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

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Abstract:

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

Introduction:

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

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

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

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

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 between these physiological metrics.

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.

Ethical Approval

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

Participants

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

Preliminary screening visit

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

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).

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 10-minutes. Following instrumentation, hemodynamic characteristics were determined (10-minutes), and venous and radial artery blood samples were taken.

Heart rate and blood pressure

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

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.

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 and immediately analyzed using point-of-care i-STAT device (Abbott Laboratories, Chicago, USA). Blood samples were analyzed using the CG4+ (lactate, pH, arterial partial pressure of carbon dioxide [$PaCO_2$], arterial partial pressure of oxygen [PaO_2], bicarbonate [HCO_3^-] and arterial oxygen saturation [SaO_2]), and CHEM8+ (glucose, urea nitrogen, creatinine, sodium, potassium, chloride, ionized calcium, TCO_2, anion gap, hematocrit and hemoglobin) test cartridges. The point of care device, i-STAT, has been validated on altitudes up to 5043 meters (30).

Renal function and urine analysis

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 before and during the 9-hour urinary collection. Urine was refrigerated until analysis (4 °C). Immediately before analysis, urine pots were shaken vigorously to ensure a homogenous mixture. Volumes were measured using graduated cylinders. Urine creatinine and urine microalbumin were quantified using a commercially available point-of-care DCA Vantage Analyzer (Siemens Healthineers Global; Germany). DCA capillary holders were submerged into urine and absorbed 40 uL of urine, then placed in a DCA microalbumin/creatinine reagent cartridge. Thereafter, the DCA System performed 48 optical, electronic, mechanical, and reagent systems checks during the course of each specimen assay. All measurements and calculations are performed automatically by the DCA Analyzer.

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

Equation 1:

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

Where Ux is urine creatinine concentration (mol/L), $\dot{\text{V}}$ 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).

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 ± SD with statistical significance set at $p < 0.05$.

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 $PaCO_2$ ($p = 0.027$), HCO_3^- ($p = 0.027$), lower pH ($p < 0.01$) and SaO_2 ($p = 0.026$), while PaO_2 ($p = 0.52$) 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 ± 18 ml • kg^{-1}; $p = 0.071$). CMS highlanders had higher total red blood cell volume (66 ± 14 vs. 46 ± 10 ml • kg^{-1} $p = 0.019$) with a smaller plasma volume (35 ± 5 vs. 43 ± 7 ml • 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*).

Discussion:

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

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.

Urinary microalbumin in chronic mountain sickness

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

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

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

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

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

Disclosures

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

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/m²)	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

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.

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

List of Abbreviations: CMS, chronic mountain sickness; $PaCO_2$, arterial partial pressure of carbon; PaO_2, arterial partial pressure of oxygen; HCO_3^-, bicarbonate and SaO_2 arterial saturation. Values are mean +/- standard deviation. Statistical comparisons performed using independent t-tests.

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

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

Figure 1: Volume regulatory hormones.

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

Figure 2: 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.

Figure 3: Glomerular filtration rate and hemoglobin

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

Figure 4: Glomerular filtration rate and plasma renin activity

There was a negative association between glomerular filtration rate and plasma renin activity (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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