Title: Global REACH 2018: Renal oxygen delivery is maintained during early acclimatization to 4330 m

Running Head: Renal oxygen delivery at high-altitude (4330 m)

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Abstract:

Early acclimatization to high-altitude is characterized by various respiratory, hematological, and cardiovascular adaptations that serve to restore oxygen delivery to tissue. However, less is understood about renal function and the role of renal oxygen delivery (RDO_2) during high-altitude acclimatization. We hypothesized that: 1) RDO_2 would be reduced after 12-hours of high-altitude exposure (high-altitude day1) but restored to sea-level values after one-week (high-altitude day7); and 2) RDO_2 would be associated with renal reactivity (RR), an index of acid-base compensation at high-altitude. Twenty-four healthy lowlander participants were tested at sea-level (344m; Kelowna, Canada), on day1 and day7 at high-altitude (4330m; Cerro de Pasco, Peru). Cardiac output, renal blood flow, arterial and venous blood sampling for renin-angiotensin-aldosterone-system hormones and NT pro-B type natriuretic peptides were collected at each time point. RR was calculated as: (Δ arterial bicarbonate)/(Δ partial pressure of arterial carbon dioxide) between sea-level and high-altitude day1, and sea-level and high-altitude day7. The main findings were: 1) RDO_2 was initially decreased at high-altitude compared to sea-level (ΔRDO_2: -22±17%, $P<0.001$), but was restored to sea-level values on high-altitude day7 (ΔRDO_2: -6±14%, $P=0.36$). The observed improvements in RDO_2 resulted from both changes in renal blood flow (Δ from high-altitude day1: +12±11%; $P=0.008$), and arterial oxygen content (Δ from high-altitude day1 +44.8±17.7%; $P=0.006$); and 2) RR was positively correlated with RDO_2 on high-altitude day7 ($r=0.70$; $P<0.001$), but not high-altitude day1 ($r=0.26$; $P=0.29$). These findings characterize the temporal responses of renal function during early high-altitude acclimatization, and the influence of RDO_2 in the regulation of acid-base.

Introduction:

High-altitude acclimatization is characterized by varying elevations in ventilation, hemoglobin concentration, heart rate, and redistribution of blood flow, which serves to restore arterial oxygen content (CaO_2) and preserve oxygen delivery to vital organs (10, 13, 44, 53, 56). Alterations in renal function are also critical during high-altitude acclimatization; however, there are few studies exploring renal acclimatization in comparison to ventilatory and hematological factors (4, 10, 22, 43). This is noteworthy since there are unique characteristics of renal oxygenation that renders the kidney susceptible to hypoxia. For example, the partial pressure of oxygen (PO_2) in renal tissue is typically tightly controlled through a coupling between renal blood flow (i.e. oxygen delivery) and sodium reabsorption load (i.e. oxygen utilization) (31). Hence, unlike other tissues, greater renal blood flow does not necessarily influence renal oxygenation since renal oxygen consumption (i.e. metabolic rate) can rapidly adapt to maintain constant oxygen delivery (31). Furthermore, portions of the medulla have a tissue PO_2 of ~10-15 mmHg, which is near the "critical PO_2", which the enzyme mitochondrial cytochrome oxidase becomes reduced, in turn limiting adenosine triphosphate production (25, 30). This, coupled with the fact that 95-99% of renal energy is via oxidative phosphorylation (30), highlights the importance of controlled renal oxygen delivery (RDO_2) for normal kidney function (7). Despite the kidney's precise maintenance of RDO_2 in normoxia, RDO_2 has not been quantified during early acclimatization to severe hypoxia (e.g. >4000 m).

While data sets are limited, renal blood flow (as indexed via the effective renal plasma flow) appears to decrease following acute (48-hours) exposure to 4350 m (37) and following a 60 day stay at 3500 m (43). Only two studies to our knowledge (37, 38) have investigated the mechanism(s) regulating renal blood flow at high-altitude. Olsen and colleagues (37) reported a

reduction in effective renal blood flow indicating a pre-existing increase in renal vascular tone. The authors from this study speculated that elevated catecholamines (e.g. noradrenaline), were responsible for the observed renal vasoconstriction and consequential reduction in renal blood flow. However, systemic hypoxia stimulates numerous factors that independently influence renal blood flow control such as natriuretic peptides (5) and RAAS hormones (6, 13). Specifically, the influence of renin during hypoxia has been unclear: some studies have reported that renin is elevated (12, 35, 40) while others have documented no change (3, 45) and others still have reported a decrease (6, 37). To our knowledge, no study has investigated the integrative mechanisms controlling renal blood flow at high-altitude following 12-hours and a week of acclimatization.

Under normal oxygen conditions (e.g. sea-level), arterial oxygen content (CaO_2) is relatively stable and RDO_2 is directly related to renal blood flow (31). However, since early exposure to high-altitude decreases both renal blood flow (37), and CaO_2 (43), this may hence effectively decrease RDO_2. Yet, RDO_2 may return to sea-level values as CaO_2 becomes restored with acclimatization (19, 43), which may offset the high-altitude related reductions in renal blood flow, and mediates the reduction in RDO_2 as demonstrated in animal studies (48). To our knowledge no study has characterized RDO_2 during acclimatization to high-altitude in humans. The contributions of renal blood flow and CaO_2 on RDO_2 has yet to be characterized at high-altitude, and may have functional consequences on acid-base acclimatization (18, 49).

The tight regulation of blood pH is critical for homeostasis and regular cellular function. High-altitude driven hyperventilation decreases the partial pressure of arterial carbon dioxide ($PaCO_2$), resulting in respiratory alkalosis (10, 47). Renal acid (H^+) retention and bicarbonate $(HCO_3)^-$ excretion aims to normalize arterial pH towards standard sea-level values (pH ~ 7.4) (9,

13, 28, 43, 45). A recent study by Zouboules and colleagues (58) proposed a novel renal reactivity index ($\Delta[HCO_3^-]/\Delta\ [PaCO_2]$), to quantify the relationship between HCO_3^- and $PaCO_2$ at high-altitude (58). While bicarbonate excretion occurs to normalize pH as a function of $PaCO_2$, this response might be linked to RDO_2, since bicarbonate reabsorption is dependent on renal cortex tissue PO_2 in hypoxic animals (49).

