

**Global REACH 2018: Volume regulation in high-altitude Andeans with and without chronic mountain sickness**

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46 **Abstract:**

47 The high-altitude maladaptation syndrome known as chronic mountain sickness (CMS) is
48 characterized by polycythemia and is associated with proteinuria despite unaltered glomerular
49 filtration rate. However, it remains unclear if indigenous highlanders with CMS have altered
50 volume regulatory hormones. We assessed N-terminal pro-B-type natriuretic peptide (NT pro-
51 BNP), plasma aldosterone concentration, plasma renin activity, kidney function (urinary
52 microalbumin, glomerular filtration rate), blood volume, and estimated pulmonary artery systolic
53 pressure (ePASP), in Andean males without (n=14; age=39±11) and with (n=10; age=40±12)
54 CMS at 4330 meters (Cerro de Pasco, Peru). Plasma renin activity (non-CMS: 15.8±7.9 vs. CMS:
55 8.7±5.4 ng/ml; p=0.025) and plasma aldosterone concentration (non-CMS: 77.5±35.5 vs. CMS:
56 54.2±28.9 pg/ml; p=0.018) were lower in highlanders with CMS compared to non-CMS, while
57 NT pro-BNP was not different between groups (non-CMS: 1394.9±214.3 vs. CMS: 1451.1±327.8
58 pg/ml; p=0.15). Highlanders had similar total blood volume (non-CMS: 90±15 vs. CMS: 103±18
59 ml • kg⁻¹; p=0.071), but Andeans with CMS had greater total red blood cell volume (non-CMS:
60 46±10 vs. CMS 66±14 ml • kg⁻¹; p<0.01) and smaller plasma volume (non-CMS 43±7 vs. CMS
61 35±5 ml • kg⁻¹; p=0.03) compared to non-CMS. There were no differences in ePASP between
62 groups (non-CMS 32±9 vs. CMS 31±8 mmHg; p=0.6). A negative correlation was found between
63 plasma renin activity and glomerular filtration rate in both groups (group: r=-0.66; p<0.01; non-
64 CMS: r=-0.60; p=0.022; CMS: r=-0.63; p=0.049). A smaller plasma volume in Andeans with CMS
65 may indicate an additional CMS maladaptation to high-altitude, causing potentially greater
66 polycythemia and clinical symptoms.

67

68 **Introduction:**

69 Globally, more than 81.6 million people live at high-altitude (>2500 m), exhibiting several
70 unique physiological adaptations incurred over millennia of hypoxic exposure (1). However, 5-
71 10% of high-altitude residents are at risk of developing a maladaptive syndrome termed chronic
72 mountain sickness (CMS) (2, 3). While this only represents a relatively small percentage of all
73 high-altitude dwellers, CMS appears to disproportionately affect men for example ~30% of long-
74 term high-altitude male residents in the Cerro de Pasco region (4, 5) and 17.8% of Han Chinese in
75 the Qinghai-Tibetan plateau suffer from CMS (6). CMS is a progressively incapacitating syndrome
76 characterized by polycythemia (hemoglobin concentration of males: 21 g/dL, and females: 19
77 g/dL) and is frequently accompanied by hypoxemia, and clinical signs and symptoms including
78 distended veins, headache, breathlessness, sleep disturbances, and cognitive impairments (7, 8).
79 There is evidence that CMS contributes to the development and severity of cardiovascular disease
80 such as hypertension. (7, 9). Numerous studies have investigated the vascular function of CMS
81 patients (10-12). However, little attention has been given to volume regulation a critical aspect of
82 blood pressure control. The present study, therefore, aimed to comprehensively assess volume
83 regulation in Andean highlanders with CMS, and to compare this with healthy highlanders (non-
84 CMS). To achieve this, we assessed volume regulatory hormones, renal function, blood volume,
85 and vascular pressure.

86 High-altitude residency is associated with elevated pulmonary artery pressure, right
87 ventricular hypertrophy, and vascular remodelling within the distal pulmonary arterial branches
88 (13-15). These physiological consequences of life-long exposure to high-altitude are exacerbated
89 in highlanders with CMS (15). Similarly, B-type natriuretic peptide (BNP) - a cardiac
90 neurohormone secreted from cardiac myocytes in response to volume and pressure overload - is

91 elevated in Tibetans with CMS (16), which was shown to be positively related to pulmonary artery
92 pressure. Nevertheless, this has yet to be examined in Andeans.

