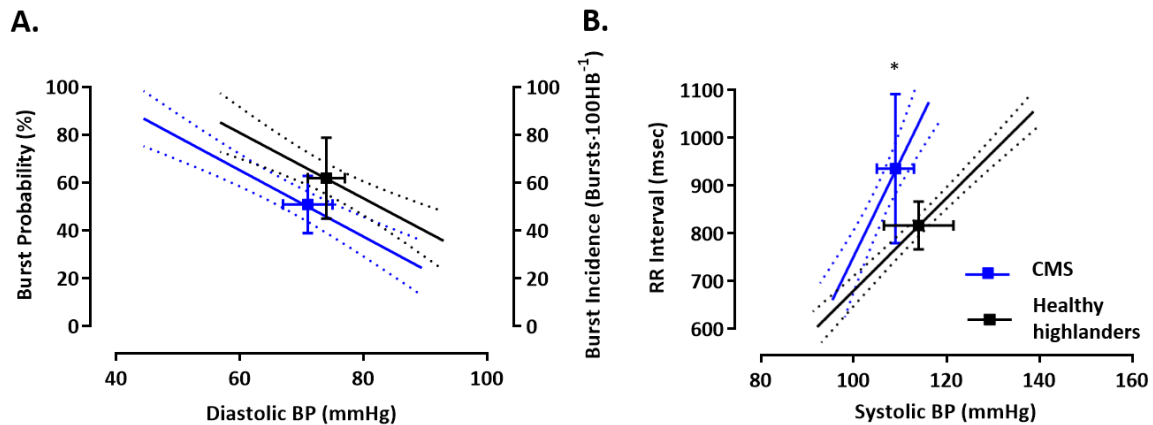
Figure 2. Arterial baroreflex function. Group average regressions between A) diastolic BP and MSNA burst probability in CMS (n=8) and healthy highlanders (n=7) and B) systolic BP and R-R interval in CMS (n=7) and healthy highlanders (n=6). The set-points of the vascular sympathetic and cardiovagal limb of the baroreflex are indicated by the symbols and error bars (mean ± SD). The arterial baroreflex set points and slopes were compared using independent t-tests. * P <0.05 versus healthy highlanders. The vascular sympathetic baroreflex set-point was similar between CMS and healthy highlanders. The slope of the relationship between diastolic BP and MSNA burst probability was also similar between groups, indicating no differences in reflex gain. The cardiovagal baroreflex operated around a similar systolic BP, but a greater RRI set-point in CMS compared to healthy highlanders. The slope of the relationship between systolic BP and R-R interval was also greater in CMS, indicating a greater reflex gain.

408 **Arterial baroreflex–peripheral chemoreflex interactions**

409 In CMS participants exposed to 100% O_2, HR significantly decreased and Qc also tended to

410 decrease, although this didn't achieve significance (d = 0.6, P = 0.07). Oxygen

411 administration had no effect on any other cardiovascular haemodynamic variable in CMS.

412 HR and Qc both significantly decreased in healthy highlanders exposed to 100% O_2. This

413 reduction in Qc, was accompanied by an increase in TPR, with no significant effect on BP.

414 The reduction in HR in both groups was accompanied by a lowering of MSNA burst

415 frequency, with no effect on burst amplitude (Table 2).

416 Administration of oxygen had no significant effect on vascular sympathetic baroreflex gain,

417 operating DBP or MSNA set-point in either CMS or healthy highlanders. Administration of

418 oxygen had no effect on cardiovagal baroreflex gain (18.8 ± 9.7 to 20.3 ± 7.4 msec·mmHg^{-1};

419 d = 0.2, P = 0.7; Figure 3) or operating SBP in CMS, but RRI was longer. In healthy

420 highlanders administration of oxygen also increased RRI; cardiovagal baroreflex gain was

421 greater (8.0 ± 2.6 to 14.1 ± 4.9 msec·mmHg^{-1}; d = 1.6, P = 0.01), with no change in

422 operating SBP (Figure 3).