The purpose of the current investigation was to assess the mechanism(s) that govern renal oxygen delivery during early high-altitude acclimatization. We hypothesized the following: 1) after rapid ascent from sea-level to high-altitude (4330 m), RDO_2 would decrease 12-hours after arrival, but thereafter following a week of acclimatization, RDO_2 would increase through an increase in arterial oxygen delivery rather than renal blood flow; and 2) an association between RDO_2 and renal reactivity would be present after 12-hours and one week of high-altitude acclimatization.

Methods:

Ethical Approval

This *a priori* study was conducted as part of the Global Research Expedition on Altitude-Related Chronic Health (REACH) expedition to Instituto de Investigacions de Altura at Cerro de Pasco, Peru (4330 m). Participants were researchers involved in the expedition and as such were in numerous studies; however, care was taken to ensure adequate washout between studies to avoid cross-over or contamination between investigations. An overview of our research team's expedition has been previously published (50).

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 100 Board (Pro00077330) and the Universidad Peruana Cayetano Heredia Comité de Ética (no. 101686). Participants were given in-depth study information and provided written consent.

Participants

Participants (20 males, 4 females) were recruited from the research expedition team and had no history of pre-existing neurological, cardiovascular or renal dysfunction prior to testing. Participants were born and lived at or near sea-level and had not traveled to high-altitude within 6 months prior to experimentation (50).

Experimental overview

Sea-level testing occurred at the University of British Columbia – Okanagan Campus, BC (altitude = ~344 m) ~three months prior to departure to high-altitude. The research team travelled to Lima, Peru (altitude = ~150m) in June 2018, spent three days in Lima before the expedition preparing to depart and then traveled via automobile directly to Cerro de Pasco, Peru (4330 m) over 6-8 hours. Participants were tested the morning immediately following ascent having spent ~12-hours at high-altitude (high-altitude day1), and again following seven days of acclimatization (high-altitude day7).

At both sea-level and high-altitude participants arrived at the laboratory between 0600 and 1030 following a 12-hour fast and having avoided caffeine, alcohol and exercise. Throughout the week of acclimatization, participants were asked to avoid exercise to not contaminate results. Participants were asked to complete a nine-hour urinary collection from the previous night, which was used to calculate glomerular filtration rate. Participants were asked to complete an acute mountain sickness questionnaire at high-altitude prior to testing on both high-altitude day1 and high-altitude day7 (i.e. Lake Louise Questionnaire) (41). Experimentation commenced with participants laying supine and resting quietly for ~ten-minutes prior to collecting measurements of renal blood flow via duplex ultrasound, venous blood samples, echocardiography, and radial artery blood samples were taken. These methodologies are discussed in further detail below.

Lake Louise acute mountain sickness scores

Acute mountain sickness was identified using the standard 2018 Lake Louis acute mountain sickness scoring system. As per the recommendations, the scoring system was not used until at least six hours prior of ascent. Acute mountain sickness is identified via four categories: headache, gastrointestinal symptoms, fatigue and/or weakness and dizziness/light-headedness with each category with a score between 0-3. Acute mountain sickness is diagnosed as a score of three with an associated headache. As per the guidelines, participants with mild symptoms of acute mountain sickness had scores between 3-5; moderate between 6-9 and severe 10-12 points [refer to (41) for more details].

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). Mean arterial pressure was subsequently calculated as: (1/3 x systolic blood pressure) + (2/3 x diastolic blood pressure). Arterial oxygen saturation was estimated by pulse oximetry (N-595; Nellcor Oximax, Boulder, USA) using an index finger sensor.

Blood measures

Venous blood samples were taken from the antecubital vein immediately centrifuged, aliquoted and frozen 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 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 analyzed using point-of-care device i-STAT (Abbott Laboratories, Chicago, USA) for blood gases using the CG4+ (lactate, pH, $PaCO_2$, arterial partial pressure of oxygen (PaO_2), $HCO_3^-$and 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 altitude up to 5043 meters (32).

CaO_2 was calculated with measures of oxygen saturation (SaO_2), [Hb] and arterial partial pressure of oxygen (mmHg) using the following formula:

Equation 1:

$$CaO_2\ (\text{ml dl}^{-1}) = \left([\text{Hb}] \times 1.36 \times \frac{SaO_2}{100}\right) + (0.003 \times PaO_2)$$

where [Hb] is the concentration of hemoglobin (g dL^{-1}), 1.36 is the affinity of oxygen to hemoglobin, SaO_2, is the percentage of hemoglobin saturated with oxygen, 0.003 is the fraction of free oxygen dissolved in the blood.

Renal reactivity on high-altitude day1 and high-altitude day7 was calculated using relative changes with respect to sea-level values as previously described (58).

Equation 2:

$$\text{Renal Reactivity} = \left(\frac{\Delta HCO_3^-}{\Delta PaCO_2}\right) = \left(\frac{(HCO_3^-)_{\text{altitude}} - (HCO_3^-)_{\text{sea-level}}}{(PaCO_2)_{\text{altitude}} - (PaCO_2)_{\text{sea-level}}}\right)$$

Where HCO_3^- is arterial bicarbonate (mmol L^{-1}) and $PaCO_2$ (mmHg) is partial pressure of arterial carbon dioxide

Transthoracic echocardiography

Echocardiography was performed using an ultrasound system (as above) and a phased-array transducer (1.5 – 3.6 MHz M4S-RS, GE Healthcare, Piscataway, NJ, USA) by the same sonographer (V.L.M.). A three-lead electrocardiograph was attached to the participant and connected to the ultrasound system to allow cardiac cycle gating. Images were acquired at end expiration over five cardiac cycles and data was stored for later offline analysis (EchoPAC, GE Medical, Horton, Norway). Measurements were made in triplicate from different cardiac cycles and averaged for use in statistical analyses. With the participant lying supine, subcostal images were acquired for assessment of inferior vena cava diameter. With the participant in the left lateral decubitus position, images were acquired for assessment of cardiac function according to current guidelines (29). Left ventricular stroke volume using end-diastolic and end-systolic volume that, were derived using the Simpson's biplane method from apical 4- and 2-chamber views. Cardiac output was calculated as stroke volume x heart rate. Total peripheral resistance was calculated as: mean arterial pressure (mmHg) / mean cardiac output (ml/min).

