Renal function and control during early altitude acclimatization

by

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Abstract

Renal acclimatization is an important aspect of high-altitude adaption coordinating diuresis, acid-base status, natriuresis, renal hemodynamics and kidney filtration. Renal blood flow, glomerular filtration rate, active renin, plasma aldosterone concentration, NT pro-brain natriuretic peptide (BNP), urine volume, urine microalbumin, HCO_3^-, PCO_2, and PO_2, were measured in twenty-four lowlanders (28 ± 7 years; 3 female) at 344 m and again at 4330 m following one (ATL1) and seven (ATL7) days of acclimatization. Renal blood flow decreased significantly from sea-level (931.4 ± 392.3 ml/min) to ATL1 (626.4 ± 364.8 ml/min*; $p<0.05$*) however rebound to sea-level values by ATL7 (892.8 ± 334.1 ml/min). Glomerular filtration rate was significantly decreased at ATL7 (86.4 ± 17.4 ml/min at ATL7) compared to sea-level (101.8 ± 21.1 ml/min; $p<0.05$). Plasma aldosterone concentration was the only hormone measured that reached significance (sea-level 121.7 ± 92.5 vs ATL7 182.7 ± 104.9 mmol/L; $p<0.05$). Lowlanders produced more urine at high-altitude (ATL1: 680.1 ml/9-hour and ALT7: 756.9 ml/9-hour) compared to sea-level (535.3 ± 277.8 ml/9-hour) reaching significance by ATL7 ($p<0.05$). Urine microalbumin was not significantly different at any time point. HCO_3^- was significantly decreased from sea-level (25.8 ± 1.7 mmol/L) and ATL1 (24.6 ± 1.9 mmol/L) compared to ALT7 (19.9 ± 2.0 mmol/L; $p<0.05$). PCO_2 was significantly higher at sea-level (38.4 ± 3.2 mmHg; $p<0.05$) compared to ATL1 (33.1 ± 3.3) and ATL7 (28.2 ± 2.6 mmHg). PO_2 was higher at sea-level (100.6 ± 18.4 mmHg; $p<0.05$) compared to ALT1 (41.5 ± 7.3 mmHg) and ATL7 (50.7 ± 3.9 mmHg) however increased during ATL7. Our findings suggest glomerular filtration rate decreases with continued high-altitude occupancy. Plasma aldosterone concentration decreases during prolonged hypoxia to cause diuresis. HCO_3^- excretion occurs to restore the acid-base status, which is evident by late acclimatization (ATL7).

Preface

This thesis is an original work by Andrew R Steele. No part of this thesis has been previously published. The research conducted for this project received ethics approval from the University of Alberta – Research Ethics Board under the project name: Sympathetic regulation of pulmonary pressure and cardiovascular function in Lowlanders and high-altitude patients with chronic mountain sickness (Pro00077330**)**.

This is a standalone study imbedded within an expedition to Instituto de Investigacions de Alture at Cerro de Pasco, Peru (4330 m) part of an international research expedition led by Professor Phillip Ainslie at the University of British Columbia Okanagan.

Dr. Craig Steinback and I contributed to conception of design, acquisition, analysis and interpretation. Dr. Victoria Meah contributed to data acquisition, in particular renal ultrasonography. Special thanks to Dr. Johnathan Moore, Dr. Michael Stembridge and Lydia Simpson for their contribution to data collection and interpretation.

Acknowledgments

Lindsey Berthelsen she knows why…

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List of Abbreviations and symbols

ADH	Antidiuretic Hormone
AMS	Acute Mountain Sickness
ANOVA	Analysis of variance
ANP	Atrial Natriuretic Peptide
ATL1	High-Altitude Day One
ATL7	High-Altitude Day Seven
BNP	Brain Natriuretic Peptide
BP	Blood Pressure
Ca^{2+}	Calcium Ion
CNP	C-type Natriuretic Peptide
CO_2	Carbon Dioxide
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbet assay
GFR	Glomerular Filtration Rate
HR	Heart Rate
H^+	Hydrogen Ion
HCO_3^-	Hydrogencarbonate (Bicarbonate)
H_2CO_3	Carbonic Acid
IV	Intravenous Catheter
Na^+	Sodium Ion
K^+	Potassium
HVR	Hypoxic Ventilation Response

MAP	Mean Arterial Pressure
NaCl	Sodium Chloride
NT-pro BNP	N-terminal (NT)-pro hormone BNP
PCO_2	Partial Pressure of Arterial Carbon Dioxide Content
PO_2	Partial Pressure of Arterial Oxygen Content
Px	Plasma Creatinine
RAAS	Renin-Aldosterone-Angiotensin-System
RBF	Renal Blood Flow
SST	Serum Separator Tubes
Ux	Urine Creatinine
V	Volume

Chapter 1 General Introduction

Oxygen is essential to normal cellular function and metabolic activity. Oxygen delivery is a complex phenomenon that is altered by diffusion limitations through the alveolicapillary membrane, ventilation mismatches between alveoli and pulmonary capillaries, limited hemoglobin concentrations and mass, diffusion limitations between capillary and skeletal muscle, and mitochondria diffusion rates (Leach & Treacher, 1994). Despite these physiological limitations, adequate oxygen delivery is maintained under normal conditions (Schober & Schwarte 2012).

High-altitude reduces barometric pressure causing hypobaric hypoxia, which is different than normoxic hypoxia. Hypobaric hypoxia is caused by decreased oxygen partial pressure, whereas normoxic hypoxia is created by decreasing fractional oxygen content (i.e O_2 13%) (West *et al*., 1962). Oxygen partial pressure at 5800 m is reduced by 50% (Goldfarb-Rumyantzev *et al.*, 2014 & Peacock, 1998). **Figure 1** outlines the typical oxygen cascade during hypobaric hypoxia, which causes a downward shift in the oxygen cascade affecting oxygen delivery.

Hypoxia sensitive organs (e.g. brain and heart) depend on constant oxygen delivery for proper metabolic function and as such hypoxia causes robust corrective and compensatory mechanisms including, increases in ventilation, cardiac output, sympathetic activity, vascular tone. High-altitude also alters fluid status, electrolyte balance and acid-base homeostasis (Goldfarb-Rumyantzev *et al.*, 2014; Maxwell *et al.,* 1993 & Menge, 1944). Proper kidney function and regulation is undoubtedly critical for high-altitude adaption. Hypoxia is a potent stimulus for polyuria occurring between 3000 and 5000 meters (Ramirez *et al.,* 1992; Koller *et al.,* 1991 & Olsen *et al.,* 1992). Hildebrant et al., (2000) demonstrated an initial hypoxic diuretic response within 90 minutes unrelated to chemoreceptor sensitivity and sodium excretion. However, following 6 hours of hypoxia (12% oxygen), humans exhibit diuresis and natriuresis related to concurrent ventilation response (Swenson *et al*., 1995). Thus, hypoxic diuresis occurs through

different mechanisms in a time-dependent manner (an early and late response), which the late response occurs through peripheral chemoreceptor activation.

Hypoxic diuresis has been documented since 1944 (Menge, 1944) however the mechanisms remain unclear. Hypoxic diuresis still occurs after renal denervation and kidney transplantation suggesting hormones not direct neural activation generates the response (Behm *et al.,* 1989); however, hormones often inconsistently correlate with hypoxic diuresis (Loeppky *et al.,* 2005; Woods *et al.,* 2011 & Bartsch *et al.,* 1991). This may relate to discrepancies between the degree and duration of altitude exposure (Swenson *et al.,* 1995; Bestle *et al* 2002; Haditsch *et al.,* 2007 & Robach *et al.,* 2000). Further complicating this mechanism is renal function may change with hypoxic duration (i.e. acclimatization) (Bestle *et al.*, 2002); thus, our aim is to quantify renal hemodynamics, blood gas metrics, renal function, volume hormones (NT pro-brain natriuretic peptide, active renin and plasma aldosterone concertation) and diuresis to better understand early (ATL1) and late (ATL7) renal acclimatization.

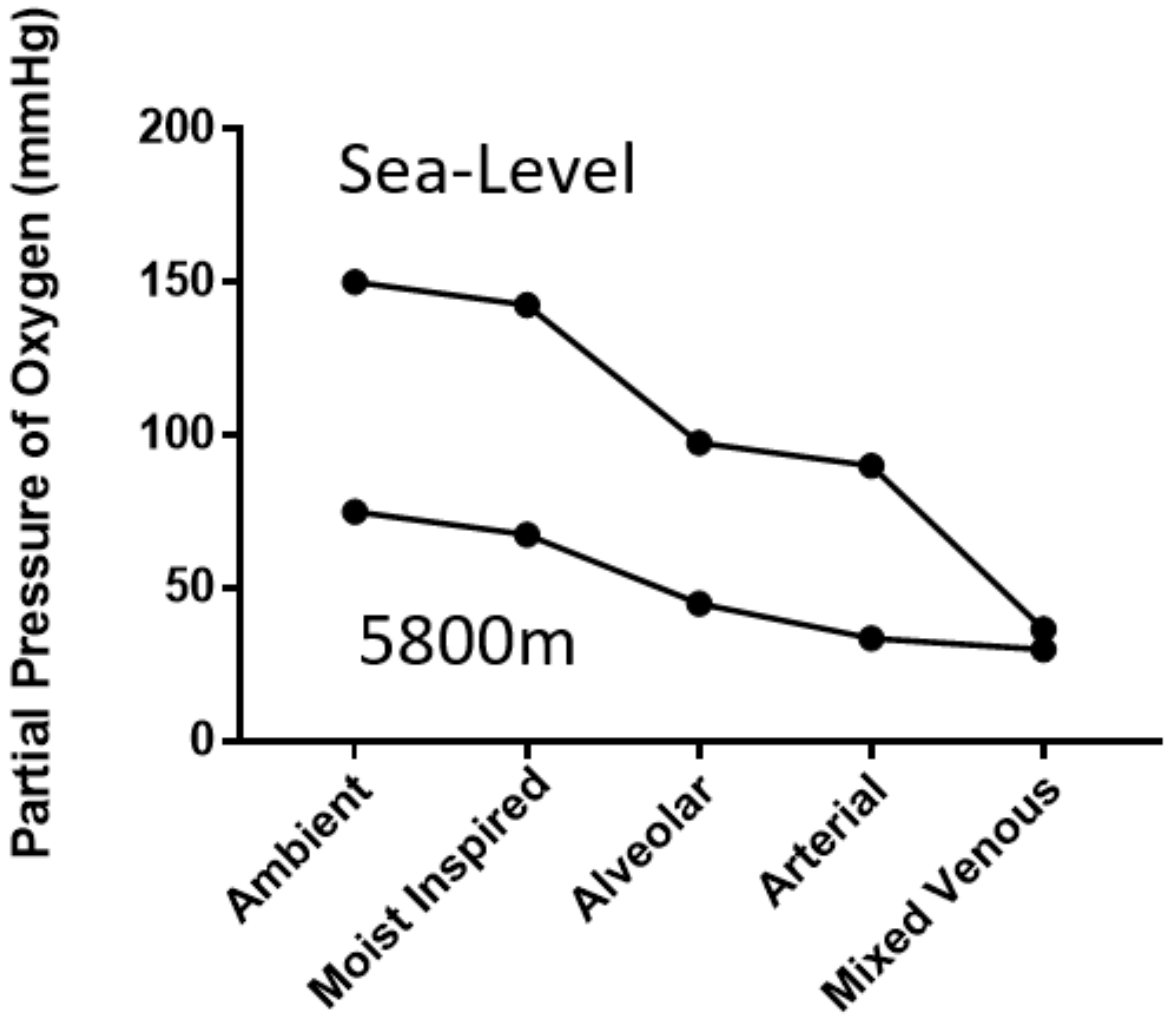

Figure 1. Changes in partial pressure at sea level and high-altitude (5800 m). Partial pressure is the driving force bringing oxygen from the atmosphere to the mitochondria. High-altitude causes every point in the cascade to be shifted downwards perturbing oxygen delivery. Data taken from Peacock (1998).

1.1 Purpose

This study aims to use a broad approach to accurately quantify multiple aspects of renal adaptation during early (<12hrs) and prolonged (~7days) acclimatization. Our methods quantify volume regulatory hormones, renal perfusion, glomerular filtration rate and arterial pH homeostasis with the same individuals to comprehensively understand the kidney at high-altitude.

1.2 Hypothesis

I hypothesize volume regulatory hormones will decrease to facilitate diuresis causing polyuria, renal blood flow and glomerular filtration rate will decrease to divert blood flow to critical tissues and HCO_3^- will decrease throughout high-altitude occupancy to normalize the acid-base status.

1.3 Significance

200 million people worldwide live at high-altitude, and 100 million travel to high-altitude annually for tourism and religious pilgrimages. Acute mountain sickness and the related life threating diseases: high-altitude pulmonary edema and high-altitude cerebral edema are becoming more prevalent. Consistent among these illnesses is fluid retention suggesting renal regulation is important at high-altitude thus by understanding volume regulation during high-altitude, may provide avenues for pharmaceutical interventions and prevention.

High-altitude exposure decreases glomerular filtration rate (Bestle *et al.,* 2002) and renal blood flow (Anand *et al.,* 1993 & Singh *et al.,* 2003) this may worsen pre-existing kidney dysfunctions. By understanding kidney acclimatization in healthy participants, we can inform future recommendations for those with chronic kidney dysfunction wanting to embark on high-altitude travel.

Chapter 2 Literature review

2.1 The Kidney during Normoxia

The kidneys are paired retroperitoneal organs, which are essential for filtering metabolic byproducts and toxins, regulating fluid status, electrolytes, acid-base balance, hormone production, and controlling long-term blood pressure. The kidney has two basic layers, an outer cortex (granular outer region) and the medulla (darker inner region) (Maxwell *et al.,* 1993 & Tammi *et al.,* 1952). Encased in the cortex is the glomerulus, which is constructed of microscopic tufts of capillaries and highly convoluted epithelial structures that form tubules. The medulla has numerous parallel arrangements of tubules and small blood vessels which feed the cortex. Each kidney is only 0.25% of total body mass but receives 10-12.5% of total cardiac output (accounting for 20-25% for total cardiac output collectively). This high blood flow is necessary to rapidly excrete toxic materials and biproducts from the circulation (Walker *et al.,* 1942 & Chertow *et al.,* 2015) (**Figure 2**).

Each kidney is comprised of functional units called nephrons, where fluid filtration occurs. Each nephron consists of a glomerulus and a tubule. The glomerulus is a cluster of blood vessels, where plasma filtrate originates. The tubule is an epithelial structure consisting of specialized sections designed to convert filtrate to urine. Plasma filtrate is forced through the glomerulus then collected in the Bowman's capsule (Walker *et al.,* 1942) where it then travels to the proximal convoluted tubule, followed by the loop of Henle, then the distal convoluted tubule and finally the collecting ducts (**Figure 2**). The loop of Henle recovers water and NaCl; however, most filtrate is reabsorbed at the proximal convoluted tubule, including NaCl, $NAHCO_3$, glucose, amino acids and water. The distal convoluted tubule and collecting ducts fine tune sodium and water excretion. These tubules are also where several hormones (e.g. aldosterone, antidiuretic hormone, atrial natriuretic peptide) exert their effects on electrolyte and water excretion (Shannon, 1935).

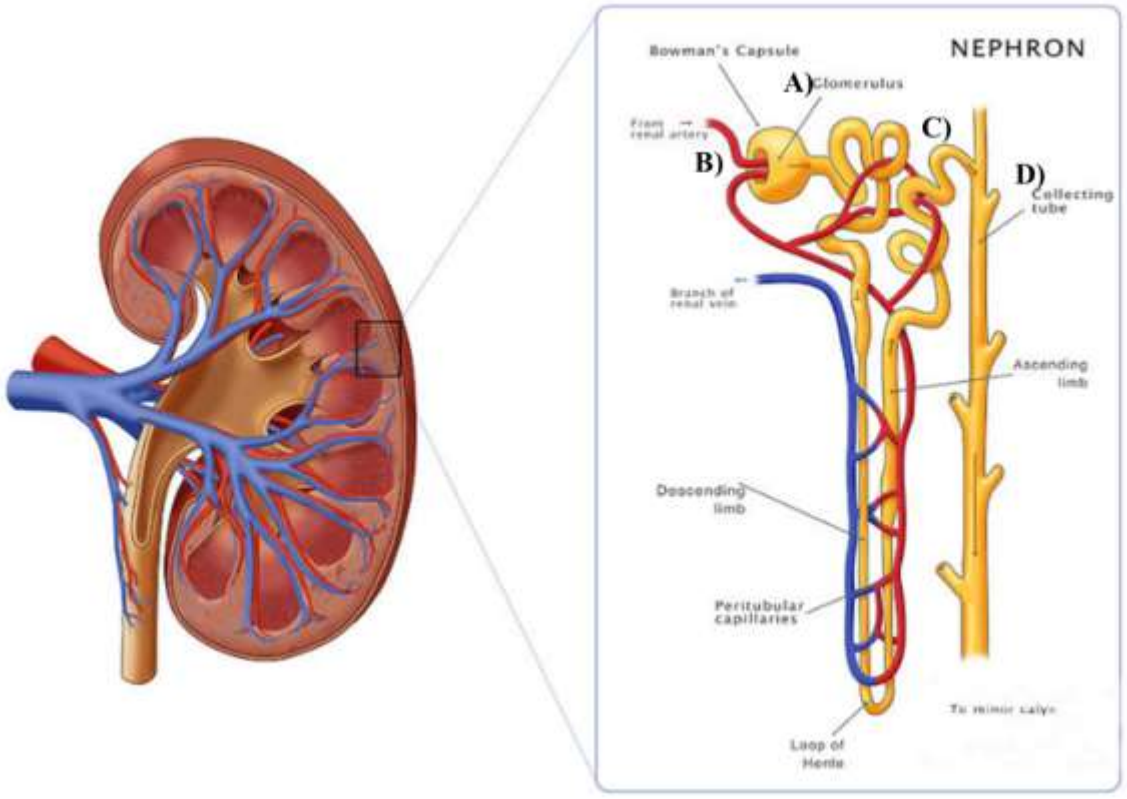

Figure 2. Morphology of the kidney including the nephron, which is the functional unit of the kidney composed of the renal corpuscle and a renal tubule used to reabsorb water, ions and small molecules. Functionally significant structures: **A)** glomerulus is where the ultrafiltrate originates **B)** renal arteries create the hydrostatic gradient to generate the ultrafiltrate **C)** & **D)** distal tubule & collecting duct where hormones change nephron permeability (Chertow *et al.,* 2015) (Brenner & Rector's The Kidney).

2.2 An Introduction into Hypoxic Diuresis

Mammals have two methods to increase hematocrit concentration during hypoxia. Hypoxia promotes the production of erythropoietin by proximal tubular fibroblasts, which stimulates erythrocyte proliferation. Erythropoietin production peaks within the first forty-eight hours and erythrocyte concentrations are significantly increased by week three of altitude exposure (Adamson, 1991). Hypoxic diuresis and natriuresis causes hematocrit concentrations to immediately increase by lowering the plasma and extracellular volume and functionally increasing the carrying capacity of a given unit of blood (Hayes *et al.,* 1982). Hypoxic diuresis is characterized by increased sodium, potassium and bicarbonate excretion and an accompanying increase in urinary pH. The increase in urinary excretion occurring in parallel with higher electrolyte excretion, causing urinary osmolarity to be unaltered and consequently serum osmolarity is also unchanged (Krafp *et al.,* 1991). The net result is hypovolemia rather than hypertonicity (Zouboules *et al.,* 2019). Substantial contractions to the total body water occur as parallel decreases in water and osmolarity. To this effect, hypobaric hypoxia decreases total body water by approximately 2-3 liters occurring equally between plasma, extracellular and intracellular spaces. In addition to renal hypoxic diuresis, decreased volume is also affected by decreased fluid intake and increased respiration (evaporative loss) (Shing *et al.,* 2003; Claybaugh *et al.,* 1989 & Westerterp *et al.,* 1996). Hypoxic diuresis is triggered during exposure to 16-10% inspired oxygen or equivalent hypobaric levels (3000 – 5000 m) (Jain *et al.,* 1980). Further hypoxia below 10% causes anti-diuresis and anti-natriuresis. Hypoxic diuresis is initiated quickly (within ninety minutes) and stabilizes by the twentieth day of high-altitude exposure (Shebnmann *et al.,* 2017) (**Figure 3.**) Diuresis occurs in two distinct phases: an initial diuretic response to local renal hypoxia (~90 minutes) and a more prolonged hormonal response triggered by neural influences from the peripheral chemoreceptors (~6 hours).

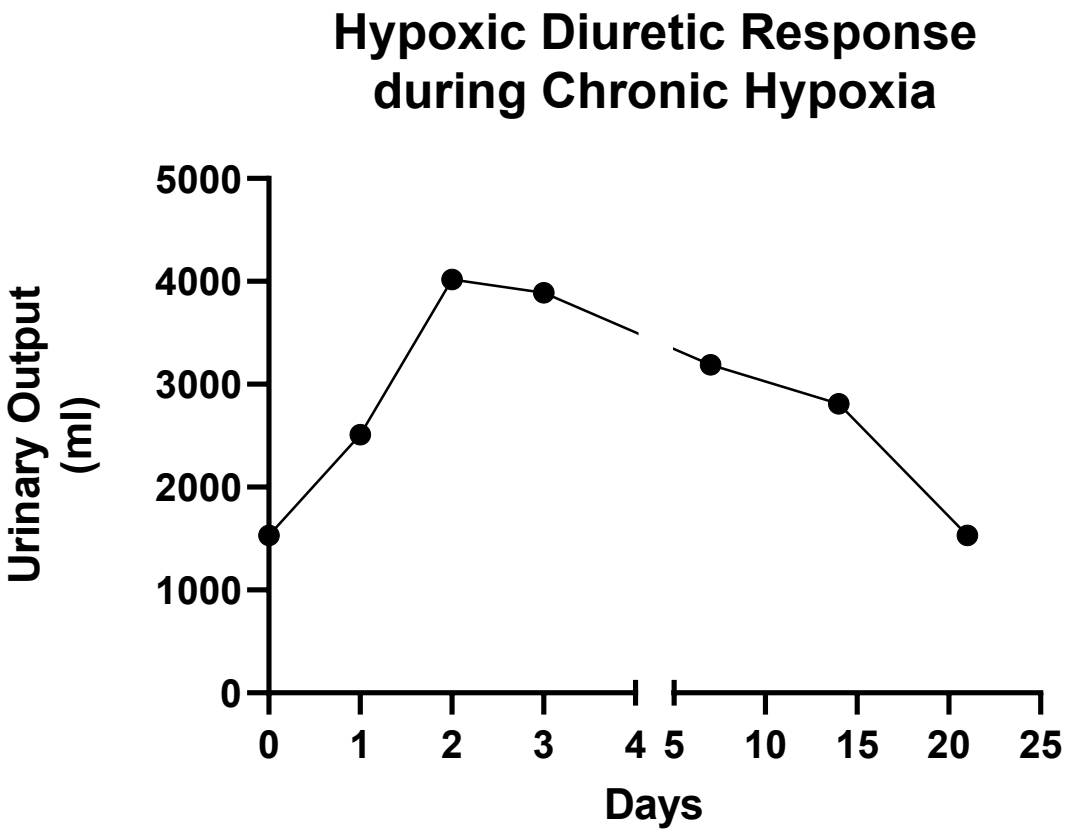

Figure 3. Data taken from Shing *et al.,* 2003; Shebnmann *et al.,* 2017; Hadistsch *et al.,* 2015 & Zaccaria *et al.* 1991 and averaged to produce this graph (3500 to 5050 m). High-altitude causes urine production to peak early (~day two) and then gradually declines to sea-level values (~day twenty-one).

2.3 Acute and Prolonged Diuretic and Natriuretic Responses

Hildebrandt *et al.,* (2000) and Hayes *et al.,* (1982) found human participants under normobaric hypoxia (fractional content 13% O_2) for ninety-minutes increased urine production. At this time-point, there was no relationship between the concurrent hypoxic ventilatory response and diuresis, suggesting it is independent from the peripheral chemoreflex arc (Hildebrandt *et al.,* 2000). Local mechanisms such as reduced ability to reabsorb chloride by the medullary thick limbs, acutely decreased antidiuretic hormone and decreased sensitivity of antidiuretic hormone at the collecting ducts are the primary mechanisms hypothesized to contribute during early diuresis (Goldfarb-Rumyantzev *et al.*, 2014).

Following the initial diuretic response, there is a hormonal response mediated through the peripheral chemoreceptor creating a prolonged diuretic and natriuretic response. When the peripheral chemoreceptor is stimulated with hypoxemia or almitrine bismesylate (peripheral chemoreceptor agonist) natriuresis and diuresis increases (Schmidit *et al.,* 1985, Smith *et al.,* 1987; & Honig, 1989). Cats with isolated carotid bodies that are perfused with hypoxic blood demonstrate an increase in sodium excretion. These results are abolished when acetic acid was used to inactivate the peripheral chemoreceptor (Schmidit *et al.,* 1985). Furthermore, rats with denervated glossopharyngeal nerves have no changes in urinary excretion when made hypoxic (Behm *et al.,* 1989).

Human research has shown similar findings. Oral ingestion of almitrine bismesylate causes renal artery vasodilation and increased sodium excretion in normoxic conditions (Ledderhos *et al.,* 1987). Hypoxic ventilatory responsiveness, a measure of peripheral chemoreceptor sensitivity, has been demonstrated to positively correlate to urinary excretion (r=0.87), and sodium excretion (r=0.76) after 6 hours of normobaric (14%) hypoxia (**Figure 4**). Across human and animal studies there is a consistent and strong implication for the involvement of the peripheral chemoreceptors in the prolonged hypoxic diuretic response (Swenson *et al.,* 1995). However, mechanisms unrelated to peripheral chemoreceptor activation contribute to hypovolemia such as bicarbonate excretion. Hypoxic diuresis is a coordinated

response dependent on multiple systems, which is too broad to only conceptualize as a peripheral chemoreceptor mediated response.

2.4 Hypoxia and Renin-Angiotensin-Aldosterone System (RAAS)

Hypoxic animals with dissected renal nerves and rats with transplanted kidneys given almitrine bismesylate have a preserved hypoxic diuretic response (Karim *et al.,* 1984 & Honig *et al., 2015*). In fact, natriuresis is augmented by 30-50% in these animals suggesting volume regulatory hormones and not direct neural activation cause hypoxic diuresis. However, hypoxic diuresis is also not prevented by adrenalectomy in rats implicating redundant pathways and multiple regulatory hormones outside the adrenal cortex contribute. (Bardsley & Suggett, 1987).

The renin-angiotensin-aldosterone-system (RAAS) is a multi-facet system regulating electrolyte metabolism, peripheral and pulmonary vascular resistance, and plasma volume (**Figure 5**) (Adamson, 1991 & Behm et al., 1989). Renin is secreted by juxtaglomerular cells in response to increased renal sympathetic nerve activity, an increase in circulating catecholamines, a decrease in sodium delivery to the macula densa cells, or a decrease in renal perfusion pressure (Persson, 2003). Renin is, in turn, inhibited by natriuretic peptides (i.e. atrial natriuretic peptide, brain natriuretic peptide, and urodilatin) and angiotensin II by negative feedback.