93 Chronic hypertension, high blood viscosity, and polycythemia may cause proteinuria often
94 prevalent among high-altitude populations (17, 18). A negative association between elevations in
95 hemoglobin concentration and estimated glomerular filtration rate has been observed in non-CMS
96 high-altitude Andean populations. CMS individuals, with higher hemoglobin concentrations, may
97 have an increased risk of impaired glomerular filtration rate. (19, 20). However, to the best of our
98 knowledge, this association has not been quantified in highlanders of any genetic lineage with
99 CMS (17).

100 Renal plasma flow is lower in highlanders with CMS compared to non-CMS highlanders
101 (21-23). Yet, glomerular filtration rate appears largely unaffected in highlanders with CMS
102 because filtration fraction is increased to compensate for a lower renal plasma blood flow (21, 22).
103 This high filtration fraction is assumed to occur by efferent arteriole vasoconstriction via elevated
104 renin-angiotensin-aldosterone-system (RAAS), although this remains debated (20). Likewise,
105 angiotensin-converting enzyme inhibitors decrease the prevalence of proteinuria in highlanders
106 with CMS again suggesting hyperfiltration and RAAS dysregulation (24). However, highlanders
107 with CMS have a greater blood volume and this may actively inhibit RAAS to compensate for
108 volume overload (25). It seems probable that differential regulation of RAAS between non-CMS
109 and CMS may alter blood volume and glomerular filtration rate. However, this has not been
110 assessed previously.

111 We hypothesized that: NT pro-BNP, plasma aldosterone and plasma active renin would be
112 higher in highlanders with CMS compared to non-CMS due to hyperfiltration. Glomerular
113 filtration rate would not differ between CMS and non-CMS, while urinary microalbumin would

114 be higher in Andeans with CMS. The secondary purpose was to explore the associations between
115 these physiological metrics.

116

117 **Methods:**

118 Data collection for the current investigation was conducted as part of the 2018 Global
119 REACH expedition to Instituto de Investigaciones de Altura at Cerro de Pasco, Peru (4330 m). An
120 overview of this research expedition can be found elsewhere (26). We have previously published
121 the blood volume data in this cohort of Andeans (25). However, the analyses performed as part of
122 this investigation address an *a priori* hypothesis and are not previously published. Specifically, the
123 current study focuses on novel analyses related to the relationship between blood volume and
124 volume regulatory hormones.

125 *Ethical Approval*

126 This study abided by the Canadian Government Tri-council Policy on Research Ethics
127 Policy Statement (TCPS2) and the Declaration of Helsinki, apart from registration in a publicly
128 accessible database. Ethical approval was obtained in advanced through the Clinical Research
129 Ethics Board of the University of British Columbia (H17-02687 and H18-01404), the University
130 of Alberta Biomedical Ethics Board (Pro00077330) and the Universidad Peruana Cayetano
131 Heredia Comité de Ética (no. 101686). Prior to participation, all experimental procedures were
132 explained to subjects in writing, and verbally in their native language and informed written consent
133 was provided.

134 *Participants*

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135 Twenty-four Peruvian Andean males with ($n = 10$) and without CMS ($n = 14$) born at an
136 altitude above 3250 meters, permanently residing in the Cerro de Pasco area and who had at least
137 two previously know generations of high-altitude Andean ancestry were recruited for the study.
138 None of the participants recruited for the study had traveled below 3000 meters in the last 6 months
139 and they did not have a history of working in the mining industry. None of the participants took
140 prescription or over-the-counter medications including any medications to treat polycythemia.
141 Participants with self-reported history of smoking, neurological, cardiovascular, cerebrovascular,
142 renal dysfunction or respiratory disease were excluded (26). Participants attended the laboratory
143 on two occasions, with a minimum of 24 hours between individual visits: 1) preliminary screening
144 visit, and 2) an experimental visit.