Renal function

Duplex ultrasound

Renal artery diameter and blood flow were measured with a convex-array transducer (2.0 – 5.5 MHz 4C-RS Probe, GE Healthcare, Piscataway, NJ, USA) on a commercially available ultrasound system (Vivid Q, GE Healthcare, Piscataway, NJ, USA) by a single trained sonographer (V.L.M). The probe was placed at the midpoint between the xiphoid process and the umbilicus where the aorta was identified in a transverse section and the origin of the renal arteries was obtained using B-mode. Images were collected for measurement of renal artery diameter and allowing subsequent calculation of cross-sectional area. Renal artery blood flow was calculated as the product of the cross-sectional area and the velocity-time integral (pulse-wave Doppler). Absolute renal blood flow and normalized renal blood flow ([renal blood flow / cardiac output] *100) are reported. Renal vascular resistance was calculated as: mean arterial pressure (mmHg) / mean renal blood flow (ml/min). All measurements were made in triplicate from different cardiac cycles and averaged for use in statistical analyses.

The product of renal blood flow (ml min^{-1}) and CaO_2 (ml dl^{-1}) was used to calculate convective RDO_2:

Equation 3:

$$RDO_2 \text{ (ml } O_2 \text{ min}^{-1}) = \frac{(\text{mean renal blood flow} \times CaO_2)}{(100)}$$

Urine collection and analysis

Participants were asked to complete a 9-hour urinary collection to calculate glomerular filtration rate. Due to limitations associated with conducting field research, were unable to control for salt and fluid intake. Participants were asked to maintain normal drinking habitats

244 throughout the week of high-altitude and we specifically requested participants to drink a
245 standardized 200 mL of water forty-five minutes before testing. Urine was refrigerated until
246 analysis (4 °C). Urine pots were shaken vigorously before analysis to ensure a homogenous
247 mixture. Volumes were measured using graduated cylinders. Urine analysis was performed using
248 a DCA Vantage Analyzer (Siemens Healthineers Global; Germany) for creatinine and
249 microalbumin. Creatinine clearance was used to calculate glomerular filtration rate using the
250 standard formula:

251 *Equation 4*:

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

252 Where Ux is urine creatinine concentration (mol L^{-1}), $\dot{V}$ is urine production rate (ml min^{-1}) and
253 Px is serum creatinine concentration (mol L^{-1}). Glomerular filtration rate was then scaled to body
254 surface area as determined through the Dubois and Dubois formula (8).

255

256 Filtration fraction was calculated using the following:

257 *Equation 5:*

$$\text{Filtraction Fraction (\%)} = \frac{\text{(glomerular filtration rate)}}{\text{(1-hematocrit)} \times \text{(mean renal blood flow)}}$$

258 Where the ratio between glomerular filtration (ml/min/1.73m^2) and renal plasma blood flow
259 (renal blood flow [ml min^{-1}] × 1- hematocrit [%]) is expressed as a percent.

260

Muscle sympathetic nerve activity

Muscle sympathetic nerve activity was measured in subset of individuals who participated in an associated study on high-altitude day7. As such basal muscle sympathetic nerve activity data and characteristics have been previously reported in nine participants (n=9; 7 males; age 25 ± 3 years and BMI 23 ± 2) (42); however, these data are presented in the current manuscript to provide novel insight related to sympathetic-mediated mechanisms governing renal blood flow at high-altitude. Muscle sympathetic nerve activity was recorded from the peroneal nerve using microneurography as previously described (42). The raw sympathetic signal was amplified (preamplifier 1000× and variable-gain, isolated amplifier (10000×), band-pass filtered (700–2000 Hz), rectified and integrated (Bio amp 16/30; ADInstruments, Australia). A suitable signal was confirmed by the presence of pulse-synchronous bursts of activity that increased in response to apnea, but not loud noise. Muscle sympathetic nerve activity raw and integrated signals were analysed using semi-automated peak detection algorithms (Labchart 8.0 Pro; ADInstruments, Australia) and bursts were confirmed by a trained observer (L.L.S. and C.D.S.) based on physiological principles. To account for differences in microelectrode position, burst amplitude data were normalized by assigning a value of 100 to the largest burst observed. Mean muscle sympathetic nerve activity was expressed as integrated burst frequency (burst min^{-1}), incidence (burst 100 cardiac $cycles^{-1}$) and total activity [mean burst amplitude × burst frequency ($a.u.\cdot min^{-1}$)].

Data and statistical analyses

Data was assessed for normality and variance using the Sharpiro-Wilk and the Bron-Forsythe test. A linear mixed-effect model analysis was performed to test for significance between sea-level *vs*. high-altitude day1 *vs.* high-altitude day7. Tukey *post-hoc* analyses were used if main effects existed. Acute mountain sickness scores were assessed using paired nonparametric tests (Wilcoxon signed-rank test). Pearson product moment correlations were used to assess associations between: Δ RDO_2 and glomerular filtration rate / renal reactivity; and renal blood flow and muscle sympathetic nerve activity. Statistical analyses were performed using Graph Pad, Prism 8.3.0. All reported data is presented as the mean ± SD with statistical significance set at $p < 0.05$

Results:

Participants

Participant demographics are presented in *table 1*. Twenty-four participants were recruited but only twenty-two full data sets across all three assessments were obtained because two participants (both male) did not complete measurements on high-altitude day7 due to unexpected departure back to Lima, Peru. The values for these two participants at sea-level and high-altitude day1 are included in the group analysis. Thirteen of the twenty-four participants had mild acute mountain sickness (Lake Louise scores between 3-5) (41) on high-altitude day1. All participants refrained from taking acetazolamide (i.e. diamox), and other medications for altitude (e.g. dexamethasone) or travel-related illness (anti-biotics). No participants experienced acute mountain sickness on high-altitude day7.

Blood gas changes with high-altitude

High-altitude caused an initial decrease in both PaO_2 and SaO_2 that improved on high-altitude day7 (*table 2*). $PaCO_2$ decreased longitudinally with high-altitude, while HCO_3^- was progressively decreased (*table 2*). Respiratory alkalosis developed on high-altitude day1 ($P<0.001$); there was partial correction to pH via renal compensation on high-altitude day7 (*table 2*). CaO_2 decreased initially with high-altitude ($P<0.001$) but improved to pre-altitude values on high-altitude day7 ($P=0.31$) through increases in PaO_2, SaO_2 and hemoglobin concentration (*figure 1B).*

Cardiovascular, sympathetic and renal responses to high-altitude

Diastolic pressure was elevated at high-altitude compared to sea-level (P=0.0092 *table 3*), but systolic and mean arterial pressure remained unchanged (*P*=0.30 *P*=0.098, respectfully *table 3*) Cardiac output increased on high-altitude day1 compared to sea-level (*P*<0.001), but fell to sea-level values on high-altitude day7 (*P*=0.67; *table 3*). Total peripheral resistance decreased on high-altitude day1 (*P*=0.018), but not high-altitude day7 (*P*=0.62; *table 3*).