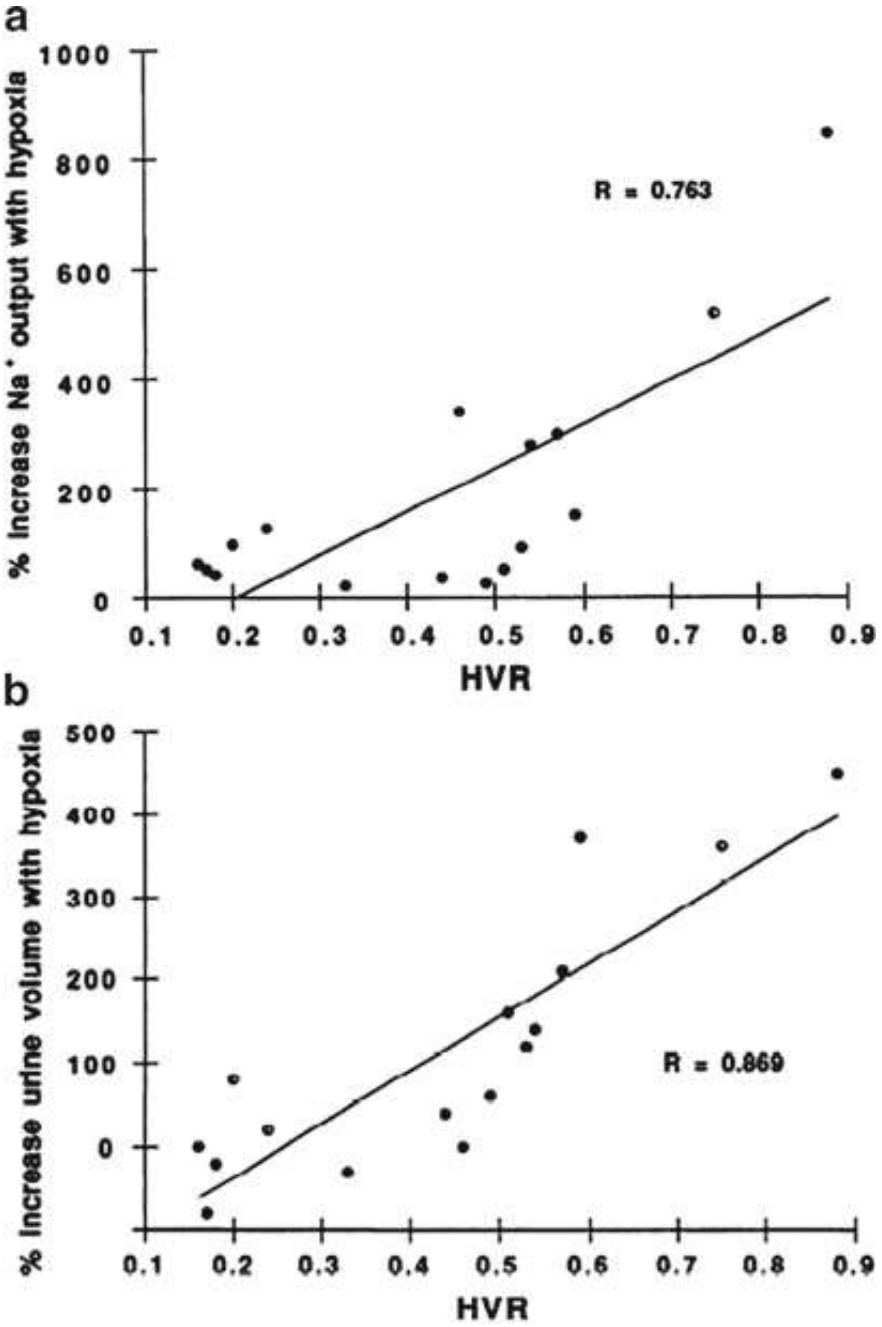

Figure 4. Correlations between isocapnic hypoxic ventilation response (HVR; L/min/% desaturation) and increases in urinary sodium excretion (a) and urinary volume (b) (both % change from normoxia) following 6 hours of hypoxia (Fraction of inspired oxygen = 14%). Peripheral chemoreceptor activation causes hypoxic diuresis after six hours of normoxic hypoxia. Taken from Swenson *et al.,* (1995).

Renin converts angiotensinogen (primarily produced by the liver) to angiotensin I, which is subsequently converted into angiotensin II by the angiotensin converting enzyme in pulmonary capillaries. Angiotensin II is a powerful vasoconstrictor and stimulates aldosterone release (Pearson, 2003). Aldosterone is a mineralocorticoid that is produced and released from zona glomerulosa cells in the outer layer of the adrenal cortex, which acts to increase potassium excretion and sodium reabsorption in the distal tubules. Aldosterone release can be influenced by multiple other pathways (Bollag, 2014); such as adrenocorticotropic hormone (ACTH), and plasma potassium, and inhibition by natriuretic peptides and increased plasma sodium concentrations (Bollag, 2014).

Hypoxia has been demonstrated to increase, (Okanski *et al*., 1984) unalter, (Souich *et al*., 1987), and decrease (Maher *et al*., 1975) plasma renin activity. These inconsistencies may relate to the differences in participants physical activity level (which stimulates renin release), degree of hypoxia/altitude exposure, or the length of altitude exposure. However, the influence of duration of hypoxia has been poorly characterized (Swenson & Bärtsch, 2013).

There are multiple potential mechanisms influencing renin concentration during hypoxia. Mildedge *et al.,* (1987) suggested angiotensin converting enzyme activity might be increased during hypoxia (causing decreased renin secretion); however, no study has found an increase in angiotensin II or angiotensin converting enzyme during hypoxia (Millar *et al.,* 1995). Almitrine bismesylate injection in cats does not change renin activity suggesting that peripheral chemoreceptor activation does not directly influence renin secretion (Honig *et al.,* 2015). However, higher cardiac output may stimulate low pressure cardiovascular receptors and the release of ANP and BNP which could decrease renin release independent of the peripheral chemoreceptor (Honig, 1989). This relationship has not been examined during acute or prolonged hypoxia.

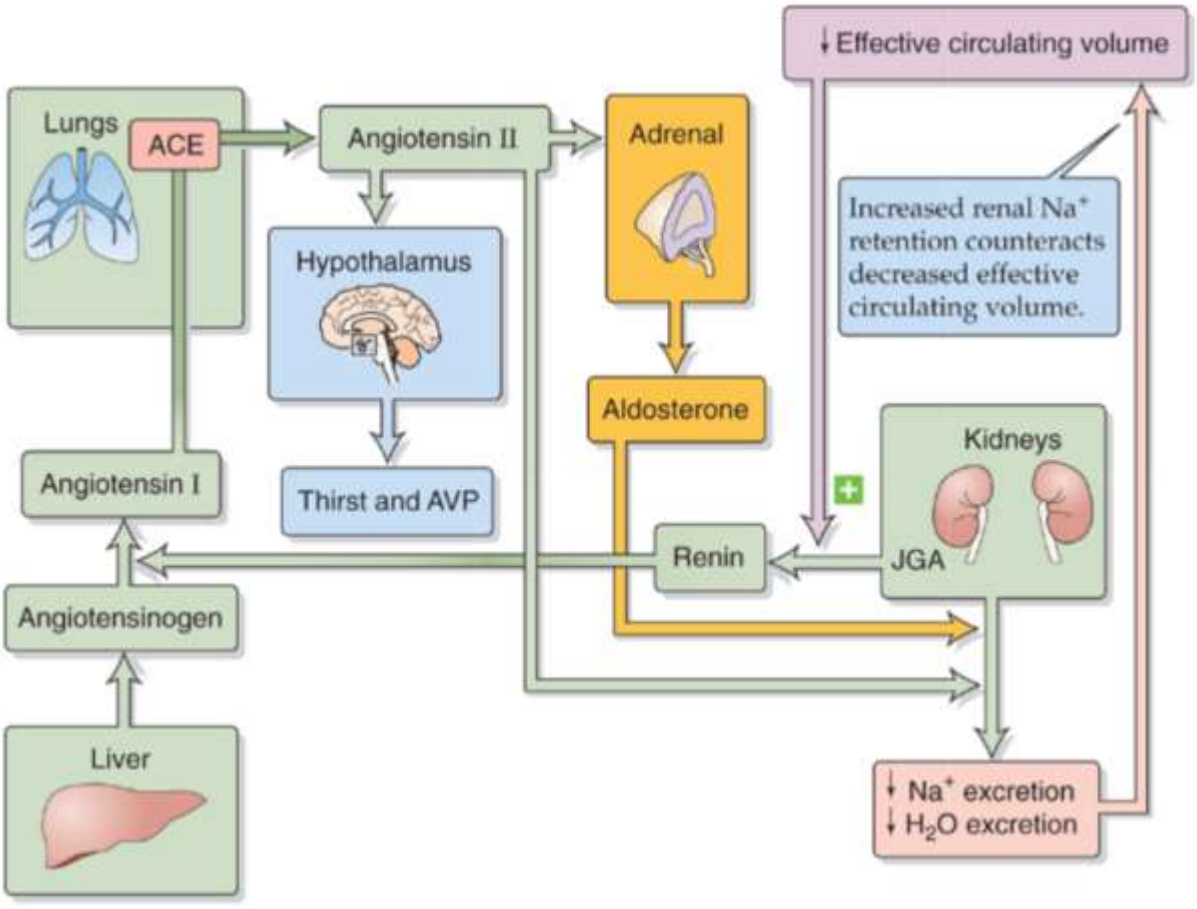

Figure 5. Renin-angiotensin-aldosterone-system (RAAS) (Taken from Brenner & Rector's The kidney, 2019). Effective circulating volume is regulated by RAAS and ADH. Renin release is stimulated by low blood pressure detected by the juxtaglomerular cells or renal sympathetic nerve activity, which triggers a signal cascade creating two functionally meaningful molecules: angiotensin II & aldosterone. Angiotensin II causes vasoconstriction, ADH release and aldosterone release. Aldosterone regulates water balance by sodium reabsorption at the distal tubule. RAAS controls blood pressure by altering volume and changing venous tone.

Unlike renin, aldosterone is consistently decreased during hypoxia in humans, which is unanimous across altitudes and hypoxic durations (Colice *et al.* 1985; Mildegde *et al.* 1987; Hadistsch *et al.,* 2015 & Zaccaria *et al.* 1991). Honig *et al.,* (2015) injected cats with almitrine bismesylate and found plasma aldosterone concentrations significantly decreased within five hours this difference was removed when the peripheral chemoreceptors were denervated. Suggesting peripheral chemoreceptor activation decreases aldosterone release. However, in vivo adrenocortical cells have been found to be directly inhibited by hypoxia. Isolated bovine adrenocortical cells secrete less aldosterone during hypoxia compared to normoxia, while cortical release is unaltered (Raff *et al.,* 1989). Thus, this may explain why the renin-aldosterone-axis is altered during hypoxia causing a decrease in the slope affecting aldosterone secretion and changing the ratio between renin and aldosterone affecting downstream natriuresis by less sodium reabsorption across the distal tubule. (Milledge *et al.,* 1987). (**Figure 6**).

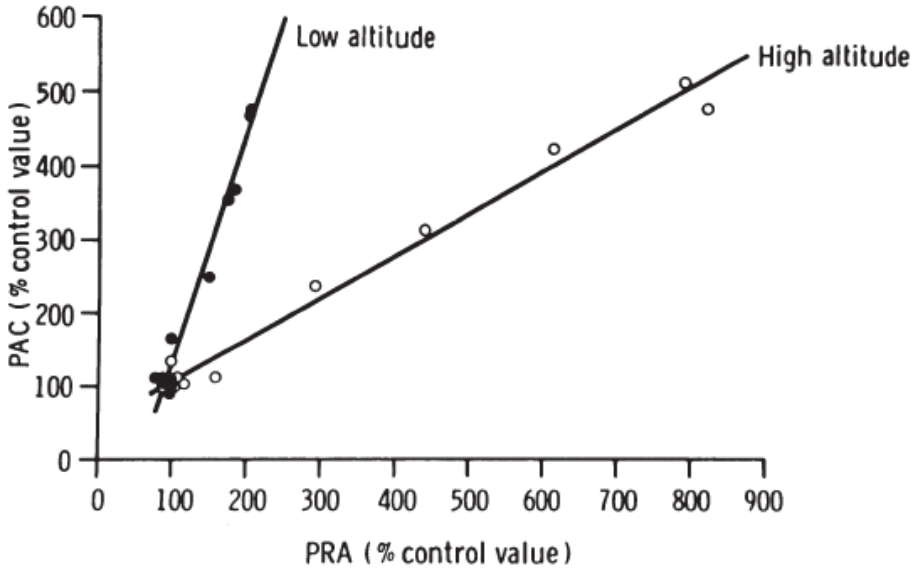

Figure 6. Plasma aldosterone concentrations in response to increased plasma renin activity during exercise. High-altitude significantly decreases the slope of aldosterone versus renin. Aldosterone release by renin is blunted during high-altitude compared to low-altitude. Taken from Milledge *et al.,* (1987).

2.5 Hypoxia and Natriuretic Peptides

Natriuretic peptides are a family of three homologous proteins: atrial (A-type) natriuretic peptide (ANP), brain (B-type) natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). ANP and BNP are important for volume regulation. ANP and BNP bind to natriuretic guanylyl cylases receptors on the kidney to influence vasodilation (and renal plasma flow), diuresis, natriuresis. ANP is synthesized and secreted by cardiac atrial muscle cells in response to stretch. ANP dilates the afferent arteriole and constricts the glomerular efferent arteriole to increase glomerular filtration rate. It reduces sodium reabsorption via effects on vasta recta and Na^+/K^+ ATPase pump activity, reduces renin secretion from juxtaglomerular kidney cells and decreases antidiuretic hormone release (Kastin, 2013). ANP also reduces both voluntary consumption of salt and water (Breuneau *et al.,* 2011). BNP is secreted by the ventricles in response to stretch and has similar actions as ANP but with a lower receptor affinity and longer half-life (Kastin, 2013).

ANP and BNP have been observed to be higher during hypoxia in the absence of measurable atrial or ventricle stretch, indicating an additional release mechanism. Chen *et al.,* (1997) showed that hypoxia stimulated ANP gene expression in cultured atrial cardiocytes and similar findings were obtained for BNP more recently (Luo *et al.,* 2006). Baertschi *et al.,* (1988) used isolated perfused rabbit hearts to demonstrate hypoxia causes a 4.6-fold increase in ANP concentrations. Toth *et al.,* (1994) demonstrated perfused hypoxic rat ventricles had a significant increase in ANP and BNP. Hypoxia also reduces ANP clearance (Sun *et al.,* 2004) and increases ANP granules (Mckenzie *et al.,* 1986) in atrial mice cells. These results demonstrate hypoxia is a direct and adequate stimulus for ANP and BNP release independent of cardiac stretch.

The degree of hypoxia and duration related to natriuretic peptide release are unclear (Arjamaa *et al.,* 2009; Swenson *et al.,* 1995). Koller *et al.,* (1989) stimulated the chemoreceptor by almitrine bismesylate in healthy young men and found no significant increase in ANP. This finding would suggest

that peripheral chemoreceptor activation on its own is not sufficient to elicit a natriuretic peptide release. Woods *et al* (2011) also demonstrated in seven healthy participants that 40 mins of 62% arterial saturation did not significantly increase BNP concentrations. However, Woods *et al*., 2012 & Fedderson *et al.,* 2009 demonstrated a three-fold increase in BNP, with participants above 5150 m (**Figure 7**). Therefore, natriuretic peptides may only change during extreme high-altitude exposure. It is also worth considering that increased BNP could also reflect an inadequate response indicating fluid retention rather than specific mechanisms for hypoxic diuresis. Fluid retention would increase cardiac load and cause BNP secretion. Woods *et al.,* (2012) failed to find a correlation between BNP and urinary sodium, urine volume, or arterial saturation across altitude supporting this interpretation.

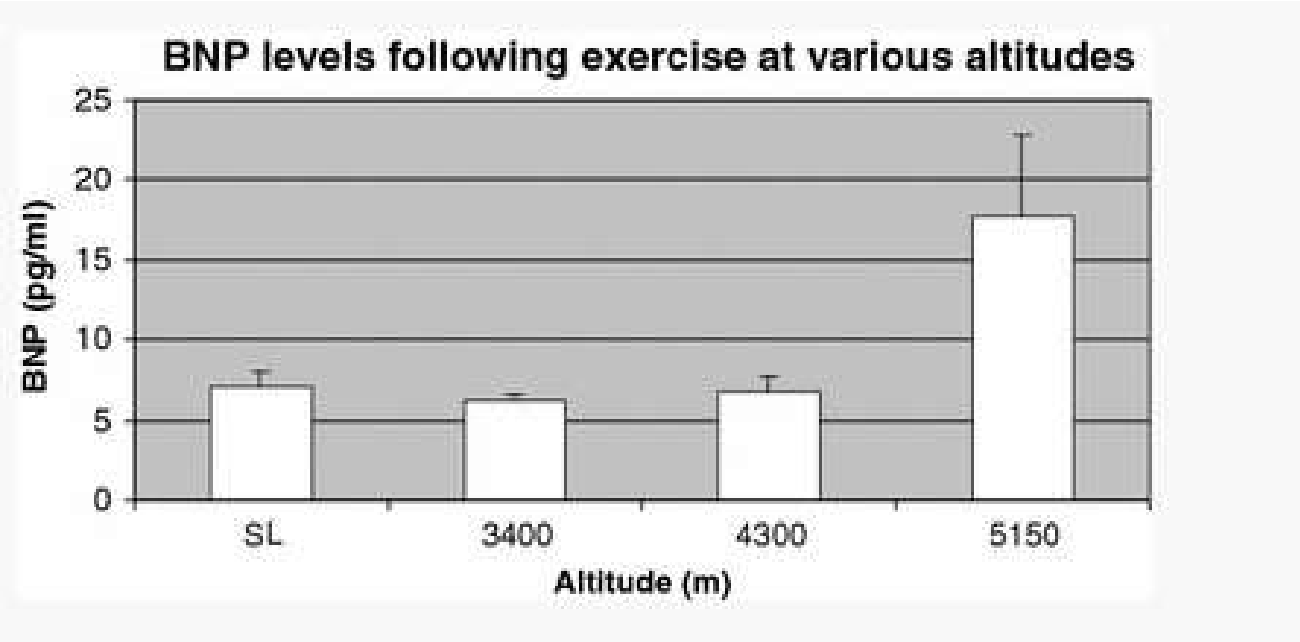

Figure 7. BNP at each of the measured altitudes (mean ± SEM are shown). BNP was significantly higher at 5150 m versus lower altitudes ($p<0.0001$ for all) Exercise was standardized across altitudes. BNP concentrations are only significant during 5150 m suggesting only extreme high-altitudes are an enough stimulus to cause BNP release. Fluid retention at 5150 m potentially could cause increased cardiac stretch leading to increased BNP release. Taken from Woods *et al.,* (2012).

2.6 Hypoxia and Central Venous Pressure

Early hypoxia (hours) significantly increases cardiac output through an increase in heart rate with unchanged or slightly increased stroke volume. However, after three days of acclimatization, cardiac output rebounds to sea-level values although heart rate is elevated and stroke volume is lower. Hypoxia increases pulmonary pressure and venous tone (Naeije *et al*., 2010). Venous centralization may stimulate low-pressure cardiovascular baroreceptors, which would increase natriuresis and dampen renal sympathetic nerve activity (Nahmod *et al.,* 1964 & Rutherford *et al.,* 1978). However, previous data have failed to consistently show a correlation between central venous pressure and diuresis during hypoxia (Koller *et al.,* 1991; Koller *et al* 1991; & Rutherford *et al*., 1978). Discrepancies between studies may reflect differences in methodology and difficulty directly measuring central venous pressure. Nonetheless, animals under artificial ventilation with cervical vagotomy still have a hypoxic diuretic response suggesting that low-pressure cardiovascular baroreceptors are not necessary but may augment the response.

2.7 Hypoxia and Hyperventilation

High-altitude decreases arterial oxygen partial pressure and ventilation increases to compensate (Bernardi *et al.,* 2006). Hypoxic ventilation is determined by the hypoxic stimulus, timing (seconds to years) and the effects of this stimulus on the physiological components of the hypoxic ventilation response (i.e. pulmonary changes in frequency and volume) (Pamenter & Powell, 2016). Hypoxic ventilation response occurs in distinct time-dependent phases increasing over the initial 10-14 days due to increased sensitivity of the peripheral chemoreceptors (Fatemian *et al.,* 2015) (Bernardi *et al.,* 2006 & Dempsey *et al.,* 2014) (**Figure 8**).

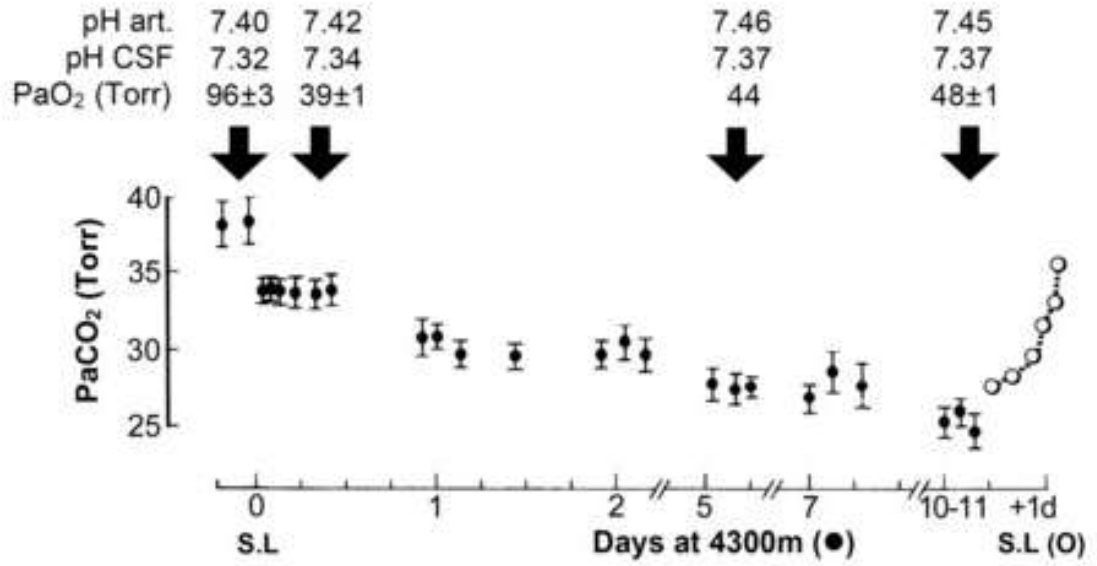

Figure 8. Time course of ventilatory acclimatization to hypoxia (over 11 days) in resting sea levels (S.L.) natives sojourning to 4,300 m. Changes in arterial PCO_2 reflect the degree of hyperventilation according to the alveolar gas equation: $PCO_2 = 863/[(\dot{V}e/\dot{V}co2) \times (1 - Vd/Vt)]$, where PCO_2 is alveolar Pco_2, $\dot{V}e$ is minute ventilation, $\dot{V}co2$ is CO2 production, Vd is dead space volume, and Vt is tidal volume (Dempsey *et al.,* 2014). Hypoxic hyperventilation increases during continuous high-altitude, caused by increased peripheral chemoreceptor activation, causing greater hypocapnia further disrupting the acid-base relationship.

Hypoxic hyperventilation causes an respiratory alkalosis until plasma bicarbonate excretion acts to normalize pH as per the Henderson-Hasselbalch equation ($CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+$) (Dempsey *et al.,* 2014). pH normalization occurs by decreasing the reabsorption of bicarbonate and decreasing secretion of hydrogen ions.

$$pH = 6.1 + (\log_{10} \frac{[HCO3]}{0.03x\ pCO2})$$

Gledhill *et al.,* (1975) demonstrated a significant increase in urinary bicarbonate excretion (by 10mEq) and significantly decreased urinary excretion of total acid (by 17.5mEq) during hypobaric hypoxia (530 mmHg) for 26 hours. Plasma bicarbonate dropped by 2.5mEq/liters and almost normalizes pH (**Figure 9**). Proximal tubular reabsorption of bicarbonate across the nephron is decreased during hypoxia causing more excretion of sodium, water and potassium further augmenting hypoxic diuresis.

Stretch receptors on venous, atrial, pulmonary vasculature and lung parenchyma can directly cause natriuresis. (Morrison *et al,* 2001; & Baekey *et al.,* 2010). Currie *et al.,* 1961 found in sixteen healthy male participants hyperventilation (>30 L/min) with normoxic isocapnia significantly increased diuresis and natriuresis. However, poikilopcapnic hypoxic ventilation (uncontrolled carbon dioxide i.e. high-altitude) is rarely above 10-15 L/min (Valli *et al.,* 2008 & Steinback & Poulin, 2008) and hypoxic diuresis occurs in animals with fixed ventilation (Honig, 1989). Hypoxic hyperventilation and diuresis have been shown to correlate (Valli *et al.,* 2008 & Swenson *et al.,* 1995), although this correlation may also reflect a common activation point (i.e. peripheral chemoreceptors).