145 *Preliminary screening visit*

146 Upon arrival to the laboratory, participants provided a detailed clinical history, history of
147 high-altitude residence and ancestral background. Venous blood samples were taken from the
148 antecubital vein to measure hematocrit, hemoglobin and to calculate total blood volume
149 (Radiometer ABL90 analyser [Radio-meter, Canada]). Total blood volume (packed cell volume
150 and plasma volume) was determined via the modified carbon monoxide rebreathing method as
151 previously described in detail (27) and previously used by our group at high-altitude (25). CMS
152 was determined using the Qinghai CMS questionnaire based on the presence and severity of eight
153 symptoms of CMS as agreed upon by international consensus (2). A score of zero (i.e. absent), to
154 three (i.e. severe) was assigned for each of the following signs and symptoms:
155 breathlessness/palpitation, sleep disruptions, cyanosis, venodilation, paresthesia, headache, and
156 tinnitus. The sum of assigned values constituted the CMS score. Participants were asked these
157 questions in their native language by a research team member. CMS was diagnosed with a summed

158 score ≥ 6 and the presence of polycythemia (hemoglobin ≥ 21 g dl⁻¹) those not meeting these
159 criteria were characterized as “non-CMS”. The complete scoring system can be obtained from
160 León-velarde and colleagues (2).

161 *Experimental measures*

162 All participants arrived at the laboratory between 0600 and 1030 following a 12-hour fast
163 and having abstained from caffeine, alcohol, and exercise for 24-hours. Participants were asked to
164 complete a nine-hour urinary collection from the previous night, which was used to calculate
165 glomerular filtration rate. Data collection commenced after an antecubital venous cannula was
166 inserted for blood draws and the participants had rested quietly in the supine position for 10-
167 minutes. Following instrumentation, hemodynamic characteristics were determined (10-minutes),
168 and venous and radial artery blood samples were taken.

169 *Heart rate and blood pressure*

170 Continuous heart rate (electrocardiogram Lead II) was recorded and integrated with a data
171 acquisition system (Powerlab 16/30; ADInstruments, Australia) and stored for subsequent analysis
172 using associated software (Labchart 8.0 Pro; ADInstruments, Australia). Systolic and diastolic
173 blood pressures were measured using an automated cuff (Omron M2 Classic; Japan) and mean
174 arterial pressure was subsequently calculated as: $(1/3 \times \text{systolic blood pressure}) + (2/3 \times \text{diastolic}$
175 $\text{blood pressure})$. Echocardiography was used to assess estimated pulmonary artery systolic
176 pressure (ePASP). Images were obtained by a highly-experienced sonographer using a
177 commercially available ultrasound system (Vivid Q, GE, Fairfield, CT, USA), as previously used
178 by our research group in high-altitude indigenous populations (28). The modified Bernoulli
179 equation ($4 V^2$) was applied to the peak systolic tricuspid regurgitation jet velocity measured via

180 continuous wave Doppler to derive the maximal systolic pressure gradient across the tricuspid
181 valve (29). ePASP was quantified as maximum systolic pressure gradient across the tricuspid valve
182 added to the right atrial pressure estimated from the collapsibility of the inferior vena cava in line
183 with the guidelines of the American Society of Echocardiography (29). Echocardiographer was
184 blinded when conducting analysis.

185 *Blood measures*

186 Blood samples were collected with EDTA tubes and then immediately centrifuged,
187 aliquoted and frozen in liquid nitrogen until analysis in Edmonton, Alberta, Canada. Frozen
188 samples were transported by a commercial company (Marken, New York, USA). Plasma
189 aldosterone concentration (LDN REF: MS E-5200) and plasma active renin (LDN REF: MS E-
190 5300) were measured using a solid phase enzyme-linked immunosorbent assay (ELISA), based on
191 the principle of competitive binding. N-terminal pro-B-type natriuretic peptide (NT pro-BNP)
192 (R&D systems REF: DY3604-05) was quantified using a sandwich solid phase ELISA.