Renal blood flow was decreased at high-altitude on high-altitude day1 by 17±15% but returned to sea-level values on high-altitude day7 (*P*=0.54; *figure 1A)*. Accordingly, renal vascular resistance was increased on high-altitude day1 (*P*=0.016), but not high-altitude day7 (*P*=0.76; *table 4*). RDO_2 was decreased by -22±17% on high-altitude day1 (*P*<0.001), due to a simultaneous reduction in both renal blood flow and CaO_2 but was normalized back to sea-level values on high-altitude day7 (-6±14%) (*P*=0.36; *figure 1C*). Total normalized sympathetic nerve activity was calculated in a subset of participants on high-altitude day7 and was negatively correlated with renal blood flow normalized to cardiac output (r=-0.69; *P*=0.039; *See figure supplemental 1)* (https://doi.org/10.6084/m9.figshare.12860744.v1). RAAS hormones: active renin, and plasma aldosterone concentration, both decreased at high-altitude (*P*=0.025 and *P*=0.018, respectively), while NT pro-BNP did not change (*P*=0.15; *table 4*).

Association between renal oxygen delivery and renal reactivity

Renal reactivity was increased between high-altitude day1 and high-altitude day7 (*P*=0.0016). A positive correlation was found between Δ RDO_2 and renal reactivity between sea-level and high-altitude day7 (r=0.70; *P*<0.001; *figure 2B*) and between high-altitude day1 and

high-altitude day7 (r=0.49; *P*=0.022; *figure 2C*), but not between sea-level and high-altitude day1 (r=0.26; *P*=0.29 *figure 2A*). No relationships were found between Δ renal blood flow (r=0.10; *P*=0.67), Δ CaO_2 (r=0.25; *P*=0.23), or Δ glomerular filtration rate (r=0.15; *P*=0.63), and renal reactivity on high-altitude day7.

Discussion:

To our knowledge, this study is the first to assess RDO_2 after rapid ascent to high-altitude in a large cohort of lowlander participants whom have refrained from taking high-altitude medications (e.g. acetazolamide). The main findings were: 1) there was a reduction in RDO_2 on high-altitude day1; however, RDO_2 was restored to sea-level values on high-altitude day7 through an increase in both CaO_2 and renal blood flow; and 2) the relative change in RDO_2 at high-altitude compared to sea-level was associated with renal reactivity on high-altitude day7, indicating that acid-base regulation is linked to renal oxygenation after exposure to severe hypobaric hypoxia. Together, these data demonstrate that RDO_2 is normalized after a week of high-altitude acclimatization and provides novel insight on the critical role of renal adaptation and acid-base balance under hypoxic conditions.

Renal blood flow control at high-altitude

Compared to ventilatory and hematological acclimatization responses (10, 13, 43, 55, 57), less is known on the impact of renal blood flow on high-altitude acclimatization. While short exposure to hypoxia (e.g. 20 minutes) augments renal blood flow (5, 51), this is not apparent during chronic hypoxia (1, 37, 38, 43). Renal blood flow has been reported as unchanged (38), and decreased (37), after 48-hours at 4350 m, but longer duration studies (weeks) have shown a decrease in renal blood flow (1, 43). Together, these findings indicate that the renal blood flow response to hypoxia is highly dependent on exposure time. We saw an early high-altitude renal vasoconstriction with a decreased renal blood flow, which normalized to sea-level values following a week of acclimatization. Numerous factors can influence renal blood

flow such as reactive oxygen species, RAAS, phosphodiesterase type 5 upregulation, renal sympathetic nerve activity, circulating catecholamines, natriuretic peptides and ET during hypoxia (11, 16, 34, 36, 43, 52). In this investigation, NT pro-BNP was unchanged during acclimatization. However, analyzed venous blood samples for RAAS hormones both renin activity and plasma aldosterone concentrations were decreased occurring on high-altitude day7, but not high-altitude day1. Prolonged hypoxia may depress RAAS to increase excretory function. This depression would counter the effects of increased renal vascular resistance and may explain the observed +12% increase in renal blood flow seen between high-altitude day1 and high-altitude day7 (37). The renal system may decrease renin secretion to preserve excretory function via decreased renal vascular resistance following a week of acclimatization (6, 38).

Sympathetic nerve activity may also influence renal blood flow at high-altitude (10, 37, 42). A previous study demonstrated that renal vascular vasodilation to dopamine at high-altitude (~48 hours at 4350 m) was attenuated, and plasma circulating norepinephrine concentrations were increased, indicating greater renal arteriole vasoconstriction potentially through increased adrenergic activity (37). Furthermore, a study conducted in dogs demonstrated an augmented renal blood flow response to hypoxia after kidney denervation (27), while another study conducted in conscious rabbits subjected to 0.14 and 0.10 fraction inspired oxygen content, had a 14% and 38% increase in renal sympathetic nerve activity, respectively, and congruent decreases in renal blood flow that were abolished following renal denervation (34). In the current study, we observed a significant negative relationship between total normalized muscle sympathetic nerve activity and normalized renal blood flow on high-altitude day7 *(See figure supplemental 1)* (https://doi.org/10.6084/m9.figshare.12860744.v1). In other words, participants with greater total normalized muscle sympathetic nerve activity had lower normalized renal blood flows.

Collectively, this latter observation and previous findings (34, 37) would suggest the level of sympathetic nerve activity is an important determinant of renal blood flow during hypoxia. We acknowledge the requirement of sea-level and high-altitude day1 muscle sympathetic nerve activity data, as well as acute manipulation of sympathetic nerve activity, to draw further conclusions.

RDO_2 at high-altitude

To date, no previous studies have calculated RDO_2 at high-altitude in humans (48). The data from the current investigation demonstrated that only 12 hours of high-altitude exposure resulted in a concomitant decrease in renal blood flow and CaO_2, resulting in a reduction in RDO_2 by 22%. The acute reduction in RDO_2 was offset by elevated CaO_2 and renal blood flow after 7 days of high-altitude acclimatization (*see figure 1*). We report similar findings as a previous animal study (48). Since sodium tubular load accounts for 99.5% of renal metabolic activity (14, 25, 31), renal blood flow may decrease in order to limit renal oxygen consumption, effectively preserving oxygen for other organs (20, 33). This is supported by reciprocal changes in cardiac output and renal blood flow observed where renal blood flow decreased was by 17%, while cardiac output was augmented by 20%. The limited oxygen supply is being directed away from the metabolic demanding kidneys conserving systemic oxygen (20, 33).