423

	Baseline	Hyperoxia				Baseline	Hyperoxia			
	CMS	CMS	P value	Cohen's d	Mean difference (95% CI)	Healthy highlanders	Healthy highlanders	P value	Cohen's d	Mean difference (95% CI)
Haemodynamics										
Heart rate (bpm)	60 ± 10	54 ± 14	**<0.01**	0.5	-6 (-10 to -3)	69 ± 7	61 ± 6	**0.03**	1.2	-8 (-14 to -1)
R-R interval (msec)	1019 164	1175 263	**0.01**	0.7	156 (52 to 262)	880 ± 89	905 ± 100	**0.03**	0.3	25 (17 to 207)
Stroke volume (mL)	92 ± 6	93 ± 12	0.95	0.1	0.6 (-16 to 18)	80 ± 21	82 ± 26	0.72	0.1	1.8 (-8 to 12)
Cardiac output (L·min^{-1})	5.6 ± 1.1	4.9 ± 1.2	0.07	0.6	-0.7 (-1.4 to 0.1)	5.4 ± 1.1	4.9 ± 1.4	**0.05**	0.4	-0.5 (-0.9 to -0.2)
TPR (mmHg·L·min^{-1})	16.0 ± 3.2	17.9 ± 4.9	0.18	0.5	1.9 (-1.3 to 5.1)	17.6 ± 5.7	20.5 ± 7.9	**0.01**	0.4	2.9 (0.3 to)
Diastolic BP (mmHg)	73 ± 12	71 ± 12	0.20	0.2	-2 (-4 to 1)	74 ± 5	77 ± 6	0.15	0.5	3 (-1 to 6)
Systolic BP (mmHg)	118 ± 8	115 ±10	0.13	0.3	-3 (-6 to 1)	115 ± 7	120 ± 12	0.13	0.5	5 (-2 to 13)
MAP (mmHg)	89 ± 9	89 ± 5	0.15	0.2	-2 (-4 to 1)	87 ± 11	92 ± 7	0.11	0.5	3 (-1 to 8)
Sympathetic neural activity										
Burst frequency (burst·min^{-1})	32 ± 13	29 ± 13	**0.02**	0.7	-3 (-6 to -1)	41 ± 16	33 ± 13	**0.04**	0.7	-8 (-16 to -0.7)
Burst incidence (bursts·100HB^{-1})	51 ± 14	53 ± 13	0.37	0.2	2 (-2 to 5)	60 ± 20	54 ± 21	0.15	0.2	-6 (-13 to 3)
Normalized burst amplitude (au)	56 ± 8	55 ± 13	0.99	0.2	2 (-10 to 9)	57 ± 5	54 ± 13	0.78	0.2	2 (-10 to 13)
Total activity (au·min^{-1})	1780 ± 801	1618 ± 922	0.19	0.4	-162 (-473 to 149)	2335 ± 900	1992 ± 1026	0.08	0.4	-343 (-728 to 42)

Table 2.

424

425   Table 2. Haemodynamics, MSNA and arterial baroreflex function at baseline and during hyperoxia in CMS (n=6) and healthy highlanders (n=6).