193 Radial artery blood samples were collected using a lithium heparin-coated auto fill syringe
194 and immediately analyzed using point-of-care i-STAT device (Abbott Laboratories, Chicago,
195 USA). Blood samples were analyzed using the CG4+ (lactate, pH, arterial partial pressure of
196 carbon dioxide [PaCO₂], arterial partial pressure of oxygen [PaO₂], bicarbonate [HCO₃⁻] and
197 arterial oxygen saturation [SaO₂]), and CHEM8+ (glucose, urea nitrogen, creatinine, sodium,
198 potassium, chloride, ionized calcium, TCO₂, anion gap, hematocrit and hemoglobin) test
199 cartridges. The point of care device, i-STAT, has been validated on altitudes up to 5043 meters
200 (30).

201 *Renal function and urine analysis*

202 Participants were asked to complete a 9-hour urinary collection to calculate glomerular
203 filtration rate (*Equation 1*). Participants were asked to maintain normal drinking and eating habits
204 before and during the 9-hour urinary collection. Urine was refrigerated until analysis (4 °C).
205 Immediately before analysis, urine pots were shaken vigorously to ensure a homogenous mixture.
206 Volumes were measured using graduated cylinders. Urine creatinine and urine microalbumin were
207 quantified using a commercially available point-of-care DCA Vantage Analyzer (Siemens
208 Healthineers Global; Germany). DCA capillary holders were submerged into urine and absorbed
209 40 uL of urine, then placed in a DCA microalbumin/creatinine reagent cartridge. Thereafter, the
210 DCA System performed 48 optical, electronic, mechanical, and reagent systems checks during the
211 course of each specimen assay. All measurements and calculations are performed automatically
212 by the DCA Analyzer.

213 Creatinine clearance was used to subsequently calculate glomerular filtration rate using the
214 standard formula:

215 *Equation 1:*

216
$$\text{Glomerular filtration rate (ml/min/1.73m}^2) = \frac{(U_x) \times (\dot{V})}{(P_x)}$$

217 Where U_x is urine creatinine concentration (mol/L), \dot{V} is urine production rate (ml/min) and P_x is
218 serum creatinine concentration (mol/L). Glomerular filtration rate was then scaled to body surface
219 area as determined through the Dubois and Dubois formula (31).

220 *Data and statistical analyses*

221 Data were assessed for normality and variance using the Shapiro-Wilk and the Bron-
222 Forsythe tests. Differences between groups (CMS vs. non-CMS) such as demographics,

223 hemodynamics, blood gases, blood volumes and urinary metrics were assessed using an unpaired
224 Student's *t* test. However, CMS scores and plasma renin activity were compared using an unpaired
225 nonparametric test (Wilcoxon rank sum test). Pearson product moment correlations were used to
226 assess associations between outcomes of interest. Statistical analyses were performed using Graph
227 Pad, Prism 8.3.0 (GraphPad Software Inc.,USA). All data are presented as the mean \pm SD with
228 statistical significance set at $p < 0.05$.

229

230 **Results:**

231 Participant demographics are presented in *Table 1*. 24 highlanders were recruited for this
232 study, including 10 who were classified as having CMS. We tested a range of CMS positive
233 participants with a score of between 6-13 on the Qinghai CMS questionnaire (2). Six were
234 classified as having mild CMS and four were considered as having moderate CMS. There were no
235 differences in age ($p = 0.79$), or body mass index ($p = 0.47$) between groups (*Table 1*). Highlanders
236 with CMS had higher PaCO₂ ($p = 0.027$), HCO₃⁻ ($p = 0.027$), lower pH ($p < 0.01$) and SaO₂ ($p =$
237 0.026), while PaO₂ ($p = 0.52$) was not different between groups (*Table 2*).

238 Plasma renin activity and plasma aldosterone concentration were lower in highlanders with
239 CMS compared to non-CMS highlanders ($p = 0.025$ and $p = 0.018$, respectively), while NT pro-
240 BNP was not different between groups ($p = 0.15$; *Figure 1*). Glomerular filtration rate was not
241 different between groups ($p = 0.47$); however, urinary microalbumin was higher in CMS compared
242 to non-CMS highlanders ($p < 0.01$; *Table 3*).