RDO_2 and acid-base acclimatization

There have been several previous studies that have characterized renal acid-base acclimatization at high-altitude. Renal alterations are initiated within two hours after the onset of hypocapnia,

405 and current data indicates incomplete pH compensation is present (metabolic alkalosis) at
406 altitudes above 2800 m (17, 20, 28, 58). Renal reactivity, an index of acid-base compensation
407 between HCO_3^- and $PaCO_2$ ($\Delta[HCO_3^-]/\Delta [PaCO_2]$), (58), has been shown to increase at altitudes
408 up to 3800 m, and then decreases with further increases in altitude (58). In the current study,
409 renal reactivity was greater on high-altitude day7 compared to high-altitude day1, indicating
410 renal reactivity has a temporal component that is influenced by early acclimatization. Compared
411 to Zouboules and colleagues (58) expedition, which was conducted at 4240 m after incremental
412 ascent over seven days, we observed similar renal reactivity response to high-altitude. It is
413 important to note, however, that the ascent profile used in this current study and Zouboules and
414 colleagues (58) expedition were very different. For example, Zouboules and colleagues (58)
415 trekked most days towards Everest basecamp where acclimatization was obviously influenced by
416 the daily changes in altitude. In our study, we ascended via automobile to 4330 m where we
417 resided for the duration of the study. Hence, the current study enabled the question of
418 acclimatization to be addressed over time at the same altitude. Therefore, to address the question
419 and to extend the data presented by Zouboules and colleagues (58), we assessed both renal
420 reactivity and RDO2 at high-altitude, and found an association between these two physiological
421 parameters on high-altitude day7 (*see figure 2B and 2C*). Interestingly, a relationship was not
422 observed between renal reactivity and renal blood flow, CaO_2 or glomerular filtration rate. One
423 interpretation of these findings is that the reduction in renal blood flow or glomerular filtration
424 rate seen at high-altitude (37, 39, 43) does not influence the kidneys capacity to filtrate and
425 excrete HCO_3^- in the urine as previously hypothesized (39, 58). Conversely, this may imply
426 RDO_2 influences the tubular handling of $HCO_3^-$and H^+ (18, 49). RDO_2 at high-altitude may
427 impact the activity of intracellular carbonic anhydrase (23), proton secretion via the Na^+-H^+

exchanger (NHE3) (2) and/or activity of intercalated cells on the collecting ducts (15). However, considering the known linkage between sodium and HCO_3^- reabsorption in the proximal (18, 54), we must acknowledge that the independent influence of sodium on acid-base regulation. That is, renal reactivity and arterial HCO_3^- may actually correlate with sodium excretion rather than changes in RDO_2. We recommend that these findings be interpreted cautiously. Future endeavours should determine the influence of sodium (and other electrolyte) excretion on acid-base regulation during acclimatization.

Experimental limitations and considerations

The current investigation was the first to assess RDO_2 at high-altitude; however, there are some experimental considerations that warrant discussion. First, para-aminohippurate would provide a more specific measure of renal perfusion. However, renal ultrasound is strongly correlated to effective renal blood flow when flows are above 280 ml min^{-1} as seen this study (46). Second, muscle sympathetic nerve activity was recorded in a subset of individuals and used as a surrogate for renal sympathetic nerve activity. We acknowledge that sympathetic vasomotor outflow to skeletal muscle vasculature may not reflect renal sympathetic nerve activity and may exhibit differential reflex responses (42). While renal sympathetic nerve activity and muscle sympathetic nerve activity are strongly correlated in animals (26), these findings should be interpreted cautiously and used to inform future research. Third, salt and fluid intake was not controlled for during testing. We acknowledge that changes in fluid and salt may have contributed to the change in renal function and renal oxygen delivery (21). However, we feel this has limited influence on our findings. Previous findings have demonstrated that high-altitude changes renal blood flow and Sprague-Dawley rats during hypobaric hypoxia have a temporal

451 RDO_2 response to our findings (48). Future endeavours should investigate this physiological
452 phenomenon while controlling salt and fluid intake. Fourth, we did not calculate metabolic
453 efficacy of sodium reabsorption across the proximal tubule or renal oxygen consumption. High-
454 altitude may change both of these to maintain normoxic filtration (48). However, this should be
455 addressed in future studies specifically looking at renal metabolic function during hypobaric
456 hypoxia. Lastly, no comparisons were made between sexes despite knowing there is a difference
457 in renal blood flow and RAAS regulation between men and women (24). However, since this
458 was a repeated measures assessment comparing within individuals and females were only a small
459 subset this should not greatly impact our findings. Future endeavours should examine the impact
460 of sex on RDO_2 at high-altitude.

461

462 *Significance and perspective*

463 Our data characterizes renal acclimatization following 12-hours and one-week exposure
464 to 4300 m. Renal oxygen delivery fell immediately with initial high-altitude exposure but was
465 restored on high-altitude day7 by increases in both CaO_2 and renal blood flow. In addition,
466 relative changes to RDO_2 from sea-level were positively correlated with renal reactivity on high-
467 altitude day7, indicating a potential link between RDO_2 and acid-base compensation during high-
468 altitude acclimatization. Together, these data demonstrate that RDO_2 is normalized following a
469 week of acclimatization and may contribute to pH normalization.