426   Data presented as mean ± SD. Statistical comparisons performed using dependent t-tests.
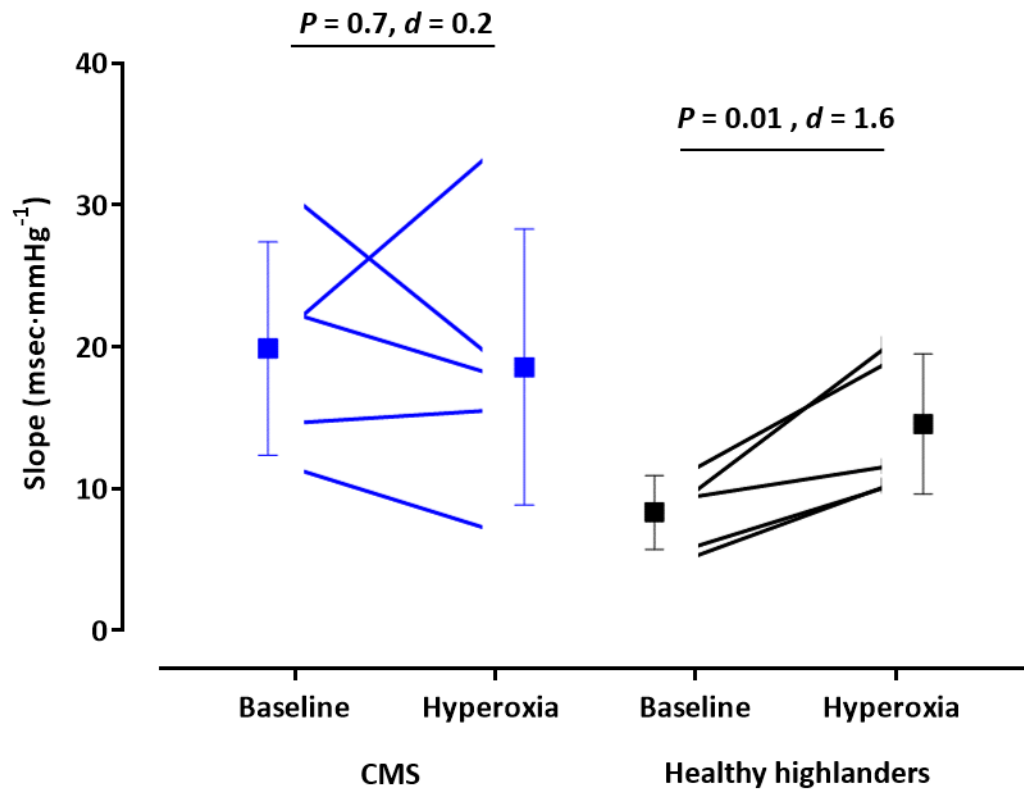
427

428 Figure 3. Cardiovagal baroreflex gain: Individual and group average slopes for the
429 relationship between RRI and systolic blood pressure at baseline and during hyperoxia in
430 CMS (n=5) and healthy highlanders (n=6). Administration of oxygen had no effect on
431 cardiovagal baroreflex gain in CMS but, increased cardiovagal baroreflex gain (8.0 ± 2.6 to
432 14.1 ± 4.9 msec·mmHg^{-1}; d = 1.6, P = 0.01) in healthy highlanders. Statistical comparisons
433 performed using dependent t-tests.

Discussion

The major findings of the present study are threefold: i) CMS individuals and healthy highlanders exhibit similar vascular sympathetic baroreflex gain (i.e. responsiveness), operating diastolic pressure, and MSNA set-point (i.e. MSNA burst incidence); ii) however, in mild CMS, the cardiovagal baroreflex operates around a longer RRI (lower heart rate) with a greater reflex gain; iii) CMS individuals have comparable cardiac output, total peripheral resistance, and thus, arterial pressure compared to healthy highlanders. However, CMS individuals exhibit a greater haemoglobin concentration, total blood volume and blood viscosity, and lower basal vasomotor sympathetic activity (i.e. MSNA burst frequency) compared to healthy highlanders. Taken together, these findings indicate adaptive changes in autonomic regulation of blood pressure homeostasis in Andean highlanders with mild CMS.

Basal sympathetic vasomotor activity in Andeans

The one previous study that has assessed resting sympathetic vasomotor activity in Andean high-altitude natives found comparable basal MSNA in healthy Bolivian Andeans (Aymara) and acclimatising lowlanders (Lundby *et al.*, 2018). However, we are the first to assess basal sympathetic vasomotor activity in Peruvian (Quechua) Andeans, including individuals with CMS. We observe a 25% *lower* basal MSNA in CMS compared to healthy highlanders, as indicated by a reduced MSNA burst frequency (Figure 1). This finding is in contrast to our hypothesis that basal sympathetic vasomotor activity would be greater in CMS, which was based upon previous studies reporting either comparable (Antezana *et al.*, 1995) or elevated (Gamboa *et al.*, 2006) plasma noradrenaline levels in individuals with CMS. A reduced glomerular filtration rate, (Lozano *et al.*, 1964) and thus noradrenaline clearance, in CMS would, however, serve to overestimate sympathetic activation using this method, and potentially explain these contradictory findings. Despite this, it might be anticipated that sympathetic vasomotor activity would be elevated in CMS individuals due to several factors. These factors include: exaggerated arterial hypoxaemia (lower PaO_2), reports of increased