243 Highlanders with CMS had a higher hematocrit ($p < 0.01$) and hemoglobin ($p < 0.01$; *Table*
244 *1*). Blood volume tended to be higher in CMS highlanders; however, this was not significant (non-

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245 CMS 90 ± 15 vs. CMS: 103 ± 18 ml \cdot kg⁻¹; $p = 0.071$). CMS highlanders had higher total red blood
246 cell volume (66 ± 14 vs. 46 ± 10 ml \cdot kg⁻¹ $p = 0.019$) with a smaller plasma volume (35 ± 5 vs. 43
247 ± 7 ml \cdot kg⁻¹ $p = 0.03$) compared to non-CMS highlanders (*Figure 2*). Heart rate ($p = 0.78$) and
248 blood pressure were not different between groups (all $p > 0.05$; *Table 1*). Similarly, there was no
249 difference in ePASP between groups ($p = 0.60$; *Table 1*).

250 Correlation analyses indicated there were no associations between: ePASP and NT-pro
251 BNP ($r = 0.32$ $p = 0.43$); NT-pro BNP, plasma aldosterone concentration, and plasma active renin
252 with plasma volume ($r = -0.063$ $p = 0.76$; $r = 0.13$ $p = 0.52$; $r = -0.069$ $p = 0.74$, respectively); or
253 hemoglobin, NT-pro BNP, plasma aldosterone concentration with glomerular filtration rate ($r =$
254 0.15 $p = 0.47$ [*Figure 3*]; $r = 0.21$ $p = 0.30$; $r = -0.10$ $p = 0.77$, respectively). A negative correlation
255 was found between plasma renin activity and glomerular filtration rate (group: $r = -0.66$; $p < 0.01$;
256 non-CMS: $r = -0.60$; $p = 0.022$; CMS: $r = -0.63$; $p = 0.049$; *Figure 4*).

257

258 Discussion:

259 The primary purpose of the current investigation was to compare volume regulatory
260 hormones, kidney function, blood volume, and vascular pressure, in both non-CMS Andean
261 highlanders and those with CMS. The main findings were: NT-pro BNP was not different between
262 groups, both plasma renin activity and plasma aldosterone concentrations were significantly lower
263 in Andeans with CMS compare to non-CMS. Glomerular filtration rate was not different between
264 groups; however, there was evidence of elevated urine microalbumin in highlanders with CMS,
265 which may suggest highlanders with CMS might be susceptible to worsening renal function.
266 Although there was a significant shift to a lower plasma volume and higher red blood cell volume

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267 in highlanders with CMS, total blood volume was not different between groups. Systemic blood
268 pressure and ePASP were similar between groups. A smaller plasma volume in CMS highlanders
269 may indicate an additional CMS maladaptation to high-altitude, causing potentially greater
270 polycythemia and clinical symptoms.

271 *Urinary microalbumin in chronic mountain sickness*

272 Urine microalbumin was higher in highlanders with CMS compared to non-CMS.
273 Microalbuminuria is common among patients living with chronic hypoxia secondary to congenital
274 cyanotic heart disease or chronic obstructive lung disease (32-34), and often occurs with reduced
275 glomerular filtration rate, although the latter was not observed in our non-CMS or CMS
276 highlanders (35). However, other high-altitude populations display a high prevalence of
277 microalbuminuria. Microalbuminuria is prevalent in 16.2% of high-altitude Tibetans without CMS
278 and is strongly associated with hematocrit (36). However, we did not see this association in our
279 cohort of Andeans. Therefore, the pathology of high-altitude microalbuminuria is most likely
280 multi-factorial and may include glomerular capillary hypertension, glomerular basement
281 membrane thickening, mesangial expansion, glomerulosclerosis, kidney parenchyma hypoxia,
282 hyper-viscosity, and notably hyperuricemia (17, 32, 37-40). Hyperuricemia develops at high-
283 altitude because of greater breakdown of adenine nucleotide and is exacerbated by impaired
284 excretion (17, 38). Uric acid induces endothelial dysfunction and oxidative stress causing
285 glomerular hypertension and related glomerulomegaly leading to greater albumin excretion (36,
286 38, 41-44). Polycythemia may also independently elevate erythrocyte turnover and further increase
287 uric acid levels (45). Jefferson and colleagues (17) found that uric acid levels were positively
288 correlated with hypertension and mild proteinuria in non-CMS and CMS highlanders. Thus,
289 microalbuminuria may occur in highlanders because of increased uric acid levels (17).