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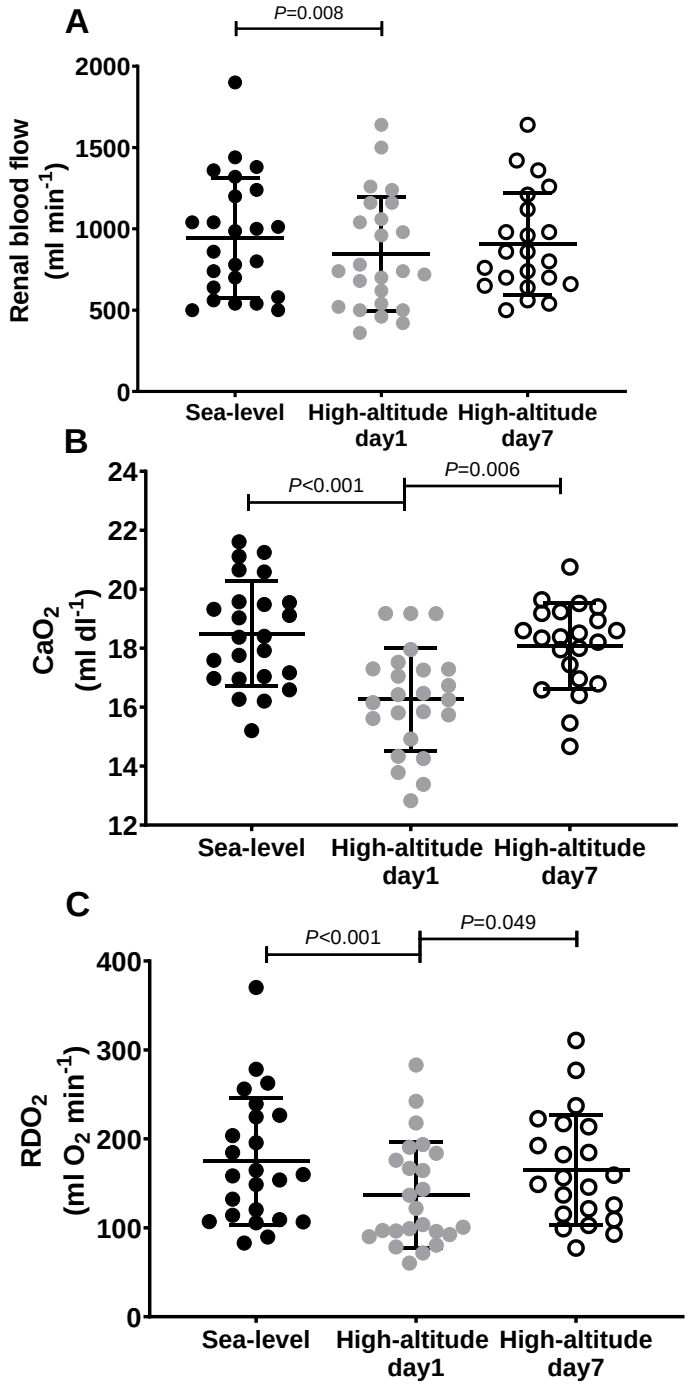
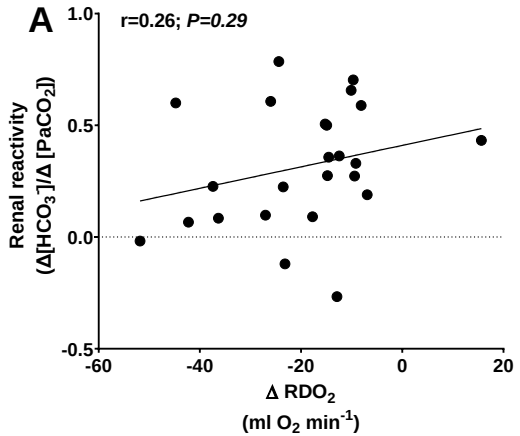
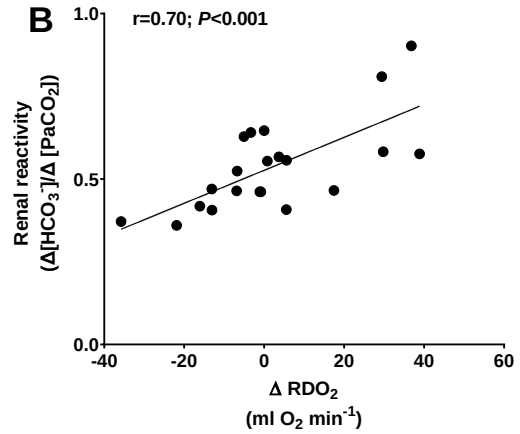
Figure 1: RDO_2 and determinants. RDO_2 is acutely decreased during initial exposure to high-altitude (4330 m), however increases to sea-level thereafter at high-altitude day7 from restored renal blood flow and CaO_2. Participants were tested at sea-level (Kelowna, BC 344 m), the morning immediately following ascent having spent ~12-hours at high-altitude (high-altitude day1) (Cerro de Pasco, Peru 4330m) and again following seven days of acclimatization (high-altitude day7) (Cerro de Pasco, Peru 4330m).

Figure 2: Renal reactivity and Δ RDO_2 at high-altitude day1, high-altitude day7 and between high-altitude day1 and high-altitude day7. While the change in renal reactivity between sea-level and high-altitude day1 was not associated with the concurrent change Δ RDO2 (A), there was a strong correlation between the changes in renal reactivity and RDO2 when considering the differences between sea-level and high-altitude day7 (B). There was also a correlation between changes renal reactivity and RDO2 during acclimatization (between high-altitude days 1 and 7) (C). Renal reactivity is higher in participants with greater RDO2 suggesting acid-base compensation is dictated by RDO2 at high-altitude. Participants were tested at sea-level (Kelowna, BC 344 m), the morning immediately following ascent having spent ~12-hours at high-altitude (high-altitude day1) (Cerro de Pasco, Peru 4330m) and again following seven days of acclimatization (high-altitude day7) (Cerro de Pasco, Peru 4330m).


A
P=0.008
Renal blood flow (ml min-1)
2000
1500
1000
500
0
Sea-level
High-altitude day1
High-altitude day7
B
P<0.001
P=0.006
CaO2 (ml dl-1)
24
22
20
18
16
14
12
Sea-level
High-altitude day1
High-altitude day7
C
P<0.001
P=0.049
RDO2 (ml O2 min-1)
400
300
200
100
0
Sea-level
High-altitude day1
High-altitude day7


A
r=0.26; P=0.29
Renal reactivity
(Δ[HCO3-]/Δ [PaCO2])
1.0
0.5
0.0
-0.5
-60
-40
-20
0
20
Δ RDO2
(ml O2 min-1)


B
r=0.70; P<0.001
Renal reactivity
(Δ[HCO3-]/Δ [PaCO2])
1.0
0.5
0.0
-40
-20
0
20
40
60
Δ RDO2
(ml O2 min-1)