461 inflammation and oxidative stress (Bailey *et al.*, 2013, Bailey *et al.*, 2019); and, a reduced

462 NO bioavailability, all of which exert known sympathoexcitatory effects (Patel *et al.*, 2001).

463 Sympathetic vasomotor outflow, however, is the net effect of the integration of both

464 excitatory and inhibitory inputs to the cardiovascular control centres in the brainstem. For

465 example, elevations in blood volume exert a sympathoinhibitory influence on basal MSNA

466 (Charkoudian *et al.*, 2004, Best *et al.*, 2014). Notably, CMS individuals in the present study

467 exhibited a 20% greater blood volume compared to healthy highlanders (Figure 1). Whilst

468 not statistically significantly different, the effect was large (Cohen's d = 0.8) and the

469 differences were comparable to those previously reported in this population (Claydon *et al.*,

470 2004). Thus, lower basal sympathetic vasomotor activity in CMS could be mediated by an

471 increase in circulating blood volume. Indeed, we have previously demonstrated a lower

472 basal sympathetic activity in high-altitude native Sherpa, compared to acclimatising

473 Lowlanders (Simpson *et al.*, 2019), with Sherpa also exhibiting a greater total blood volume

474 (Stembridge *et al.*, 2019). Despite this, however, there was no significant correlation

475 between these factors in the present study (data not shown). It is also important to note,

476 however, that individuals with CMS were on average ~5 years younger than healthy

477 highlanders. Although, the reported ~3 bursts·min^{-1} increase in basal MSNA per decade of

478 life (Narkiewicz *et al.*, 2005) would not exclusively explain the observed 12 burst·min^{-1}

479 difference in basal MSNA.

480 **Arterial baroreflex function in Andeans**

481 This is the first study to assess baroreflex control of MSNA in Andean high-altitude natives.

482 In addition, it is the first to simultaneously assess the vascular sympathetic and cardiovagal

483 limbs of the arterial baroreflex in the same group. We demonstrated that both CMS and

484 healthy highlanders exhibit a similar ability to increase and decrease MSNA in response to

485 transient, pharmacologically induced changes in blood pressure (i.e. the vascular

486 sympathetic baroreflex gain was unchanged). This is consistent with one previous report of a

487 similar reflex gain for carotid baroreflex control of forearm vascular resistance (Moore *et al.*,

488 2006) in both CMS and healthy highlanders. Contrary to our hypothesis, resting heart rate

489 was lower, and the ability to alter RRI during the modified Oxford test was greater in CMS

490 compared to healthy highlanders. The operating diastolic pressure for the vascular

491 sympathetic baroreflex and the operating systolic pressure for the cardiovagal baroreflex

492 were similar in CMS and healthy highlanders. Furthermore, MSNA burst incidence (i.e.

493 vascular sympathetic baroreflex set-point) was also not significantly different between

494 groups, meaning the probability of a burst occurring per cardiac cycle was similar between

495 CMS and healthy highlanders. Therefore, our data indicate that CMS does not influence the

496 arterial baroreflex control and gating of sympathetic bursts. Importantly, however, a lower

497 resting heart rate in CMS reduces the opportunities (i.e. cardiac cycles) for a burst to occur.