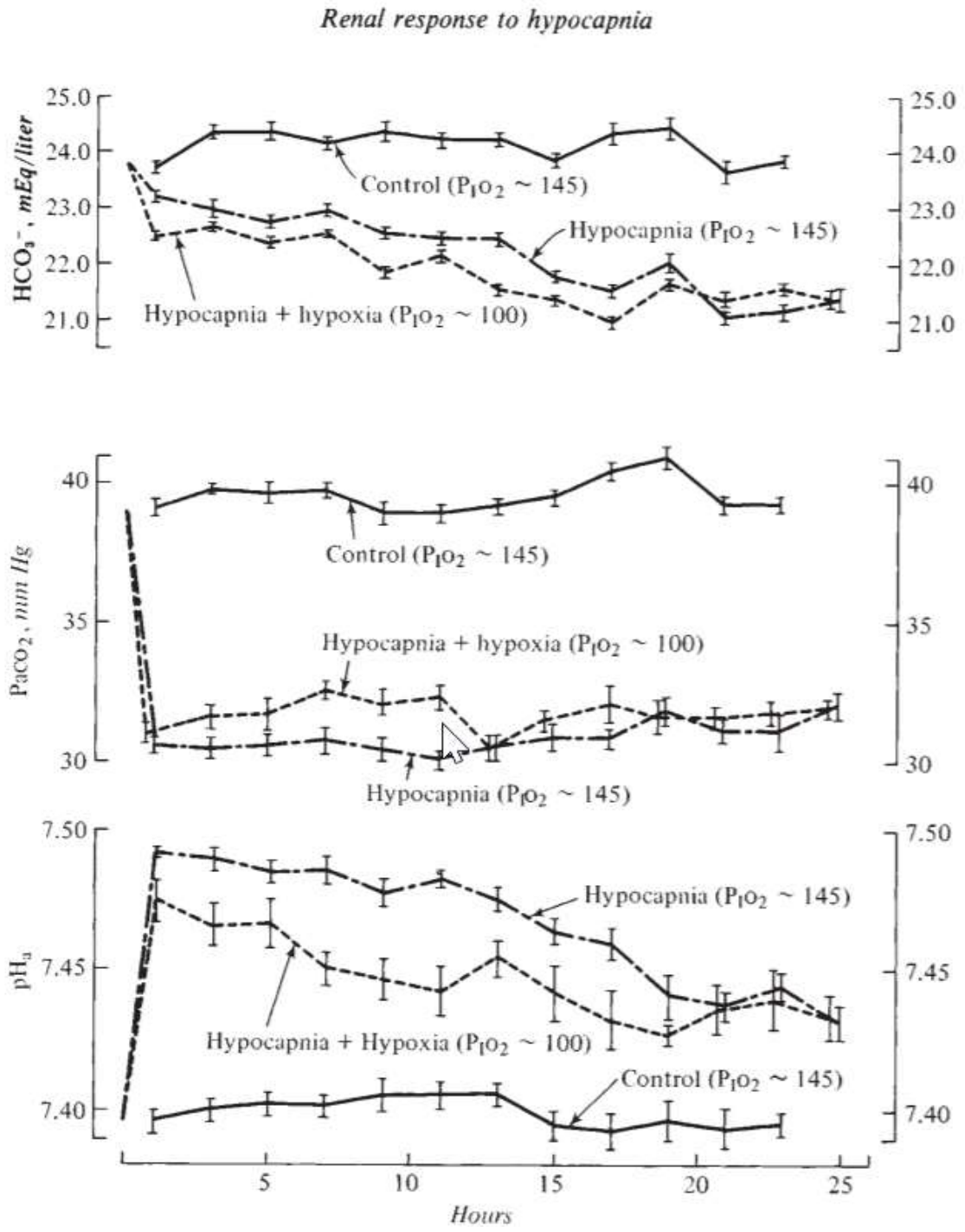

Figure 9. Changes in arterial pH, PCO_2, and HCO_3^- in control, hypercapnia, and hypobaric hypoxia conditions during a 26-hour protocol. Taken from Gledhill *et al.,* (1975). Participants began to normalize the acid-base status within hours during hypobaric hypoxia.

2.8 Hypoxia and Renal Hemodynamics

Renal blood flow is the volume of blood delivered to the kidney per time unit. During rest, the kidney receives 20% of cardiac output (1 L/min). All renal blood flow is delivered to the renal cortex with 10% of the cortical blood flow then redirected to the renal medulla (Carlstrom *et al.,* 2015). Glomerular filtration rate describes the flow rate of filtrate (mL/min) across the kidneys. Glomerular filtrate production, ultrafiltrate, has a similar composition as plasma, however, proteins and other high-molecular weight compounds (> 50 to 60 nm) are present in reduced amounts (Carlstrom *et al.,* 2015). Ultrafiltrate is produced in high amounts due to high Starling forces (hydrostatic forces & oncotic pressure) and high permeability. Under normal conditions, glomerular filtration rate is 125 mL/min/1.73 m^2 or 180 L/day/1.73 m^2. The kidneys clean the plasma ten times a day (Brenner *et al.,* 1972). **Figure 10** demonstrates the hyperbolic relationship between renal plasma flow and glomerulus filtration rate this relationship maintains glomerular filtration rate even when renal plasma flow is reduced (Tucker *et al.,* 1977). Filtration fraction is the proportion of renal plasma flow filtered across the glomerulus. Under normal conditions, filtration fraction is about 20%, which indicates the remaining 80% continues unimpeded by the glomerulus to the renal vein (Carlstrom *et al.,* 2015).

Autoregulation maintains renal plasma blood flow and glomerulus filtration rate within narrow limits under variable mean arterial pressures (80 and 170 mmHg; **Figure 11**). Renal blood flow autoregulation (**Figure 11**) is largely controlled through afferent arteriole resistance (Carlstrom *et al.,* 2015). Efferent arteriole resistance, capillary resistance and venous resistance all change very little during changes in renal arterial pressures (**Figure 11**). Volume regulatory factors and sympathetic outflow (e.g. Renin-angiotensin-aldosterone, Sympathetic nerve activity, Antidiuretic hormone, & Natriuretic factors) may all influence renal blood flow and glomerulus filtration rate; which, as discussed above change significantly during hypoxia (Brenner *et al.,* 1972).

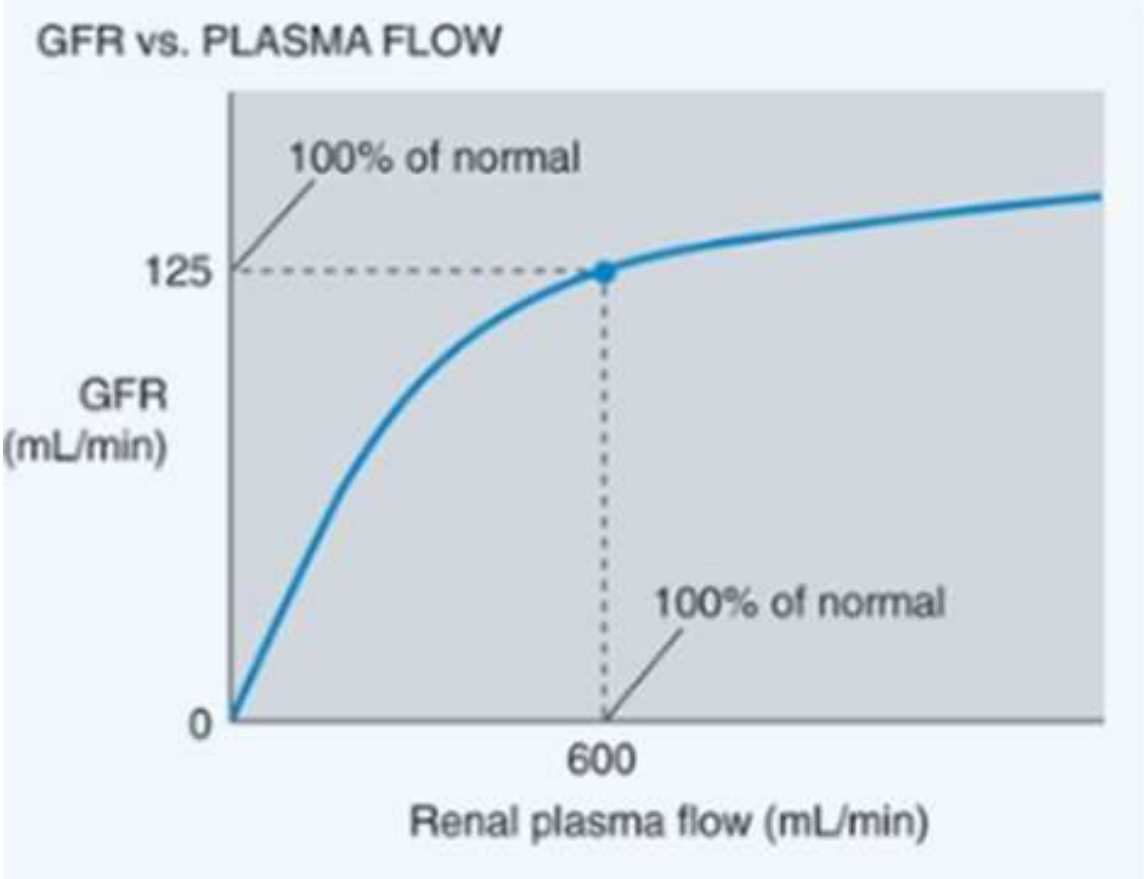

Figure 10. The relationship between renal plasma flow (mL/min) and glomerulus filtration rate (GFR). Glomerular filtration rates and renal plasma flow have a hyperbolic relationship to minimize changes in filtration when renal plasma flow is altered. Taken from Brenner & Rector's The kidney.

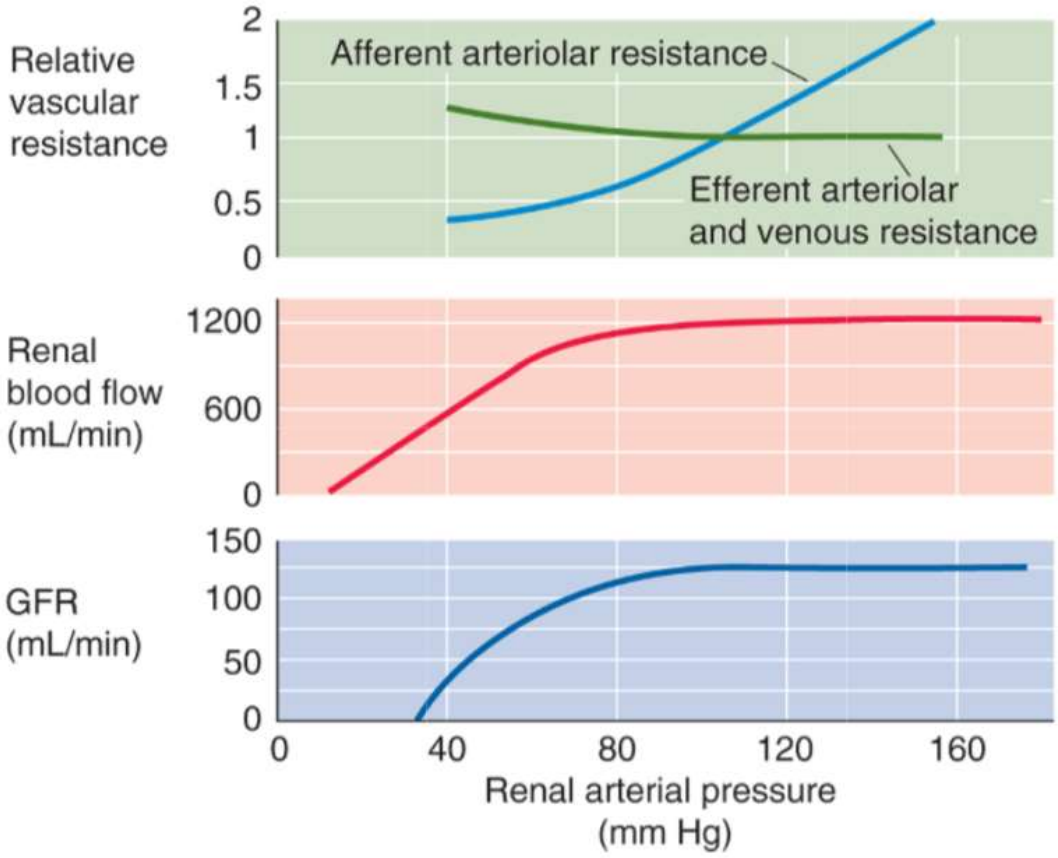

Figure 11. Changes in relative vascular resistance (afferent arteriole resistance, efferent arteriole and venous resistance), renal blood flow (mL/min) and glomerular filtration rate (GFR) (mL/min) as a function of renal arterial pressure (mmHg). Afferent arteriole resistance and not efferent arteriole or venous resistance maintains renal blood flow and glomerulus filtration rate. Taken from Brenner & Rector's The kidney

Short-term exposure to hypoxia has demonstrated inconsistent glomerular filtration rates and renal blood flows. Similar to other aspects of renal acclimatization differences may occur during prolonged duration. Olsen *et al.,* (1985) and Swenson *et al.,* (1995) found acute hypoxia (~48 hours) does not change glomerular filtration rates, which is supported by effective renal blood flow data (Singh *et al.,* 2003). Chronic high-altitude studies have found consistent significant reductions in glomerular filtration rates (Haditsch *et al* 2015; Bestle *et al.,* 2002 & Pichler *et al.,* 2008). One large study found participants trekking to 6865 m for fourteen days had a linear decrease of 3.1 mL/min/1.73 m^2 per 1000 m, which is supported by effective renal blood flow data. Singh *et al.,* (2003) found that participants exposed to high-altitude (3500 m) over sixty days demonstrate a linear decrease in effective renal plasma flow, which became significant after ten days and continued to decrease until day sixty (924.5 to 715.2 mL/min/1.73 m^2). Renal hemodynamics and filtration capacity are undoubtedly dependent on hypoxic stimulus and duration.

As noted, mechanisms governing renal function during high-altitude remain speculative. Swenson & Bärtsch (2013) postulated that renal blood flow is increased during hypoxia to facilitate urinary excretion by local release of adenosine, nitric oxide and prostaglandins with blunted renal sympathetic nerve outflow by hypocapnia (Fukuda *et al.,* 1989). Renal function, much like the hypoxic ventilation response, may augment during acclimatization through peripheral chemoreceptor sensitization (Bernardi *et al.,* 2006; Pamenter & Powell, 2016; Fatemian *et al.,* 2015 & Karim *et al.,* 1987). Renal function during high-altitude is undoubtably more complicated than a single monophasic response and likely changes to match metabolic and homeostatic needs. Future studies must understand the nuanced relationship between hypoxic stimulus and duration on renal function.

Nonetheless, reduced renal blood flow provides potential physiological advantages during hypoxia. More cardiac output could be diverted to other more essential tissues (e.g. brain and coronary tissues) and decreased renal blood flow would lessen renal oxygen consumption (90% of renal oxygen

consumption is through sodium reabsorption). Glomerular filtration rate may be maintained by higher filtration fraction (filtration fraction = glomerular filtration rate / Renal blood flow) (Goldfarb-Rumyantzev *et al.,* 2014). That is, glomerular filtration rate is largely maintained by extracting more plasma in the glomerulus. This data highlights the necessity to measure both renal blood flow and glomerulus filtration rate to conceptualize the relationship.

2.9 Hypoxia and Microalbumin

At altitude renal capillary permeability is increased and proteinuria is common during rapid ascent to 3000 m or greater (Winterborn *et al.,* 1987; Hansen *et al.,* 1994 & Rennie *et al.,* 1970). Hansen *et al.,* (1994) used a double-blind randomized design with dihydropyridine calcium antagonist israpidine (which normalized the hypoxic-induced changes in arterial pressure) to determine the mechanism(s) that cause proteinuria. Participants rapidly ascended to 4,300 m and were tested three days after acclimatization. ^{125}I-labeled albumin in the urine was significantly increased in both groups. Placebo participants had a (non-significantly) higher glomerular filtration rate compared to sea level and israpidine. β_2-microgloblin clearance, a measure of proximal tubular protein clearance, was non-significantly changed in both groups. Therefore, proteinuria appears to be caused by changes in renal capillary permeability and not changes in arterial pressure, or glomerular filtration rates.

2.10 Summary

Hypoxia stimulates multiple physiological responses related to volume regulation and pH homeostasis. Hypoxic diuresis occurs within ninety minutes and is sustained for roughly two weeks (Hildebrandt *et al.,* 2000; Hayes *et al.,* 1982 & Goldfarb-Rumyantzev et al., 2014) Thus, hypoxic diuresis represents an immediate and effective means to reduce plasma volume and functionally increase hematocrit concentration without altering erythropoietin mass. Hypoxic diuresis is mediated through multiple mechanisms including renal sympathetic innervation, antidiuretic hormone, renin, aldosterone secretion and increased natriuretic peptide release (Hong, 1989). Mechanisms independent of the hypoxia

per se including respiratory alkalosis, bicarbonate excretion and low-pressure cardiovascular baroreceptors may also augment hypoxic diuresis (Zouboules *et al.,* 2018; Morrison et al, 2001 & Koller et al., 1991). Renal blood delivery and filtration may change throughout hypoxia to match physiological need (Bestle et al., 2002). Hypoxia augments glomerular capillary permeability causing microalbuminuria during rapid ascent to altitude (Winterborn et al., 1987). Fundamentally, urine production is simply determined by renal plasma delivery, filtration across the kidney and nephron permeability. However, this becomes complicated because hypoxia appears to alter all of these. Hypoxic diuresis represents the new relationship between these factors, which are continuously changing throughout the hypoxic duration.

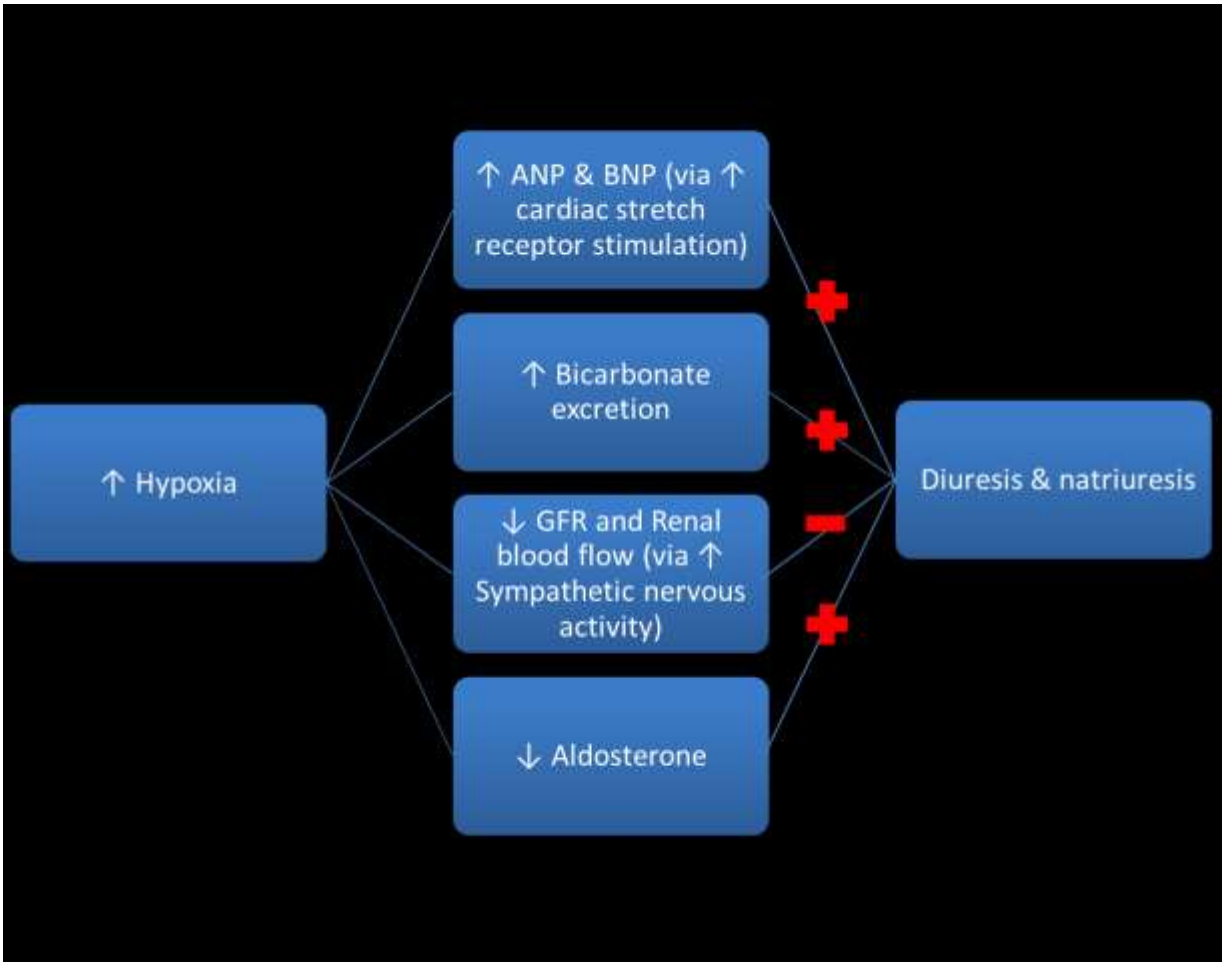

Figure 12. Mechanisms that contribute to hypoxic diuresis. Hypoxic diuresis is coordinated by multiple mechanisms involving and independent of the peripheral chemoreceptor. Cardiac stretch receptors are stimulated, releasing ANP and BNP, during early acclimatization due to increased cardiac output. Hypoxic hyperventilation causes hypocapnia causing an increase in bicarbonate excretion and consequently greater diuresis. Glomerular filtration rate decreases during continued high-altitude occupancy decreasing renal filtration capacity and thus hypoxic diuresis. Aldosterone release is inhibited by hypoxia directly and peripheral chemoreceptor mediated pathways.

Chapter 3 Methods

The following chapter provides a detailed account of the techniques, instrumentation and data analysis. All methods described within the following chapter were either performed during the research expedition, subsequently as part of biochemical analyses or were used for post-hoc quantification of physiological parameters. Dr. Victoria Meah performed renal ultrasound nd analysis.

This study abided by the Canadian Government Tri-council Policy on Research Ethics Policy Statement (TCPS2) and the declaration of Helsinki. Ethical approval was obtained in advanced through the University of Alberta Biomedical Ethics 100 Board (Pro00077330; see Appendix I), the Clinical Research Ethics Board of the University of British Columbia (H17-02687 and H18-01404), and the Universidad Peruana Cayetano Heredia Comité de Ética (no. 101686). Participants were given in-depth study information and provided written consent (see Appendix III).

3.1 Research Design

The research questions and hypothesis have been previously mentioned. Limited data sets are available on renal acclimatization; thus, this study used a comprehensive approach to detail renal adaptation during high-altitude. This study used a quasi-experimental approach (i.e. lacking true randomization). This is a standalone study imbedded within an expedition to Instituto de Investigacions de Alture at Cerro de Pasco, Peru (4330 m). Participants were researchers involved in the expedition and as such were in numerous studies.

3.2 Testing Locations

Testing occurred in three separate times at two different locations (**Figure 13**). Low-altitude testing occurred at the University of British Columbia - Okanagan, Kelowna, Canada, 344m and the high-altitude portion of the study was carried out in the Peruvian Andeas at the Instituto de investigacions de Alture Research Facility (Cerro de Pasco, Peru, 4330 m).

In Peru, participants drove from Lima, Peru (154 m) to Cerro de Pasco, Peru (4330 m) ascending rapidly reaching 4800 meters within two hours and then remaining plateaued above 4000 meters until destination (**Figure 13**). At altitude, Lowlanders were tested the morning immediately following ascent having spent 12-15 hours at high-altitude prior to participating (ATL1). Special attention was taken at this time to observe any serious complications related to mountain sickness (high-altitude pulmonary edema, or high-altitude cerebral edema). Participants all had the same ascent profile but arrived on three different consecutive days this dispersed testing. Lowlanders acclimatized to high-altitude for seven days and were retested thereafter (ATL7).

3.3 Study Participants

Healthy Lowlanders with no underlining diseases were recruited. Lowlanders prior to enrolment were administered a healthy history questionnaire that determined pre-existing neurological, cardiovascular or renal dysfunction before testing (see appendix V). Health history questionnaire were administered to reduce confounding factors and co-morbidities that may alter renal function and volume regulation during low and high-altitude. Renal dysfunction was identified using: The Kidney Disease Questionnaire (Tran *et al.,* 2017) (see appendix VI). Participants had no identified kidney dysfunction. Lowlanders were research members associated with the expedition residing below 2500 m and had not visited high-altitude (>2500 m) for a minimum of three months prior to testing. Lowlanders resided in several different countries: United Kingdoms, Austria, New Zealand, United States and Canada with the majority coming from Western Canada. Twenty-four participants (28 ± 7 years; 3 female) were tested.

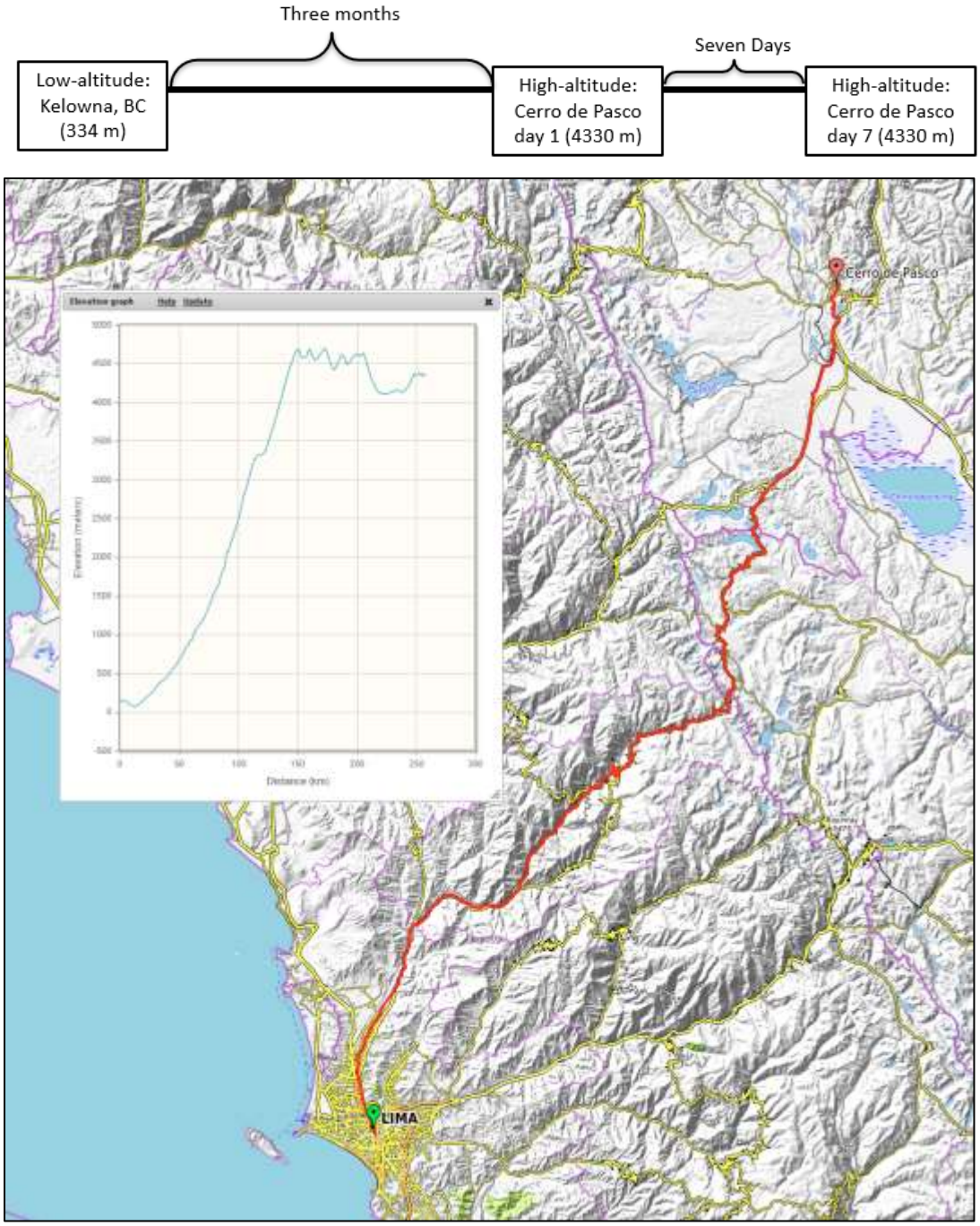

Figure 13. Overview of study timeline (Top Panel) and ascent profile from Lima, Peru to Cerro de Pasco, Peru (Bottom Map). Highest point during ascent was 4800m, thereafter ascent plateaus above 4000 m till Cerro de Pasco (4430 m).