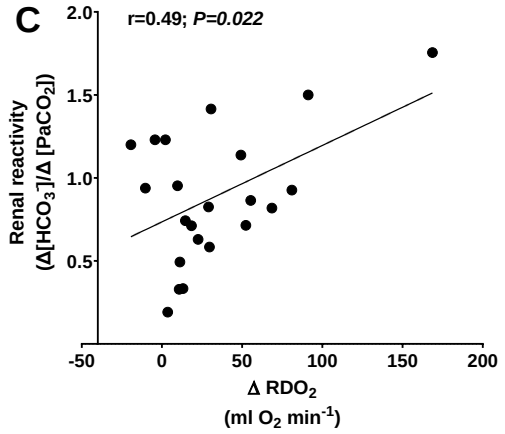

C
r=0.49; P=0.022
Renal reactivity
(Δ[HCO3-]/Δ [PaCO2])
2.0
1.5
1.0
0.5
-50
0
50
100
150
200
Δ RDO2
(ml O2 min-1)

Table 1: **Participant demographics and acute mountain sickness scores.**

	Low altitude	High-altitude		
	Sea-level (n=24)	**High-altitude day1 (n=24)**	**High-altitude day7 (n=22)**	**P-Value**
Age	28 ± 6.4	-	-	-
Weight (kg)	74 ± 8	73 ± 10	72 ± 10	0.57
Height (cm)	176 ± 10	-	-	-
BMI (kg m^{-1})	24.3 ± 2.4	23.6 ± 9.6	22.8 ± 3.5	0.19
AMS scores	-	3.0 ± 1.9	0.4 ± 0.9 #	0.046

List of Abbreviations: **BMI, body mass index; and AMS; acute mountain sickness.**

Participants were tested at sea-level (Kelowna, BC 344 m), the morning immediately following ascent having spent ~12-hours at high-altitude (high-altitude day1) (Cerro de Pasco, Peru 4330m) and again following seven days of acclimatization (high-altitude day7) (Cerro de Pasco, Peru 4330m).

P-value for a linear mixed-effect model analysis (effect of time) indicated for each variable. Symbols indicate significant post-hoc comparisons,

 # Represents a significant difference between Sea-level vs High-altitude day7 ($p<0.05$),

Table 2: **Arterial blood data**

	Low altitude	High-altitude		
	Sea-level (n=24)	**High-altitude day1 (n=24)**	**High-altitude day7 (n=22)**	**P-Value**
pH	7.43 ± 0.033	7.48 ± 0.034 *	7.45 ± 0.031 #	<0.001
Bicarbonate (mmol L^{-1})	25.8 ± 1.7	24.6 ± 1.9	19.9 ± 2.0 #†	<0.001
$PaCO_2$ (mmHg)	38.4 ± 3.2	33.1 ± 3.3 *	28.2 ± 2.6 #†	<0.001
Renal reactivity ($\Delta[HCO_3^-]/\Delta[PaCO_2]$)	-	0.098±0.75	0.54±0.14†	0.0016
PaO_2 (mmHg)	100.6 ± 18.4	41.5 ± 7.3 *	50.7 ± 3.9 #†	<0.001
SaO_2 (%)	97.6 ± 1.2	78.9 ± 8.4 *	87.6 ± 2.1	<0.001
Hemoglobin (g dl^{-1})	14.2 ± 1.3	15.2 ± 1.1	15.6 ± 1.2 #	<0.001
Hematocrit (%)	42.3 ± 4.4	44.3 ± 2.7	46.5 ± 2.4 #	<0.001
CaO_2 (ml dl^{-1})	15.2 ± 1.8	12.8 ± 1.7*	18.1 ± 1.4 †	<0.001

***List of Abbreviations*: PaO_2, arterial partial pressure of oxygen and $PaCO_2$, arterial partial pressure of carbon**

Participants were tested at sea-level (Kelowna, BC 344 m), the morning immediately following ascent having spent ~12-hours at high-altitude (high-altitude day1) (Cerro de Pasco, Peru 4330m) and again following seven days of acclimatization (high-altitude day7) (Cerro de Pasco, Peru 4330m).

P-value for a linear mixed-effect model analysis indicated for each variable. Symbols indicate significant post-hoc comparisons,

*** Represents a significant difference between Sea-level vs High-altitude day1 ($p<0.05$),**

 # Represents a significant difference between Sea-level vs High-altitude day7 ($p<0.05$),

† Represents a significant difference between High-altitude day1 vs High-altitude day7 ($p<0.05$).

Table 3: Cardiovascular hemodynamics and muscle sympathetic nerve activity				
	Low altitude	**High-altitude**		
	Sea-level (n=24)	**High-altitude day1 (n=24)**	**High-altitude day7 (n=22)**	**P-Value**
Cardiovascular hemodynamics				
Heart rate (beats min^{-1})	56 ± 12	77 ± 13 *	66 ± 13	<0.001
Cardiac output (L min^{-1})	4.0 ± 0.8	5.0 ± 1.1 *	4.1 ± 0.9 †	<0.001
Mean arterial pressure (mmHg)	88 ± 6	89 ± 7	90 ± 8	0.098
Systolic pressure (mmHg)	117 ± 9	118 ± 8	119 ± 10	0.30
Diastolic pressure (mmHg)	70 ± 7	78 ± 7 *	76.± 7	0.001
Total peripheral resistance (mmHg L^{-1} min^{-1})	21.9 ± 3.9	18.9 ± 4.1 *	22.7 ± 4.6 †	0.001
Muscle sympathetic nerve activity (n = 9)				
Burst frequency (bursts min^{-1})	-	-	32 ± 15	-
Burst incidence (bursts $100HB^{-1}$)	-	-	42 ± 15	-
Mean burst amplitude (a.u.)	-	-	39 ± 9	-
Total activity (a.u. min^{-1})	-	-	1284 ± 411	-

***List of Abbreviations*: HB, heartbeat and a.u, arbitrary units**

Participants were tested at sea-level (Kelowna, BC 344 m), the morning immediately following ascent having spent ~12-hours at high-altitude (high-altitude day1) (Cerro de Pasco, Peru 4330m) and again following seven days of acclimatization (high-altitude day7) (Cerro de Pasco, Peru 4330m).

P-value for a linear mixed-effect model analysis indicated for each variable. Symbols indicate significant post-hoc comparisons,

*** Represents a significant difference between Sea-level vs High-altitude day1 ($p<0.05$),**

 # Represents a significant difference between Sea-level vs High-altitude day7 ($p<0.05$),

† Represents a significant difference between High-altitude day1 vs High-altitude day7 ($p<0.05$).