498 Thus, the interaction of a similar MSNA burst incidence (i.e. vascular sympathetic baroreflex

499 set-point) and lower resting heart in CMS reduces MSNA burst frequency (i.e. basal

500 sympathetic vasomotor activity). It should be noted that, in contrast to our findings, several

501 studies report higher, rather than lower, resting heart rates and/or blood pressures for CMS

502 (Keyl *et al.*, 2003; Claydon *et al.*, 2004; Richalet *et al.*, 2005, Corante *et al.*, 2018). The

503 reasons for these differences are unclear, although differences in posture, measurement

504 technique, time of day for measurement, and the duration of the measurement period, all

505 might contribute. In addition, CMS severity may also be important; indeed, studies that report

506 higher resting heart rates and blood pressures for CMS also report greater average Hct and

507 CMS scores than those observed in the present study. Moreover, a positive correlation has

508 been observed between [Hb] and blood pressure in the Cerro de Pasco population

509 (Gonzales & Tapia, 2013). Importantly in the present study, cardiac output, total peripheral

510 resistance, and thus, arterial pressure for CMS are comparable to healthy highlanders; this

511 is despite greater blood viscosity and total blood volume, secondary to an increase in red

512 blood cell volume, in CMS. Thus, the lower sympathetic vasomotor outflow and heart rate in

513 CMS appear to balance the haemodynamic effects of EE, which maintains blood pressure

514 homeostasis, at least in mild CMS studied here.

Influence of peripheral chemoreflex on arterial baroreflex function in Andeans

Lower PaO_2 in CMS individuals would be expected to increase peripheral chemoreflex activation and potentially reset the arterial baroreflex to operate at higher heart rates, arterial pressures and level of MSNA (Halliwill and Minson *et al.*, 2002, Steinback *et al.*, 2009). Importantly, however, peripheral chemoreceptor ventilatory responsiveness to hypoxia is reported to be blunted in CMS individuals (León-Velarde & Richalet, 2006, Severinghaus *et al.*, 1966), contributing to alveolar hypoventilation (higher $PaCO_2$) reported in this population (León-Velarde & Richalet, 2006). Despite a blunted ventilatory responsiveness reported in CMS the peripheral chemoreflex mechanism did not appear to contribute to the lower HR in CMS, as acutely eliminating peripheral chemoreceptor drive, via 100% oxygen administration, had comparable effects on HR in both groups. Interestingly, MSNA burst incidence remained unchanged for CMS during acute hyperoxia, whilst it was reduced (~ 6 bursts·$100HB^{-1}$) for healthy highlanders. However, this reduction in MSNA burst incidence occurred alongside a small increase in both arterial pressure (~3mmHg) and stroke volume; therefore, such reductions were likely arterial baroreflex-mediated. In addition, whilst other hemodynamic responses to hyperoxia were comparable between groups, there was a significant reduction in TPR in healthy highlanders; this was not observed in CMS. This may indicate different intrinsic control and regulation of vascular tone; that is healthy highlanders possess a greater vascular responsiveness to hypoxia compared with CMS. Indeed, this could contribute, in part, to the observed difference in basal MSNA (i.e. extrinsic control) under ambient hypoxic conditions. However, any potential difference in local control cannot be determined from the data presented here.

An inhibitory relationship exists between the peripheral chemoreflex and baroreflex mechanisms (Somers *et al.*, 1991), whereby an acute increase in peripheral chemoreflex activation is consistently shown to inhibit baroreflex control of the heart (Heistad & Wheeler, 1971; Sagawa *et al.*, 1997; Steinback *et al.*, 2009; Niewinski *et al.*, 2014; Mozer *et al.*, 2016) with inconsistent effects on baroreflex control of MSNA (Halliwill & Minson, 2002; Simpson *et*

542 *al.*, 2019). In the present study, during ambient air breathing, we observed a greater

543 cardiovagal baroreflex responsiveness for CMS compared to healthy highlanders.

544 Furthermore, we observed no change in cardiovagal baroreflex responsiveness for CMS

545 during acute hyperoxia, but demonstrated a 75% increase in reflex gain for healthy

546 highlanders. These findings indicate a peripheral chemoreflex-mediated inhibition of

547 cardiovagal baroreflex responsiveness in healthy Andeans at high-altitude, which does not

548 appear to be present in CMS. This raises an interesting possibility that whilst a blunted

549 peripheral chemoreflex responsiveness may contribute to the exaggerated arterial

550 hypoxaemia in CMS, it may, paradoxically, prevent the reduced cardiovagal baroreflex gain

551 normally observed during sustained high-altitude exposure (Yazdani *et al.*, 2016; Bourdillon

552 *et al.*, 2018; Simpson *et al.*, 2019).