290 *ePASP and NT Pro-BNP in chronic mountain sickness*

291 NT pro-BNP concentrations and ePASP were not different between groups (13, 16). It is
292 well known that a degree of mild-to-moderate pulmonary artery hypertension is common among
293 healthy individuals born and living at high-altitude (13, 46, 47), which is thought to be exacerbated
294 by CMS; however, this was not apparent in this cohort. It possible that greater differences in
295 hypoxemia, hematocrit, and/or viscosity, beyond those observed in this study, are needed to see a
296 difference in pulmonary artery pressure between groups (48). Similarly, NT pro-BNP was not
297 elevated in CMS highlanders. Conversely, Ge and colleagues (16) found that Tibetan and Hans
298 Chinese highlanders with CMS had higher BNP blood concentrations compared to non-CMS,
299 which were positively associated with elevated ePASP (16). Circulating BNP levels are often
300 elevated in conditions with ventricular pressure overload, and often occurs with diagnosis of heart
301 failure (49). BNP is also relevant within the context of pulmonary artery hypertension with or
302 without ventricular dysfunction (50, 51). Given that there was no difference between total blood
303 volume and PASP between groups it seems plausible that BNP would not be different in CMS
304 (16). Likewise, low-altitude participants during ascent to high-altitude (5150 m) demonstrate that
305 those with a ePASP ≥ 40 mmHg vs. <40 mmHg at various altitudes had associated higher BNP
306 and NT-pro BNP levels (52), suggesting that elevated BNP appears to occur with elevated ePASP
307 (16, 52) at high-altitude. However, the relationship between CMS, pulmonary artery pressure, and
308 NT pro-BNP may occur differently in Peruvian Andeans compared to Tibetans and Hans Chinese
309 (16). Therefore, we cannot rule out that disparities within the literature and with our data are not
310 due to genetic variation.

311 *Blood volume in chronic mountain sickness*

312 The widespread underlying assumption is that polycythemia in Peruvian Andeans occurs
313 via hypoxic induced erythrocyte expansion (3, 5). The theory states that age results in a blunting
314 of ventilatory chemosensitivity leading to central hypoventilation and a subsequent polythemia
315 response (3, 5). However, recent evidence has emphasised the importance of plasma volume for
316 balancing erythropoiesis in response to hypoxia (25). For example, Himalayan Sherpa have a
317 larger plasma volume than Peruvian Andeans, resulting in a comparable total blood volume at a
318 lower hemoglobin concentration. Sherpa are able to benefit from the increased oxygen delivery
319 that comes from an expansion of their hemoglobin mass but are not restricted by an increase in
320 blood viscosity (25). Conversely, our cohort of Andeans with CMS had a smaller plasma volume
321 compared to healthy controls. It seems probable that a lower plasma volume may cause a greater
322 degree of polycythemia. CMS polycythemia may occur by two distinct mechanisms erythrocyte
323 expansion and plasma volume contraction (25). With the current data, we cannot determine if this
324 lower plasma volume occurs as a consequence from CMS or is a predisposing risk factor for
325 development of the condition. CMS polycythemia does however seem to be exacerbated by plasma
326 volume contraction. One potential mechanism causing this plasma volume contraction could be an
327 adaptive decrease in RAAS activity (17). We theorized that in response to CMS blood volume
328 expansion RAAS activity may adaptively decrease to mitigate the risk of hypertension.
329 Importantly, lower RAAS activity would not just lower blood volume, but also lower angiotensin-
330 II concentration thereby effectively decreasing vasoconstriction and sympathetic nerve activity
331 (53).