1

Table 4: Renal function and volume regulatory hormones				
	Low altitude	**High-altitude**		
	Sea-level (n=24)	**High-altitude day1 (n=24)**	**High-altitude day7 (n=22)**	**P-Value**
Renal function				
RDO_2 (ml O_2 min^{-1})	174.8 ± 71.7	137.9 ± 59.2*	164.9 ± 61.9†	<0.001
Renal blood flow (ml min^{-1})	924 ± 366	795 ± 351*	907 ± 312	0.019
Normalized renal blood flow (%)	23 ± 3	16 ± 3*	22 ± 4†	<0.001
Renal vascular resistance (mmHg ml^{-1} min^{-1})	110 ± 50	129 ± 64 *	116 ± 47	0.046
Glomerular filtration rate (ml/min/1.73^2)	102 ± 20	91 ± 31 *	86 ± 17 #	0.005
Filtration fraction (%)	21 ± 10	28 ± 9 *	24 ± 9	0.005
Volume regulatory hormones				
Active renin (pg ml^{-1})	59.2 ± 23.1	49.4 ± 38.9	37.2 ± 24.1	0.025
Plasma aldosterone concentration (pg ml^{-1})	212.7 ± 104.9	175.1 ± 162.4	111.7 ± 92.5 #	0.018
NT-pro-BNP (pg ml^{-1})	1753.1 ± 600.2	1909 ± 970.6	1460 ± 764.6	0.15
Urinary volume (ml) (9 hours)	510 ± 198.5	680.1 ± 405.8	754.6 ± 255.8 #	0.022
Urinary microalbumin (mg L^{-1})	5.9 ± 1.5	10.2 ± 3.5*	6.4 ± 2.0	0.012

***List of Abbreviations*: NT pro-BNP, N-terminal pro-B-type natriuretic peptide and pg, picogram**

Participants were tested at sea-level (Kelowna, BC 344 m), the morning immediately following ascent having spent ~12-hours at high-altitude (high-altitude day1) (Cerro de Pasco, Peru 4330m) and again following seven days of acclimatization (high-altitude day7) (Cerro de Pasco, Peru 4330m).

P-value for a linear mixed-effect model analysis indicated for each variable. Symbols indicate significant post-hoc comparisons,

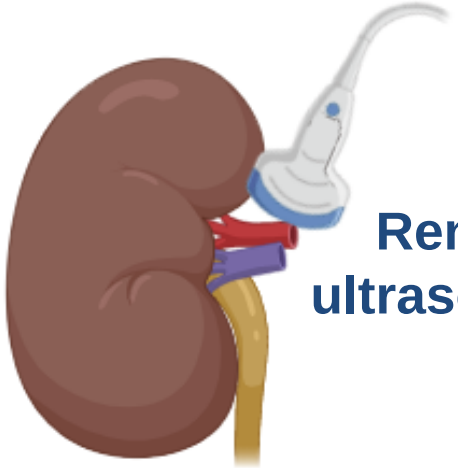
*** Represents a significant difference between Sea-level vs High-altitude day1 ($p<0.05$),**

 # Represents a significant difference between Sea-level vs High-altitude day7 ($p<0.05$),

† Represents a significant difference between High-altitude day1 vs High-altitude day7 ($p<0.05$).

Global REACH 2018: Renal oxygen delivery is maintained during early acclimatization to 4330 m

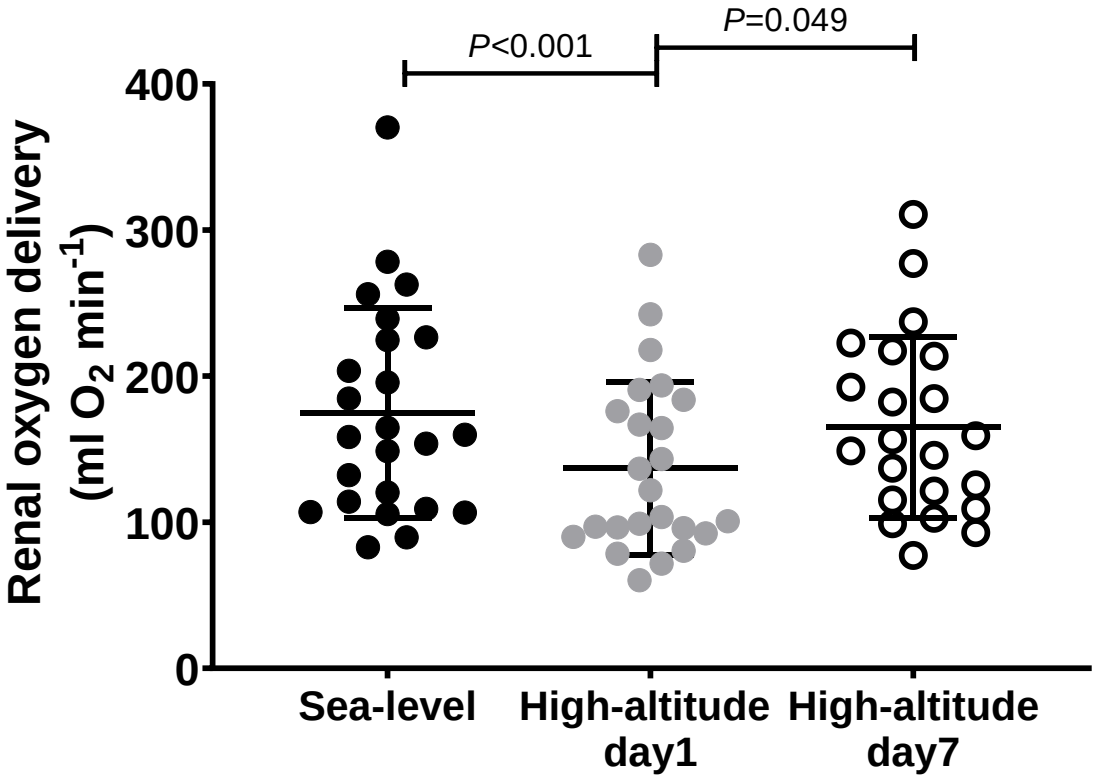
METHODS



Renal ultrasound

Radial arterial blood samples

Renal oxygen delivery at sea-level (344 m), after 12 hours and 7 days at high-altitude (4330 m)



CONCLUSION: Renal oxygen delivery is maintained at 4330 meters after a week of acclimatization.