3.4 Instrumentation

All instrumentation and testing occurred with the participants positioned in a resting, supine position. Participants were asked to fast for twelve hours prior to testing avoiding caffeine, alcohol and strenuous exercise with minimal water consumption in order to obtain a good renal artery ultrasound. Participants arrived in the lab between 06.30 and 10.30 a.m. with an overnight urine collection (~ 9-hours) Participants laid in a supine resting position for ten minutes prior to assessment. An overview of the protocol is depicted in **Figure 14**.

3.41 Urinary Volume and Microalbumin

Participants were instructed to void their bladder in the evening and record the time. Thereafter, all urine was collected in a container (pot) to get an overnight (9hr) volume. Urine was refrigerated until analysis could be completed (4 °C). Urine pots were shaken vigorously before analysis to ensure a homogenous mixture. Volumes were measured using graduated cylinders. Urine analysis was performed using a DCA Vantage Analyzer (Siemens Healthineers Global; Germany) for creatinine and microalbumin. Capillary glass tubes were submerged into urine pots till full (100 µL) and then placed into reagent cartridges. Siemens DCA vantage quantifies microalbumin and creatinine by optic density analysis.

3.42 Cardiovascular measurements

Hemodynamics were collected in triplicate. Heart rate was measured through electrocardiogram (ECG) (Lead II) and blood pressure was obtained through an automated blood cuff (Omron; Japan). Peripheral oxygen saturation was collected by a pulse oximetry (Nellcore Medtronics; United States) from the index finger. Cardiovascular measurements were assumed unchanged throughout the protocol but were not collected throughout.

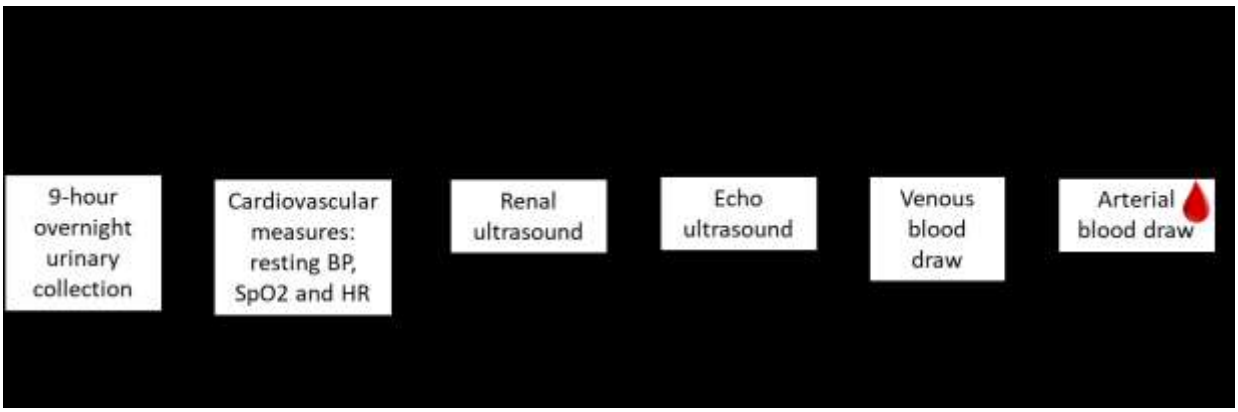

Figure 14. Overview of the timeline of the protocol at Kelowna, Canada and Cerro de Pasco, Peru.

3.43 Venous Collection and Peripheral Venous Pressure

A trained phlebotomist inserted a shielded intravenous (IV) catheter (BD; United States) (**Figure 15)** into the antecubital vein which was connected to a three-way stopcock (Smiths medical; United States). The three-way stopcock allowed researchers to switch between venous collection and peripheral venous pressure without two IVs. Fasted (minimum 12 hours) venous blood draws were taken directly from the extension line (**Figure 15, C**) using a multi-sample needle and vacutainers. Four tubes were collected per participant (two serum separator tubes [SST] and two Ethylenediaminetetraacetic acid [EDTA] tubes) (BD; United States). A small portion of venous blood was immediately analyzed for chemistry (glucose, urea nitrogen and creatinine) electrolytes (sodium, potassium, chloride, ionized calcium, TCO_2 and anion Gap) and hematology (hematocrit and hemoglobin) using an automated point-of-care analyzer (CHEM8+ Cartridge, i-STAT; Abbot; United States).

The i-STAT has been on the market since the early 1990s allowing a single cartridge to provide multiple measures. CHEM8+ analyses were run within three minutes without the need for central clinical laboratory test. I-STAT has strong reproducibility for electrolytes and creatinine (<2%), but poor reproducibility for hematocrit (21%). Clinical agreement between gold-standard tests (i.e. creatinine ELISA) and i-STAT for all metrics were within acceptable measures (Papadea *et al.,* 2002).

Remaining samples were immediately centrifuged at 2500 rpm at 4 °C for 15 minutes then aliquoted into 1.2 mL internally threaded cryotubes (500 µL per vial) and then frozen at -196.2°C in liquid nitrogen during the expedition and at -80 °C in Kelowna until they could be shipped on dry ice to Edmonton for analysis.

An extension line connected the pressure transducer to the antecubital vein to measure peripheral venous pressure continuously. The arm was maintained below heart level (11 +/- 2.7 cm) to maintain an open circuit with the central circulation. This hydrostatic difference was measured and subtracted during post-analysis to provide an index of central venous pressure (Hutchinson & Shaw, 2016). The pressure

transducer was calibrated with a manual pressure gauge before testing. Peripheral venous pressure was taken continuously during baseline and renal ultrasound (see below). Peripheral venous pressure trace was recorded by a BP amp using Labchart (Chart Pro v8.3.1, ADInstruments; Australia). Only eight participants had peripheral venous pressure on ATL1 due to equipment malfunction.

3.44 Arterial puncture and blood gases

Radial artery punctures (2-3 mL) were collected by a clinician using heparinized auto-fill syringes. Samples were immediately analyzed using a point of care device, I-STAT (CG4+ Cartridge, Abbot; United States), for lactate, pH, PCO_2, PO_2, bicarbonate, and oxygen saturation.

3.45 Renal Ultrasound

Renal blood flow was measured using duplex ultrasonography using a 4 MHz curvilinear transducer (abdominal probe, Vivid Q, GE Healthcare). Examinations were performed in a supine position with hands on the chest and knees slightly bent. Renal arteries originate from the anterolateral aspect of the aorta and dive posteriorly to course beneath the inferior vena cava. To locate, the probe was placed at a midpoint between the xiphoid process and the umbilicus. The aorta was identified in a transverse section where the origin of the renal arteries was obtained (Zubarev 2001 & Granata *et al.,* 2009). Diameter and flow were assessed at the origin. Previous research has demonstrated renal ultrasound is strongly correlated with para-aminohippurate extraction in participants with normal flows above 280mL/min per 1.48m^2. Therefore, renal ultrasound is a valid method for quantifying renal artery flows in healthy participants (Takano *et al.,* 2006). Primarily, the right renal artery was measured, unless imagining was unsuccessful (low-altitude n=3; ATL1 n=4 & ATL7 n=5) then left renal artery was used. Some participants had unusable images (low-altitude n=1 & ATL1 n=1). Renal blood flow was calculated by the mean velocity signal times the cross-sectional area ($\prod$ times radius^2) (**Figure 16 Bottom**).

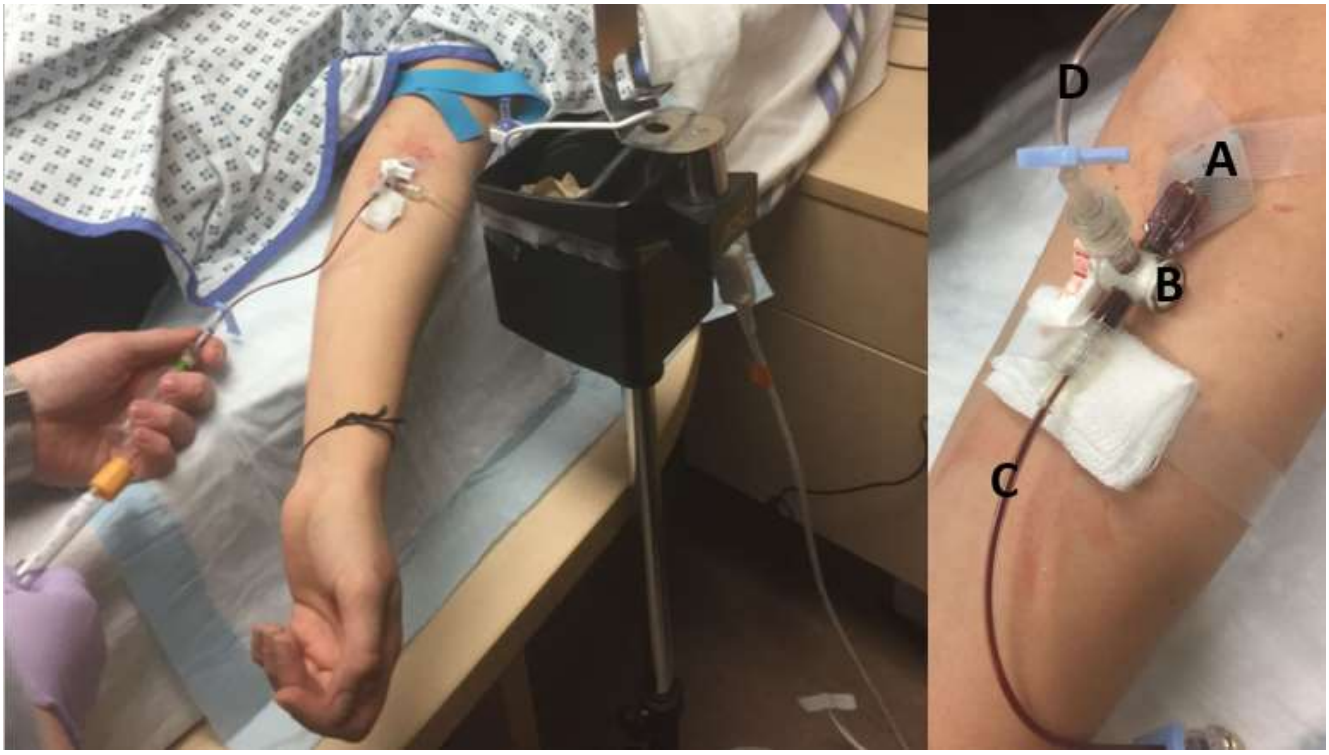

Figure 15. Peripheral venous pressure and venous collection set-up. An intravenous catheter **(A)** is inserted into the antecubital vein and attached to a three-way stop cock **(B)**. Extension line **C** was used to sample venous blood and extension line **D** is attached to a pressure transducer.

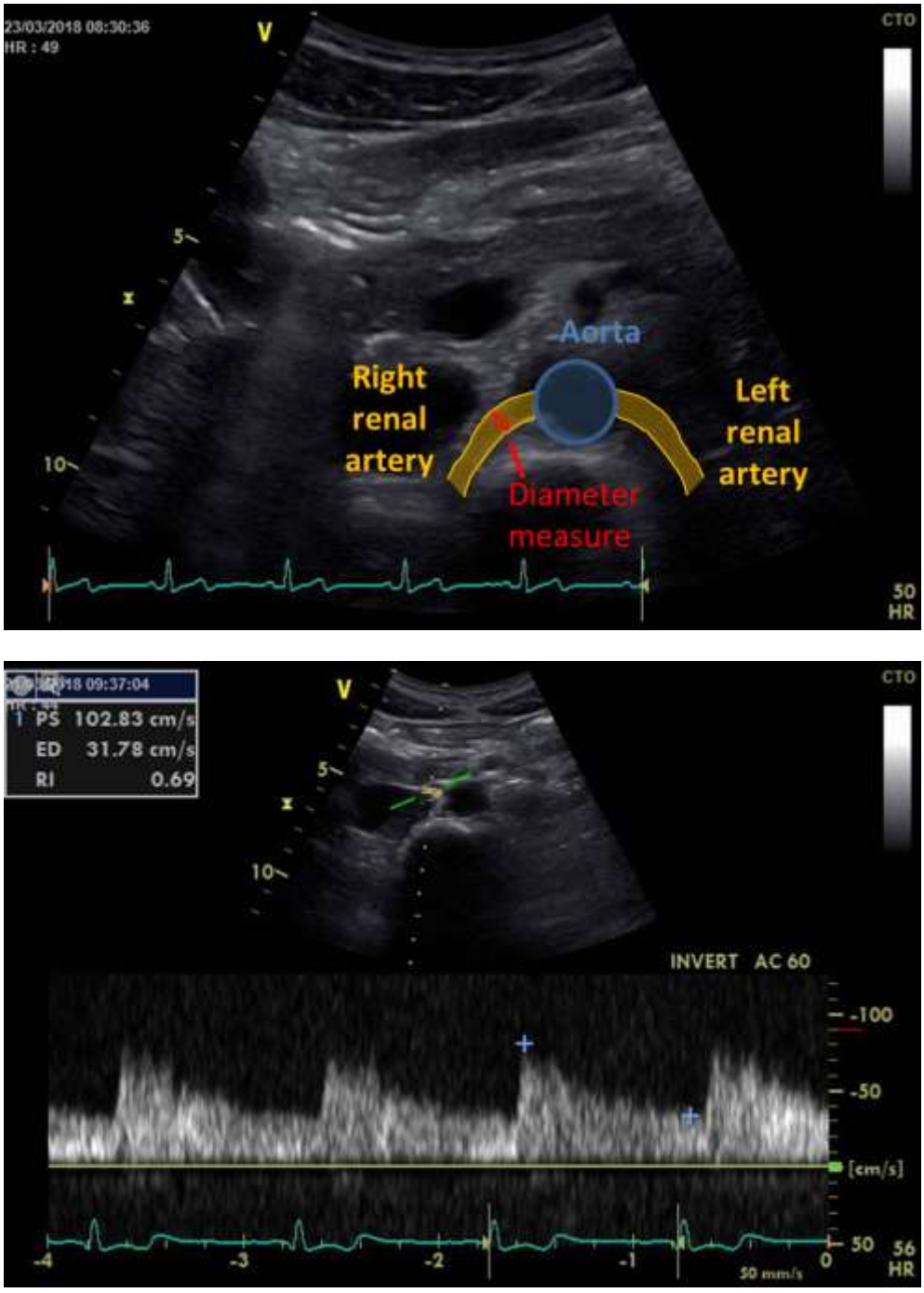

Figure 16. Top panel depicts an ultrasound of the renal arteries perfusing the kidney. The bottom panel illustrates a renal artery flow trace during high-altitude (Cerro de Pasco, 4330).

3.5 Data Analysis and Statistics

Venous pressure trace was averaged over five-minutes during baseline. Blood pressure and S_pO_2 was averaged from three consecutive values taken during baseline. Automated analysis for urine and blood was run internally in duplicate. Renal artery diameter and flow was taken in triplicate during ultrasound.

Hormone analyses used only plasma samples. Solid phase ELISAs (enzyme-linked immunosorbet assay) were used for all hormone detection. Aldosterone was quantified with a competition assay with a pre-coated plate (LDN-Immunoassays and service). Active renin was quantified with a sandwich assay with a pre-coated plate (LDN-Immunoassays and service). NT pro-BNP was obtained through a sandwhich ELISA (LDN-Immunoassays and service). Samples were run in duplicate and averaged. Standard samples were run on each assay to correct for differences between plates.

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

$$GFR = \frac{U_X \times \dot{V}}{P_X} \qquad \frac{mL}{min} = \frac{(mg/mL)\times(mL/min)}{(mg/mL)}$$

Where Ux is urine creatinine concentration, V is urine production rate and Px is serum creatinine concentration. Creatinine clearance may change during the first 24-hours of hypoxia and a shorter period is more specific to early acclimatization. Previous work has shown creatinine clearance for 9-hours is strongly correlated to 24-hours (Devanand *et al.,* 2013 & Tomé da Silva *et al.,* 2010). Recent published work employed similar methods (Haditsch *et al.,* 2015).

Data is presented as a mean ± standard deviation. One-way repeated measures ANOVA (Analysis of variance) was used to compare time points. Tukey pos-hoc analysis was used when effects existed. Regression analysis were run to determine correlations between renal function (glomerular filtrate rate and renal blood flow) urinary excretion, volume hormones and hematology (Graph Pad, Prism 5.0). Differences were considered significant if $p<0.05$ with a two-tailed analysis.

Chapter 4 Results

4.1 Participant Demographics

Twenty-four individuals participated in this study, including 21 men and 3 women. Repeated measures were obtained in all individuals at ALT1. However, two participants (both male) did not complete ATL7 because one had to depart the expedition early and the other developed pneumonia. These participants were still included in the analysis of low-altitude and ATL1. Demographics and acute mountain sickness scores are listed in **Table 1.** Lake Louise acute mountain sickness scores significantly decreased from ATL1 (3.2 ± 1.9) to ATL7 (0.4 ± 0.9; $p<0.05$). Body mass index (sea-level 24.3 ± 2.4 & ATL7 22.8 ± 3.5) decreased but not significantly.

4.2 Hematology and Cardiovascular Parameters

Basal hematology and cardiovascular measures are listed in **Table 2**. Hemoglobin saturation decreased dramatically at ATL1 from sea-level (sea-level 97.6 ± 1.2% & ATL1 78.9 ± 8.4%; $p<0.05$) but subsequently increased by ATL7 (87.6 ± 2.1%) such that it was no longer statistically lower. Hematocrit was elevated at altitude (main effect, $p<0.05$) and was significantly different at ALT7 (ATL7 46.5 ± 2.4%; $p<0.05$) compared to sea-level (42.3 ± 4.4%).

There was a main effect of time on resting heart rate ($p<0.05$), with values during ATL1 (77 ± 13.8 beats/min) being significantly higher than at sea-level (56 ± 12.1 beats/min; $p<0.05$). However, heart rate was no longer different that sea-level values by ATL7 (66 ± 13.4 beats/min). There was no influence of altitude on arterial pressure; however, diastolic pressure was higher during early acclimatization (sea-level 69.8 ± 6.9 mmHg vs ATL1 77.9 ± 7.4 mmHg; $p<0.05$). Systolic pressure remained unchanged at altitude. Peripheral venous pressure decreased from sea-level (12.2 ± 3.8 mmHg) during high-altitude (main effect, $p<0.05$), reaching significance at ATL7 (9.4 ± 3.0 mmHg; $p<0.05$) (**Figure 17**).

Table 1: Participant demographics and acute mountain sickness scores.

	Sea-level (n=24)	**ATL1 (n=24)**	**ATL7 (n=22)**	**P-Value (ANOVA)**
Age	28 ± 6.4	-	-	0.374
Weight (kg)	74.4 ± 8.2	73.3 ± 9.6	71.6 ± 9.9	0.569
Height (cm)	176 ± 10	-	-	0.371
BMI	24.3 ± 2.4	23.6 ± 9.6	22.8 ± 3.5	0.189
AMS scores	-	3.2 ± 1.9	0.4 ± 0.9#	0.0465

Participants included 21 males and 3 females. Repeated measures were obtained in all participants at ALT1 and in 22 participants (19 males, 3 females at ALT7. At ALT1, 17 individuals reported mild-moderate AMS and 2 participants reported severe AMS (0-6). Symptoms were subsequently reduced by ALT7, with only 2 individuals reporting mild-moderate AMS (0-3). P-value for repeated measures ANOVA (effect of time) indicated for each variable. Symbols indicate significant post-hoc comparisons,

 # Represents a significant difference between ATL1 vs ATL7 ($p<0.05$)

Abbreviations: **ATL1, High-Altitude After 1 Day; ATL7, High-Altitude After 7 Days; BMI, Body Mass Index; and AMS; Acute Mountain Sickness.**

Table 2: Cardiovascular and hematological measurements.

	Sea-level (n=24)	ATL1 (n=24)	ATL7 (n=22)	P-Value (ANOVA)
HR (beats/min)	56 ± 12.1	77 ± 13.8 *	66 ± 13.4	<0.001
MAP (mmHg)	84.8 ± 7.5	89.1 ± 7.1	90.4 ± 7.7	0.098
Systolic Pressure (mmHg)	116.5 ± 8.7	117.7 ± 8.3	118.6 ± 9.8	0.302
Diastolic Pressure (mmHg)	69.8 ± 6.9	77.9 ± 7.4 *	76.2 ± 7.2	0.001
Peripheral Venous Pressure (mmHg)	12.2 ± 3.8	10.1 ± 1.5	9.4 ± 3.0 †	0.014
O_2 Saturation (%)	97.6 ± 1.2	78.9 ± 8.4 *	87.6 ± 2.1	<0.001
Hemoglobin (g/dL)	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

Participants had a moderate increase in mean arterial pressure during high-altitude that was caused by diastolic pressure rather than systolic pressure. Peripheral venous pressure decreased throughout high-altitude occupancy indicating central venous pressure is decreasing. O_2 saturation begins to improve by ATL7 higher hematocrit concentrations contribute to this change.

P-value for repeated measures ANOVA (effect of time) indicated for each variable. Symbols indicate significant post-hoc comparisons**,**

* *Represents a significant difference between Sea-level vs ATL1 (p<0.05),*

† *Represents a significant difference between Sea-level vs ATL7 (p<0.05).*

Abbreviations: **ATL1, High-Altitude After 1 Day; ATL7, High-Altitude After 7 Days; HR, Heart Rate and MAP, Mean Arterial Pressure**

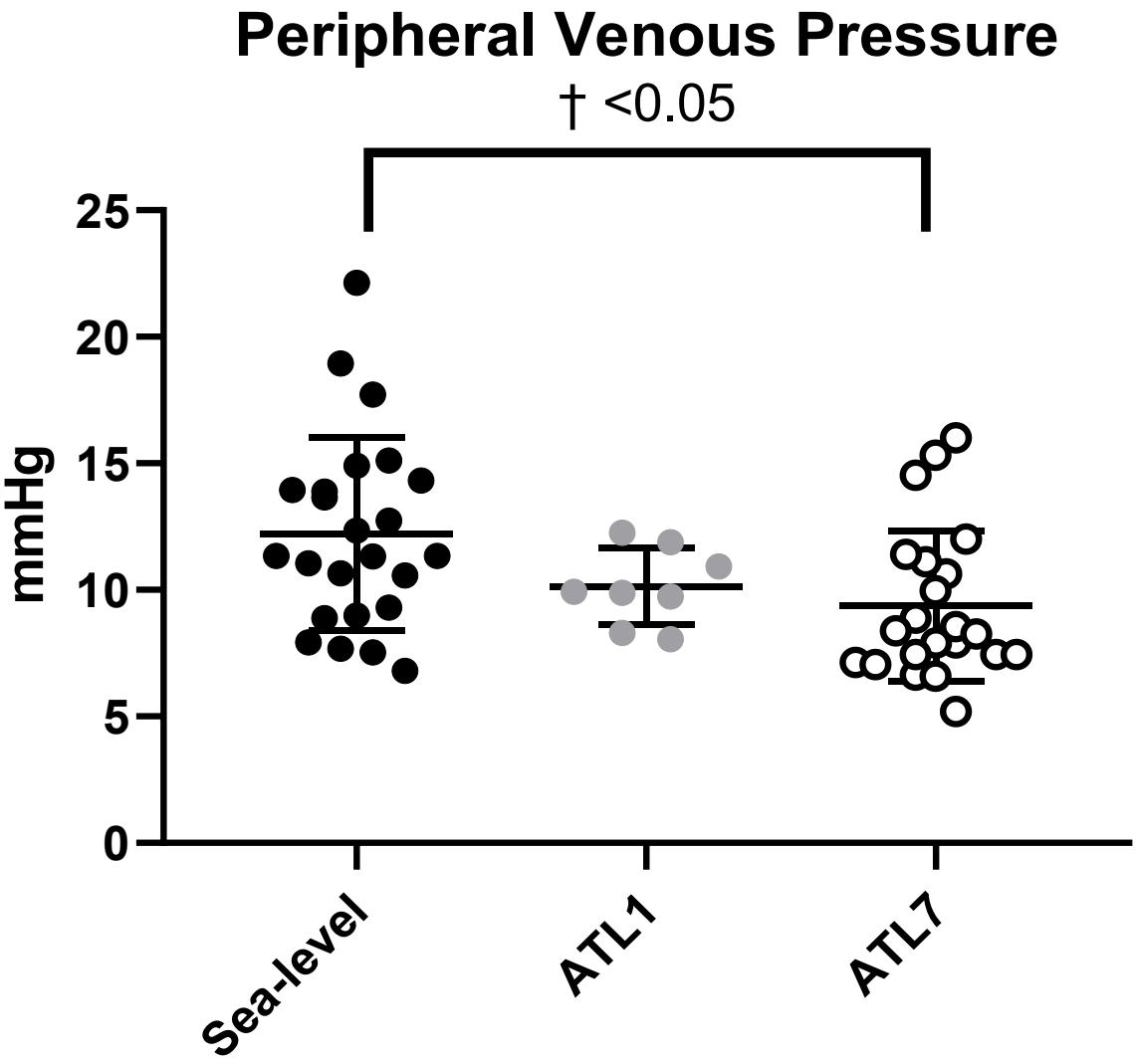

Figure 17. Peripheral venous pressure during low (340 m) and high-altitude (4330 m). Peripheral venous pressure decreased during high-altitude reaching significance by ATL7.

† *Represents a significant difference between Sea-level vs ATL7 ($p<0.05$)*

4.3 Renal Function and Urinary Parameters

Renal blood flow significantly decreased from sea-level (931.4 ± 392.4 ml/min) to ATL1 (626.1 ± 364.8 ml/min; $p<0.05$) (**Figure 18 Top Panel**). Re-measurement at ATL7 (892.8 ± 334.1 ml/min) renal blood flow had increased to sea-level values. These differences are maintained when renal blood flow is corrected with cardiac output (**Figure 18 Bottom Panel**) Glomerular filtration rate was significantly decreased by ATL7 (86.4 ± 17.4 ml/min $p<0.05$) compared to sea-level (101.8 ± 21.1 ml/min). Although lower, glomerular filtration rate at ATL1 (91.4 ± 21.6 ml/min) failed to reach significance. Renal blood flow (**Figure 18 Top Panel**) had a larger percent decrease (-27.4%) compared to glomerular filtration rate (-9.6%) (**Figure 19**) at ATL1. Renal blood flow and glomerular filtration rate did not correlate at any time point.

4.4 Volume Regulatory Hormones

Volume regulatory hormones responded differently during high-altitude. Of the three tested, only aldosterone reached significance. Aldosterone was significantly reduced by ATL7 (121.7 ± 92.5 mmol/L) decreased from sea-level (182.7 ± 104.9 mmol/L; $p<0.05$) (**Figure 20**). Active renin, unlike aldosterone, does not reach significance at any time point but decreases non-significantly. NT-pro BNP non-significantly increased at ATL1 (1909 ± 970.6 mmol/L), however decreased below sea-level (1753 ±600.2 mmol/L) at ALT7 (1460 ± 764.6 mmol/L) (**Figure 21 Bottom Panel**).