553 **Implications**

554 Our findings imply that elevated sympathetic vasomotor outflow and arterial baroreflex

555 dysfunction do not contribute to the elevated cardiovascular disease risk reported in mild

556 CMS, since autonomic control of blood pressure is well maintained in the group studied

557 here. Therefore, other factors may predispose individuals with CMS to cardiovascular

558 disease. However, we cannot exclude the possibility that elevated sympathetic vasomotor

559 outflow and/or arterial baroreflex dysfunction may develop in more severe CMS, with

560 elevated pulmonary arterial pressure (Simpson *et al.*, 2020), which may contribute to the

561 greater cardiovascular disease risk reported in moderate and severe CMS.

562 **Experimental limitations**

563 There are several limitations in the present study that should be acknowledged. First, due to

564 time constraints, only small opportunistic samples could be studied. Therefore, meaningful

565 differences between groups may not have been detected due to low statistical power.

566 Indeed, insufficient statistical power likely prevented a meaningful 20% difference in both

567 total blood volume between groups from being detected, despite a similar magnitude of

568   difference to previous studies (Claydon *et al.*, 2004). Second, given the time constraints

569   associated with expedition research, it was not possible to control for the time of day that

570   participants were tested; therefore, diurnal variations in basal MSNA, blood pressure and

571   cardiovagal baroreflex gain (Taylor *et al.*, 2011) are a consideration in our interpretation.

572   Notably, our analysis indicates that time of day was not a significant covariate. Third, two

573   CMS individuals were light to moderate smokers. It is reported that tobacco smoking leads to

574   increased basal MSNA and attenuates vascular sympathetic baroreflex gain (Middlekauff *et*

575   *al.,* 2013), which may have influenced our results. However, this would have potentially

576   overestimated resting MSNA in CMS, which would not have altered the interpretation of our

577   results (i.e. lower sympathetic vasomotor outflow in CMS). Fourth, due to a lack of CMS

578   positive female volunteers, we only studied males; meaning that the findings cannot be

579   generalised to females, who likely exhibit differences in blood pressure control mechanisms.

580   Last, we did not assess vascular sympathetic baroreflex gain to rising and falling pressure

581   independently, due to an insufficient number of data points to construct baroreflex slopes

582   that met the criteria for inclusion. We acknowledge that this fails to take baroreflex hysteresis

583   into account (Rudas *et al.*, 1999).

584   **Conclusion**

585   Contrary to our hypotheses, elevated sympathetic vasomotor outflow and arterial baroreflex

586   dysfunction are not apparent in mild CMS. In fact, basal sympathetic vasoconstrictor drive

587   and heart rate are lower in CMS, with enhanced cardiovagal baroreflex gain, compared to

588   healthy highlanders. Such changes appear to be adaptive physiological responses to the

589   elevations in red blood cell volume, which allow blood pressure homeostasis to be

590   maintained. Furthermore, whilst a blunted peripheral chemoreflex is reported to be a

591   possible mechanism responsible for accentuated arterial hypoxaemia in CMS, it may,

592   paradoxically, augment cardiovagal baroreflex responsiveness compared to healthy

593   highlanders.

594   **ADDITIONAL INFORMATION**

Competing Interests

None

Author Contributions

LLS, JPM, MS, CDS, PNA, FCV contributed to conception and design of the work. LLS, JPM, MS, CDS, JSL, GM, VLM, CG, AS, SAB, TGD, GAV, RJF, CH, MMT, SJO contributed to acquisition analysis, or interpretation of the data. LLS, JPM, MS, CDS, FCV, PNA, JSL, GM, MMT contributed to the drafting of the work or revising it critically for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons included as an author qualify for authorship, and all those who qualify for authorship are listed.

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