332 *Glomerular filtration rate in chronic mountain sickness*

333

334 Andeans with CMS have a lower renal plasma flow compared to healthy controls (21-23).
335 However, glomerular filtration rate is maintained because of a high filtration fraction (21, 22).
336 Filtration fraction linearly increases with hematocrit in Andeans with CMS (22), and polycythemic
337 rats (54). Prolonged elevated filtration fraction causes glomerular hypertension and inevitable
338 chronic kidney impairments (55). Of note, high-altitude populations demonstrate similar
339 characteristics as patients with diabetics kidney disease (56) including glomerulomegaly (57),
340 hyperfiltration and polyuria (17). Likewise, high-altitude dwellers (i.e. Peruvian and Bolivian)
341 have a higher prevalence of impaired estimated glomerular filtration rate (< 60 ml/min/1.73 m²).
342 Two epidemiology studies have also reported an inverse associated between hemoglobin and
343 estimated glomerular filtration rate (19, 20). We and another study did not observe this relationship
344 in healthy and CMS Andeans (17). It remains unknown how polycythemia interacts with renal
345 function. However, we did find renin was inversely related to glomerular filtration rate. Lower
346 renin, and presumably angiotensin-II, may suggest a lower efferent arteriole vasoconstriction and
347 supports a previous study that found angiotensin-converting enzyme inhibitors decrease the
348 prevalence of proteinuria in highlanders with CMS (24). RAAS dysregulation might contribute to
349 renal dysfunction in high-altitude Andeans.

350 *Experimental limitations and considerations*

351 There are some methodological considerations that warrant further comment. First, salt and
352 fluid intake were not strictly controlled during testing. We acknowledge that differences in fluid
353 and salt intake between participants may have contributed to our findings. Notably, Andeans with
354 CMS have a lower fractional excretion of lithium suggesting an increased sodium reabsorption
355 capacity (17), which may impact salt sensitivity, RAAS and overall fluid regulation (53). Second,
356 creatinine clearance was used as a metric of glomerular filtration rate rather than a gold-standard

357 measure such as inulin clearance. Creatinine clearance is however strongly correlated with inulin
358 clearance when participants have a glomerular filtration rate ≥ 42 ml / min. Third, urine creatinine
359 concentration was not used to determine the overall quality of urine collection. Urine creatinine
360 excretion is expected to be within a specific range over a given period of time. Renal excretion of
361 creatinine is relatively constant (58). This provides a method to determine the overall quality of
362 the urine collection and indicates if urine was over-or-under collected. Urine collection quality
363 was not determined in this cohort because the rate of creatinine excretion across the kidney might
364 be different in high-altitude dwellers compared to lowlanders. Future studies should determine 24-
365 hour urine creatinine excretion. Forth, erythrocyte labelling methods (i.e. CO rebreathing) assumes
366 that there is an even distribution of erythrocytes in the body. However, because of the Fåhræus
367 effect, venous hematocrit is higher than capillary hematocrit. Therefore, plasma derived from CO
368 rebreathing underestimates plasma volume by 5-10% (59). While this would be consistent across
369 participants; it remains unknown if this is further exacerbated in populations with polycythemia.
370 Finally, because female Andean volunteers were prioritized for expedition investigations that
371 focused on sex differences, and CMS preferentially affects Andean males (3, 5), all participants in
372 this investigation were male. However, male and female kidneys exhibit dimorphic patterns of
373 transporter expression and salt handling, the implications of which could be profound in terms of
374 renal function (60). Future studies should examine the impact of sex on renal function and volume
375 regulation in high-altitude populations.

376 *Significance and perspective*

377 Our data demonstrate that CMS highlanders compared to healthy controls have a smaller
378 plasma volume, which may further exacerbate polycythemia and clinical symptoms of CMS. One
379 potential mechanism causing this plasma volume contraction could be an adaptive decrease in

Volume regulation in high-altitude Andeans

380 RAAS activity. We theorized that in response to CMS blood volume expansion RAAS activity
381 may adaptively decrease to mitigate the risk of hypertension. Further comparison of volume
382 regulating factors and renal function between indigenous high-altitude populations, including
383 those that develop CMS, may shed further light on divergent patterns of (mal)adaptation.

384 *Disclosures*

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Table 1: Demographics and hemodynamics characteristics.