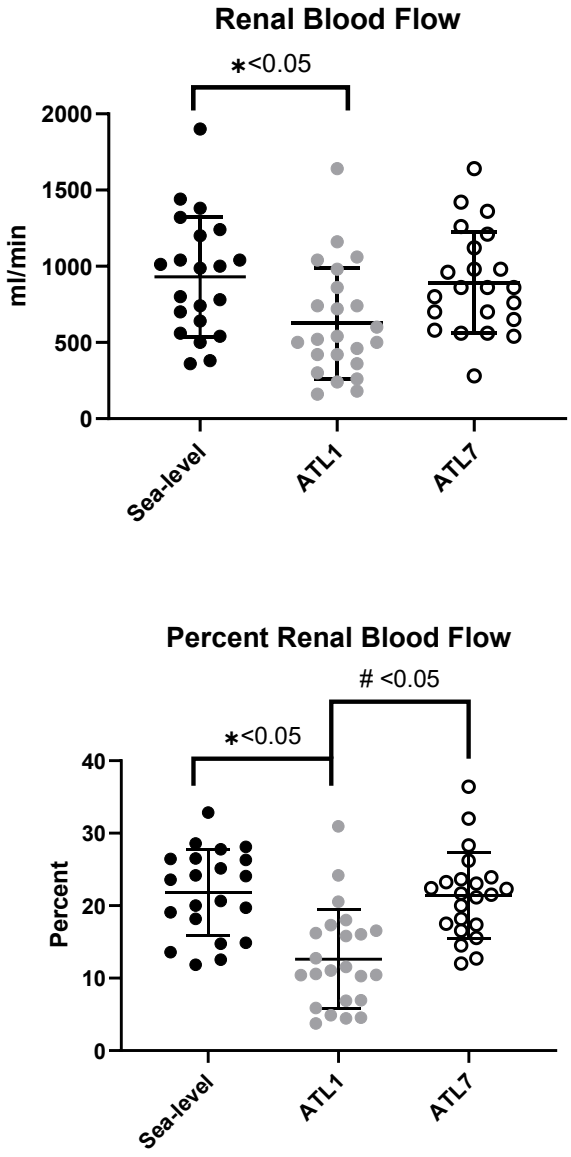

Figure 18. Renal blood flow raw (**Top Panel**) and corrected (**Bottom Panel**) with percent of cardiac output during low (340 m) and high-altitude (4330 m). Renal blood flow significantly decreased at ATL1 but rebounds towards sea-level.

** Represents a significant difference between Sea-level vs ATL1 ($p<0.05$)*

 # Represents a significant difference between ATL1 vs ATL7 ($p<0.05$)

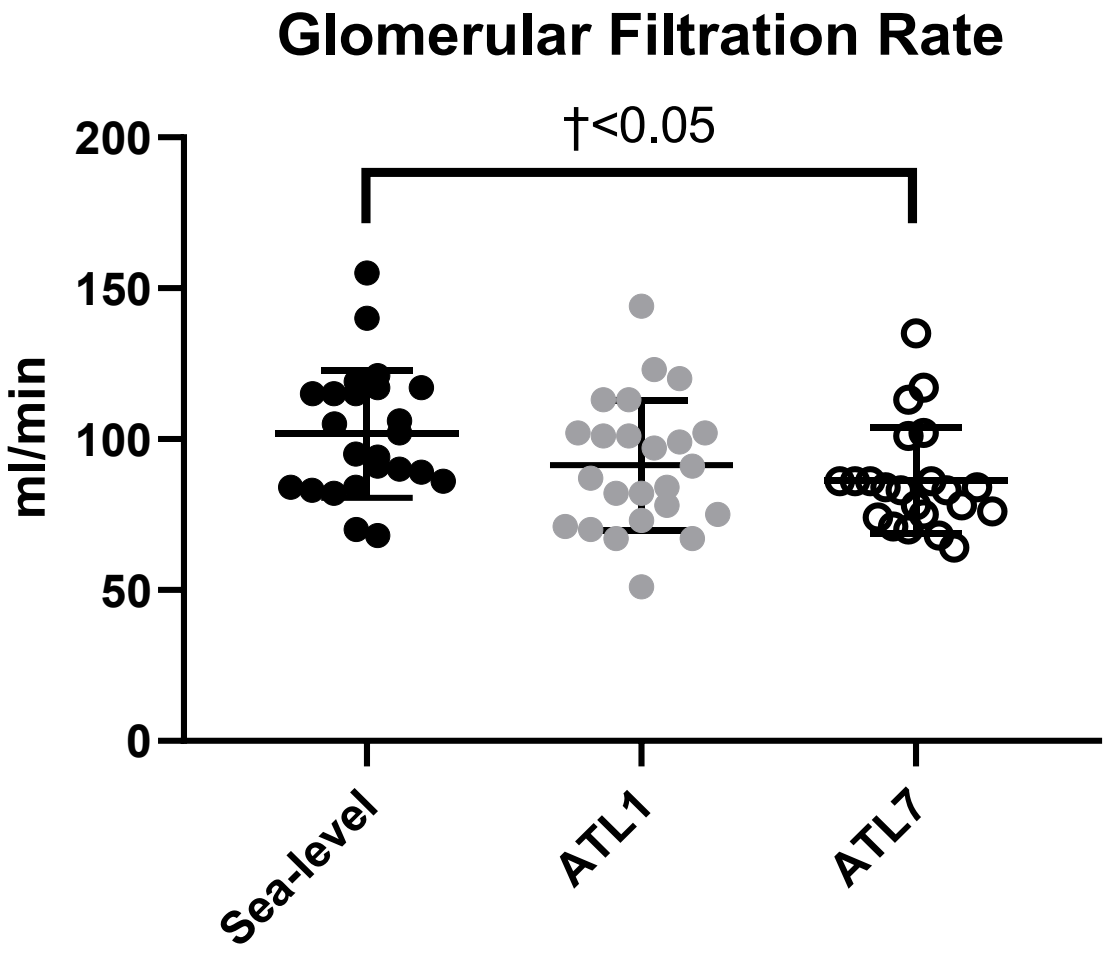

Figure 19. Glomerular filtration rate during low (340 m) and high-altitude (4330 m). Glomerular filtration trended downward reaching significance by ATL7.

† *Represents a significant difference between Sea-level vs ATL7 ($p<0.05$)*

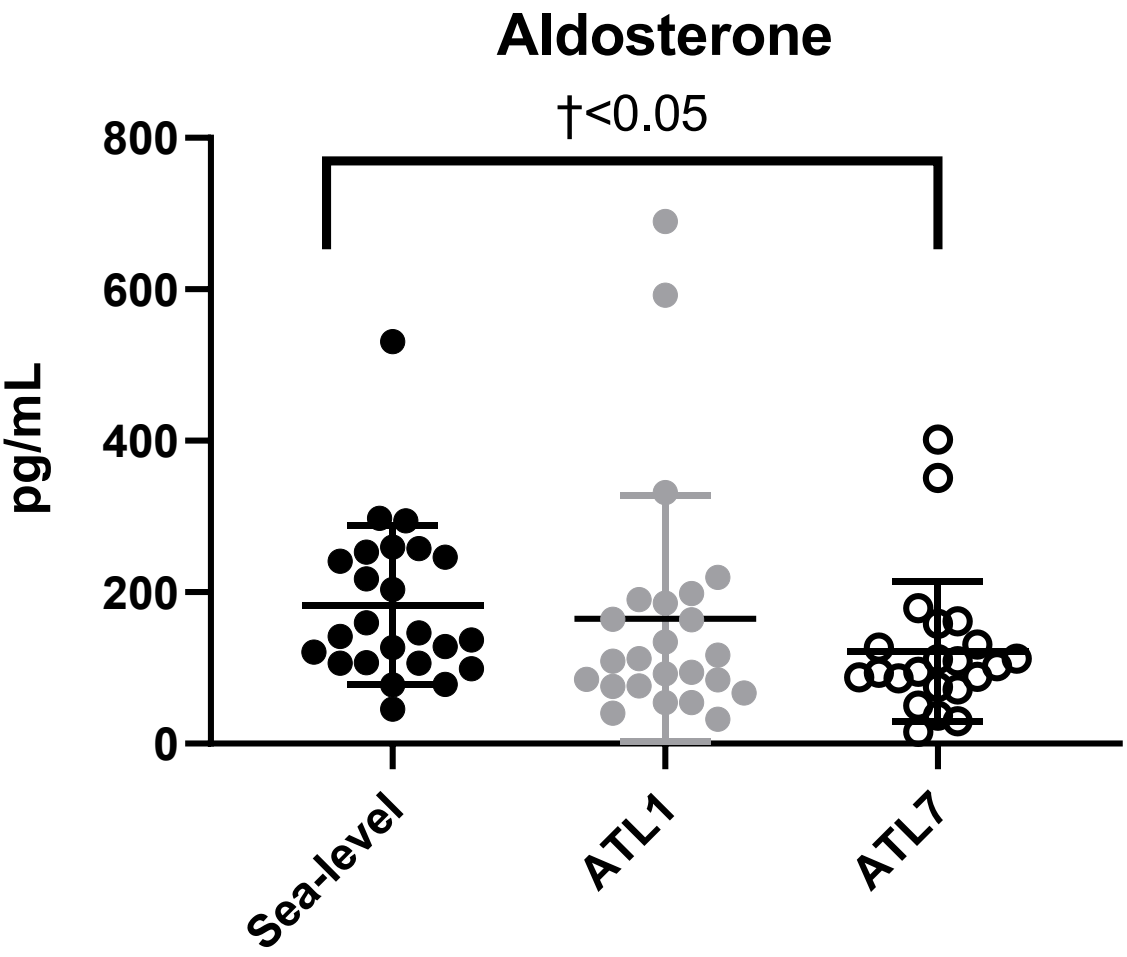

Figure 20. Plasma Aldosterone Concentration during low (340 m) and high-altitude (4330 m). Aldosterone decreased during high-altitude reaching significance by ATL7.

† *Represents a significant difference between Sea-level vs ATL7 ($p<0.05$)*

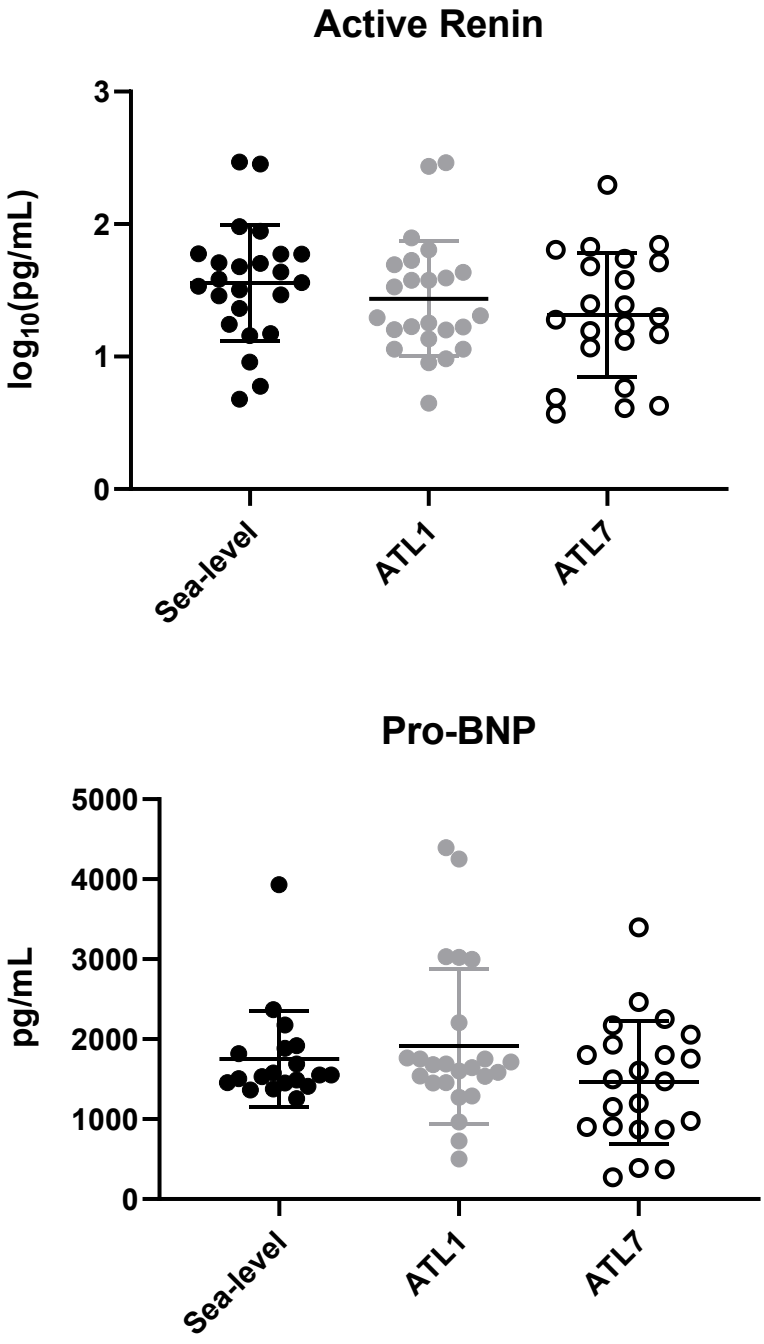

Figure 21. Active renin corrected using $\log_{10}$ (**Top Panel**) and NT-Pro BNP (**Bottom Panel)** during low (340 m) and high-altitude (4330 m). There was no main effect for either hormone.

4.5 Urine Excretion

Overnight 9-hour urinary output significantly increased during high-altitude (sea-level 535.3 ± 277.8 ml/9-hour increased to 680.1 ml/9-hour at ALT1 $p>0.05$ & 756.9 ml/9-hour at ATL7 $p<0.05$). Urinary output was highly variable with 8 participants not increasing urinary excretion (± 405.8 ml) However, this variability decreased by ATL7 (± 255.8 ml) with only 3 participants not exhibiting increased urinary excretion versus sea-level (± 198.5) (**Figure 22 Top Panel**). At all time points, renal blood flow, glomerular filtration rate and urinary excretion did not correlate. Urinary microalbumin was non-significantly different across time: sea-level (5.9 ± 3.5 mg/L), ATL1 (9.2 ± 3.5 mg/L) and ALT7 (6.4 ± 2.0 mg/L) (**Figure 22 Bottom Panel**).

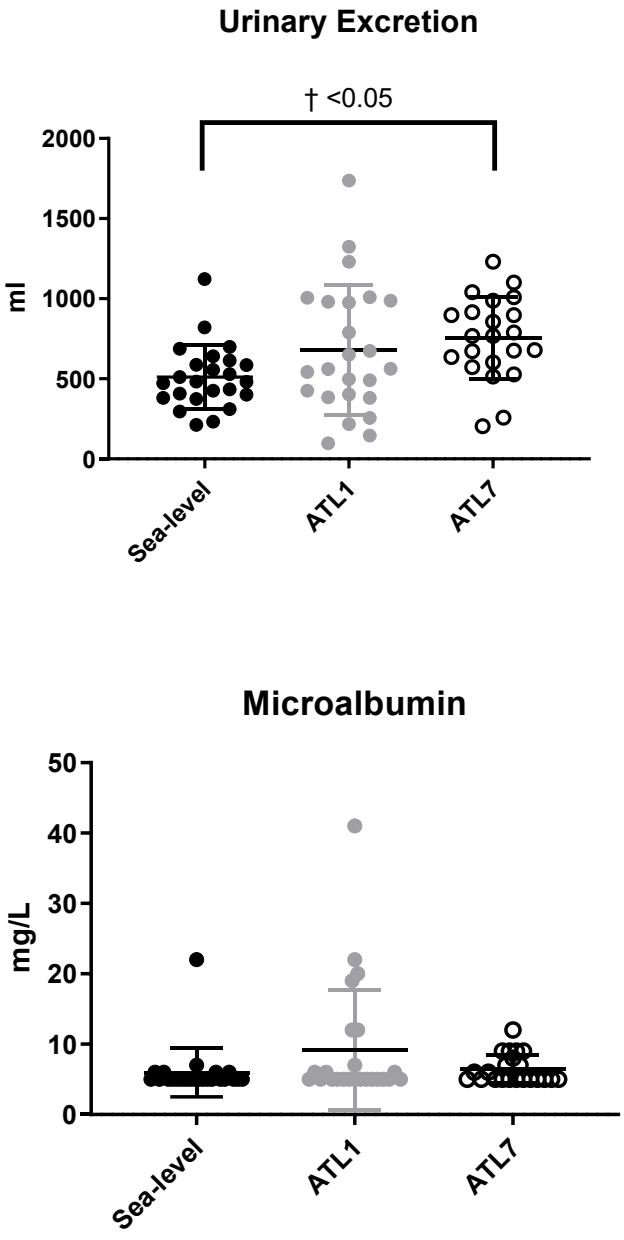

Figure 22. Urinary excretion (**Top Panel**) and microalbumin (**Bottom Panel**) during low (340 m) and high-altitude (4330 m). Urinary excretion increased throughout high-altitude becoming significant at ATL7. Microalbumin had a transient non-significant increase at ATL1, which disappears at ATL7.

† *Represents a significant difference between Sea-level vs ATL7 ($p<0.05$)*

4.6 Blood Gas Metrics

Oxygen partial was significantly decreased at ATL1 (41.5 ± 7.3 mmHg) and ATL7 (50.7 ± 3.9 mmHg) compared to sea-level (100.6 ± 18.4 mmHg). Plasma lactate significantly increased during ATL1 (1.18 ± 0.28 mmol/L) and returned to sea-level (0.69 ± 0.27 mmol/L) at ATL7 (0.8 ± 0.15 mol/L). PCO_2 significantly decreased during acclimatization, as expected, declining from 38.4 ± 3.2 mmHg at sea-level to 33.1 ± 3.3 and 28.2 ± 2.6 mmHg during ATL1 and ATL7 ($p<0.05$), respectively. pH was significantly increased at ATL1 (7.48 ± 0.03; $p<0.05$) compared to sea-level (7.43 ± 0.03) and ATL7 (7.45 ± 0.03). Bicarbonate in turn was significantly decreased at ATL7 (19.9 ± 2.0 mmol/L; $p<0.05$) compared to sea-level (25.8 ± 1.7 mmol/L) and ATL1 (24.6 ± 1.9 mmol/L) (**Table 2** and **Figure 23**).

Table 3: Blood gases during low and high-altitude.

	Sea-level (n=24)	ATL1 (n=24)	ATL7 (n=22)	P-Value (ANOVA)
pH	7.43 ± 0.03	7.48 ± 0.03 *	7.45 ± 0.03 #	<0.001
Bicarbonate (mmol/L)	25.8 ± 1.7	24.6 ± 1.9	19.9 ± 2.0 †#	<0.001
PO_2 (mmHg)	100.6 ± 18.4	41.5 ± 7.3 *	50.7 ± 3.9 †#	<0.001
PCO_2 (mmHg)	38.4 ± 3.2	33.1 ± 3.3 *	28.2 ± 2.6 †#	<0.001
Lactate (mmol/L)	0.69 ± 0.27	1.18 ± 0.28 *	0.8 ± 0.15 †#	<0.001

Hypobaric hypoxia causes an immediate decrease in PO_2, which is mitigated by ATL7. Early acclimatization uses more lactate compared to sea-level or ATL7 suggesting more energy is being generated from anaerobic processes. pH, PCO_2 and bicarbonate are in a dynamic balance and change to when one is perturbated.

P-value for repeated measures ANOVA (effect of time) indicated for each variable. Symbols indicate significant post-hoc comparisons,

* *Represents a significant difference between Sea-level vs ATL1 ($p<0.05$),*

† *Represents a significant difference between Sea-level vs ATL7 ($p<0.05$),*

Represents a significant difference between ATL1 vs ATL7 ($p<0.05$)

Abbreviations: PO_2, partial pressure of oxygen; PCO_2, partial pressure of carbon; O_2, oxygen saturation; mmHg, millimetres of mercury & mmol/L, millimoles per litre.

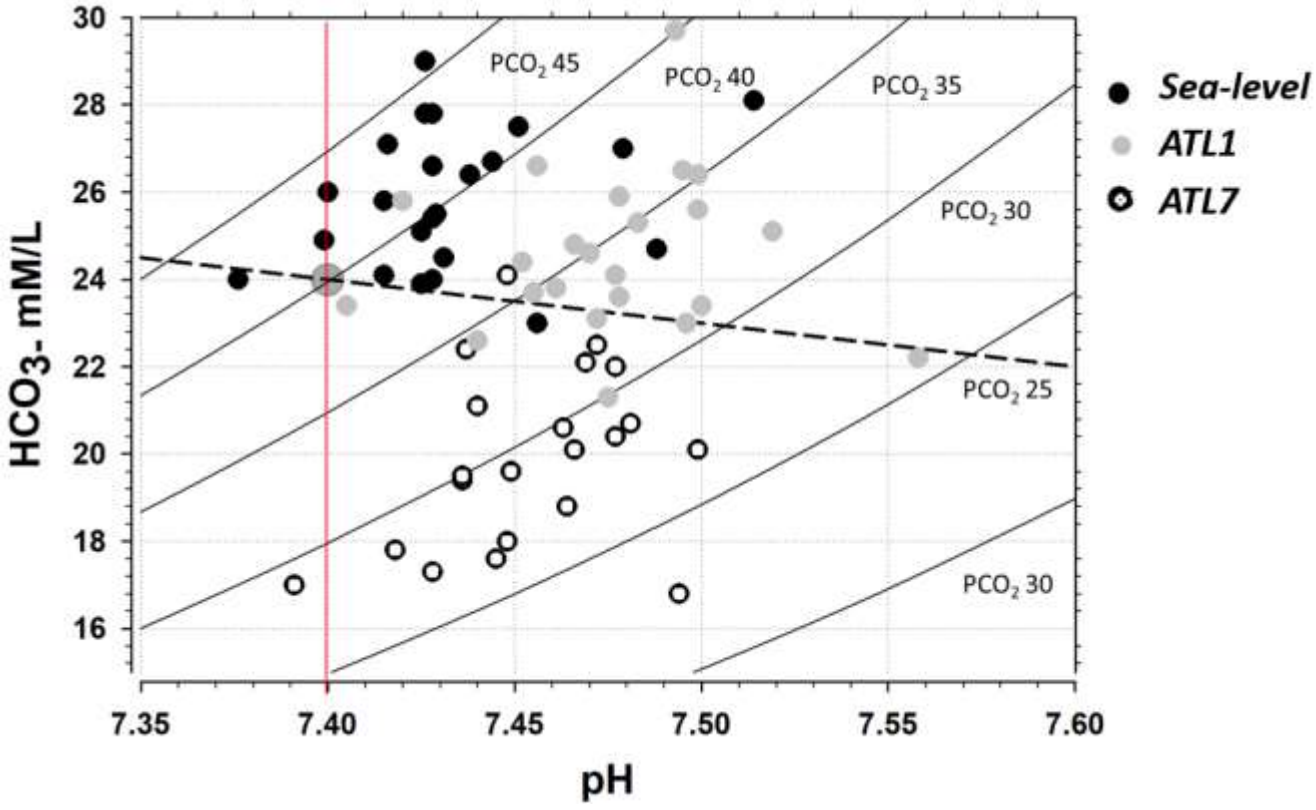

Figure 23. Davenport plot depicting a three-dimensional surface describing all possible states of chemical equilibrium between PCO_2, pH and HCO_3^- (plus non-bicarbonate buffering). Davenport plots provide a conceptual tool to envision the effects of physiological changes on acid-base chemistry. Hypoxic hyperventilation causes hypocapnia (respiratory alkalosis) at ATL1, which is partially normalized by HCO_3^- excretion by ATL7. Black dots represent sea-level, grey dots represent ATL1 and open circles represent ATL7. The larger grey symbol indicates the physiological normal point for pH, bicarbonate, and PCO_2 and the red line highlights normal pH. The dashed line represents the buffer line. Curvilinear isopleths represent corresponding range of PCO_2 within 5mmHg bands.

4.7 Correlations

Notably at all-time points there was no correlations between renal blood flow, glomerular filtration rate, active renin, aldosterone and urinary output. NT-pro BNP had a significant correlation with renal blood flow (r=0.514) and peripheral venous pressure (r=0.440) at ATL1 (p<0.05), this relationship had disappeared by ALT7. Active renin and aldosterone had no significant correlation at any time point.

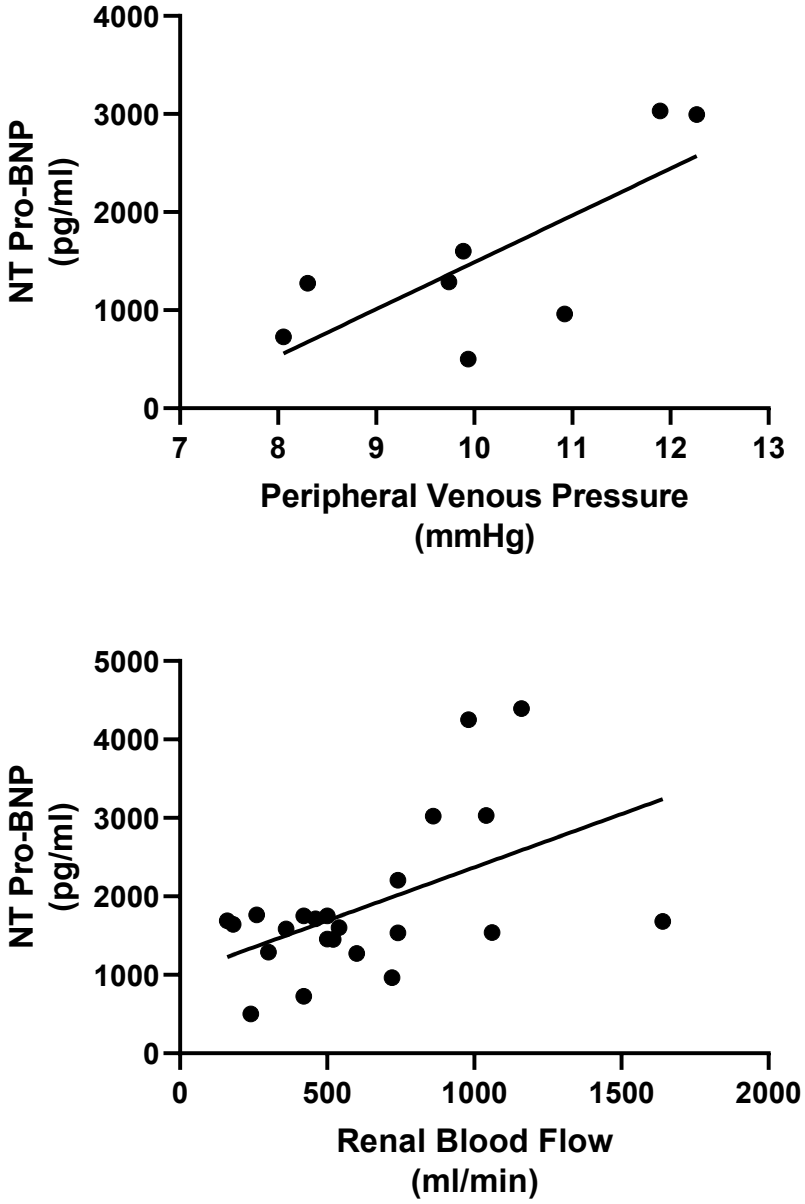

Figure 24. NT-pro BNP had a significant correlation with renal blood flow (r=0.514) (**Top Panel)** and peripheral venous pressure (r=0.440) (**Bottom Panel**) at ATL1 (p<0.05).

