- Title: Global REACH 2018: Volume regulation in high-altitude Andeans with and 1 without chronic mountain sickness 2 3 Running Head: Volume regulation in high-altitude Andeans American Journal of Physiology – Regulatory, Integrative and Comparative 4 Journal: Physiology 5 Andrew R Steele¹, Michael M Tymko¹, Victoria L Meah^{1,10,11} Lydia L Simpson², 6 Authors: Christopher Gasho³, Tony G Dawkins⁴, Alexandra M Williams^{5,6} Francisco C 7 Villafuerte⁷, Gustavo A Vizcardo-Galindo⁸, RómuloJ.Figueroa-Mujíca⁷, Philip N 8 Ainslie⁸, Michael Stembridge⁴, Jonathan P Moore⁹, Craig D Steinback^{1,10,11,12} 9 ¹Neurovascular Health Lab, Faculty of Kinesiology, Sport, & Recreation, Affiliations: 10 University of Alberta, Canada; 11 ²Department of Sport Science, Division of Physiology, University of Innsbruck, 12 13 Austria; ³Division of Pulmonary and Critical Care, School of Medicine, Loma Linda 14 University, Loma Linda, CA, USA; 15 ⁴Cardiff School of Sport and Health Sciences, Cardiff Metropolitan University, 16 17 Cardiff, UK; ⁵Department of Cellular and Physiological Sciences, Faculty of Medicine, 18 University of British Columbia, Canada 19 ⁶International Collaboration on Repair Discoveries, University of British 20 Columbia, Vancouver, Canada 21 ⁷Department of Biological and Physiological Sciences, Universidad Peruana 22 Cayetano Heredia, Lima, Peru; 23 24 ⁸Centre for Heart, Lung, and Vascular Health, University of British Columbia Okanagan, Kelowna, Canada: 25 ⁹Extremes Research Group, School of Sport, Health and Exercise Sciences, Bangor 26 University, Bangor, United Kingdom; 27 ¹⁰Women and Children's Health Research Institute, University of Alberta, Canada; 28 ¹¹Alberta Diabetes Institute, University of Alberta, Canada; 29 ¹²Neuroscience and Mental Health Institute, University of Alberta, Canada. 30 31 **Correspondence:** 32 Craig D. Steinback, PhD Associate Professor 33 Faculty of Kinesiology, Sport, and Recreation 34 University of Alberta 35
- 36 1-059D Li Ka Shing Centre for Health Research Innovation
- 37 Edmonton, Alberta, Canada

- T6G 2E1 38
- 39
- Tel: (780) 492-5553 Fax: (780) 492-4249 40
- 41
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46 Abstract:

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

68 Introduction:

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

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



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

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

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

be higher in Andeans with CMS. The secondary purpose was to explore the associations betweenthese physiological metrics.

116

117 Methods:

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

125 *Ethical Approval*

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

134 *Participants*

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

145 Preliminary screening visit

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

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

161 *Experimental measures*

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

169 *Heart rate and blood pressure*

Continuous heart rate (electrocardiogram Lead II) was recorded and integrated with a data 170 acquisition system (Powerlab 16/30; ADInstruments, Australia) and stored for subsequent analysis 171 using associated software (Labchart 8.0 Pro; ADInstruments, Australia). Systolic and diastolic 172 blood pressures were measured using an automated cuff (Omron M2 Classic; Japan) and mean 173 arterial pressure was subsequently calculated as: $(1/3 \times \text{systolic blood pressure}) + (2/3 \times \text{diastolic})$ 174 blood pressure). Echocardiography was used to assess estimated pulmonary artery systolic 175 pressure (ePASP). Images were obtained by a highly-experienced sonographer using a 176 commercially available ultrasound system (Vivid Q, GE, Fairfeild, CT, USA), as previously used 177 by our research group in high-altitude indigenous populations (28). The modified Bernoulli 178 equation (4 V²) was applied to the peak systolic tricuspid regurgitation jet velocity measured via 179

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

185 *Blood measures*

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

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

201 *Renal function and urine analysis*

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

213 Creatinine clearance was used to subsequently calculate glomerular filtration rate using the214 standard formula:

215 *Equation 1*:

216 Glomerular filtration rate (ml/min/1.73m²) =
$$\frac{(Ux) \times (\dot{V})}{(Px)}$$

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

220 Data and statistical analyses

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

hemodynamics, blood gases, blood volumes and urinary metrics were assessed using an unpaired Student's *t* test. However, CMS scores and plasma renin activity were compared using an unpaired nonparametric test (Wilcoxon rank sum test). Pearson product moment correlations were used to assess associations between outcomes of interest. Statistical analyses were performed using Graph

Pad, Prism 8.3.0 (GraphPad Software Inc.,USA). All data are presented as the mean \pm SD with statistical significance set at p < 0.05.