	Non-CMS (n=14)	CMS (n=10)	Main effect of group p- value
Demographics			
Age (yrs)	39 ± 11	40 ± 12	0.79
Height (cm)	158 ± 6.0	159 ± 5.9	0.57
Weight (kg)	65 ± 11	69 ± 14	0.42
BMI (kg/m ²)	26 ± 3.5	27 ± 4.1	0.47
CMS scores	2.1 ± 1.7	7.5 ± 2.3	<0.01
Hemodynamics			
Heart rate (bpm)	71 ± 12	73 ± 14	0.78
Mean arterial pressure (mmHg)	92 ± 10	89 ± 11	0.51
Systolic arterial pressure (mmHg)	118 ± 9.9	115 ± 11	0.55
Diastolic arterial pressure (mmHg)	79 ± 10	76 ± 12	0.54
ePASP (mmHg)	32 ± 9	31 ± 8	0.60
Hematocrit (%)	51 ± 5.1	66 ± 5.7	<0.01
Hemoglobin (g/dl)	18.1 ± 1.3	23.8 ± 1.7	<0.01

386 *List of Abbreviations:* CMS, chronic mountain sickness; BMI, body mass index; ePASP, estimated
387 pulmonary artery systolic pressure. Values are mean +/- standard deviation. Statistical
388 comparisons performed using independent t-tests except for CMS scores a Wilcoxon rank-sum
389 test was used.

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Table 2: Blood gases and electrolytes

	Non-CMS (n=14)	CMS (n=10)	Main effect of group p- value
pH	7.44 ± 0.02	7.39 ± 0.03	<0.01
PaCO ₂ (mmHg)	30 ± 3.8	37 ± 5.6	0.03
PaO ₂ (mmHg)	47 ± 8.3	45 ± 2.6	0.52
HCO ₃ ⁻ (mmol/L)	20 ± 2.1	22 ± 1.0	0.01
SaO ₂ (%)	84.7 ± 4.7	80.0 ± 2.6	0.026
Lactate (mmol/L)	1.5 ± 0.61	1.5 ± 0.65	0.89
Arterial potassium (mmol/L)	4.0 ± 0.26	4.1 ± 0.29	0.30
Arterial sodium (mmol/L)	65.4 ± 14.3	63.6 ± 14.5	0.79
Arterial glucose (mmol/L)	5.3 ± 0.34	5.7 ± 0.83	0.57

400 *List of Abbreviations:* CMS, chronic mountain sickness; PaCO₂, arterial partial pressure of carbon;
 401 PaO₂, arterial partial pressure of oxygen; HCO₃⁻, bicarbonate and SaO₂ arterial saturation. Values
 402 are mean +/- standard deviation. Statistical comparisons performed using independent t-tests.

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Table 3: Glomerular filtration rate, urine volume and microalbumin.

	Non-CMS (n=14)	CMS (n=10)	Main effect of group p-value
Blood creatinine (mg/dL)	64 ± 15	65 ± 14	0.79
Urine creatinine (mg/L)	105 ± 61	95 ± 54	0.71
GFR (ml/min/1.73 ²)	105 ± 14	107 ± 22	0.47
9-hour urinary volume (mL)	623 ± 279	770 ± 313	0.29
Microalbumin (mg/L)	10 ± 6.7	76 ± 43	<0.01

417 *List of Abbreviations:* CMS, chronic mountain sickness; and GFR, glomerular filtration rate.
 418 Values are mean +/- standard deviation. Statistical comparisons performed using independent t-
 419 tests.

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422 **Figure 1: Volume regulatory hormones.**

423 NT pro-B-type natriuretic peptide (panel A) was not different across groups, but renin-angiotensin-
424 aldosterone-system (RAAS) was lower evident in both plasma renin activity (panel B), and plasma
425 aldosterone concentration (panel C). Values are mean \pm standard deviation unless otherwise
426 specified. Statistical comparisons performed using independent t-tests except for plasma renin
427 activity a Wilcoxon rank-sum test was used.

428 **Figure 2: Blood volumes**

429 Total blood volume (panel A) was not different between groups however CMS Andeans had a
430 greater red blood cell volume (panel B) and smaller plasma red volume (panel C) compared to
431 non-CMS. Values are mean \pm standard deviation unless otherwise specified. Statistical
432 comparisons performed using independent t-tests.

433 **Figure 3: Glomerular filtration rate and hemoglobin**

434 There was no association between glomerular filtration rate and hemoglobin ($r = 0.15$; $p = 0.47$).

435 **Figure 4: Glomerular filtration rate and plasma renin activity**

436 There was a negative association between glomerular filtration rate and plasma renin activity
437 (group: $r = -0.66$; $p < 0.01$; non-CMS: $r = -0.60$; $p = 0.022$; CMS: $r = -0.63$; $p = 0.049$).

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