Chapter 5 Discussion

Within this current project, we have carried out a comprehensive assessment of kidney function and fluid regulation during high-altitude acclimatization. By broadly evaluating renal hemodynamics, glomerular filtration rate and volume regulatory hormones we have systematically quantified early and prolonged renal acclimatization. This study supported these conclusions: aldosterone decreases to facilitate hypoxic diuresis, glomerular filtration rate decreases with prolonged hypoxic occupancy and HCO_3^- decreases to normalize acid-base status. Hypoxia causes renal blood flow to significantly decrease however this is only transient (significant only within the first 12 hours of exposure) and rebounds after seven days of acclimatization.

5.1 High-Altitude and systemic hemodynamics

Hypoxia temporarily increases cardiac output to increase cardiac output, which stabilizes within a few days. Stroke volume decreases, but heart rate increases to maintain cardiac output (Klausen 1966 & Naeije *et al.,* 1982). Our participants had a dramatic increase in heart rate, which fell thereafter by ATL7 however still above sea-level, consistent with previous reports. Peripheral venous pressure, a surrogate for volume status and central venous pressure was decreased during acclimatization (**Figure 17**). Peripheral venous pressure has been validated against central venous pressure by numerous studies (Amar et al., 2001l; Munis et al., 2001; Anter et al., 2004; Sahin et al., 2005 & Hoftman et al., 2006). For example, peripheral venous pressure was found to be within 1.62 ± 0.84 mmHg (mean difference) of direct central venous pressure in thirty patients in the Burns Intensive Care Unit during a 10-hour period (Kim et al., 2011). Measurements remained in a constant range over the 10-hour period during changes in hemodynamics indicating peripheral venous pressure is accurate enough to dictate changes in central venous pressure. Across all studies, peripheral venous pressure was found to be between 6.89 and 12.87. This corresponds to the measurement values obtained in this study (range = 7.67 – 15.31 mmHg). Decreased peripheral venous pressure suggests that low-pressure cardiovascular baroreceptors may be

less active and contribute less to hypoxic diuresis than previously hypothesized (Koller *et al* 1991). Some participants had a higher peripheral venous pressure and greater NT pro-BNP concentration during ATL1 **(Figure 24**) suggesting these individuals are experiencing greater atrial stretch. We observed no change in mean arterial pressure at high-altitude, however, diastolic pressure was significantly increased (**Table 2**). Changes in diastolic pressure may therefore be a more sensitive measure of peripheral hemodynamics and due to a greater sympathetic nervous system activation (Simpson *et al.,* 2019; Hansen *et al.,* 2003 & Wolfel *et al.,* 1994).

5.2 High-Altitude Decreases Renal Blood flow and Glomerular Filtration Rate

Our findings indicate that renal blood flow measured using ultrasonography was reduced within the initial 12-hours of altitude exposure and rebounds following 7 days of acclimatization (**Figure 18**). Very few studies have reported renal hemodynamics during chronic hypoxia. Singh *et al* (2003) found effective renal plasma flow, estimated by para-aminohippurate clearance, was linearly decreased throughout a sixty-day occupancy at 3500 m becoming significant on day 10 compared to sea-level. However, effective renal blood flow was not significantly changed on day three, which was supported by another study (Olsen *et al.* 1992). Values for low-altitude renal blood flow (expressed in absolute terms or as a percent of cardiac output) were "text-book" with similar variability in measurement at low and high-altitude, indicating that the responses observed represent a true phenomenon. I speculate that this difference may relate to the rapid ascent profile, previous studies had participants trekking and ascending slowly (Anand *et al.,* 1993 & Singh *et al.,* 2003). Furthermore, high-altitude decreases plasma volume (Young *et al.,* 2019), so effective renal blood flow may inaccurately measure renal hemodynamics during hypoxia. Renal ultrasound may provide the best method to quantify renal blood flow during high-altitude.

Hypoxia mediated increased renal arterial pressure ($\Delta P = 4.3$ mmHg) may cause autoregulatory vasoconstriction at the afferent arteriole increasing renal vascular resistance normalizing the volumetric flow rate (Drew & Charkoudian, 2019).

Numerous sources could have generated the increase in renal vascular resistance including reactive oxygen species and renal sympathetic nerve activity. Hypobaric hypoxia generates reactive oxygen species such as superoxide, hydroxyl radical, peroxynitrite and hydrogen during exposure to 4300 meters (Tran et al., 2017). However, arterial hypoxaemia and local renal hypoxia increases nitric oxide and nitric oxide synthase activity suggesting hypoxia is protective for renal blood flow (Chen et al., 1997). High altitude occupancy may also alter the relationship between reactive oxygen and nitric oxide changing renal vascular resistance. Further research is needed to understand this mechanism (Blantz et al., 2007). Regardless, with disparity between techniques and timing, the current literature is too small to make a decisive conclusion regarding the time-course of renal-blood flow at altitude.

Glomerular filtration rate responded differently during hypoxia compared to renal blood flow and did not correlate. High-altitude caused a significant drop in glomerular filtration rate, which became significant by ATL7. Suggesting hypoxia has a time-effect on glomerular filtration rate, which may continue to decrease with further hypoxic duration. Acutely (less than ~48-hours), glomerular filtration rate has been shown to be unchanged or minorly decreased during hypoxia (Anderson *et al.,* 1978; Olsen *et al.,* 1992; Olsen *et al.,* 1993; Swenson *et al.,* 1995; Hildebrant *et al.,* 2000 & Bestle *et al.,* 2002). Authors postulated that glomerular filtration rate may increase to promote diuresis during hypoxia (Hogan *et al.,* 1973 & Maher *et al.,* 1975) this has not been supported by the current data or previous studies. Animal studies have demonstrated glomerular filtration rates and renal blood flow have been shown to significantly correlate with renal sympathetic nerve activity during hypoxia indicating increased vasoconstriction of renal arterioles may decrease renal blood delivery (Malpas *et al.,* 1996).

Studies investigating glomerular filtration rate during prolonged acclimatization are rare. Bestle *et al.,* (2002); Pichler *et al.,* (2007) & Haditsch *et al* (2015) all found after several days at high-altitude (range = 7 – 15 days) glomerular filtration rates were significantly decreased. Pichler *et al.,* 2007 & Haditsch *et al* (2015) had participants exposed to hypoxia for fourteen days and glomerular filtration rates

remained decreased after three days. Future endeavors should capture glomerular filtration rate and renal blood flow beyond this point. High-altitude Peruvian populations have low effective renal plasma flow but relatively maintained glomerular filtration rates because of high filtration fraction (Becker *et al.,* 1957). Lowlanders may employ similar methods to maintain adequate glomerular filtration rates during chronic hypoxic exposure. Contrasting these populations would shed light on this hypothesis.

5.3 High-Altitude Diuresis is Regulated by Volume Regulatory Hormones

Active renin did not significantly change throughout hypoxia. Data on active renin has conflicting results with most studies demonstrating a slight decrease or no change regardless of duration (Swenson *et al.,* 1995; Millar *et al.,* 1995 & Zaccaria, 1998). Haditsch *et al* (2015), the most comparable to our study, found participants did not change renin activity during a two-week hypoxic bout supporting our results. Common to most studies is a consistent decrease in circulating aldosterone with hypoxia. Aldosterone decreased during early acclimatization becoming significant by ATL7. Aldosterone may continue to decrease throughout hypoxia to promote diuresis. However, urinary output and aldosterone did not correlate at any-time point. Hypoxia may change receptor sensitivity (Swenson *et al.,* 1995). Aldosterone and renin receptor sensitivity may be blunted causing an inhibited response. Previous work has indicated that the renin-aldosterone-axis becomes less steep during high-altitude (Milledge *et al.,* 1987). Suggesting, at least for aldosterone, that receptor sensitivity is decreased, while hormone concentration is unaltered. Future endeavors should consider this while interpreting findings.

In this current study, NT pro-BNP was not significantly different during acclimatization. Suggesting that BNP did not contribute to hypoxic diuresis. While duration at altitude did not appear to influence this interpretation in the current study, Woods *et al.,* found BNP was significantly increased during high-altitude exposure at 5150 m but not at 4300 m. This study suggest that the degree of hypoxia may have an important influence on BNP. Presumably, a higher altitude (e.g. 5150 m) would be expected to cause a more robust hypoxic response, including greater cardiac output, pulmonary pressure and

venous pressure stimulating the low-pressure baroreceptors producing BNP. It is also worth considering that we did not measure ANP in the current investigation, which may have been influenced. NT pro BNP may not contribute to hypoxic diuresis but may help maintain renal blood flow during early acclimatization. Renal blood flow and NT pro BNP correlated during ATL1 (**Figure 24)**, suggesting BNP increases renal blood flow. However, this correlation may occur because NT pro BNP and renal blood flow are each correlated to cardiac output.

5.4 High-Altitude Causes a Rapid Diuretic Response

High-altitude caused an immediate increase in urinary output that was augmented with persistent hypoxia. Urinary output remained elevated during ATL7 when glomerular filtration rate has decreased indicating how robust the natriuretic response is. Unlike glomerular filtration rates, findings on urinary excretion are consistent across studies. Studies as early as 1944 have observed the hypoxic diuretic response (Menge*,* 1944). Hypoxic diuresis occurs in a dose-dependent relationship between urinary output, sodium excretion and acute hypoxia during 10-16% inspired oxygen. Antidiuresis and sodium retention occurs below <10% oxygen (Luks *et al.,* 2008 & Sanchez-Lozada *et al.,* 2002). Urine volumes significantly increase within 90 minutes (Hildebrant *et al.,* 2000) and reach peak urine output within two days (Bestle *et al.,* 2002) completing hypoxic diuresis within three weeks (Goldfarb-Rumyantzev *et al.,* 2014; Stokke *et al.,* 1986 & Jones *et al 1981*). Hypoxic diuresis provides a rapid method to increase hematocrit concentration without erythropoiesis, which in comparison takes three weeks to manifest (Adamson, 1991). This is supported by the current data, indicating that by ATL1 participants had increased hematocrit concentration and continued to increase at ALT7. Jain *et al.,* (1980) found participants had a significant higher hemoglobin concentration without an increase in red blood cell mass specifically indicating decreased plasma and extracellular fluid caused the change.

Although the means were non-significant, multiple participants exhibited higher urinary microalbumin concentrations during ATL1 (n = 8) compared to sea-level, which decreased by ATL7 (n =

3). Proteinuria is common during rapid ascent caused by changes in renal capillary permeability (Winterborn *et al.,* 1987; Hansen *et al.,* 1994 & Rennie *et al.,* 1970). The current data suggest renal capillary permeability may transiently change in some individuals at altitude and appear to normalize within seven days of hypoxic exposure. In those participants with higher microalbumin concentrations, there could be acute mountain sickness scores because changes in systemic capillary permeability contributes to the illness prevalence (Goldfarb-Rumyantzev *et al.*, 2014). However, changes in renal capillary permeability, as denoted by higher microalbumin concentrations, did not translate to higher acute mountain sickness scores in the current study. Potentially, renal capillary permeability changes are isolated to the kidney and do not reflect a systemic change.

5.5 Acid-Base Set-point is Changed during High-altitude

The acid-base relationship changed dramatically throughout high-altitude as evident in the Davenport plot (**Figure 23.**). Participants hyperventilated during early acclimatization (ATL1) causing hypocapnia. Hypocapnia affects homeostasis by increasing pH and blunting blood buffer capacity. However, acid-base status is quickly corrected by secreting more bicarbonate and reabsorbing more hydrogen (Zouboules *et al.,* 2018). Glenhill *et al* (1975) found hypoxic participants begin correcting hypocapnia within twenty-four hours. In our study, there is a minor decrease in bicarbonate at ATL1 suggesting acid-base normalization is already beginning. By ATL7, participants have significantly decreased bicarbonate and normalized pH close to sea-levels. However, as Krapf *et al* (1991) demonstrated, even at lower altitudes (3450 m) bicarbonate normalization fails to completely restore acid-base status. It is hypothesized that renal luminal cells may reach a physiological ceiling for bicarbonate excretion (Zouboules *et al.,* 2018).

Chapter 6 General Conclusions

6.1 Main Findings

In summary, our findings have quantified the renal acclimatization difference during early and late high-altitude exposure. For the first time, renal acclimatization has been presented using broad approaches to intrinsically understand the kidney. Mean arterial pressure was not significantly altered by high-altitude but diastolic pressure was suggesting a strong sympathetic activation response. High-altitude causes a decrease in renal blood flow initially, which rebounds within seven days of acclimatization. Glomerular filtration rate linearly decreases during high-altitude exposure and may continue to decrease with further residency. Hypoxic diuresis occurs early and remains high even seven days after ascent. Active renin, aldosterone and NT pro-BNP all changed during high-altitude however only aldosterone reached significance. Collectively, this study helps further comprehend the complex physiological processes coordinating hypoxic diuresis this study has highlighted how glomerular filtration rate decreases to lower energy expenditure and reduces kidney function. Urinary production however is not inhibited because renal volume regulatory hormones, specifically aldosterone, are decreased leading to diuresis and functionally increasing hematocrit and hemoglobin concentration. Hypoxic diuresis is a dynamic process involving changes in renal blood flow, filtration and regulatory hormones.

6.2 Strengths

High-altitude acclimatization occurs as a coordinated action dependent on multiple systems. However, previous studies have investigated the kidney in isolation. Our approach quantifies the systemic environment the kidney operates under to provide novel insight into high-altitude acclimatization. No study has systematically quantified renal blood delivery, filtration rate, downstream urinary output, blood metrics and volume regulatory hormones. Furthermore, few studies have used a rapid ascent model to isolate mechanisms to capture the true effect of hypobaric hypoxia. This study will be paramount for isolating mechanisms and developing future kidney acclimatization studies.

6.2 Limitations

There are several considerations that should be acknowledged when interpreting these results because field research expeditions are exposed to unavoidable limitations. Salt intake and fluid consumption was allowed ad libitum. Nonetheless, ad libitum is the standard for field research expeditions, as well as trekking or other natural altitude exposures, making these findings more applicable to "real-world" scenarios. A short coming of our study was the fact that no gold standard assessment for measuring glomerular filtration rate was employed. Creatinine clearance can be affected by age, gender, physical activity, ethnicity, dietary protein intake and lean muscle mass. Baxmann *et al.,* (2008) found creatinine clearance was substantially modified by physical exercise. However, all participants were tested in the morning, so they had an over-night fast and were rested. In addition, participants were their own controls, and so even if variations occurred due to demographics this was conserved across time-points. Renal ultrasound was difficult in some individuals due to abdominal gas following rapid ascent. However, the standard deviation of the reported measures was not different between low altitude, ALT1 and ALT7, so this may not have a significant influence on our interpretations.

6.3 Future Directions

High-altitude travel continues to increase due to high-altitude industries, trekking and military deployment. Thus, understanding kidney function during hypoxia is critically important. Future studies should use gold standard methods like ^{125}I-Iothalamate-clearance and other clinically relevant methods like cystatin C to quantify glomerular filtration rate. Renal blood flow should be reinvestigated using effective renal plasma flow to validate our data. Our study and others (Pichler *et al.,* 2008 & Haditsch *et al.,* 2015) have demonstrated glomerular filtration rate decreases during high-altitude. Future research should aim to understand the mechanism(s) causing glomerular filtration rate to decrease. Specifically, kidney norepinephrine spillover and vascular reactivity studies. Long-term altitude studies should be

pursued to better understand the fully acclimatized kidney. Studies quantifying glomerular filtration rate and renal blood flow for six months or greater.

Renal acclimatization studies have never conclusively isolated the hypoxic diuretic response. Antagonist infusion could help isolate the major contributors. Hypoxic diuresis has multiple redundant pathways isolation may be impossible, however previous work by Modesti *et al.,* (2006) has demonstrated that bosentan, an endothelin-1 blocker, reduces urinary production during acute hypoxia suggesting the work is possible. Previous work across different altitudes, ascent profiles, high-altitude occupancies, genders and age could all be quantified providing direction for future work.

6.4 Conclusions

Hypobaric hypoxia is a potent stimulus for diuresis. Hypoxic diuresis occurs early (minutes) and continues for weeks decreasing the plasma volume to functionally increase hematocrit concentrations. This study has demonstrated that only specific hormones, aldosterone, contributes to hypoxic diuresis. Glomerular filtration rate decreases non-significantly during acute altitude exposure (< 24 hours) and continues to decrease at day seven supporting previous work (Hadistsch *et al.,* 2015). Polyuria is not inhibited during decreased glomerular filtration rate highlighting the robust diuretic stimulus that occurs during high-altitude.

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Appendix I: Ethics Approval for Human Subjects (University of Alberta)

Health Research Ethics Board

308 Campus Tower
University of Alberta, Edmonton, AB T6G 1K8
p. 780.492.9724 (Biomedical Panel)
p. 780.492.0302 (Health Panel)
p. 780.492.0459

Approval Form

Date:	April 11, 2018
Principal Investigator:	Craig Steinback
Study ID:	Pro00077330
Study Title:	Sympathetic regulation of pulmonary pressure and cardiovascular function in Lowlanders and high altitude patients with chronic mountain sickness
Approval Expiry Date:	April 10, 2019
Approved Consent Document:	Approval Date: 4/11/2018 — Approved Document: Peru 2018 Consent Form - Lowlanders and Andeans.pdf
Funding/Sponsor:	NSERC - Natural Sciences And Engineering Research Council

Thank you for submitting the above study to the Health Research Ethics Board - Biomedical Panel.

We acknowledge that your application has received approval from the University of British Columbia Office of Research Ethics, with a current approval expiry of November 14, 2018. Your application is approved on behalf of our committee as we accept the ethical approval from University of British Columbia under the Western Provinces Harmonization Agreement. The following form part of this approval:

- Protocol, Version 6, 23 Jan 2018;
- Informed Consent Form, Version 6, 23 Jan 2018; and
- Study Health Questionnaire, Version 2, 21 Oct 2017.

A renewal report must be submitted next year prior to the expiry of this approval if your study still requires ethics approval. If you do not renew on or before the renewal expiry date (March 5, 2019), you will have to re-submit an ethics application.

The membership of the Health Research Ethics Board - Biomedical Panel complies with the membership requirements for research ethics boards as defined in Division 5 of the Food and Drug Regulations and the Tri Council Policy Statement. The HREB - Biomedical Panel carries out its functions in a manner consistent with Good Clinical Practices and the Canadian General Standards Board (CAN/CGSB-101.1-2013).

Approval by the Health Research Ethics Board does not encompass authorization to access the patients, staff or resources of Alberta Health Services or other local health care institutions for the purposes of the research. Enquiries regarding Alberta Health administrative approval, and operational approval for areas impacted by the research, should be directed to the Alberta Health Services Research Administration office, #507 College Plaza, email nactrc.contracts@albertahealthservices.ca.

Sincerely,

Donald W. Morrish, MD, PhD, FRCPC
Associate Chair, Health Research Ethics Board – Biomedical Panel

Note: This correspondence includes an electronic signature (validation and approval via an online system).

ENVIRONMENT, HEALTH & SAFETY

3-107 Research Transition Facility
8410-114 Street
Edmonton, Alberta, Canada T6G 2V2
www.ualberta.ca/environment-health-safety

January 31, 2018

Research Ethics Office (REO)
308 Campus Tower, 8625 – 112 Street
University of Alberta
Edmonton, AB, Canada T6G 1K8

RE: BIOHAZARDS APPROVAL; HUMAN ETHICS APPLICATION; DR. CRAIG STEINBACK; PHYSICAL EDUCATION AND RECREATION; PRO00077330

Please be advised that the Human Ethics Application Pro00077330 as submitted by Dr. Craig Steinback, Department of Biochemistry, and titled "Sympathetic regulation in Lowlanders and high-altitude patients with CMS" has been reviewed for biosafety purposes.

This project meets the necessary biosafety regulations and the research group is currently in compliance with the University of Alberta biosafety program. **Therefore, this project is approved for work within research and clinical facilities operated by the University of Alberta.**

This Research Safety Compliance review was based upon the documentation provided to the University of Alberta's Department of Environment, Health and Safety (EHS) by the Principal Investigator. In case of significant changes to experimental strategies that may have an impact on possible personnel and/or environmental exposures, the Principal Investigator shall immediately reapply to EHS for project approval. EHS must also be notified immediately if any new personnel are added to this project. Furthermore, the pertinent international, federal, provincial, and university regulations and policies must be implemented and followed by everyone associated with the project for the duration of this award.

Luke Price, M.Sc.
EHS Operations Team

Appendix II: Consent Forms for Participants

Global Research Expedition on Altitude related Chronic Health (Global REACH): a scientific expedition to the Andean mountains

Principal Investigator:

[5]Philip N Ainslie, PhD

Co-Investigators:

[1]David MacLeod, MD; [2]Myp Sekhon, MD; [3]Christopher Gasho, MD; [3]James Anholm, MD; [4]Mike Stembridge, PhD; [5]Brad Monteleone, MD; [5]Lindsey Boulet, MSc; [5]Matt Rieger, MSc; [6]Anthony Bain PhD; [6]Christopher DeSouza, PhD; [5]Daniela Flueck, PhD; [5]Ryan Hoiland, MSc; [7]Joshua Tremblay, MSc; [5]Michael Tymko, MSc; [5]Alexander Hansen, MSc; [8]Jonathan Moore, PhD; [9]Craig Steinback, PhD; [9]Andrew Steele, BSc; [10]Joseph Donnelly, MD; [5]Ali McManus, PhD; [11]Justin Lawley, PhD; [12]Francisco Villafuerte, PhD; [5]Geoff Coombs, MSc; [5]Alex Patrician, MSc; [5]Connor Howe, BHK; [5]Hannah Caldwell, BHK; Benjamin S. Stacey[13], BSc; M. Bailey[13], PhD; Robert Brothers, PhD[14]

Investigator affiliation:

[1] Duke Clinical Research Unit, Duke University School of Medicine
[2] Faculty of Medicine, Department of Critical Care, University of British Columbia
[3] Loma Linda University, School of Medicine, California
[4] Cardiff Metropolitan University, Cardiff, United Kingdom
[5] Centre for Heart Lung and Vascular Health, University of British Columbia (Okanagan)
[6]. University of Colorado, Boulder, Colorado
[7].Queen's University, Kingston, Ontario
[8] Bangor University, Bangor, Gwynedd, Wales
[9] University of Alberta, Edmonton, Canada
[10] Department of Clinical Neurosciences, Cambridge University, United Kingdom
[11] University of Texas Southwestern Medical Center, Dallas, Texas
[12] Universidad Peruana Cayetano Heredia, Lima, Peru
[13] Neurovascular Research Laboratory, Faculty of Life Sciences and Education, University of South Wales, Glamorgan, UK
[14] University of Texas Arlington, USA

Investigation Sites:

The University of British Columbia, Okanagan Campus; Universidad Peruana Cayetano Heredia, Lima, Peru; Cerro de Pasco, Peru.

1. INVITATION

Please read the following information carefully before deciding to participate in the study. If you have any questions, please do not hesitate to ask. You are being invited to take part in this research study because you are a healthy volunteer free of any cardiovascular or pulmonary disorders, and are joining the research expedition to Cerro de Pasco, Peru, located at 4380m above sea level, in June of 2018.

2. YOUR PARTICIPATION IS VOLUNTARY

Your participation is entirely voluntary. Before you decide to volunteer, it is important for you to understand what this research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study, and the possible benefits, risks and discomforts associated with your participation. If you wish to participate, you will be asked to sign this form. Please take time to read the following information carefully and to discuss it with your family, friends, and doctor before you decide. If you choose not to participate in this study, you will not be penalized in any way. You do not need to disclose why you have chosen not to participate, and you will still receive complete clinical care if your still want to join the research expedition.

3. WHO IS CONDUCTING THE STUDY

The research team includes investigators from the Centre for Heart, Lung and Vascular Health at the University of British Columbia (Okanagan), University of British Columbia (Vancouver campus), University of Colorado, Bangor University, University of Cambridge, University of Texas Southwestern Medical Center, University of Texas Arlington, University of Alberta, the Duke University Medical Center, Cardiff Metropolitan University, University of South Wales, Loma Linda University, and the Universidad Peruana Cayetano Heredia (Lima, Peru). The research is, in part, supported by the National Science and Engineering Research Council of Canada and Canada Research Chairs program.

4. BACKGROUND

Many respiratory and cardiovascular diseases involve exposure to low levels of oxygen (hypoxia), high blood pressure in the lung, and difficulty in breathing. Some examples of these diseases include cerebral stroke, sleep apnea, chronic obstructive pulmonary (lung) disease, and congestive heart failure. Ascent to high altitude provides an excellent means to examine physiological adaptation to acute and chronic hypoxia. Because of the time necessary to study any chronic adaptation (i.e. several weeks of exposure), the profound limitations on quality of life, and related expense, studying the effects of high altitude at sea level using hypobaric (low barometric pressure) or hypoxic chambers is not feasible. This research expedition entails 14 distinct studies that will be performed at sea level (at the Centre for Heart, Lung and Vascular Health) and during ~30 days in Cerro de Pasco, in a laboratory supported by the Universidad Peruana Cayetano Heredia. The 14 studies are outlined in section five (below) and in more detail in section eight of this consent form.

5. WHAT IS THE PURPOSE OF THE STUDY?

Very few studies have taken an integrative (i.e. whole body) research approach to investigating biological changes to acute and chronic hypoxia. These 14 main research studies and related purposes take an integrative approach to study chronic hypoxic physiology, with particular focus on the cerebrovascular functioning (i.e. brain blood flow functioning).

NOTE: YOU WILL BE PERFORMING SOME BUT NOT ALL OF THESE STUDIES. THE INCLUSION OF CERTAIN STUDIES WILL BE ASSIGNED BASED ON AVAILABILITY (WHEN
YOU ARE NOT ACTING AS A RESEARCHER YOURSELF), FOR LOGISTIC PURPOSES. YOU MAY ALSO DECIDE ON WHAT STUDIES YOU MAY WANT OR NOT WANT TO VOLUNTEER FOR.