229

230 **Results:**

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

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

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

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

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

257

258 **Discussion:**

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

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

271 Urinary microalbumin in chronic mountain sickness

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

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

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

311 Blood volume in chronic mountain sickness

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

332 Glomerular filtration rate in chronic mountain sickness

Andeans with CMS have a lower renal plasma flow compared to healthy controls (21-23). 334 However, glomerular filtration rate is maintained because of a high filtration fraction (21, 22). 335 Filtration fraction linearly increases with hematocrit in Andeans with CMS (22), and polycythemic 336 rats (54). Prolonged elevated filtration fraction causes glomerular hypertension and inevitable 337 chronic kidney impairments (55). Of note, high-altitude populations demonstrate similar 338 characteristics as patients with diabetics kidney disease (56) including glomerulomegaly (57), 339 hyperfiltration and polyuria (17). Likewise, high-altitude dwellers (i.e. Peruvian and Bolivian) 340 have a higher prevalence of impaired estimated glomerular filtration rate ($< 60 \text{ ml/min}/1.73 \text{ m}^2$). 341 Two epidemiology studies have also reported an inverse associated between hemoglobin and 342 estimated glomerular filtration rate (19, 20). We and another study did not observe this relationship 343 in healthy and CMS Andeans (17). It remains unknown how polycythemia interacts with renal 344 function. However, we did find renin was inversely related to glomerular filtration rate. Lower 345 renin, and presumably angiotensin-II, may suggest a lower efferent arteriole vasoconstriction and 346 supports a previous study that found angiotensin-converting enzyme inhibitors decrease the 347 prevalence of proteinuria in highlanders with CMS (24). RAAS dysregulation might contribute to 348 renal dysfunction in high-altitude Andeans. 349

350 *Experimental limitations and considerations*

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

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

376 *Significance and perspective*

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

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*

385 No conflicts of interest, financial or otherwise, are declared by the authors

			Main effect of
	Non-CMS (n=14)	CMS (n=10)	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

 Table 1: Demographics and hemodynamics characteristics.

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

	~~~~	effect of
Non-CMS (n=14)	CMS (n=10)	group p- value
$7.44\pm0.02$	$7.39\pm0.03$	< 0.01
$30\pm3.8$	$37\pm5.6$	0.03
$47\pm8.3$	$45\pm2.6$	0.52
$20\pm2.1$	$22\pm1.0$	0.01
$84.7\pm4.7$	$80.0\pm2.6$	0.026
$1.5\pm0.61$	$1.5\pm0.65$	0.89
$4.0\pm0.26$	$4.1\pm0.29$	0.30
$65.4 \pm 14.3$	$63.6\pm14.5$	0.79
$5.3\pm0.34$	$5.7\pm0.83$	0.57
	Non-CMS (n=14) $7.44 \pm 0.02$ $30 \pm 3.8$ $47 \pm 8.3$ $20 \pm 2.1$ $84.7 \pm 4.7$ $1.5 \pm 0.61$ $4.0 \pm 0.26$ $65.4 \pm 14.3$ $5.3 \pm 0.34$	Non-CMS (n=14)CMS (n=10) $7.44 \pm 0.02$ $7.39 \pm 0.03$ $30 \pm 3.8$ $37 \pm 5.6$ $47 \pm 8.3$ $45 \pm 2.6$ $20 \pm 2.1$ $22 \pm 1.0$ $84.7 \pm 4.7$ $80.0 \pm 2.6$ $1.5 \pm 0.61$ $1.5 \pm 0.65$ $4.0 \pm 0.26$ $4.1 \pm 0.29$ $65.4 \pm 14.3$ $63.6 \pm 14.5$ $5.3 \pm 0.34$ $5.7 \pm 0.83$

## Table 2: Blood gases and electrolytes

*List of Abbreviations:* CMS, chronic mountain sickness; PaCO₂, arterial partial pressure of carbon;
 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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	Non-CMS (n=14)	CMS (n=10)	Main effect of group p-value
Blood creatinine (mg/dL)	$64 \pm 15$	$65\pm14$	0.79
Urine creatinine (mg/L)	$105\pm61$	$95\pm54$	0.71
GFR (ml/min/1.73 ² )	$105 \pm 14$	$107 \pm 22$	0.47
9-hour urinary volume (mL)	$623\pm279$	$770\pm313$	0.29
Microalbumin (mg/L)	$10 \pm 6.7$	$76\pm43$	< 0.01

<b>TADIE J.</b> OTOMETUTAL INITIATION FAILS, UTIME VOTUME AND INICIDATOUNIN	Table 3:	Glomerular	filtration rate.	urine	volume	and	microal	bumin
-----------------------------------------------------------------------------	----------	------------	------------------	-------	--------	-----	---------	-------

417 List of Abbreviations: CMS, chromic mountain sickness; and GFR, glomerular filtration rate.

418 Values are mean +/- standard deviation. Statistical comparisons performed using independent t419 tests.

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# 422 <u>Figure 1</u>: 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 <u>Figure 2</u>: Blood volumes

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

# 433 **<u>Figure 3</u>**: Glomerular filtration rate and hemoglobin

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

# 435 **<u>Figure 4</u>**: 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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