1. The common carotid artery vasomotor response to the cold pressor test at sea-level and high-altitude in low-landers and Andeans: the role of oxygen

Aim: To determine the effects of altitude on the common carotid artery vasomotor response to a cold pressor test at sea-level and high-altitude in low-landers and high-altitude natives

Synopsis: the common carotid artery blood vessel response to a cold pressor test has established itself as a valuable clinical test to provide information on current cardiovascular health. Currently, there is evidence indicating that blood vessel function is reduced at high-altitude via flow-mediated dilation, but it is unknown if the same is observed during a cold pressor test. Furthermore, the common carotid artery response to the cold pressor test may be

different between high-altitude Andeans with and without CMS, and potentially, this test could be used as a complementary diagnostic test for CMS.
2. Nitric oxide-mediated endothelium-dependent vasodilation in high altitude natives with and without chronic mountain sickness (CMS)
Aim: The purpose of this study is to determine: 1) if nitric oxide-mediated endothelium-dependent vasodilation is impaired in high altitude natives with CMS; and if so 2) whether the impairment in NO-mediated endothelium-dependent is due, at least in part, to increased oxidative stress.
Synopsis: High altitude natives with CMS have been shown to have reduced blood vessel function, however, the mechanisms that govern these changes in blood vessel function have yet to be fully elucidated. Using a sophisticated approach to measure endothelial health at high-altitude, this study aims to determine the mechanisms responsible for reduced blood vessel function associated with CMS.
3. Investigating the role of hemoglobin concentration, plasma volume, and absolute blood volume on cardiac function and exercise capacity in high altitude natives.
Aim: To explore whether hemoglobin mass or absolute blood volume is associated with exercise performance in the Andean population, and whether differences in performance are related to cardiac structure and function.
Synopsis: Data from our Nepal 2016 high-altitude research expedition indicates that exercise performance is directly related to hemoglobin mass at in high-altitude Sherpa. However, it is currently unknown if the same relationship between hemoglobin mass and exercise performance is found in Andean high altitude natives.
4. Sympathetic function in high altitude Andean's with and without chronic mountain sickness
Aim: To investigate sympathetic nervous system activity (SNA) in Peruvian high altitude natives and identify a specific link between sympathetic hyperactivity and elevated pulmonary arterial pressure (PAP).
Synopsis: Currently, the only effective treatment for CMS is to descend from high-altitude; however, this is a challenges for many Andean's living in Cerro de Pasco, since they collect their primary source of income working in the mine located in the heart of Cerro de Pasco. This study will focus on the pulmonary (lung) response to high-altitude exposure in Andean natives, and assess if pharmacological approaches that use sympathetic nervous blockades are viable as a potential treatment route in this population.
5. The effects of oxidative stress on cutaneous vasodilation at sea level and high altitude.
Aim: The purpose of this study is to determine the role of oxidative stress on cutaneous vascular function at sea level and high altitude in lowlanders compared to high altitude residents with and without CMS.
Synopsis: To date, there is little information known on the effects of hypobaric hypoxia (i.e. high-altitude) on conduit blood vessel function, and even less known on the microvasculature (e.g. skin blood vessels). This study aims for the first time determine the effects of high-altitude on skin blood vessel function, and tease out the mechanisms responsible for any observed changes in skin microvascular function.
6. The effects of hemodilution on peripheral and central vascular function in Andeans with CMS.
Aim: The purpose of this study is to quantify the influence of reductions in hematocrit (via hemodilution) on central and peripheral vascular function in Andean high altitude residents with CMS.
Synopsis: Unfortunately, it is common for high-altitude Andean natives living in Cerro de Pasco to suffer from CMS, which is commonly associated with an increase in red blood cell concentration. One of the treatment options available for Andeans suffering from CMS is to remove ~20% of their blood volume, which temporary lowers their hematocrit levels. The objective of this research project is to determine whether blood-letting improves vascular function in the brachial and common carotid arteries.
7. The factors effecting resting and active (exercising) skeletal muscle blood flow through the process of acclimatization, adaptation and maladaptation to high-altitude.
Aim: The purpose of this study is to provide a broad assessment of the integrative factors influencing skeletal muscle blood flow through the process of acclimatization, adaptation and maladaptation to high-altitude.
Synopsis: Upon ascent to high-altitude, nervous activity is dramatically elevated, which has a multitude of downstream effects on the respiratory, cardiovascular, and cerebrovascular system. The objective of this investigation is to characterize the extent of the contribution that the nervous system plays in altering these respiratory and circulatory systems.
8. Pulmonary vascular changes to acute and chronic high altitude hypoxia
Aim: To study pulmonary vascular responses at rest and during exercise, at both low and high altitude in non-acclimatized lowlanders and in Andeans with and without CMS and to compare these responses to those previously observed in Sherpas.

Synopsis: Our data collected in Sherpa upon ascent to high-altitude in October 2016 indicated that the pulmonary vascular response to oxygen was distinctly different between Sherpa that were un-acclimatized to high-altitude, and to those that were living at high-altitude chronically. The primary objective of this investigation is to further explore the mechanisms responsible for high altitude related changes in pulmonary (lung) vascular function, and to determine whether the results found in the Sherpa high altitude natives are similar in high-altitude Andean natives.

9. Endothelial function and shear stress in high altitude Andeans with and without CMS

Aim: To first aim is to characterize resting shear stress patterns and assess endothelial function via flow-mediated dilation in response to transient and sustained elevations in shear stress in high altitude native Andeans with and without CMS. The second aim is to assess the influence of descent and re-ascent to high altitude on endothelial function in Andeans with and without CMS

Synopsis: Brachial artery blood vessel function measured via flow-mediated dilation has been previously assessed in high-altitude Andeans with and without CMS, and it was determined that Andeans with CMS have an impaired blood vessel function compared to Andeans without CMS. However, a major caveat to this previous investigation is that the researchers did not account for differences in blood thickness between the two groups of Andeans. This project intends on determining any differences in blood vessel function between Andeans with and without CMS while additionally accounting for the likely large difference in blood thickness between these two groups.

10. The global cerebral blood flow and intracranial pressure response to hypobaric hypoxia in high altitude Andeans with and without chronic mountain sickness.

Aim: To determine the effects of high altitude on cerebral blood flow and intracranial pressure in high-altitude Andeans with and without CMS.

Synopsis: In October 2016, we determined the global cerebral blood flow and intracranial pressure response (both non-invasive measurements) upon ascent to high-altitude in both low-landers and Sherpa. We intend on repeating a similar experiment in high-altitude Andeans with and without CMS to compare their physiological responses to our existing Sherpa data.

11. The effects of high altitude on neurovascular coupling in low landers and high altitude Andeans

Aim: The purpose of this study is to assess the effects of hypobaric hypoxia on the brain blood flow response to passive reading (i.e. neurovascular coupling) in low-landers and high-altitude native Andeans at 4380m.

Synopsis: After acclimatizing to high-altitude, there have been reports of significant cognitive decline, and although the mechanisms responsible for this reduction in cognitive
performance are likely due to the drastic reductions in the partial pressure of oxygen in the atmosphere, it remains unclear if the brain blood flow response to simple cognitive tasks is impaired while at high altitude. Furthermore, this investigation intends to compare the brain blood flow responses to a simple cognitive test between low-landers and the high-altitude Andean natives.

12. Influence of menstrual cycle on hematology, iron status, and vascular function in high altitude Andeans.

Aim: To describe the hematological and iron status changes during follicular and luteal phases of the menstrual cycle in premenopausal permanent residents of Cerro de Pasco. In the same group, we will assess whether these potential cyclic changes elicit functional changes in the systemic and pulmonary vasculatures, and in clinically-relevant CMS scores.

Synopsis: Recent evidence high-lights the importance of iron status on blood vessel function, however, most of these studies have altered iron status through iron infusions. The natural menses cycle provides the unique opportunity to explore reductions in iron status immediately after menses where red blood cell, hemoglobin, and iron status are likely decreased.

13. Central effects of exercise in children at high altitude

Aim: To determine the cerebral blood flow and ventilatory responses in Andean children at rest and exercise at high-altitude.

Synopsis: In October 2016, our research group was successful in collecting respiratory and brain blood flow responses during exercise in 30+ Sherpa children. Our research objective for the current project is to collect similar exercise data in Andean children and to compare it to our existing Sherpa children data set.

14. The effects of iron supplementation on vascular function between low-landers and high-altitude Andeans.

Aim: To determine the effect of iron supplementation on peripheral vascular function between healthy low-landers and Andean natives at high-altitude.

Synopsis: Iron supplementation has been shown to reduce pulmonary (lung) pressure during exposure to high altitude, thus, it is being considered as a treatment for hypoxia related elevations in pulmonary (lung) pressures.

However, there is also evidence that iron supplementation may reduce blood vessel function assessed via flow-mediated dilation. The objective of this research investigation is to characterize the effects that iron supplementation has on blood vessel function in order to determine its potential as a treatment option for high-altitude related health conditions.

1) 6. WHO CAN PARTICIPATE IN THE STUDY?

Healthy English speaking human volunteers, ages between 18 and 50 years (inclusive), who will be a part of the research team to Cerro de Pasco, Peru, can participate in this study. In total, we will have 20 research subjects. All subjects, however, will not complete each study.

2) 7. WHO SHOULD NOT PARTICIPATE IN THE STUDY?

You will not be permitted to participate in this study if you are below 18 or above 50 years of age, obese (body mass index greater than 30), diabetic, are taking any medications, have a history of smoking or have a history of pulmonary or cardiovascular disease. The investigators will directly assess such exclusion criteria during your initial laboratory visit. A clinician will screen you for systemic hypertension, obstructive coronary artery disease, or structural heart disease, assessed with resting and exercise ECG and echocardiograms. If you suspect you may be pregnant, or are trying to become pregnant, you should not participate in this study. We will be issuing a pregnancy test to females.

Participants will be excluded from studies if taking any nitrate medications. All members will be carefully screened by an independent clinician for co-morbidities, including sleep disordered breathing, systemic hypertension, obstructive coronary artery disease, or structural heart disease, assessed with resting and exercise ECG, and echocardiograms. You will be excluded if they are obese (body mass index greater than 30 kg•m-2), have a history of smoking, or have poor pulmonary function based on spirometry measurements (i.e. FEV1/FVC ratio less than 0.75).

3) 8. WHAT DOES THE STUDY INVOLVE?

The sea-level studies will be conducted in the Integrative Cardiovascular and Respiratory Laboratory at the University of British Columbia (Room 118, Health Science Centre; Okanagan Campus). The research team will then fly to Lima, Peru, and ascend to Cerro de Pasco via automobile. The research team will then be conducting the proposed high-altitude investigations in both low-landers and in high-altitude Andean natives in a laboratory supported by the Universidad Peruana Cayetano Heredia. The research team will then spend ~30 days at the research lab conducting the proposed experiments below.

4) Overview of the Study

Depending on the studies you volunteer for, your participation in this study will involve between two and six visits to the laboratory at sea level in Kelowna, BC (altitude = 344m) prior to your departure to Peru. The first visit will be to ensure you meet the necessary criteria for participation and will involve medical history, pulmonary function testing (spirometry), ultrasound measurements of your heart and arteries, and an exercise stress test on a bicycle. This testing session will last 1-2 hours. The next visits will last 1-6 hours, depending on the study. The specific details of primary measurements that will be performed are detailed below.

5) Procedures of each study

6) The common carotid artery vasomotor response to the cold pressor test at sea-level and high-altitude in low-landers and Andeans: the role of oxygen

You will visit the laboratory on either one or two occasions for the assessment of the common carotid blood vessel response to the cold pressor test (as described below) while 1) breathing room air at sea-level (low-landers); and 2) following acclimatization to 4380m at Cerro de Pasco (low-landers and high-altitude Andeans). The approximate time commitment will be ~one-hour at sea-level, and two-hours at high-altitude.

General Protocol

a. You will be laying supine and breathing normally through a mouthpiece and nose clip.

b. Ultrasound technicians will non-invasively measure the blood flow through your common carotid artery.

c. Your right foot will then be gently placed in a bucket of ice water for three minutes.

d. At sea-level (in low-landers), this protocol will only be completed once while breathing room air. At high-altitude (in low-landers and Andeans), this protocol will be conducted twice, once while breathing room air, and then repeated while breathing 100% oxygen.

Specific Methods
Respiratory and Cardiovascular Measurements: During all procedures we will record tidal volume (size of breath), respiratory frequency, minute ventilation (how many liters of air you breathe per minute), end-tidal O2 and CO2, heart rate, and blood pressure parameters. To do this you will breathe through a mouthpiece with nose clip, will wear electrodes on your chest, and will wear pressure cuffs on your finger and upper arm. All of these procedures are non-invasive.
Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your blood vessels within your brain, and within your neck. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of blood vessels, and measurement of blood flow through arteries.
End-tidal gas control: The partial pressures of oxygen and carbon dioxide in your lungs will be controlled by manipulating the concentrations of those gases you inspire. You will breath through a mouth piece and instructed to avoid any irregular breathing. After a 45 minute break you will repeat this procedure.
7) Nitric oxide-mediated endothelium-dependent vasodilation in high altitude natives with and without chronic mountain sickness
You will visit the laboratory once at high-altitude in Cerro de Pasco. This study aims to recruit high-altitude Andeans with (n=15), and without (n=15) chronic mountain sickness. The approximate time commitment for this investigation will be ~six hours in one visit.
General Protocol
a. You will be instructed to lay down on a hospital bed, and an arterial catheter will be placed into the brachial artery of your non-dominant arm as well as a venous catheter. More specific details on the placement of an arterial catheter is outlined below.
b. In order to assess blood vessel function, several different drugs (in very small doses) will be infused into your forearm through the brachial artery catheter.

Specific Methods
Placement of catheters: Following local anaesthetic (similar to what a dentist would use), we will place one flexible sterile catheter into an artery in your arm. There may be a brief burning or stinging sensation on immediate insertion of the local anaesthetic; afterwards you should experience minimum discomfort. Highly experienced physicians (Drs David MacLeod, Myp Sekhon or Chris Gasho) who are specialists in these techniques will complete all procedures. Strict adherence to full sterile procedures will be followed at all times. The placement of these catheters allows us to deliver the drugs to your arm throughout the experiment. You will not experience any pain, or be aware, when we do this.
8) Investigating the role of haemoglobin concentration, plasma volume and absolute blood volume on cardiac function and exercise capacity in high altitude natives
You will visit the laboratory on two separate occasions at high-altitude in Cerro de Pasco. This study aims to recruit high-altitude Andeans with (n=15), and without (n=15) CMS. The approximate time commitment for this investigation will be a total of ~4 hours on the first laboratory visit, and ~one-hour during the second laboratory visit.
General Protocol
a. The first experimental visit will consist of a max exercise test on a semi-recumbent bicycle.
b. You will then have a three-hour rest period after the exercise, and then you will undergo a blood volume measurement (described in more detail below).
c. The second visit involves a resting measurement of your heart using a non-invasive, painless, ultrasound technique called “echocardiography”.
d. After the resting measurements, you will undergo a sub-maximal exercise test, and ultrasound measurements of your heart will be taken once again.

Specific Methods:
9) Blood volume measurement: You will be seated for 15 minutes before one blood sample is drawn from the forearm to assess baseline concentrations of carbon monoxide in the blood. You will then be fitted with a nose clip and asked to breathe normally through your mouth for 2 minutes. After this you will exhale maximally and we will place a re-breathing mouthpiece in your mouth. You will then inhale maximally and then hold your breath for 10

seconds. Prior to the maximal inhalation, you will be switched on to the rebreathing apparatus, which will contain a calculated volume of carbon monoxide (based on body weight) and a small reservoir (~3-5L) of 100% oxygen. You will rebreathe from this system for 1 min and 50 seconds, after which the mouthpiece is removed and you will resume breathing room air. A second blood sample (2 ml) will be drawn from the forearm at minute 7 (from the start of the procedure). Your expired carbon monoxide levels (ppm) will also be measured by simply expiring onto an analyzer.
Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your heart. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of the chambers in your heart, allowing us to determine the overall function of your cardiovascular system.
10) Sympathetic function in high altitude Andean's with and without CMS
You will visit the laboratory on one occasion at high-altitude in Cerro de Pasco. This study aims to recruit high-altitude Andeans with (n=20), and without (n=20) CMS. The approximate time commitment for this investigation will be a total of ~4 hours.
General protocol:
a. You will be lying supine on a hospital bed, breathing through a mouthpiece and nose clip
b. A very small, tungsten needle (the width of a human hair), will then be gently placed into a nerve in your leg. During this process, you may feel tingle and "pin and needles" sensations.
c. We will then assess how your body responds to changes in blood pressure through a number of brief tests that include infusing drugs that temporarily and safely alter your blood pressure, and exercising by squeezing a small metal bar.

Specific Methods:
11) Rhythmic handgrip exercise flow-mediated vasodilation: This test is also done to assess the function of your blood vessels. You will hold a handgrip device and squeeze it in time to an audio and visual cue for six minutes while the blood flow in your arm is measured using an ultrasound machine.
Respiratory and Cardiovascular Measurements: During all procedures we will record tidal volume (size of breath), respiratory frequency, minute ventilation (how many liters of air you breathe per minute), end-tidal O2 and CO2, heart rate, and blood pressure parameters. To do this you will breathe through a mouthpiece with nose clip, will wear electrodes on your chest, and will wear pressure cuffs on your finger and upper arm. All of these procedures are non-invasive.
Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your heart and blood vessels. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of the chambers in your heart, allowing us to determine the overall function of your cardiovascular system.
Microneurography: A very thin tungsten needle will be inserted into a nerve on your leg, just below your knee. This allows for the measurement of nervous activity that is travelling to your blood vessels, as this activity changes at altitude. Insertion of this needle causes very slight pain and may sometimes cause a tingling or hot sensation in your foot. The needle will remain in place for the duration of the experiment and be removed after the experiment is complete or at any time you wish should you feel discomfort of any kind.
12) The effects of oxidative stress on cutaneous vasodilation at sea level and high altitude
This study aims to recruit low-landers (n=10), high-altitude Andeans with (n=10) and without (n=10) CMS. The approximate time commitment for this investigation will be a total of ~4 hours. Low-landers will complete the protocol on three difference occasions: 1) at sea-level breathing room air, 2) at sea-level breathing hypoxia, and 3) at high-altitude (Cerro de Pasco). High-altitude Andeans will only complete the protocol once while at high-altitude.
General protocol:
a. You will be undergoing a painless procedure that involves mild local heating on the surface of the skin on your arm in four different, very small areas.
b. Each of these small areas will contain a solution that may result in an increase in blood flow in the area. It is highly likely that you will not feel any changes, or sensations, as opposed to mild heating in the areas that we will be experimenting on.

Specific methods:

Skin microdialysis: This technique is used to measure small blood vessel function. A very small tube (1/100th of an inch in diameter) will be placed into the top layer of your skin. The drugs being administered will be through this small tube, and no pain will be associated with their placement.
Cardiovascular measurements: During all procedures we will record heart rate, and blood pressure parameters. To do this you will wear electrodes on your chest, and will wear pressure cuffs on your finger and upper arm. All of these procedures are non-invasive.
Laser Doppler flowmetry: This method is used to measure blood flow in the skin. A laser is emitted from the end of a probe, and the reflection of the laser off of blood cells in your small blood vessels records information on the amount of blood flowing through your small blood vessels. This technique is harmless, and you will not feel anything.
Skin heating: Skin temperature will be increased in specific sites on your arm. During this time, you will feel those areas on your arm warming up, however, the sites that will be heated will be smaller than the size of a quarter, and it will not be painful.
13) The effects of hemodilution on peripheral and central vascular function in Andeans with CMS.
This study aims to recruit high-altitude Andeans with CMS (n=10). The approximate time commitment for this investigation will be a total of ~one hour on four separate days.
General protocol:
a. You will be laying in the supine position breathing through a mouthpiece and nose-clip
b. You will then do two separate tests that measure blood vessel health, separated by 10 minutes.
c. On the second visit to the laboratory, your blood volume will be measured, and then 20% of your blood volume will be removed.
d. You will then visit the laboratory two more times, on days 3 and 7 after blood removal. On both days 3 and 7, we will then re-assess blood vessel health using the same protocol.

Specific Methods:
14) Blood volume measurement: You will be seated for 15 minutes before one blood sample is drawn from the forearm to assess baseline concentrations of carbon monoxide in the blood. You will then be fitted with a nose clip and asked to breathe normally through your mouth for 2 minutes. After this you will exhale maximally and we will place a re-breathing mouthpiece in your mouth. You will then inhale maximally and then hold your breath for 10 seconds. Prior to the maximal inhalation, you will be switched on to the rebreathing apparatus, which will contain a calculated volume of carbon monoxide (based on body weight) and a small reservoir (~3-5L) of 100% oxygen. You will rebreathe from this system for 1 min and 50 seconds, after which the mouthpiece is removed and you will resume breathing room air. A second blood sample (2 ml) will be drawn from the forearm at minute 7 (from the start of the procedure). Your expired carbon monoxide levels (ppm) will also be measured by simply expiring onto an analyzer.
Hemodilution via venesection: During your visit to the laboratory in Cerro de Pasco 15% of your total measured blood volume will be removed, and afterward, an equivalent amount of saline will be infused into your through a venous catheter to restore your blood volume. An intensive care physician will closely monitor you during the entire study and if at any time your heart rate or blood pressure varies too much your blood will be immediately re-infused and the study stopped. If at any time you feel uncomfortable or simply wish to stop the study your blood will be immediately re-infused and the study will be stopped.
Flow mediated dilation: This test is done to assess the function of your blood vessels. A blood pressure cuff will be placed around your left forearm, and pumped up to a level that will reduce blood flow for 5-minutes. After 5-minutes the cuff pressure will be released, and using an ultrasound machine, we will monitor the return of blood flow in the arm.
Respiratory and Cardiovascular Measurements: During all procedures we will record tidal volume (size of breath), respiratory frequency, minute ventilation (how many liters of air you breathe per minute), end-tidal O2 and CO2, heart rate, and blood pressure parameters. To do this you will breathe through a mouthpiece with nose clip, will wear electrodes on your chest, and will wear pressure cuffs on your finger and upper arm. All of these procedures are non-invasive.
Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your blood vessels within your brain, and within your neck. Ultrasound emits very high frequency sound

(which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of blood vessels, and measurement of blood flow through arteries.
End-tidal gas control: The partial pressures of oxygen and carbon dioxide in your lungs will be controlled by manipulating the concentrations of those gases you inspire. You will breath through a mouth piece and instructed to avoid any irregular breathing. After a 45 minute break you will repeat this procedure.
15) The factors effecting resting and active (exercising) skeletal muscle blood flow through the process of acclimatization, adaptation and maladaptation to high-altitude
This study aims to recruit low-landers (n=10), high-altitude Andeans with (n=10), and without CMS (n=10). The approximate time commitment for this investigation will be a total of ~5 hours. Low-landers will be tested on four separate occasions: 1) sea-level (Kelowna, BC), 2) week one at high-altitude (Cerro de Pasco), 3) week two at high-altitude, and 4) week three at high-altitude. The high-altitude natives (both healthy and those with CMS) will be tested on one occasion at high-altitude.
General protocol:
a. You will complete a maximal exercise test at both sea-level and high-altitude.
b. You will then undergo a series of exercise tests that include handgrip exercise, which involves squeezing a metal bar, and bicycle exercise.
c. During these tests, measurements of your nerve activity and blood flow in one of your blood vessels will be recorded (explained in more detail below).
d. These tests will be repeated after the administration of a drug that are used to treat high blood pressure. During this time, you may feel symptoms such as light-headedness, and nausea; however, medical professionals will be monitoring you closely.

Specific methods:
16) Rhythmic handgrip exercise flow-mediated vasodilation: This test is also done to assess the function of your blood vessels. You will hold a handgrip device and squeeze it in time to an audio and visual cue for six minutes while the blood flow in your arm is measured using an ultrasound machine.
Respiratory and Cardiovascular Measurements: During all procedures we will record tidal volume (size of breath), respiratory frequency, minute ventilation (how many liters of air you breathe per minute), end-tidal O2 and CO2, heart rate, and blood pressure parameters. To do this you will breathe through a mouthpiece with nose clip, will wear electrodes on your chest, and will wear pressure cuffs on your finger and upper arm. All of these procedures are non-invasive.
Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your heart and blood vessels. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of the chambers in your heart, allowing us to determine the overall function of your cardiovascular system.
Microneurography: A very thin tungsten needle will be inserted into a nerve on your leg, just below your knee. This allows for the measurement of nervous activity that is travelling to your blood vessels, as this activity changes at altitude. Insertion of this needle causes very slight pain and may sometimes cause a tingling or hot sensation in your foot. The needle will remain in place for the duration of the experiment and be removed after the experiment is complete or at any time you wish should you feel discomfort of any kind.
Placement of catheters: Following local anaesthetic (similar to what a dentist would use), we will place one flexible sterile catheter into an artery in your arm. There may be a brief burning or stinging sensation on immediate insertion of the local anaesthetic; afterwards you should experience minimum discomfort. Highly experienced physicians (Drs David MacLeod, Myp Sekhon or Chris Gasho) who are specialists in these techniques will complete all procedures. Strict adherence to full sterile procedures will be followed at all times. The placement of these catheters allows us to deliver the drugs to your arm throughout the experiment. You will not experience any pain, or be aware, when we do this.
Muscle microdialysis: This technique is used to measure function of muscle tissue. A very small tube (1/100th of an inch in diameter) will be placed into the muscle in your thigh. The drug being administered will be through this small tube. A small amount of pain may be associated with the placement of this needle into the muscle tissue, similar to what you might feel when getting blood drawn when donating blood.
17) Pulmonary vascular changes to acute and chronic high altitude hypoxia

This study aims to recruit low-landers (n=10), high-altitude Andeans with (n=10), and without CMS (n=10). Additionally, high-altitude Andeans whom have been de-acclimatized at sea-level for ~10 days will be recruited (n=10). The approximate time commitment for this investigation will be a total of ~two hours. Low-landers will be tested at sea-level, upon ascent to high-altitude, and at high-altitude (Cerro de Pasco). High altitude Andeans will be tested upon ascent to high-altitude and at high-altitude.
General protocol:
a. You will be laying supine on a bed, and ultrasound images of your heart will be taken. b. You will then be administered oxygen through a breathing mask, and the same ultrasound images will be recorded once again. c. After a 15 minute recovery period breathing room air, you will be asked to exercise on a bicycle, and the same images will be repeated. d. Following a one-hour rest period, you will be asked to do the same exercise protocol, but you will be administered oxygen once again through a breathing mask. The same heart measurements will be repeated. Specific methods: Respiratory and Cardiovascular Measurements: During all procedures, we will continuously record blood pressure, pulse, oxygen saturation, respiratory rate, EKG, end-tidal O2 and CO2. To do this you will wear electrodes on your chest, and will wear pressure cuffs on your finger and upper arm. All of these procedures are non-invasive. Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your blood vessels within your chest and heart. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of blood vessels, and measurement of blood flow through arteries. In addition the use of a contrast agent which utilizes small bubbles will be administered to improve the image quality and perform further calculations.
18) Endothelial function and shear stress in high-altitude Andeans with and without CMS
This study aims to recruit high-altitude Andeans with (n=15), and without CMS (n=15). The approximate time commitment for this investigation will be a total of ~one-hour. High altitude Andeans will be tested upon ascent to high-altitude and at high-altitude.
General protocol:
a. You will be asked to lay down in the supine position on a bed, and relax for 30 minutes prior to testing
b. You will then undergo two protocols to assess blood vessel function, separated by 10-minutes. Each protocol is painless, and uses Doppler ultrasound to measure both blood vessel diameter and blood flow.

Specific methods:
Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your blood vessels. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of blood vessels, and measurement of blood flow through arteries.
19) Rhythmic handgrip exercise flow-mediated vasodilation: This test is also done to assess the function of your blood vessels. You will hold a handgrip device and squeeze it in time to an audio and visual cue while the blood flow in your arm is measured using an ultrasound machine.
Flow mediated dilation: This test is done to assess the function of your blood vessels. A blood pressure cuff will be placed around your left forearm, and pumped up to a level that will reduce blood flow for 5-minutes. After 5-minutes the cuff pressure will be released, and using an ultrasound machine, we will monitor the return of blood flow in the arm.
20) The global cerebral blood flow and intracranial pressure response to hypobaric hypoxia in high-altitude Andeans with and without CMS.
This study aims to recruit high-altitude Andeans with (n=15), and without chronic mountain sickness (n=15). The approximate time commitment for this investigation will be a total of ~one-hour. High altitude Andeans will be tested upon ascent to high-altitude and at high-altitude.
General protocol:
a. You will be asked to lay down in the supine position on a bed, and asked to rest for 30 minutes b. After 30-minutes you will ultrasound measurements completed on two blood vessels in your neck, and shortly afterwards, you will have another ultrasound measurement done gently over a closed eye-lid. Specific methods:
b. Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your blood vessels. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of blood vessels, and measurement of blood flow through arteries.

Optic nerve sheath diameter: an ultrasound probe will be placed over your eye to measure the diameter of your optic nerve. This is a painless and safe procedure that is regularly carried out clinically.

21) The effects of high-altitude on neurovascular coupling in low-landers and high-altitude Andeans

This study aims to recruit low-landers (n=10), high-altitude Andeans with (n=10), and without CMS (n=10). The approximate time commitment for this investigation will be a total of ~one-hour for each session. Low-landers will be tested at both sea-level and high-altitude, while high altitude Andeans will be tested at high-altitude.

General procedures:

a. You will be asked lay down in the supine position and breath through a mouthpiece and nose clip.

b. We will then place ultrasound probes on the side of your head to measure blood flow in your brain, and ask you do complete a protocol that involves you opening and closing your eyes in sync with audio cues.

Specific methods:

Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your blood vessels. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of blood vessels, and measurement of blood flow through arteries.

Respiratory and Cardiovascular Measurements: During all procedures we will record tidal volume (size of breath), respiratory frequency, minute ventilation (how many liters of air you breathe per minute), end-tidal O2 and CO2, heart rate, and blood pressure parameters. To do this you will breathe through a mouthpiece with nose clip, will wear electrodes on your chest, and will wear pressure cuffs on your finger and upper arm. All of these procedures are non-invasive.

End-tidal gas control: The partial pressures of oxygen and carbon dioxide in your lungs will be controlled by manipulating the concentrations of those gases you inspire. You will breath through a mouth piece and instructed to avoid any irregular breathing. After a 45 minute break you will repeat this procedure.

22) Influence of menstrual cycle on hematology, iron status, and vascular function in high altitude Andeans

This study aims to recruit 15 healthy female high-altitude Andeans. The approximate time commitment for this investigation will be a total of ~one-hour. High altitude Andeans will be tested upon ascent to high-altitude and at high-altitude.

General protocol:

a. You will be asked to specific information on the recent history of your menstrual cycle, and you will be asked to use an ovulation kit that will give us information on the hormone levels in your body.

b. In between 3-7 days after your last menstruation, you will be asked to come into the lab in Cerro de Pasco and lay down in the supine position on a bed, and relax for 30 minutes prior to experimentation.

c. You will then undergo two protocols to assess blood vessel function, separated by 10-minutes. Each protocol is painless, and uses Doppler ultrasound to measure both blood vessel diameter and blood flow.

Specific methods:

Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your heart and blood vessels. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of blood vessels, and measurement of blood flow through arteries.

Flow mediated dilation: This test is done to assess the function of your blood vessels. A blood pressure cuff will be placed around your left forearm, and pumped up to a level that will reduce blood flow for 5-minutes. After 5-minutes the cuff pressure will be released, and using an ultrasound machine, we will monitor the return of blood flow in the arm.

23) Central effects of exercise in children at high altitude

This study aims to recruit 60 healthy high-altitude Andeans children between the ages 7-14 years old. The approximate time commitment for this investigation will be a total of ~two-hours.

General protocol:

a. Upon arrival at the lab, we will be recording simple parameters such as height, weight, age.

b. You will then be asked to perform a maximal effort exercise test. During the exercise test, we will be measuring blood flow in your brain using ultrasound, and your how much you are breathing by collecting data through a mask.

Specific methods:
Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your blood vessels. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of blood vessels, and measurement of blood flow through arteries.
Respiratory and Cardiovascular Measurements: During all procedures we will record tidal volume (size of breath), respiratory frequency, minute ventilation (how many liters of air you breathe per minute), end-tidal O2 and CO2, heart rate, and blood pressure parameters. To do this you will breathe through a mouthpiece with nose clip, will wear electrodes on your chest, and will wear pressure cuffs on your finger and upper arm. All of these procedures are non-invasive.
24) The effects of iron supplementation on vascular function between low-landers and high-altitude Andeans.
This study aims to recruit healthy high-altitude Andeans without chronic mountain sickness (n=20). The approximate time commitment for this investigation will be ~6 hours on the first experimental session, and then ~one-hour for the next three session. Both Low-landers and high altitude Andeans will be tested only at high-altitude.
General protocol:
a. You will be asked to lay down in the supine position and rest for 30 minutes
b. Two tests will then be administered: a flow-mediated dilation test, and a cold pressor test, both provide information on blood vessel health
c. Immediately afterwards, you will be infused with iron sucrose over ~4 hours into a vein located on your arm.
d. After the infusion is complete, we will then repeat the experimental protocols on you that same day.
e. You will then be asked to come back to the laboratory for the following three days, where we will repeat the protocol once again.

Specific methods:
Flow mediated dilation: This test is done to assess the function of your blood vessels. A blood pressure cuff will be placed around your left forearm, and pumped up to a level that will reduce blood flow for 5-minutes. After 5-minutes the cuff pressure will be released, and using an ultrasound machine, we will monitor the return of blood flow in the arm.
Doppler Ultrasound: We will be using non-invasive tests to assess the health and function of your blood vessels within your brain, and within your neck. Ultrasound emits very high frequency sound (which you cannot hear), then records the resultant echoes from the tissue and moving red blood cells. This allows imaging of blood vessels, and measurement of blood flow through arteries.
End-tidal gas control: The partial pressures of oxygen and carbon dioxide in your lungs will be controlled by manipulating the concentrations of those gases you inspire. You will breath through a mouth piece and instructed to avoid any irregular breathing. After a 45 minute break you will repeat this procedure.
9. WHAT ARE THE POSSIBLE HARMS AND DISCOMFORTS?
A physician will be either on-sight (studies involving arterial catheters; Drs Macleod, Gasho and/or Sekhon) or on-call during all experimental sessions should any complications arise. In the unlikely event of any complication, such as cardiac arrest or syncope (fainting), an emergency medical response will be immediately initiated. All investigators are certified to perform cardiopulmonary resuscitation and in the use of an automated external defibrillator and will follow standard emergency protocols. However, complications are very unlikely given the rigorous screening you will first undertake prior to admission to the study.
You are asked to report any unusual symptoms during each of the tests. You can stop any test at any time if you are feeling uncomfortable. Every effort will be made to conduct the tests in such a way to minimize discomfort and risk.
Female participants in this study must avoid pregnancy. Failure to do so may result in potential harm to your fetus. You should discuss the issues surrounding this necessity (of not being or becoming pregnant during the course of the study) with your study doctors, and find an acceptable solution that will address this matter.
It must be noted that individual responses to the experimental procedures exist and you are encouraged to report any unusual sensations or symptoms to the investigator. You are permitted to end testing at any time for any reason. If you do experience undesirable symptoms during the experiment, the onsite physician(s) will provide immediate care. All procedures used to collect physiological data will pose no risk to your continued health and well-being.

These procedures have been performed around the world since the 1960's. However, the following risks should be considered:
Arterial catheters: A catheter is a thin plastic tube placed in a blood vessel. Qualified, highly experienced physicians who are specialists in such procedures will make all the arterial and venous catheterizations. These procedures are part of routine clinical care for patients in intensive care, but differ from those you would normally expect to receive in normal clinical care. There is typically brief pain on immediate insertion of the local anaesthetic. Once the needle is in place, the pain should subside. Blood draws through the needles should not be painful, and there should only be minor swelling at the site. At the end of the study, the needle will be withdrawn and a sterile dressing will be applied.
The most common complications of inserting a small needle into an artery is a small bruise and pain at the site of the needle location, which may last several days after removal of the catheter. Other complications of radial artery canalization include temporary occlusion/spasm, permanent ischemia, pseudoaneurysm, thrombosis, AV fistula, air embolism, compartment syndrome, carpal tunnel syndrome, and median nerve paralysis. These are very rare however, and these risks will be minimized by; 1) the use of ultrasound; 2) having an experienced physician (Dr. Sekhon), and; 3) adhering to strict aseptic techniques. The latter includes full surgical scrub, and the use of chlorohexidine or iodine-based cleaning solutions and sterile towels. Having lines in situ for short periods of time (<5 h) further minimizes risks.
Approximately 20 mL (4 teaspoons) of blood will be withdrawn over the course of the experiment in order to measure arterial blood concentration of oxygen and carbon dioxide. Two mL of blood will be frozen for subsequent analysis of standard markers of metabolism; any remaining blood will be discarded immediately using normal bio-safety procedures. Experienced physicians who have appropriate resources available to manage most complications will supervise experiments. In the unlikely event of an emergency that cannot be adequately managed in Cerro de Pasco, there are two nearby hospitals within ~20 minutes' drive of the laboratory. However, as in our normal operating procedures, we will be largely self-contained: For example, we will have ample amounts of oxygen and advanced resuscitation drugs will be available. All experiments will be supervised by experienced ICU physician Dr Sekhon, Dr Gasho and Dr MacLeod (senior staff anesthetist). These experienced physicians have carried out >1000 of these procedures with no adverse-effects, including identical interventions during our Nepal research expeditions in 2012 and 2016.
Acute Hypoxia at Sea-level: There are few risks associated with mild exposures to high altitude, a condition that will be simulated in this experiment. The level of low oxygen (hypoxia) that you will be exposed to is equal to approximately 4000 m. This is approximately equal to being at the summit of Pike's Peak, Colorado. At this level of simulated high altitude you will breathe more quickly and more deeply. You may feel shortness of breath, dizzy or faint, and you may develop a temporary headache. These sensations will go away very quickly when you breathe room air. Your responses to the exposures to low levels of oxygen will be monitored during the test, and the test will be terminated if abnormal responses are observed (not anticipated). There is no risk of developing altitude illness. You may feel discomfort from lying in the same position for two to four hours. These discomforts will be alleviated once the testing is terminated and you are permitted to move around.
Ultrasound: Ultrasound is non-invasive, painless technique used for measuring blood flow in this study. It poses no risk.
Exercise in normoxia or hypoxia: The American Thoracic Society and the American College of Chest Physician statements associated with cardiopulmonary testing for patients with respiratory and cardiovascular disease (ATS/ACCP Statement on Cardiopulmonary Exercise Testing (2001), AJRCCM, pp. 213) state that the estimated risk of sudden cardiac death in testing in patients being assessed for a variety of medical reasons is stated to be 2 to 5 per 100,000 tests. No prospective study has ever been performed to assess the risk of exercise in any disease, however, this statement comes from a review of all studies that have provided estimates of sudden cardiac death from 100,000s of tests performed in medical centers across the World assessed in patients with high risk of cardiovascular complications to exercise. In general, exercise testing is considered extremely safe in healthy individuals, but risk of sudden cardiac death is higher in people with history of cardiovascular disease. As stated in our exclusion criteria, any participants with cardiovascular or cerebrovascular complications will not be eligible to participate in this study and as such the risk of an adverse event is considered extremely low.
Ascent to high altitude and altitude illness: The planned ascent to the laboratory in Cerro de Pasco will be done over a period of a day via automobile, starting the ascent from Lima, Peru. The risk of moderate to severe acute mountain sickness is less than 15%, however, low-lander participants will be taking oral acetazolamide prior, and during

ascent to reduce the risk of acute mountain sickness. The risk of High Altitude Pulmonary or Cerebral Edema is less than 0.05%. Normally high altitude medications (e.g., acetazolamide, dexamethasone, etc) and oxygen will be available at all times in case of an emergency. The Principal Investigator, Prof. Philip Ainslie, will also be carrying a regular cell phone at all times in case arrangements at the local hospital need to be made. Finally, as outlined, participants will have detailed physiological monitoring during their first 48 hours at high altitude; this monitoring will allow for any early detection of any serious AMS complications. Please note that should you need emergency evacuation back down to Lima, Peru, and depending on the severity of the injury you will be accompanied by one of the team physicians or researchers. We will make sure you have made full recovery before you travel home.
Blood volume measurement: There have been few, if any, complications for those participating in this test. A small amount of pain may be associated with routine blood sampling from a vein and skin puncture. It is possible that some individuals may experience lightheadedness, fainting, and/or nausea from the blood collections. You may experience localized bruising and swelling at or near the puncture site. You may also experience petechiae (small, non raised red spots on skin) where the tourniquet was applied. Occasionally, some individuals are allergic to the antiseptic used in skin preparation, the glue used in adhesive bandages, or latex. In this case, an alternate antiseptic, paper tape, and non-latex gloves will be used. Carbon monoxide is known to compete strongly for oxygen binding sites in the blood. This attribute is the basis on which the CO-rebreathe technique has been developed. This 'optimised' procedure has been further refined to reduce exposure in terms of the total quantity (~100ml) of carbon monoxide to which you will be exposed. The blood concentration will be minimal (5%), which is related to a small (3%) reduction in your maximal oxygen consumption. The CO has a short half-life of 90 minutes. This study should not leave you with any long-term adverse effects. Qualified technicians will be present for all the testing sessions to reduce discomfort and monitor your condition.
Hemodilution via venesection: Blood volume removal has previously been well tolerated in similar studies in both healthy volunteers and CMS patients. The risks associated with blood volume removal in this study are therefore only related to changes in heart rate and blood pressure. The blood pressure and heart rate will be continuously monitored by the research team and if any significant changes occur the study will be stopped, and you will be given oxygen by facemask and saline to restore your blood volume.
α-adrenoreceptor and □-adrenoreceptor blockers, Phenotolamine and Propranolol: With intravenous administration of these drugs, participants may feel may feel faint and potentially nauseas, due to an expected decrease in blood pressure. The most common side-effect symptoms of these two drugs include mild diuresis (increased urine production and therefore need to urinate), a mild decrease in blood pressure (hypotension), or feelings of dizziness and sleepiness. After administration of these drugs, participants will remain in supine position to reduce the risk of hypotension. In any case, a researcher will be available to assist the volunteer should begin to feel faint from the hypotensive effect of the drug intervention. The small and acute dose of drug we are using in this study means that any side effects are very short lasting; 2-3 hours. All participants will be continually and closely monitored.
Cold Pressor Test: During the CPT participants may feel slight discomfort and mild pain having their foot submerged in cold-ice water for 120 seconds. These symptoms will alleviate shortly after removal from ice bath
10. WHAT ARE THE BENEFITS OF PARTICIPATING IN THIS STUDY?
You will not directly benefit from this study. However, you will gain information regarding your physiological makeup, including the structure and function of vessels that feed your brain, and your unique tolerance to heat stress.
11. WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO PARTICIPATE?
You may withdraw from this study at any time without giving reasons. If you choose to enter the study and then decide to withdraw at a later time, you have the right to request the withdrawal of your information collected during the study. This request will be respected to the extent possible. Please note however that there may be exceptions where the data will not be able to be withdrawn for example where the data is no longer identifiable (meaning it cannot be linked in any way back to your identity) or where the data has been merged with other data. If you would like to request the withdrawal of your data, please let your study doctor know.
12. AFTER THE STUDY IS FINISHED
The tests performed in this study are not intended to be diagnostic and are not performed under diagnostic conditions. However, if any medical issue (incidental finding) is presumed, you will be notified. You will be recommended to contact your medical doctor, and we will provide you with a written letter detailing our observations. If the information is thought to be serious by the research physician (Drs. Anholm, Gasho, Sekhon or MacLeod), we will follow the emergency procedure (see below), which may involve contacting emergency service

and transporting you to the emergency department at Kelowna General Hospital (for sea level testing) or Cerro de Pasco hospital at high altitude.

13. WHAT WILL THE STUDY COST ME?

You will not be paid for participation in this study. Transportation to Cerro de Pasco from Lima, Peru will be covered.

14. WILL MY TAKING PART IN THIS STUDY BE KEPT CONFIDENTIAL?

Your confidentiality will be respected. However, research records and health or other source records identifying you may be inspected in the presence of the Investigator, Health Canada, UBC Clinical Research Ethics Board, and the Natural Sciences and Engineering Research Council of Canada (NSERC; the funding agency) for the purpose of monitoring the research. No information or records that disclose your identity will be published without your consent, nor will any information or records that disclose your identity be removed or released without your consent unless required by law.

You will be assigned a unique study number as a subject in this study. Only this number will be used on any research-related information collected about you during the course of this study, so that your identity [i.e. your name or any other information that could identify you such as your Personal Health number] as a subject in this study will be kept confidential. Information that contains your identity will remain only with the Principal Investigator and/or designate. The list that matches your name to the unique study number that is used on your research-related information will not be removed or released without your consent unless required by law.

Your rights to privacy are legally protected by federal and provincial laws that require safeguards to insure that your privacy is respected and also give you the right of access to the information about you
that has been provided to the sponsor and, if need be, an opportunity to correct any errors in this information. Further details about these laws are available on request to your study doctor.

A trained research assistant will be available on every occasion to explain the procedure and answer any questions.

Disclosure of Race/Ethnicity: Studies involving humans now routinely collect information on race and ethnic origin as well as other characteristics of individuals because these characteristics may influence how people respond to different medications. Providing information on your race or ethnic origin is voluntary.

15. WHAT HAPPENS IF SOMETHING GOES WRONG?

By signing this form, you do not give up any of your legal rights and you do not release the study doctor, participating institutions, or anyone else from their legal and professional duties. If you become ill or physically injured as a result of participation in this study, medical treatment will be provided on-site by one of the expedition intensivists (James Anholm, Myp Sekhon, Prajan Subedhi). If you require evacuation and further medical care the expedition and University of British Columbia Okanagan will pay for any costs associated with your medical treatment that are not covered by travel insurance.

In an event of a medical emergency, Drs. MacLeod, Gasho, Sekhon, and Anholm will guide the research team.

25) In the event of emergency during sea level testing:

• The individual present with the highest level of medical training will guide the research team – this will very likely be one of the critical care physicians listed above, unless the medical emergency takes place outside of an experimental session.

• A member of the research team will dial 911 on the laboratory phone and contact emergency services for their help.

• A second member of the research team will dial campus security and summon a university-designated first aid to attendant the research laboratory. The first aid dispatch is ~1min walk to the research laboratory. These first aid attendants are equipped with a Level 2 kit including oxygen and an automated external defibrillator, and can assist with interim treatment while waiting for emergency services. They will also facilitate transport of emergency services to the building.

• The research laboratory is 15-20 minutes away from the emergency department at Kelowna General Hospital, and one of the research team will accompany the participant at all times

26) In the event of emergency during high altitude testing:

• The high altitude physicians will provide acute care (Drs Sekhon, Gasho, Aholm,). All first aid amenities, including an automated external defibrillator, will be on site. The physicians will also be equipped with a standard emergency room crash cart (including epinephrine, oxygen etc.).

• If required, study participants can be transported to the hospital in Cerro de Pasco located within 20 minutes of the research laboratory.

16. WHO DO I CONTACT IF I HAVE QUESTIONS ABOUT THE STUDY DURING MY PARTICIPATION?
If you have any questions or desire further information about this study before or during participation, or if you experience any adverse effects, you can contact Dr. Phil Ainslie at 001-250-807-8089. In the event of a research related injury post the experimental testing, please speak to your doctor and contact the Dr. Phil Ainslie about the event on the above number.
17. WHO DO I CONTACT IF I HAVE ANY QUESTIONS OR CONCERNS ABOUT MY RIGHTS AS A SUBJECT DURING THE STUDY?
If you have any concerns or complaints about your rights as a research subject and/or your experiences while participating in this study, contact the Research Participant Complaint Line in the University of British Columbia Office of Research Ethics by e-mail at RSIL@ors.ubc.ca or by phone at 604-822-8598 (Toll Free: 1-877-822-8598).
18. SUBJECT CONSENT TO PARTICIPATE
Global Research Expedition on Altitude related Chronic Health (Global REACH): a scientific expedition to the Andean mountains
In signing this form you are consenting to participate in this research project. Furthermore, signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.
• I have read and understood the subject information and consent form.
• I have had sufficient time to consider the information provided and to ask for advice if necessary.
• I have had the opportunity to ask questions and have had satisfactory responses to my questions.
• I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
• I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.
• I understand that I am not waiving any of my legal rights as a result of signing this consent form.
• I understand that there is no guarantee that this study will provide any benefits to me
• I have read this form and I freely consent to participate in this study.
• I have been told that I will receive a dated and signed copy of this form.
• I understand the procedure that will be followed in an event of a medical emergency

SIGNATURES

_____
Printed name of subject Signature Date

_____
Printed name of witness Signature Date

_____
Printed name of principal investigator/
designated representative Signature Date

Appendix III: Sample "Day Of" Sheet and checklists for protocol

Daily Pressure (mmHg) _____ Temperature _____

Date of birth_____ Age _____

Height_____ Weight_____

Hours of sleep_____ Sleep quality_____

Time since last meal_____ Size of last meal_____

Time since last beverage_____ Size of last beverage_____

Gum: Y / N Smoking: Y / N

Females Only: First date of last menstruation_____

Blood sample: Y / N, Fasted: Y / N Duration of Fast:_____

Time of blood sample:_____ Freezer Location:_____

Urine sample: Y / N Time of evening urination_____

Time of morning urination_____ Duration of urinary

collection_____

Y / N Abstained from caffeine for the past 12 hours, if not how long

_____

Y / N Abstained from alcohol for the past 12 hours, if not how long

_____

Y / N Abstained from strenuous exercise for the past 12 hours, if not how long

_____

Y / N Any changes in personal or familial history of HHQ? If yes, complete new form.

Forms Filled Out:

Y / N Consent Form **Urine Collection:**

_____

Y / N Health History Questionnaire **Bloods:**

_____

Y / N AMS Score (Post-Test) **Aliquot:**

_____

Baseline Measures

Automated BP			
Heart Rate			
Arterial Saturation			
Urine Volume			
Urine Creatinine			
Urine Microalbumin			

iStat

	CHEM8+
Sodium	
Potassium	
Chloride	
Anion Gap	
Ionised Calcium	
Glucose	
Urea Nitrogen	
Creatinine	
Haematocrit	
Haemoglobin	
TCO_2 (measured)	

ULTRASOUND & CVP

Sonographer _____ VIVID ID

_____

Renal US

LabChart Cmd	**Image**	**#**	Depth	FPS	Remarks
F4	RA Diameter				
F5	RA Flow				

Probe: *Abdominal* Position:

_____

Echocardiography

LabChart Cmd	**Image**	**#**	Depth	FPS	Remarks
F6	IVC Diameter				
F7	PW Hepatic Vein Flow				
F8	LV 4-Ch				
F9	LV 2-Ch				
F10	RV 4-Ch				
F11	PW Tricuspid Inflow				
F12	PW TDI – Septum				

Probe: *Cardiac* Position:

_____

Appendix V: Health History Questionnaire

Date of Birth: _____/_____/_____ Height: _____ Weight: _____

Ethnic Background: _____ Family Physician: _____

ALL PARTICIPANTS

Please check any and all that apply Personal History Family History

Stroke □ □

Hypertension □ □

Heart Attack □ □

Heart Murmur □ □

Blood clots □ □

Anemia □ □

Congestive Heart Failure or Heart Failure □ □

Other cardiovascular disorders (please specify) □ □

_____

Personal History Family History

Type I Diabetes □ □

Type II Diabetes □ □

Obesity □ □

Circulation Disease in legs □ □

Protein in Urine □ □

Other metabolic disorders (please specify) □ □

_____

Personal History Family History

Asthma □ □

Sleep Apnea □ □

COPD □ □

Other respiratory/breathing disorders (please specify) □ □

_____

Personal History Family History

Alzheimers □ □

Cognitive impairment □ □

Parkinsons □ □

ALS (Lou Gerhigs Disease) □ □

Seizures □ □

Other neurological disorders (please specify) □ □

_____

Yes No

Any other major surgery, illness or injury not listed above? □ □

(If yes, please Specify)

_____

Yes No Unknown

Were you born pre-mature (before 37wks) □ □ □

Yes No

Do you smoke? □ □

(If yes, how many cigarettes per day?)

_____

(If you have quit, how long since your last cigarette?)

_____

Yes No

Have you ever fainted before? □ □

(If yes, under what circumstances?)

_____

Yes No

Are you currently taking any medications? □ □

(If yes, please list medications)

_____

_____

_____

What have your eating habits been like in the past month? Check all that apply:

□ One meal per day, specify when _____

□ Two meals per day, specify when _____

□ Three meals per day

□ Snack(s) every day, specify when _____

□ Special diet, please specify name _____

□ Trying to follow Canada's Food Guide to Healthy Eating

□ Other nutrition plan, please specify _____

Strenuous Intensity (heart beats rapidly; e.g. running, jogging, vigorous swimming, vigorous long distance cycling).

During a typical 7-Day period (a week) in the past year, in your leisure time, how often do you engage in any regular activity long enough to work up a sweat (heart beats rapidly)? often sometimes never/rarely

WOMEN

Please check any and all that apply

Yes No

Are you post-menopausal? □ □

(If not, how long since the first day of your last period?)

_____

Yes No

Chapter 1 Are you on hormone replacement therapy? □ □

Yes No

Are you currently using oral contraceptives? □ □

(If yes, what is the brand?)

_____

Yes No

Are you pregnant? □ □

(If yes, how many weeks?)

_____

Yes No

Have you been pregnant previously? □ □

(If yes, please indicate the number of previous pregnancies.

Were there any complications, including pregnancy related hypertension, gestational diabetes, or pre-eclampsia?)

_____

_____

Do you have any other health concerns you think we should be aware of?

_____

_____

Appendix VI: The Kidney Disease Questionnaire

Do You Have Kidney Disease? Take This Test and Know Your Score.

Find out if you might have silent chronic kidney disease now. Check each statement that is true for you. **If a statement is not true or you are not sure, put a zero.** Then add up all the points for a total.

- Age:
  1. I am between 50 and 59 years of age.....Yes 2 _____
  2. I am between 60 and 69 years of age.....Yes 3 _____
  3. I am 70 years old or older.....Yes 4 _____
- I am a woman.....Yes 1 _____
- I had/have anemia.....Yes 1 _____
- I have high blood pressure .....Yes 1 _____
- I am diabetic.....Yes 1 _____
- I have a history of heart attack or stroke .....Yes 1 _____
- I have a history of congestive heart failure or heart failure .....Yes 1 _____
- I have circulation disease in my legs .....Yes 1 _____
- I have protein in my urine.....Yes 1 _____

Total _____

If You Scored 4 or More Points

You have a 1 in 5 chance of having chronic kidney disease. At your next office visit, a simple blood test should be checked. Only a professional health care provider can determine for sure if you have kidney disease.

If You Scored 0-3 Points

You probably do not have kidney disease now, but at least once a year, you should take this survey.